The background of the book cover is a high-magnification fluorescence micrograph showing the complex branching structure of dendrites in green. Superimposed on this are numerous small, bright blue spots representing synaptic boutons or vesicles. The overall image has a grainy, scientific quality.

dendrites

third edition

edited by

greg stuart

nelson spruston

michael häusser

OXFORD

Dendrites

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THIRD EDITION

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Foreword to the Third Edition

The new edition of *Dendrites*, by now a well-established book in the neurosciences research community, presents another substantially updated version on what is presently known about dendrites. Six of the chapters of the previous edition have been extensively revised. They document the rapid advance in the elucidation of molecular and biochemical pathways that govern the building of dendrites, mostly of their spines, and that control long term changes in structural and functional connectivity (*vulgo*: plasticity). Here the invertebrate brain is beginning to yield important insights on genetic pathways that drive such changes. The four new chapters indicate that one future avenue of research on dendrites is to investigate the cell-type specificity of dendritic structures, by classification based on e.g. their synaptic input maps and their geometric shapes. One may hopefully find (or not find) structural principles that allow classifications which eventually will facilitate modeling of complete neuronal networks, for example of a sensory or motor system. The new chapters also emphasize the need to measure functions of dendrites, spines, and specific molecules therein, when they act as components of a functioning network, meaning at the molecular level: observations of networks of extensively connected signaling molecules, and at the cellular level: observations of ensembles of dendrites and spines in the intact brain.

Many of the additional new findings depend on recent, spectacular methodological advances that have impacted the study of dendrites. These will be more widely used and may allow, in the not so distant future, more detailed ensembles of dendrites as they function in the intact brain, integrating insights from the level of dendritic molecules to ensembles of spines and ultimately to neurons.

Bert Sakmann
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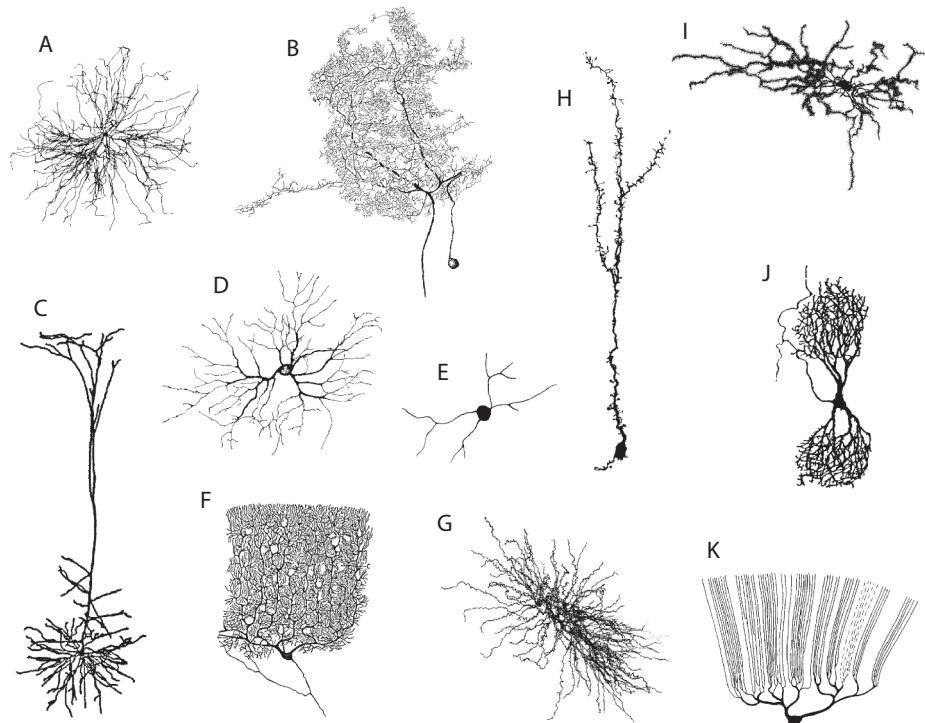


Figure 1 Dendritic trees come in different shapes and sizes. (A) Cat motoneuron. (B) Locust mesothoracic ganglion spiking neuron. (C) Rat neocortical layer 5 pyramidal neuron. (D) Cat retinal ganglion neuron. (E) Salamander retinal amacrine neuron. (F) Human cerebellar Purkinje neuron. (G) Rat thalamic relay neuron. (H) Mouse olfactory granule neuron. (I) Rat striatal spiny projection neuron. (J) Human nucleus of Burdach neuron. (K) Fish Purkinje neuron. Modified from Mel, B.W. (1994) Neural Computation 6:1031–1085.

Preface to the Third Edition

Welcome to the third edition of *Dendrites*. For the reader new to this field, dendrites are the major receiving element of neurons, and represent the targets for synaptic input from thousands of other neurons. Dendrites do more than simply collect and funnel this input to the soma and axon: they shape and integrate synaptic inputs in complex and powerful ways. Thus, it is within these complex branching structures that the real work of the nervous system takes place.

Despite being discovered more than a century ago, dendrites received little attention until the early 1950's (for a historical perspective see Chapter 14). Recently there has been a resurgence of interest in dendrites, spurred on in part by major technical advances, which has led to substantial new information on their properties and role in neuronal function. The main aim of this book is to gather this new information into a single volume covering a wide range of topics on dendrites, from their morphology and development, through to their biochemical, electrical and computational properties. The third edition of *Dendrites* contains fully revised and updated chapters from earlier editions, with six chapters completely rewritten by new authors. In addition, there are four new chapters on molecular machinery in spines, dendritic integration *in vivo*, dendritic connectomics, and generation of synthetic dendrites. The addition of these new topics provides ample evidence that the field continues to expand and grow into new areas.

Our own interest in dendrites dates back to our time as graduate students and postdocs. All three of us did our PhDs in laboratories with a keen interest in dendrites, and we thank Steve Redman, Dan Johnston and Julian Jack for introducing us to this fascinating subject. The main motivation for this book, however, comes from our time together in Bert Sakmann's laboratory, one of the places where the "new age" of dendritic research began. We thank all the authors for their outstanding contributions to the new edition, and Martine Groen and Patrycja Dzialecka for help with the index. We are particularly grateful to the various funding agencies that have supported our work and that of others in the field. We hope that the new edition, like those before it, serves as both a valuable resource to the neuroscience community, and an impetus for further research on these beautiful structures (Figure 1, left).

Greg Stuart
Nelson Spruston
Michael Häusser
September 2015

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List of Abbreviations

2LM	two-layer model	AP5	d-2-amino-5-phosphonopentanoic acid
2pFLIM	two-photon fluorescence lifetime imaging	ARF6	small GTPase
2PLU	two-photon laser uncaging	ASDs	autism spectrum disorders
2PLSM	two-photon laser scanning microscopy	ATD	amino-terminal domain
3DEM	three-dimensional reconstructions from serial section electron microscopy	AZ	active zone
4-AP	4-aminopyridine	BAC	backpropagating action potential-activated Ca^{2+} spike
$A(x_i, x_d)$	steady voltage attenuation from location x_i to location x_d (dimensionless)	BACE1	beta-secretase 1
AA	arachidonic acid	BAF	Brg/Brm associated factor; chromatin-remodeling complex
Abp1	actin-binding protein 1	Bantam	microRNA regulating PKB
AC	association commissural	bAP	backpropagating action potential
ACC	anterior cingulate cortex	BAPTA	1,2-bis(o-aminophenoxy)ethane- N,N,N',N' -tetraacetic acid
Acj6	abnormal chemosensory jump 6 (POU-domain homeobox transcription factor)	BAI1	brain-specific angiogenesis inhibitor
ACPD	1-amino-1,3-dicarboxycyclopentane	BAR	bin amphiphysin rvs
ACSF	artificial cerebrospinal fluid	Bcr	a Rac-GAP
AD	Alzheimer's disease	BDNF	brain-derived neurotrophic factor; ligand for Trk receptor
ADHD	attention-deficit/hyperactivity disorder	bHLH	basic helix-loop-helix (motif in a family of transcription factors)
AHA	azidohomoalanine	BK	large conductance Ca^{2+} and voltage-activated K^+ channel
AHP	Afterhyperpolarization potential	BMP	bone morphogenetic protein
AIS	axon initial segment	BONCAT	bio-orthogonal non-canonical amino acid tagging
AKAP	A-kinase anchor protein	Brg	SWI/SNF2 subunit
ALS	amyotrophic lateral sclerosis	Brm	SWI/SNF2 subunit
Akt	original name for protein kinase B	BSA	bovine serum albumin
AMP	adenosine monophosphate	BTB	domain of zinc finger protein
AMPA	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid	C	input capacitance
AMPAR	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor	C_i	i th coefficient of a multi-exponential function
AN2	auditory ascending neuron	Ca^{2+}	calcium ion
Arc	activity-regulated cytoskeleton-associated protein	C/A	commissural/associational
AP	action potential	CA1	cornu ammonis (Ammon's horn), subregion 1
APC	anaphase-promoting complex (E3 ubiquitin ligase)	CA2	cornu ammonis (Ammon's horn), subregion 2
APN	anterior pagoda neuron	CA3	cornu ammonis (Ammon's horn), subregion 3
APP	amyloid precursor protein		
AP-1	activator protein 1		

CACNAG1	gene encoding the T-type calcium channel subunit Ca _v 3.1	CPEB	cytoplasmic polyadenylation element binding
CAGE	cap analysis of gene expression	CREB	cAMP response element-binding protein
CAM	cell adhesion molecule	CREST	calcium-responsive transactivator
CaMK	calcium/calmodulin-dependent protein kinase	CTD	carboxy-terminal domain
CaMKIIα	calcium/calmodulin-dependent protein kinase 2 alpha	CUB	complement, Urchin EGF, BMP protein domain
CaMKK	calcium/calmodulin-dependent protein kinase kinase	Cut	a homeodomain protein
cAMP	cyclic adenosine monophosphate	CV	coefficient of variation
CaN	calcineurin	cVA	11- <i>cis</i> -vaccenyl acetate (a sex pheromone)
Ca _v	voltage-gated calcium (channels)	Cux	members of the homeodomain family of DNA-binding proteins
CBC	cone bipolar cell	<i>d</i>	diameter
CCD	charge-coupled device	<i>da</i>	dendritic arborization (neuron)
CCK	cholecystokinin	DA	dendritic arborization
Cdc42	cell division cycle 42	DAG	diacylglycerol
Cdk	cyclin-dependent kinase	Dbl	diffuse B-cell lymphoma
CDI	corollary discharge interneuron	DCC	deleted in colorectal carcinoma (netrin receptor; homolog of Frazzled and UNC-40)
cDNA	complementary DNA (deoxyribonucleic acid)	DCMD	descending contralateral motion detector
Celsr1–3	atypical seven transmembrane cadherins 1–3 (orthologs of Drosophila flamingo)	DCPEB	cytoplasmic polyadenylation-binding protein
CF	climbing fiber	DG	dentate gyrus
CFP	cyan fluorescent protein	DHGP	(RS)-3,5-dihydroxyphenylglycine
cGMP	cyclic guanosine monophosphate	DIFO	difluorinated cyclooctyne
Chinmo	chronologically inappropriate morphogenesis (zinc-finger protein)	DIGE	2D-difference gel electrophoresis
ChR2	channelrhodopsin 2	DiI	1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate
CICR	calcium-induced calcium release	DLAs	diffusion-limited aggregates
CKAMP44	cysteine-knot AMPAR modulating protein	dLGN	dorsal lateral geniculate nucleus
CLIMP63	63-kDa cytoskeleton-linking membrane protein	DLM	dorsal longitudinal wing depressor muscle
<i>c_m</i>	membrane capacitance per unit length (μF/cm)	DM-nitrophen	1-(2-nitro-4,5-dimethoxyphenyl)- <i>N,N,N',N'</i> -tetrakis[(oxycarbonyl)methyl]-1,2-ethanediamine
<i>C_m</i>	specific membrane capacitance (μF/cm ²)	DN	dominant negative
CNIH	cornichon (AMPAR regulatory protein)	DR	delayed rectifier
CNQX	6-cyano-7-nitroquinoxaline-2,3-dione	DS	direction selective
CNS	central nervous system	Dscam1	Down syndrome cell adhesion molecule 1
COE	Collier/Olf1/EBF family of transcription factors		
CP	cortical plate		
CPE	cytoplasmic polyadenylation element		

DSGC	direction-selective ganglion cell	FRIL	SDS-digested freeze-fracture replica immunogold labeling
Dsh	disheveled, Wnt signaling protein (same as Dvl)	fry	<i>furry</i> , <i>Drosophila</i> gene
Dvl	disheveled, Wnt signaling protein (same as Dsh)	Fry	furry protein
EC ₅₀	concentration giving half-maximal response	FtTM	fluorescent tRNA for translation monitoring
ECF	extracellular cerebral fluid	Fyn	member of the Src family of non-receptor tyrosine kinases
eEF2	eukaryotic translation elongation factor 2	FXS	fragile X syndrome
EFA6A	exchange factor for ARF6	GABA	gamma-aminobutyric acid
EGFP	enhanced green fluorescent protein	GABA _A R	GABA receptor, type A (ionotropic)
eIF	eukaryotic initiation factor	GABA _B R	GABA receptor, type B (metabotropic)
E-LTP	early-phase long-term potentiation	GAD	glutamic acid decarboxylase
Elk	ETS domain-containing protein (transcription factor)	GAP	GTPase activating (or accelerating) protein
EM	electron microscopy	GC	granule cell; ganglion cell
Eph	ephrin	GCaMP	genetically encoded calcium indicator
EphB	ephrin B receptor	GDP	guanine diphosphate
EphR	ephrin receptor	GEF	guanine nucleotide exchange factor
EPL	external plexiform layer	GF	giant fiber
EPSC	excitatory postsynaptic current	GFP	green fluorescent protein
EPSP	excitatory postsynaptic potential	<i>g_i</i>	steady synaptic conductance perturbation (S)
<i>E_{rev}</i>	reversal potential	GKAP	guanylate kinase-associated protein
ER	endoplasmic reticulum	GluA	AMPA receptor subunit
ERK	extracellular signal-related kinase (same as MAPK)	GluN	NMDA receptor subunit
es	external sensory (neuron)	GluR	glutamate receptor
<i>E_{syn}</i>	synaptic reversal potential	GPCR	G protein-coupled receptor
<i>E(Spl)</i>	Enhancer-of-Split, <i>Drosophila</i> gene	GPe	globus pallidus
ETC	external tufted cells	GR	geometric ratio
F-BAR	FCH Bin-Amphiphysin-Rvs protein dimerization domain	GSG1 L	germ-cell-specific gene 1-like
FAK	focal adhesion kinase	GSK-3β	glycogen synthase kinase 3 beta
FAS	fetal alcohol syndrome	<i>G_{syn}</i>	synaptic conductance
Fez1	fasciculation and elongation protein zeta 1	GTP	guanosine triphosphate
FGF	fibroblast growth factor	GTPase	guanosine triphosphatase
<i>f-I</i>	frequency-current (plot)	h	hour
FMRI	gene encoding FMRP	HA	hemagglutinin
FMRP	fragile X mental retardation protein	<i>ham</i>	<i>hamlet</i> , <i>Drosophila</i> gene
FUNCAT	fluorescent non-canonical amino acid tagging	HCN	hyperpolarization-activated and cyclic nucleotide-gated channels
FRAP	fluorescence recovery after photobleaching	HD	Huntington's disease
FRET	fluorescence resonance energy transfer	HDAC	histone deacetylase (HDAC6, HDAC11)
		HPG	homopropargylglycine
		HSP	homeostatic synaptic plasticity

5-HT	5-hydroxytryptamine	L-LTP	late-phase long-term potentiation
HVA	high-voltage activated	LM	light microscopy
HVC	high vocal centre	LN	local neuron
ICAM5	intracellular adhesion molecule 5	LPTC	lobula plate tangential cell
ICL	peptide containing the intracellular loop of the γ_2 subunit of GABA _A R	LRRTM	leucine-rich repeat transmembrane proteins
ID	intellectual disability	LTD	long-term depression
Id1	centrosomal helix-loop-helix protein	LTP	long-term potentiation
I_{dend}	dendritic membrane current	LVA	low-voltage activated
I&F	integrate and fire	MA	mercaptopropionic acid
Ig	immunoglobulin	mAChRs	muscarinic acetylcholine receptors
IGF-1	insulin like growth factor-1	MAGUK	membrane-associated guanylate kinase
I_h	hyperpolarization-activated current	MALDI-TOF MS	matrix-assisted laser desorption/ionization time of flight mass spectrometry
IP3	inositol 1,4,5-trisphosphate	MAP2	microtubule associated protein-2
IP3 R	inositol 1,4,5-trisphosphate receptor	MAPK	mitogen-activated protein kinase
IPSC	inhibitory postsynaptic current	MAPKK	mitogen-activated protein kinase kinase
IPSP	inhibitory postsynaptic potential	MARCKS	myristoylated alanine-rich C-kinase substrate
IRES	internal ribosomal entry site	MB	mushroom body
IRK	inwardly rectifying potassium (channels)	md	multiple dendrite (neuron)
Jnk	Jun-terminal kinase	mDia	mammalian homolog of diaphanous
k	special unit normalizing constant	MEF2A	myocyte enhancer factor 2A
K _{Ca}	calcium-activated potassium (channels)	MEGAP	Slit-Robo Rho-GAP (also known as SRGAP3)
K _{ir}	inwardly rectifying potassium (channels)	mEGFP	monomeric enhanced green fluorescent protein
K _v	voltage-gated potassium (channels)	MEK	mitogen-activated protein kinase kinase (same as MAPKK)
KA	kainate	mEPSC	miniature excitatory postsynaptic current
KC	Kenyon cell	MetRS	methionyl-tRNA synthetases
KCNK9	gene encoding TASK3 channels	MF	mossy fiber
KCNQ2	gene encoding K _v 7.2 channels	MG	medial giant interneurons
KCNQ3	gene encoding K _v 7.3 channels	mGluR	metabotropic glutamate receptor
KCNT1	gene encoding KCa4.1 (a sodium-activated K ⁺ channel)	mHRP	membrane-targeted horseradish peroxidase
kDa	kilodalton	mICL	mutant form of ICL
λ	space constant (cm)		
l	length		
L	electrot tonic length		
L5	layer 5		
LBD	ligand-binding domain		
LG	lateral giant interneurons		
LGMD	lobula giant motion detector		
LIM	domain of homeobox transcription factors		
Lim1	LIM-domain homeobox transcription factor 1		
LKB1	liver kinase B1 (also known as STK11)		

min	minutes	Nur77	transcription factor
mIPSC	miniature inhibitory postsynaptic current	ODE	ordinary differential equation
MMP	matrix metalloproteinase	O-LM	oriens lacunosum-moleculare
MN	motor neuron	ON1	omega neuron 1
MNI-glutamate	4-methoxy-7-nitroindolinyl-caged L-glutamate	OPHN1	oligophrenin-1
mRNA	messenger ribonucleic acid	OP-puro	O-propargyl-puromycin
mRFP	monomeric red fluorescent protein	ORN	olfactory receptor neuron
MRX	X-chromosome-linked mental retardation	P_o	channel open probability
MSB	multiple synapse bouton	$P_{o,\max}$	maximal channel open probability
MST	minimum spanning tree	P13 K	phosphatidylinositol 3-kinase
mTOR	mammalian target of rapamycin	PA	phosphatidic acid
MTs	microtubules	PABP	poly(A)-binding protein
M/T	mitral/tufted cell	paGFP	photoactivatable green fluorescent protein
Na _v	voltage-gated sodium (channels)	Paip2	PABP-interacting protein 2
nBAF	neuronal BAF	PAK	p21 protein (Cdc42/Rac)-activated serine/threonine kinase
NBQX	2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide	PALM	photoactivated localization microscopy
NCAM	neural cell adhesion molecule	Par	partitioning-defective gene
NDD	neurodevelopmental disorder	PC	phosphatidylcholine; pyramidal cell
NETO	neuropilin toll-like protein	Pcdh	protocadherin; families α (Pcdha), β (Pcdhb), and γ (Pcdhg)
Netrin	axon guidance protein (same as UNC-6)	PCR	polymerase chain reaction
Neuropilin	receptor for Sema-3A	PD	pyloric dilator (neuron)
NF1	neurofibromin	PD	Parkinson's disease
NGF	nerve growth factor; ligand for Trk receptor	PDE	partial differential equation
NMDA	<i>N</i> -methyl-D-aspartic acid	PDZ	PSD-95/disks large (Dlg)/zona occludens
NND	neurodevelopmental disorder	PERK	protein kinase R-like endoplasmic reticulum kinase
NO	nitric oxide	PF	parallel fiber
Notch	single-pass transmembrane receptor	PFC	prefrontal cortex
npBAF	neural progenitor BAF	PG	periglomerular (cell)
n-PIST	neuronal isoform of protein-interacting specifically with TC10	P13 K	phosphoinositide 3 kinase
NRSF	neuron restrictive silencing factor	PICK1	protein interacting with C-kinase 1
NS	non-spiking	PKA	protein kinase A
NSFA	non-stationary fluctuation analysis	PKB	protein kinase B (also known as Akt)
NT	neurotrophin; ligand for Trk receptor	PKC	protein kinase C
NT-3	neurotrophin-3	PKG	cGMP-dependent protein kinase
NT-4	neurotrophin-4	PKR	protein kinase R
		PLC	phospholipase C
		PLD1	phospholipase D1
		PN	projection neuron
		PNH	point neuron hypothesis
		PNS	peripheral nervous system

POU	pituitary, octamer, unc (domain of homeobox transcription factors)	Rnd2	Rho family GTPase 2
PP	perforant path	RNP	ribonucleoprotein
PP1	protein phosphatase 1	Robo	Roundabout (receptor for Slit)
PP2B	protein phosphatase 2B	ROCK	Rho-dependent kinase
PS1	presenilin1	RPM	ribopuromycylation method
PSA	polysialic acid	RyR	ryanodine receptor
PSC	postsynaptic current	s	seconds
PSD	postsynaptic density	SAC	starburst amacrine cell
PSD-93	postsynaptic density protein 93	SAM	sterile alpha motif
PSD-95	postsynaptic density protein 95	SBFI	sodium-binding benzofuran isophthalate
PSD-95-GFP	a fusion protein containing PSD-95 and GFP	SC	Schaffer collateral
PSP	postsynaptic potential	SDS-FRL	SDS-digested freeze-fracture replica immunogold labeling (also known as FRIL)
PV	parvalbumin	SE	status epilepticus
QuaNCAT	quantitative non-canonical amino acid tagging	Sem3A	semaphorin 3A
r_i	intracellular resistivity per unit length (Ω/cm)	Sema	semaphorin
R_i	specific intracellular resistivity ($\Omega \text{ cm}$)	SEP	super ecliptic-pHluorin (a pH-dependent variant of GFP)
r_m	membrane resistance per unit length ($\Omega \text{ cm}$)	SER	smooth endoplasmic reticulum
R_m	specific membrane resistance ($\Omega \text{ cm}^2$)	SF	scaling factor
R_N	input resistance (Ω)	sGC	soluble guanylate cyclase
ΔR_N	change in R_N due to synaptic conductance perturbation (Ω)	SH3	Src homology 3 domain
Rab5	member RAS oncogene family, 5	shRNA	short hairpin RNA
Rac1	Ras-related C3 botulinum toxin substrate 1	SIE	synaptic information efficacy (bits/s)
Raf(1)	mitogen-activated protein kinase kinase kinase	SILAC	stable isotope labeling by amino acids in cell culture
Rap	ras-related protein	siRNA	small interfering RNA
Ras	rat sarcoma gene	SK	calcium-dependent small-conductance K (channel)
RBD	Ras-binding domain	SL	shunt level: $\Delta R_N/R_N$ due to activation of single or multiple conductance perturbations ($0 \leq SL \leq 1$; dimensionless)
RBP	RNA-binding proteins	$SL(x_i, x_d)$	attenuation of SL from x_i to x_d [$SL(x_d)/SL(x_i)$] for a single conductance perturbation at x_i ($0 \leq SL(x_i, x_d) \leq 1$; dimensionless)
RFP	red fluorescent protein	Slack	sequence like a Ca^{2+} activated K^+ channel
RGC	retinal ganglion cell	Slit	ligand for Robo receptor
RGK	small Ras-related proteins that inhibit VSCCs	SN-MN	sensory-motor synapse
Rho	Ras homolog gene family	SNC	substantia nigra pars compacta
RhoA	Ras homolog gene family, member A	SOL	suppressor of lurcher, <i>C. elegans</i> gene
RhoGAP	Rho GTPase activating protein	SPAR	Rap-specific GTPase activating protein
RNA	ribonucleic acid	sptPALM	single-particle tracking photoactivated localization microscopy
RNAi	RNA interference	Src	family of non-receptor tyrosine kinases

SRGAP	Slit-Robo Rho-GAP	TOP	terminal oligopyrimidine
SSA	stimulus-specific adaptation	TPEN	<i>N,N,N',N'</i> -tetrakis-(2-pyridylmethyl)-ethylenediamine
STDP	spike-timing-dependent plasticity	TRAP	translating ribosome affinity purification
STED	stimulated emission depletion microscopy	Trio	triple functional domain
STK11	serine/threonine kinase 11 (also known as LKB1)	Trc	tricornered (kinase)
STORM	stochastic optical reconstruction microscopy	Trk	neurotrophin receptor tyrosine kinase
Su(H)	suppressor-of-hairless	TRP	transient receptor potential (channel)
SWI/SNF2	switch/sucrose non-fermentable nucleosome remodeling complex	TRUP8b	tetratricopeptide repeat-containing Rab8b-interacting protein
SVZ	subventricular zone	TS	Timothy's syndrome (a monogenetic form of ASD)
SynCAM	synaptic cell adhesion molecule 1	TSP	thrombospondin
SynDIG1	synapse differentiation induced gene 1	TTC	thick-tufted cells (in L5 of the cortex)
SynGAP	synaptic GTPase activating protein	TTX	tetrodotoxin
<i>t</i>	time	UNC-5	netrin receptor
τ_0	zeroth (slowest) time constant of a multi-exponential function	UNC-6	axon guidance protein (same as netrin)
τ_{act}	activation time constant	UNC-40	netrin receptor (homologue DCC)
τ_{deact}	deactivation time constant	UTR	untranslated region
τ_{decay}	decay time constant	$\langle V \rangle$	average membrane potential
τ_H	homeostatic plasticity time constant	V_{com}	command potential
τ_i	<i>i</i> th time constant of a multi-exponential function	V_{dend}	dendritic membrane potential
τ_{inact}	inactivation time constant	V_m	membrane potential
τ_m	membrane time constant	V_{rest}	resting potential
τ_{rise}	time constant of rising exponential	V_{soma}	somatic membrane potential
TAMRA	tetramethylrhodamine	V_{syn}	synaptic membrane potential
TARP	transmembrane AMPAR regulatory protein	VACC	voltage-activated calcium current
TASK	two pore acid-sensitive K ⁺ channel	V_e	extracellular voltage
TbR2	type II receptor for TGF- β	VGCC	voltage-gated calcium channels
TEA	tetraethylammonium	VMN	ventromedial nucleus of the hypothalamus
TGF- α	transforming growth factor alpha	VSCC	voltage-sensitive calcium channel
TGF- β	transforming growth factor beta	VZ	ventricular zone
Tiam1	T-lymphoma invasion and metastasis-inducing protein 1	w	weeks
TL	thresholded linear	Wnt	family of secreted proteins
TLE	temporal lobe epilepsy	Wnt7b	member of Wnt family
TLN	telencephalin (same as ICAM5)	<i>x</i>	dendritic location
TMD	transmembrane domain	X	electrotonic distance
TN1	an auditory ascending neuron	YFP	yellow fluorescent protein
TNIK	Traf2- and Nck-interacting kinase	ZBP1	zipcode-binding protein 1
		Zfp312	zinc finger protein 312 (also known as Fez1)

Chapter 1

Dendrite structure

Kristen M. Harris and Josef Spacek

Summary

Dendrites extend from the cell body of the neuron and are specialized for processing synaptic information. Dendritic arbors assume diverse forms, branching in characteristic spatial domains where they receive specific synaptic inputs. Synaptic inputs occur directly on the shaft of some dendrites, but others have dendritic spines or specialized enlargements that host synapses. These specializations also occur in many different forms related to both local connectivity and the need for compartmentalization of molecular signaling. The use of three-dimensional reconstructions from serial section electron microscopy (3DEM) has shown that these dendritic synaptic specializations differ widely in dimensions, distribution, and intracellular composition. The shape and composition of dendrites and their synaptic specializations are influenced throughout life by genes, environment, learning, memory, and neuropathological conditions. Therefore, understanding the structure of dendrites is essential to understanding their function.

Introduction

What is the purpose of dendrites? How are their diverse shapes related to neuronal function? Why are specialized compartments formed at the sites of synaptic contacts? The Spanish neuroanatomist Santiago Ramón y Cajal posed, and to a remarkable extent answered, these questions more than 100 years ago, as summarized in his compendium *Histology of the Nervous System of Man and Vertebrates* (Ramón y Cajal, 1995). He used a variety of experimental approaches, especially the technique developed by the Italian neuroanatomist Camillo Golgi to stain individual neurons. From this work, he proposed that axons and dendrites do not anastomose in continuity between different neurons (Ramón y Cajal, 1954) as was originally suggested by Golgi (Golgi, 1908). The importance of the Golgi technique, together with Ramón y Cajal's astute analyses, won them the Nobel prize in physiology or medicine in 1906 (Shepherd, 1991). Their Norwegian contemporary, Fridtjof Nansen, took a comparative approach, also using the Golgi technique to study the structure of the nervous system in diverse organisms from crayfish to hagfish and mammals, to conclude that the complexity of the dendritic processes and the so-called "dotted substance" (neuropil), where axons and dendrites communicate, is "more complicated and extensive the higher an animal is mentally developed" (Nansen, 1887). Similarly, Ramón y Cajal argued that phylogenetic differences in specific neuronal morphologies support the relationship between dendritic complexity and the number of connections. For example, the complexity of dendritic arbors increases with increasingly complex nervous systems on different types of vertebrate neurons, including cerebellar Purkinje cells, cortical pyramidal cells, and mitral cells of the olfactory bulb (see Chapter 2 for more phylogenetic differences). Although the work of Ramón y Cajal favored what became known as the neuron doctrine, namely that communication occurred at junctions between discrete neurons,

definitive proof awaited the advent of the electron microscope and the demonstration of a synaptic cleft between the presynaptic axon and the postsynaptic dendrite (Palade and Palay, 1954; De Robertis and Bennett, 1955).

Modern approaches to the evaluation of the properties of dendrites, axons, and glia provide reasons to reconsider the strictly unidirectional, axo-dendritic patterns of synaptic activation championed in the original neuron doctrine (Shepherd, 1991; Guillery, 2005; Kruger and Otis, 2007). As Ramón y Cajal and Nansen correctly surmised, dendrites usually comprise the receptive surfaces of a neuron, while axons usually deliver signals from other neurons. However, dendrites can also be output devices (see Chapter 21). For example, dendrites make reciprocal synapses having both pre- and postsynaptic components on corresponding pairs of dendrites in the retina, olfactory bulb, lateral geniculate nucleus, some cortical neurons, and some peripheral sensory neurons (Price and Powell, 1970; Lieberman, 1973; Sloper and Powell, 1978; Ellias and Stevens, 1980). Conversely, axons also provide receptive surfaces, forming axo-axonic synapses in many places throughout nervous systems (Cuello, 1983). Furthermore, dendrites, axons, and glia all form gap junctions—bidirectional sites of communication through channels that are arranged so as to span the adjacent membranes and bridge the extracellular space (Bennett and Zukin, 2004). While such dendro-dendritic, axo-axonic, and gap junction-mediated communications are relatively rare compared with axo-dendritic communication, they nevertheless play important roles in both direct neuron-to-neuron communication and modulation of that communication.

More complex dendritic arbors have the potential to host more synapses. A neuron with a roughly spherical cell body has a very limited surface area for receiving inputs. By extending dendrites, the neuron increases its surface area without excessively increasing cell volume. For example, the dendrites of cat spinal motoneurons have a volume of $300,000 \mu\text{m}^3$ and provide a surface area of $370,000 \mu\text{m}^2$ for synaptic input (Ulhfake and Kellerth, 1981). To provide an equivalent surface area, a spherical cell body would have to have a diameter of $342 \mu\text{m}$ and a volume of more than $20,900,000 \mu\text{m}^3$, a factor of 70 greater than the dendrite-bearing motoneurons.

The convolution of the cell surface into a dendritic arbor also facilitates the packing of a larger number of neurons in close proximity and extends their reach to more axons. However, the expanded dendritic arbor does not necessarily correspond one-to-one with increased synapse density. For example, only 20% of the axons within reach of or touching a dendrite of a hippocampal pyramidal cell actually form a synapse on that dendrite; furthermore, the summed surface area occupied by those synapses is only about 10% of the total dendritic surface area (Harris and Stevens, 1988a, 1989; Mishchenko et al., 2010). Thus, 3DEM findings suggest that enhancing synaptic connectivity is probably not the primary function of dendrites, their spines, and other synaptic specializations (Peters and Kaiserman-Abramof, 1970; Swindale, 1981; Gray, 1982).

Dendrites have a relatively local reach compared with the axon. The axon, emerging either from the soma or from a proximal dendrite, may extend to distant targets a meter or more away from the cell body (e.g., motoneurons and corticospinal projection neurons in large mammals). In contrast, dendrites are rarely longer than 1–2 mm, even on the largest neurons, and are often much smaller (Table 1.1). The diameter of dendrites at their origin from the cell body is proportional to that of the cell body itself. Dendrites taper and ramify in proportion to their size, such that the total length and number of branches are also correlated with dendrite caliber. Thus, larger neurons typically have both larger cell bodies and more extensive dendritic fields. Compare and contrast, for example, the dendritic arbor of a Purkinje cell that integrates input from multiple cell types with the tiny granule cell that typically forms just four small dendritic branches (on average), each ending in a claw that forms synapses (Fig. 1.1).

Table 1.1 Typical dimensions of dendrites for a few types of neuron

Neuron	Average soma diameter (μm)	Number of dendrites at soma	Proximal dendrite diameter (μm)	Number of branch points	Distal dendrite diameter (μm)	Dendrite extent* (μm)	Total dendritic length (μm)
Cerebellar granule cell (cat)	7	4	1	0	0.2–2	15	60
Starburst amacrine cell (rhesus)	9	1	1	40	0.2–2	120	—
Dentate gyrus granule cell (rat)	14	2	3	14	0.5–1	300	3,200
CA1 pyramidal cell (rat)	21						11,900
basal dendrites		5	1	30	0.5–1	130	5,500
stratum radiatum		1	3	30	0.25–1	110	4,100
stratum lacunosum-moleculare				15	0.25–1	500	2,300
Cerebellar Purkinje cell (guinea pig)	25	1	3	440	0.8–2.2	200	9,100
Principal cell of globus pallidus (human)	33	4	4	12	0.3–0.5	1,000	7,600
Meynert cell of visual cortex (macaque)	35						15,400
basal dendrites		5	3	—	—	250	10,200
apical dendrites		1	4	15	2–3	1,800	5,200
Spinal α -motoneuron (cat)	58	11	8	120	0.5–1.5	1,100	52,000

*The average distance from the cell body to the tips of the longest dendrites.

Sources: Palay (1978), Ulphake and Kellerth (1981), Ito (1984), Yelnik et al. (1984), Mariani (1990), Claiborne et al. (1990), Rapp et al. (1994), Bannister and Larkman (1995a).

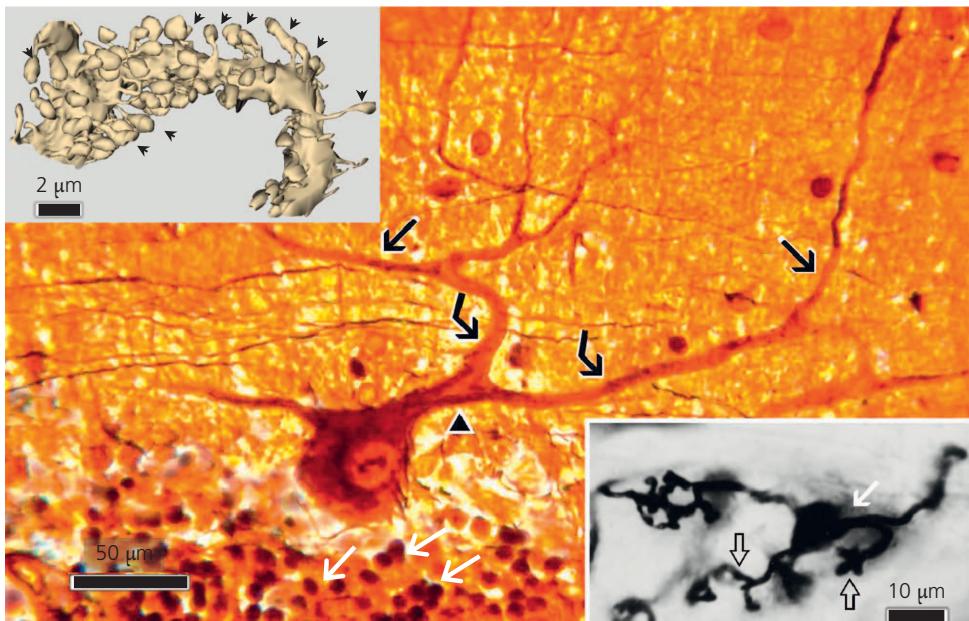


Fig. 1.1 Dendrites often look like tree branches, and their name derives from the Greek word for tree, *dendron*. In this image of the cerebellar cortex of a mouse impregnated with silver using the Bielschowsky method, a thick primary dendrite (triangle, main image) extends from the upper right of the cell body of a Purkinje neuron. The primary dendrite branches into secondary (curved arrows) and tertiary (straight arrows) dendrites within the plane of the section. Terminal dendritic branchlets are not visible with this method. In the upper inset 3DEM reveals the high density of protrusions, known as dendritic spines (small arrows). Beneath the large Purkinje cell can be seen a layer of smaller granule cell bodies (white arrows). In the lower inset higher magnification of a Golgi-impregnated granule cell (white arrow) reveals that this neuron possesses just a few, short dendrites ending in claw-like formations (open arrows).

In this chapter we consider the functional significance of the diversity of dendritic arbor structures together with diversity in the structure and subcellular composition of individual dendrites, dendritic spines, and other synaptic specializations. We show that dendrites contain subcellular structures including smooth endoplasmic reticulum, microtubules, endosomes, Golgi apparatus, polyribosomes, and others that can rapidly direct resources to sites of synaptic activation and plasticity. We end with an analysis of ultrastructural changes that occur in synaptic specializations during long-term potentiation, a cellular mechanism of learning, and various neuropathological conditions.

Dendritic arbors

Both the geometry (Fig. 1.2 and Table 1.2) and the density of dendritic branching (Figs 1.2 and 1.3) define the dendritic arbor and are important for understanding connectivity in the nervous system. Dendritic arbors are shaped to receive inputs in particular spatial domains. At one extreme, a dendritic arbor connects a single remote target to the rest of the neuron, providing a highly

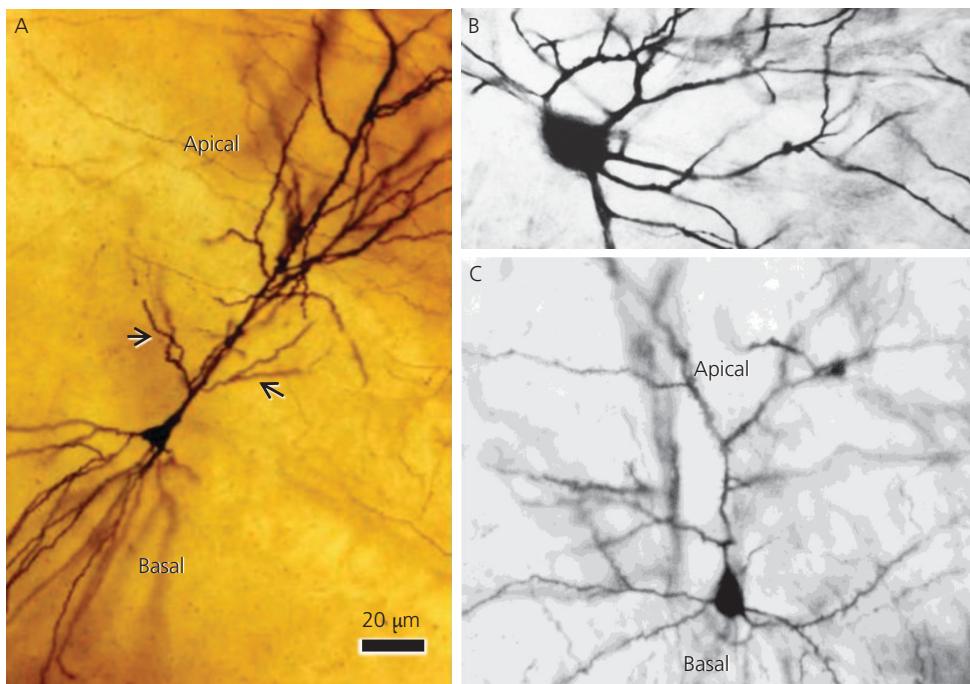


Fig. 1.2 Neurons impregnated with silver by the Golgi method. (A) A pyramidal neuron from hippocampal area CA1 of the rat, in which basal and apical dendrites ramify in separate conical domains. Between these two denser domains, a few dendrites extend obliquely (arrows) from the apical dendrite as it passes through stratum radiatum. (B) A rat thalamic projection neuron exhibits many primary dendrites extending from the cell body with a spherical radiation. (C) A pyramidal neuron from the parietal area of mouse cerebral cortex has a sparsely branching apical dendrite and a few dendrites that extend almost horizontally from the base of the cell body. All three images have been scaled to match the magnification and scale bar in A.

Part A adapted from *Journal of Neuroscience Methods*, 2(4), Kristen M. Harris, William L.R. Cruce, William T. Greenough, and Timothy J. Teyler, A Golgi impregnation technique for thin brain slices maintained in vitro, pp. 363–71, Figures 6a and 5c, Copyright 1980, Elsevier. With permission from Elsevier. Part B reproduced from Josef Spacek and A.R. Lieberman, Ultrastructure and three-dimensional organization of synaptic glomeruli in rat somatosensory thalamus, *Journal of Anatomy*, 117(3), p. 489, Figure 2a © 1974, John Wiley and Sons Ltd.

selective arborization. At the other extreme, dendritic branches occupy most of the domain in a *space-filling arborization*. The majority of dendritic arborizations lie between these extremes and are considered to be *sampling arborizations*. Here we describe various geometrical patterns as a basis for understanding the consequences of diversity in dendritic arborization on synaptic connectivity.

Adendritic neurons have no dendrites and usually have a single branched axon arising from the soma (Table 1.2). Examples of adendritic neurons occur in the trigeminal, nodose, and dorsal root ganglia. These neurons conduct sensory input from the periphery to the central nervous system. Although GABAergic and glutamatergic axons have been found to surround the soma of these

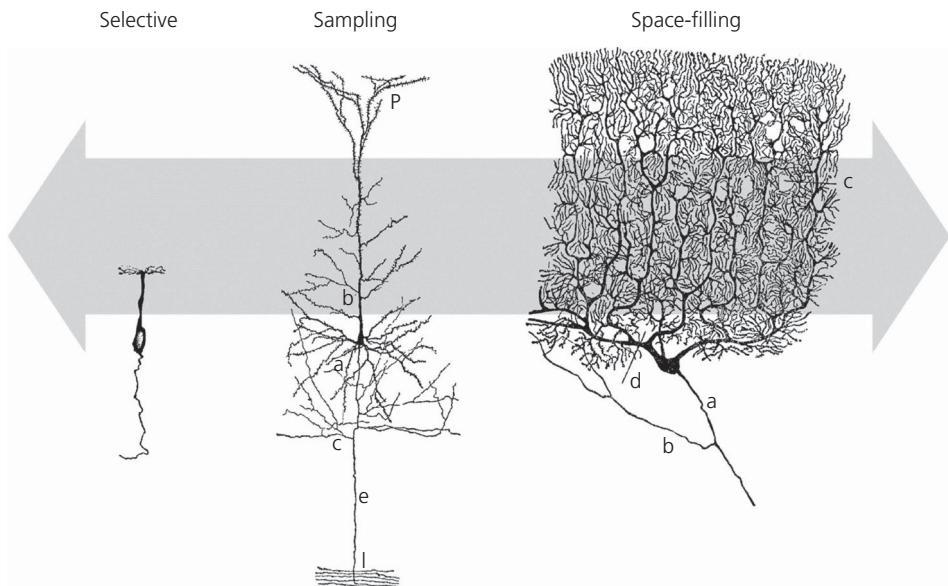


Fig. 1.3 Differences in dendritic arbor density reflect differences in connectivity. At one extreme are selective arborizations in which each dendrite connects the cell body to a single remote target. An olfactory sensory cell illustrates this pattern. At the other extreme lie the space-filling arborizations in which the dendrites cover a region, as with the cerebellar Purkinje cell. Intermediate arbor densities are referred to as sampling arborizations, as demonstrated by a pyramidal cell from cerebral cortex.

The material in this figure has been adapted from Ramón y Cajal's *Histology of the Nervous System of Man and Vertebrates*, translated by Neely Swanson and Larry W. Swanson pp. 52–4, figures 7, 8, and 9 © 1995, Oxford University Press.

adendritic neurons (Sholl, 1953, 1967; Stoyanova et al., 1998), to our knowledge no electron microscopy studies have been done to determine whether these processes make synapses on them.

Dendritic arbors of many slender neurons found throughout the brain have a *spindle radiation*, with two sparsely branching dendrites emerging from opposite poles of the soma. Examples include Lugano cells of the cerebellar cortex (Palay and Chan-Palay, 1974) and bipolar cells of the cerebral cortex, which are usually interneurons expressing calretinin in combination with neuropeptides (Cauli et al., 2014). This arborization would perform sparse sampling of two distinct input domains.

Stellate neurons of the central nervous system have a dendritic arbor with a *spherical* or *partial spherical radiation* depending on their position with respect to the boundaries of the region in which they reside. The dendrites of the cerebellar granule cells have spherical radiations that make synapses selectively at their claw-like ends (Fig. 1.1). Other examples include spinal cord neurons and principal neurons in non-laminated nuclei such as the inferior olive, pontine nuclei, striatum, and thalamus. Interneurons are defined as having axons that terminate locally within a particular brain region, and many interneurons have dendritic arbors with spherical radiations. Although attempts have been made to describe in general terms the variety of stellate arbor types

Table 1.2 Some characteristic dendritic arborization patterns

Pattern	Characteristics	Examples
Adendritic	Cell body lacks dendrites	Dorsal root ganglion cells Sympathetic ganglion cells
Spindle radiation	Two dendrites emerge from opposite poles of the cell body and have few branches	Lugaro cells Bipolar cells of cortex
Spherical radiation	Dendrites radiate in all directions from cell body	Spinal neurons Neurons of subcortical nuclei (e.g., inferior olive, pons, thalamus, striatum) Cerebellar granule cells
Partial	Dendrites radiate from cell body in directions restricted to a part of a sphere	Neurons at edges of "closed" nuclei (e.g., Clarke's column, inferior olive, vestibular nuclei)
Laminar radiation		
Planar	Dendrites radiate from cell body in all directions within a thin domain	Retinal horizontal cells
Offset	Plane of radial dendrites offset from cell body by one or more stems	Retinal ganglion cells
Multi	Cell has multiple layers of radial dendrites	Retinal amacrine cells

Table 1.2 (continued) Some characteristic dendritic arborization patterns

Pattern	Characteristics	Examples
Cylindrical radiation	Dendrites ramify from a central soma or dendrite in a thick cylindrical (disk-shaped) domain	Pallidal neurons Reticular neurons
Conical radiation	Dendrites radiate from cell body or apical stem within a cone or paraboloid	Granule cells of dentate gyrus and olfactory bulb Primary dendrites of mitral cells of olfactory bulb Semilunar cells of piriform cortex
Biconical radiation	Dendrites radiate in opposite directions from the cell body	Bitufted, double bouquet, and pyramidal cells of cerebral cortex Vertical cells of superior colliculus
Fan radiation	One or a few dendrites radiate from cell body in a flat fan shape	Cerebellar Purkinje cells

(Ramón-Moliner, 1968), classification often comes down to individual characteristics. For example, in the ventral cochlear nucleus, descriptive morphologies of the dendritic arbor include spherical bushy, globular bushy, stellate, bushy multipolar, elongate, octopus, and giant (Ostapoff et al., 1994). These descriptors are not readily applicable to stellate neurons in other areas of the brain where, for example, in the cerebral cortex the primary distinguishing characteristic of the many stellate interneurons is not their dendritic arbors but rather their axonal arborization pattern (Jones, 1975). Overall, the morphology of stellate dendritic arbors suggests that they perform selective or sparse sampling of axonal inputs that congregate locally within the neuropil of a circumscribed region.

Dendritic arbors with a *laminar radiation* spread in arbitrary directions from the cell body but are restricted to a *planar* region, as seen, for example, in horizontal cells of the retina (Kolb et al., 1994) and in some interneurons of the hippocampus (Parra et al., 1998). Nearly 20 kinds of retinal ganglion cells are distinguished by their planar dendritic arborization patterns that are *offset* by one or more dendritic stems (Fukuda et al., 1984; Sterling and Demb, 2004; Wingate et al., 1992). These dendrites can ramify into one or more layers to access multiple types of afferents and provide either a selective or sampling arborization that gives rise to distinct physiological functions.

Multi-laminar radiation characterizes the dendritic arborizations of retinal amacrine cells. At least 26 different types of amacrine cell can be identified based on their dendritic arborization and retinal tiling patterns alone (Mariani, 1990; Kolb et al., 1992; MacNeil and Masland, 1998). As with ganglion cells, the morphological differences in the extent of the dendritic arbors of amacrine cells also denote differences in the computational properties of these neurons.

Some dendritic arbors have a strictly *cylindrical radiation*. For example, in the globus pallidus of primates, the dendrites of the large pallidal neurons have a cylindrical radiation that fills spatial domains approximately 1,000–1,500 μm in diameter and 250 μm thick (Yelnik et al., 1984). These dendrites run parallel to the boundaries of the globus pallidus and thus perpendicular to incoming striatal axons, such that each neuron receives a broad distribution of input in their dense-sampling to space-filling arbors.

Many types of granule cells have a dendritic arbor with a *conical radiation*, where the dendrites radiate from one side of the neuron in a conical or paraboloidal fashion. This selective but dense sampling pattern characterizes granule cells in the hippocampal dentate gyrus, for example, where axonal input is strictly layered. Here, the primary excitatory input from the lateral entorhinal cortex synapses in the outer third, input from the medial entorhinal cortex synapses in the middle third, and from the contralateral hippocampus in the inner third of the dendritic arbor (Steward, 1976; Amaral et al., 1990; Claiborne et al., 1990).

The dendritic arbors of pyramidal cells often have two distinct conical arbors, one at the apex and the other at the base of the pyramid-shaped cell body. This configuration corresponds to a *biconical radiation* and may be characterized by different afferents contacting the basal versus apical domains. Furthermore, the length of an apical dendrite of a cortical pyramidal cell depends on how far the cell body is from the outermost layer in which it ramifies its apical tuft. Cells very near the outermost layer do not usually have an apical stem at all, since one is not required to reach the appropriate axons (Ramón y Cajal, 1995). Other pyramidal cells may have three distinct spatial domains, including the apical and basal cones as well as a central cylinder. This pattern occurs, for example, in the large pyramidal cells of hippocampal area CA1 (Fig. 1.2A). There the apical tuft arborizes in stratum lacunosum-moleculare to receive perforant path input from entorhinal cortex. The middle cylindrical arbor in stratum radiatum receives the Schaffer collaterals from CA3 pyramidal cells. The basal cone extends into stratum oriens where it receives afferents from a more proximal part of CA3 (Amaral and Witter, 1989). A similar pattern is frequently seen among other neocortical pyramidal cells (Feldman and Peters, 1978; Prieto and Winer, 1999).

The *fan radiation* is basically an elaboration of the conical radiation that is more space-filling, and is flatter. This pattern is best characterized by the dendritic arbor of cerebellar Purkinje cells that synapses with a large fraction of the parallel fiber axons that pass through it (Palay and Chan-Palay, 1974; Harvey and Napper, 1991).

This summary of common arborization patterns is far from exhaustive. Many other arbors can be characterized by elaborations or combinations of the basic patterns outlined in Table 1.2. Mitral cells of the olfactory bulb, for example, can exhibit a number of variations, such as laminar radiation of secondary dendrites from the soma or a branch in the apical stem, giving rise to two

separate dendritic tufts (Kishi et al., 1982). The apical stem of pyramidal cells in CA1 may likewise bifurcate midway through stratum radiatum, giving rise to a pair of conical tufts (Bannister and Larkman, 1995a). Some types of neocortical pyramidal cells have an essentially stellate or planar arbor around the cell body, rather than a conical arbor of basal dendrites (Ramón y Cajal, 1995; Prieto and Winer, 1999).

Several approaches have been developed for estimating the density of dendritic arborizations (Uylings and van Pelt, 2002; Scorcioni et al., 2004). The simplest scheme is to count the number of branch points in the entire dendritic arbor (Table 1.1). The *centrifugal method* identifies dendrites by their branching order, with primary dendrites emerging from the cell soma, their first branches being classified as secondary branches, and with increasing order until the tips are reached; the number of dendrite segments of each order characterizes arbor branching (Fig. 1.4). Sholl analysis is the most widely used centrifugal analysis method (Sholl, 1953, 1967), and modern, computer-assisted analyses are available that are well documented and referenced (see http://fiji.sc/Sholl_Analysis). Such schemes show how branched a neuron is but do not measure the degree to which the branches fill the space of the dendritic arbor.

The *fractal dimension* is an estimate of the degree to which an arborization fills its spatial domain. From basic geometry, linear objects have a dimension of one; planar objects have a dimension of two; solid objects, such as a sphere, have a dimension of three; and *fractal* objects fill a fraction of the space in which they are embedded. Dendritic arbors are not fractal objects in the strict mathematical sense, but the concept of a fractal dimension is useful for quantifying their space-filling properties (Smith et al., 1989; Knifflki et al., 1994; Panico and Sterling, 1995; Fernández and Jelinek, 2001). Selective arborizations have fractal dimensions close to one whereas sampling arborizations have fractal dimensions greater than one but less than the dimension of the spatial domain in which they arborize. Space-filling arborizations have fractal dimensions closer to the dimension

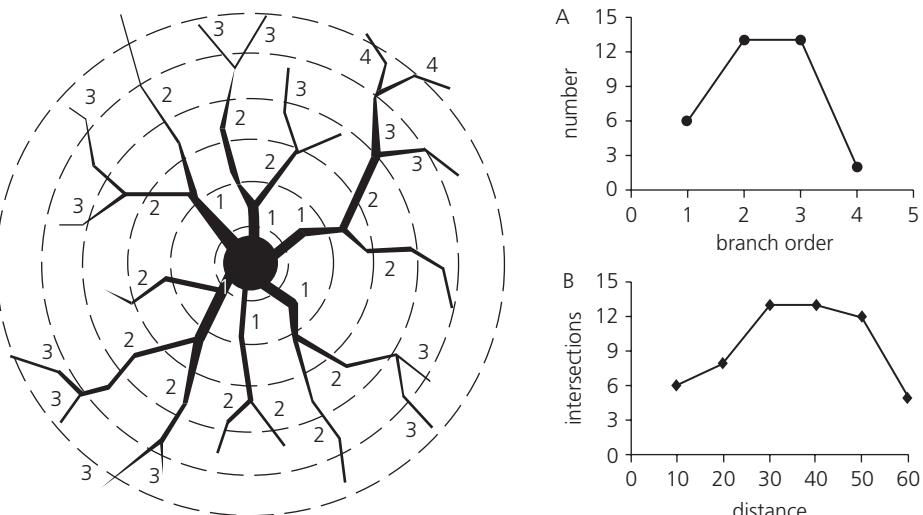


Fig. 1.4 Methods for quantifying dendritic arbors. (A) A plot of the number of branches of each order using the centrifugal method of branch ordering. (B) A Sholl plot with counts of the number of intersections the dendritic tree makes with circles of increasing radius from the center of the soma. When three-dimensional data are available, concentric spheres are used.

of the geometrical domain they occupy, namely close to two for planar domains and closer to three for solid domains such as spheres. As with Sholl analysis, there are modern, computer-assisted approaches available to perform fractal analyses of dendritic arbors that are well documented and referenced (see <http://rsb.info.nih.gov/ij/plugins/fraclac/fraclac.html>).

Some examples illustrate the effect of the branching pattern and fractal dimension on connectivity. Pyramidal neurons have selective arborizations in a three-dimensional volume with fractal dimensions of about 1.4–1.5 relative to 3.0 that would fill the volume they occupy (Porter et al., 1991; Scorcioni et al., 2004). In contrast, retinal ganglion cells have essentially two-dimensional arbors, with a fractal dimension of about 1.5 (Wingate et al., 1992; Fernández and Jelinek, 2001). Fractal dimensions of the dendritic arbors of Purkinje cells range from a value of 1.13 in lampreys up to 1.86 in humans, showing an increase with phylogeny to a nearly complete coverage of the two-dimensional area they occupy (Takeda et al., 1992). To understand how the differences in fractal dimension relate to differences in connectivity, consider the retinal ganglion cell, which has a *sampling* planar arbor covering 25,000 μm^2 and a fractal dimension of 1.5 but receives only 2,000 synapses (Sterling and Demb, 2004). In contrast, a Purkinje cell with a *space-filling* planar arbor covering an area of 50,000 μm^2 with a fractal dimension above 1.5 receives about 160,000 synapses (Smith et al., 1989; Harvey and Napper, 1991). Somewhere in between, the dendritic arbors of hippocampal CA1 pyramidal cells receive about 20,000–30,000 synapses (Shepherd and Harris, 1998).

These analyses suggest that the complexity of the dendritic arbor reflects a propensity for connectivity; however, the actual connectivity also depends on the axonal arborization pattern and the direction of axonal projections relative to the dendritic arbor. For example, parallel fiber axons are orthogonal to the dendritic trees of Purkinje cells, permitting only a few synapses per axon per dendritic arbor (Palay and Chan-Palay, 1974; Harris and Stevens, 1988a; Xu-Friedman et al., 2001). In contrast, the climbing fiber from the inferior olive arborizes within the plane of the dendritic arbor of a single Purkinje cell, wrapping itself around the dendrite and forming many synapses (Harvey and Napper, 1991; Xu-Friedman et al., 2001). Similarly, pyramidal cells may receive many synapses from a single axon which runs parallel to a dendritic segment or a few synapses from axons which traverse its dendrites perpendicularly (Sorra and Harris, 1993). Thus, dendritic arbors provide a rich array of patterns that provide capacity for both specificity and diversity in connectivity. The pattern of the dendritic arbor is also sensitive to experience (Volkmar and Greenough, 1972; Greenough et al., 1973; Chang and Greenough, 1982). How particular arbor morphologies affect physiology, behavior, and the capacity for learning is examined in other chapters.

Intracellular structure of dendrites

Dendrites contain numerous subcellular structures that provide local resources at a distance from the soma. These structures include the Golgi apparatus, rough endoplasmic reticulum with ribosomes, free polyribosomes, smooth endoplasmic reticulum, mitochondria, cytoskeletal elements, smooth vesicles, and organelles of the endosomal pathways. These structures provide dendrites with the resources needed to respond rapidly to local changes in synaptic efficacy.

Electron microscopy reveals that the contents of large proximal dendrites are similar to those of the cell soma (Fig. 1.5A). The *Golgi apparatus* (Fig. 1.5A) and the *rough endoplasmic reticulum* (Fig. 1.5B) extend well into proximal dendrites. These also form mobile units called Golgi outposts that occur particularly at dendritic branch points but can also move into the thinner more distal dendrites (Horton and Ehlers, 2003, 2004; Horton et al., 2005; Cui-Wang et al., 2012). The Golgi apparatus is involved in posttranslational modifications of proteins that are synthesized

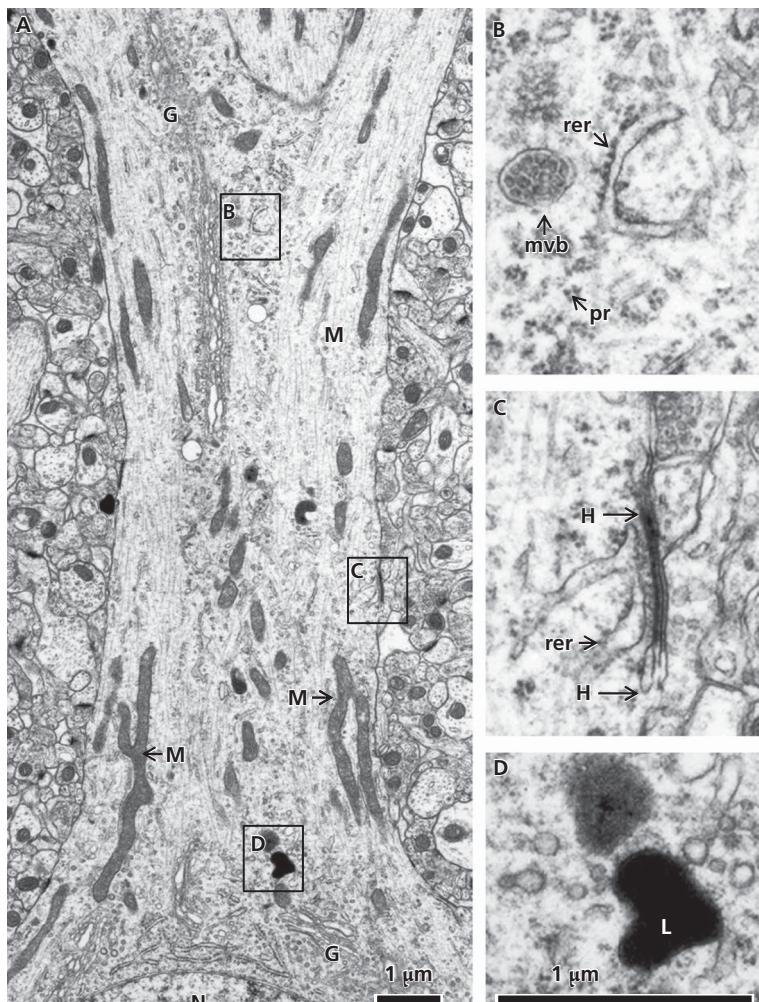


Fig. 1.5 Organelles extend from soma into the apical dendrite of an adult rat CA1 pyramidal cell: (A) nucleus (N), Golgi apparatus (G), mitochondria (M, branch points indicated by an arrow); (B) detail of rough endoplasmic reticulum (rer), polyribosomes (pr), multivesicular body (mvb); (C) two arrows mark the extent of a hypolemmal cisterna of the endoplasmic reticulum (H) that also has rer in close proximity; (D) lysosome (L).

by ribosomes on the rough endoplasmic reticulum. As discussed later, the spine apparatus has a structure and molecular composition suggesting that it too may be a Golgi outpost that occurs in some dendritic spines (Spacek, 1985a; Spacek and Harris, 1997; Pierce et al., 2000, 2001).

Polyribosomes are clusters of free ribosomes that occur throughout the dendritic cytoplasm (Fig. 1.5B) and in some dendritic spines and other dendritic synaptic specializations as discussed later. Whereas the rough endoplasmic reticulum synthesizes transmembrane proteins, free polyribosomes synthesize cytoplasmic proteins. Thus, proteins can be synthesized locally in the dendrite, although the ribosomes themselves are synthesized in the nucleolus and ribonucleic acids

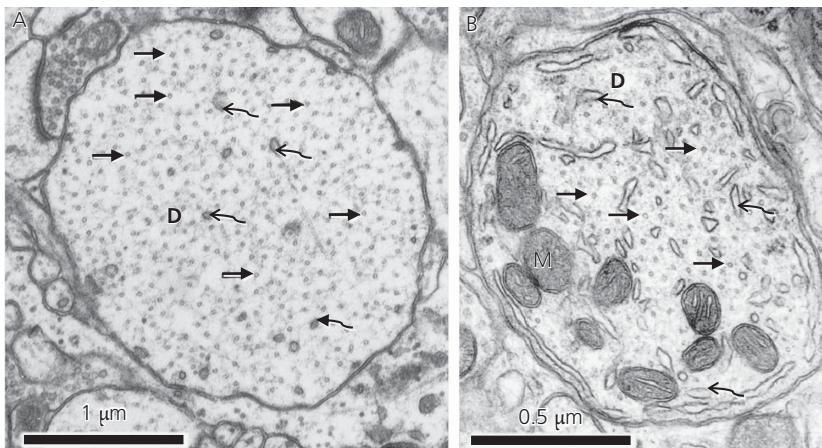


Fig. 1.6 The effect of subcellular organelles on the distribution of microtubules in the cytoplasm. **(A)** An apical dendrite (D) in the middle of hippocampal CA1 stratum radiatum from an adult rat has a regular array of microtubules (arrows) interspersed with a few, narrow tubules of SER (wavy arrows), and no mitochondria. **(B)** A secondary dendrite (D) of an adult mouse Purkinje cell with microtubules (horizontal arrows) interspersed between many elements of SER (wavy arrows) and nine mitochondrial profiles (M). Note that the magnification of part B is exactly twice that of part A.

(RNAs) are transcribed in the nucleus and both are subsequently transported into the dendrites (Bassell et al., 1998; Krichevsky and Kosik, 2001).

Smooth endoplasmic reticulum (SER) is the largest subcellular organelle, forming an essentially continuous network throughout the neuron (Harris and Stevens, 1988a, 1989; Martone et al., 1993; Spacek and Harris, 1997; Cooney et al., 2002). Hypolemmal cisternae (Fig. 1.5C) form junctions between the SER and the plasma membrane (Henkart et al., 1976) where store-operated calcium channels regulate the replenishment of calcium in the SER from the extracellular space, among other functions (Majewski and Kuznicki, 2015). In single sections, the SER usually appears as tubules or flattened cisternae with a clear interior that is bounded by a wavy membrane (Fig. 1.6). In a three-dimensional view obtained by reconstruction from serial sections the continuous reticulum becomes apparent throughout dendrites and some spines (Fig. 1.7). The SER regulates calcium locally and provides posttranslational modification and trafficking of integral membrane proteins (Higley and Sabatini, 2008; Ehlers, 2013). In the dendritic shaft, the SER forms local areas of complexity that retain and enhance the delivery of cargo to nearby synapses (Cui-Wang et al., 2012).

Mitochondria in dendrites are typically rod-shaped organelles that run parallel to the long axis of the dendrite or curve into a dendritic branch (Fig. 1.5). They vary greatly in length, with single mitochondria extending more than 10 μm while others can form a branched network more than 25 μm long (Popov et al., 2003, 2005). In thin dendrites ($<0.5 \mu\text{m}$ in diameter), a single mitochondrion usually lies in the center of the SER network (Fig. 1.7), and the SER is often found to surround individual mitochondria (see Fig. 1.9; Spacek and Lieberman, 1980) in a similar way to how it surrounds the nucleus. This intimate relationship between the SER and mitochondrial and nuclear membranes suggests inter-organelle communication, perhaps to regulate calcium during RNA synthesis, which occurs in both the mitochondria and the nucleus. In stratum radiatum of

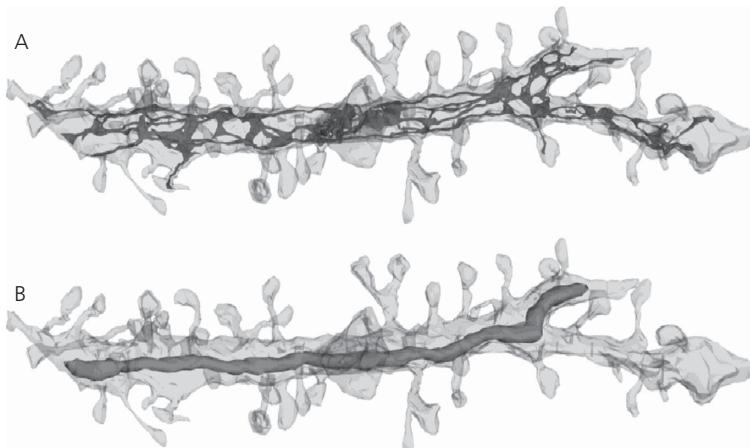


Fig. 1.7 Reconstruction of a segment of a lateral dendritic branch (12.5 μm long) from a CA1 pyramidal cell in the stratum radiatum of the hippocampus of an adult rat: (A) network of SER; (B) a mitochondrion runs down the center of the dendrite and is surrounded by the SER network.

area CA1, mitochondria comprise about 2% of the intracellular volume in the apical stem dendrites and fill 13% of the thinnest dendritic branches of the apical tuft (Nafstad and Blackstad, 1966). Mitochondria accumulate in dendrites at large synaptic specializations, for example in thalamic nuclei (Lieberman and Spacek, 1997) and in highly branched synaptic specializations of the CA3 pyramidal neurons known as thorny excrescences (Chicurel and Harris, 1989; Rollenhagen et al., 2007). As is known from time-lapse movies in cell culture (Overly et al., 1996), mitochondria are highly dynamic structures and can be redistributed to regions of synaptogenesis or enlarging synapses in response to metabolic demands (Li et al., 2004; Kraev et al., 2009).

The *cytoskeleton* of dendrites is composed of *microtubules*, *neurofilaments (intermediate filaments)*, and *actin filaments (microfilaments)*. Microtubules are long, thin structures, ~25 nm in diameter and ~90 μm long (Fiala et al., 2003), that are usually oriented parallel to the longitudinal axis of the dendrite, although they can be found to curve into dendritic branches from the parent dendrite. In regions of the dendrite that are relatively free of organelles, the microtubules are packed in a regular array at a density of 50–150 per μm^2 , typically spaced at 80–200 nm (Fig. 1.6A). The number of microtubules in a dendritic cross section is proportional to the caliber of the dendrite (Fiala et al., 2003), which is also proportional to the number of spines per micron length of dendrite (Harris et al., 2007; Bourne and Harris, 2011). Microtubules are the “railroad tracks” of the cell, and they play an important role in the transport of mitochondria and other organelles (Overly et al., 1996; Ehlers, 2013). Microtubules transport SER which leads to local elaboration and redistribution of dendritic SER (Cui-Wang et al., 2012). In regions of a dendrite with a lot of SER, the microtubules are more widely dispersed (Figure 1.6B). The 63-kDa cytoskeleton-linking membrane protein (CLIMP63) is an integral membrane protein in the SER that when phosphorylated by protein kinase C (PKC) causes the SER to dissociate from microtubules and become more elaborate (Klopfenstein et al., 1998; Vedrenne et al., 2005; Cui-Wang et al., 2012). In contrast, where CLIMP63 is dephosphorylated, the SER is associated with microtubules and becomes straighter and more tubular (Cui-Wang et al., 2012). As already indicated, when the SER is more elaborate, cargo is slowed and can be offloaded to support synapses and

other structures in the neighborhood, whereas simplification of the SER enhances the movement of proteins and other cargo and slows offloading. Three-dimensional reconstructions reveal that the SER is a rather continuous network that often surrounds the mitochondrion in thin dendritic branches (Fig. 1.7).

Neurofilaments (10 nm in diameter) occur in dendrites but are more common in axons, where they are important for radial growth (Yuan et al., 2012). *Actin filaments* (7 nm in diameter) constitute the bulk of the cytoskeleton between microtubules and, as discussed later, the actin filaments tend to be more highly concentrated in dendritic synaptic specializations, especially filopodia and dendritic spine necks, where they provide a means of rapidly changing shape.

Organelles of the endosomal pathway are involved in membrane protein sorting and recycling and are commonly found in dendrites (Cooney et al., 2002; Park et al., 2006). *Coated pits* and *coated vesicles* represent the initial step in endocytosis and are frequently seen at the plasma membrane of dendrites (Fig. 1.8A). The cytoplasmic coat is composed of *clathrin*, giving it a distinctive periodic structure (Fig. 1.8C). Coated vesicles and coated pits occur more frequently in dendrites during development (Altman, 1971) and periods of synaptic remodeling (McWilliams and Lynch, 1981). *Recycling endosomes* appear as tubular compartments that can be distinguished from SER by their darker interior, more uniform diameter, smooth (as opposed to wavy) membrane, and the frequent occurrence of specialized coats at the ends of the tubule. These coated ends represent budding sites of recycling vesicles that are bound for the cell membrane. Thus, single *smooth vesicles* are generated when these vesicles lose their coats and are then recycled back to the plasma membrane. In addition, smooth vesicles of varying dimensions can be transported from the soma or other parts of the cell along microtubules. *Sorting endosomes* can be identified by the occurrence of similar tubular compartments connected to larger, spherical organelles with interior vesicles. These spherical compartments mature into *multivesicular bodies* that separate from the sorting endosome and eventually become late endosomes and lysosomes (see Fig. 1.8B, D). Thus, multivesicular bodies in dendrites occur alone or in conjunction with the sorting endosome compartments (Fig. 1.8B, D).

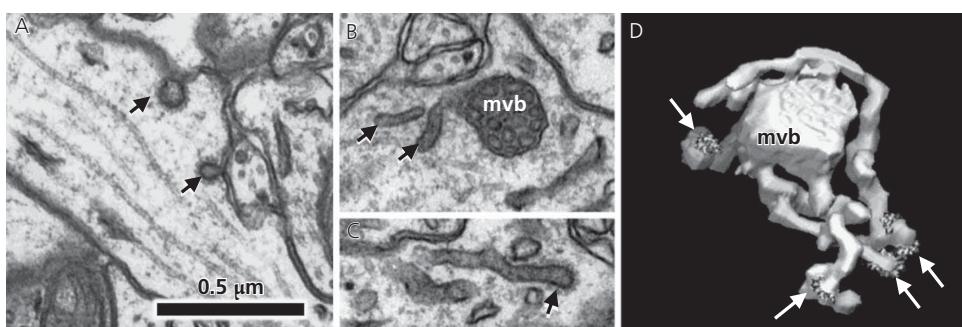


Fig. 1.8 Endosomes in lateral dendrites in stratum radiatum of area CA1 from the hippocampus of an adult rat. (A) Clathrin-coated pits (arrows) are the initial step in the endosomal pathway. (B) A multivesicular body (mvb) with its connected tubular compartments of a sorting endosome (arrows). (C) A tubular endosome associated with a sorting complex has a coated tip at one end (arrow). (D) Three-dimensional reconstruction of the sorting complex reveals a multivesicular body with many tubules. The top of the multivesicular body is removed to reveal the interior vesicles. Clathrin-coated tips are identified at the ends of a few tubules (arrows).

Autophagy is a normal process that occurs in dendrites, as evidenced by the presence of *macroautophagosomes*, *autophagosomes*, and *autophagosomal complexes* (Nixon, 2007, 2013; McBrayer and Nixon, 2013; Wolfe et al., 2013). Depending on the stage in the degradation process, these structures are characterized by a SER-like structure that forms a double-walled delimiting membrane and surrounds other organelles (e.g., see “vac” in Fig. 1.12), and ultimately sends them to the lysosome.

Dendrites contain other membranous subcellular structures. Amorphous vesicular clumps of membrane are common in dendritic growth cones and at sites of synaptogenesis along dendrites and in dendritic growth cones (Fiala et al., 1998). They also appear to be critical for the growth and formation of dendritic spines during development and synaptic plasticity (Park et al., 2006).

Structure of synaptic specializations of dendrites

The potential for connectivity is established primarily by the patterns of dendritic and axonal arborization and secondarily by the formation of a variety of synaptic specializations emerging from the dendrites (Table 1.3).

Shaft synapses reside directly on the surface of the dendrite without obvious changes in the dimensions of the dendrite at the synapses (e.g., Fig. 1.9). Both excitatory and inhibitory axons can form synapses directly on the dendritic shafts throughout the dendritic arbor. In the hippocampus and elsewhere, the interneuronal dendrites have most of their synapses directly on the dendritic shafts (~95%) with only a small fraction on dendritic specializations or protrusions (~5%) (Harris et al., 1985; Harris and Landis, 1986; Ascoli et al., 2008). In contrast, 95% of synapses onto the dendrites of CA1 pyramidal cells are located on dendritic spines, while shaft synapses make up only 5% (Harris et al. 1992; Kirov et al. 1999).

Varicosities in the dendrite are one type of synaptic specialization that can be found in certain neurons, such as amacrine cells of the retina, where dendritic varicosities both receive synapses from rod bipolar cells and make reciprocal synapses back onto the bipolar cells (Ellias and Stevens, 1980). Under normal, non-pathological conditions, many of the cortical, hippocampal, and cerebellar non-spiny interneurons also have dendritic varicosities containing subcellular components that support axo-dendritic synapses (DiFiglia and Carey, 1986; Harris and Landis, 1986; Ascoli et al., 2008).

Filopodia are transient synaptic specializations of dendrites. All neurons exhibit dendritic filopodia during development when extracellular space separates potential synaptic partners (Morest, 1969). Filopodia are identified in 3DEM by their length (often exceeding more than 2 μm) and a somewhat denser cytoplasm than mature spines; when visualized in culture, they are highly dynamic, extending and retracting within a few minutes (Dailey and Smith, 1996; Fischer et al., 1998), which could explain the diversity of lengths captured in 3DEM (Fiala et al., 1998). After the developmental period, filopodia diminish (Dunaevsky et al., 1999; Grutzendler et al., 2002) and then are usually distinguished from dendritic spines only by the absence of synapses (Bourne and Harris, 2011). Even during synaptogenesis most filopodia bear no synapses, but some filopodia make small synaptic contacts along their lengths with multiple presynaptic axons (Fiala et al., 1998). Multiple synapses with different axons can also be found surrounding the base of filopodia (Fiala et al., 1998), consistent with the rapid migration of synaptic proteins along filopodia in culture (Marrs et al., 2001). Long filopodia ($>1 \mu\text{m}$) are rarely seen on dendrites in the normal rat hippocampus beyond 2 weeks after birth, possibly because the more mature neuropil is densely packed with axonal boutons; hence, even a short dendritic outgrowth would encounter multiple potential synaptic partners. During maturation, filopodia are generally replaced with shaft synapses, stubby spines, or other types of synaptic specialization (Harris, 1999).

Table 1.3 Synaptic specializations of dendrites

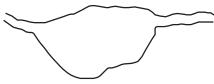
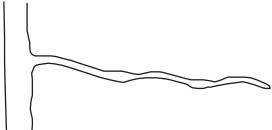
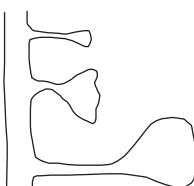
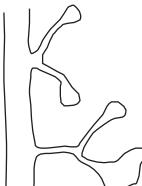
Pattern	Characteristics	Examples
Shaft	Synapses occur directly on the surface of the dendritic shaft without obvious swelling or specialization	Interneurons
		
Varicosity	An enlargement in a thinner dendrite associated with synaptic contacts	Retinal amacrine cells
		
Filopodium	A long, thin protrusion with a dense actin matrix and few internal organelles	All neurons during developmental synaptogenesis
		
Simple spine		
Sessile	Synaptic protrusions without a neck constriction Sessile spine Stubby spine Crook thorn	Cerebral pyramidal cells Cerebral pyramidal cells Neurons of dentate nucleus
		
Pedunculated	Bulbous enlargement at tip Thin spine Mushroom spine Gemmule	Cerebral pyramidal cells Cerebral pyramidal cells Olfactory bulb granule cell
		
Branched spine	Each branch has a unique presynaptic partner and each branch has the shape characteristics of a simple spine	CA1 pyramidal cells Granule cells of dentate gyrus Cerebellar Purkinje cells
		

Table 1.3 (continued) Synaptic specializations of dendrites

Pattern	Characteristics	Examples
Synaptic crest	Crest-like protrusion with a synapse on either side of a thin lamellar neck region	Cerebral pyramidal cells Neurons of habenula, subfornical organ, and interpeduncular nucleus
Claw ending	Synaptic protrusions at the tip of the dendrite associated with one or more glomeruli	Granule cells of cerebellar cortex and dorsal cochlear nucleus
Brush ending	Spray of complex dendritic protrusions at the end of the dendrite that extends into glomerulus and contains presynaptic elements	Unipolar brush cells of cerebellar cortex and dorsal cochlear nucleus
Thorny excrescence	Densely lobed dendritic protrusion into a glomerulus	Proximal dendrites of CA3 pyramidal cells and dentate gyrus mossy cells Proximal dendrites of thalamocortical relay cells
Racemose appendage	Twig-like branched dendritic appendages that contain synaptic varicosities and bulbous tips	Inferior olfactory neurons Relay cells of lateral geniculate nucleus
Coralline excrescence	Dendritic varicosity extending numerous thin protrusions, velamentous expansions and tendrils	Neurons of dentate nucleus and lateral vestibular nucleus

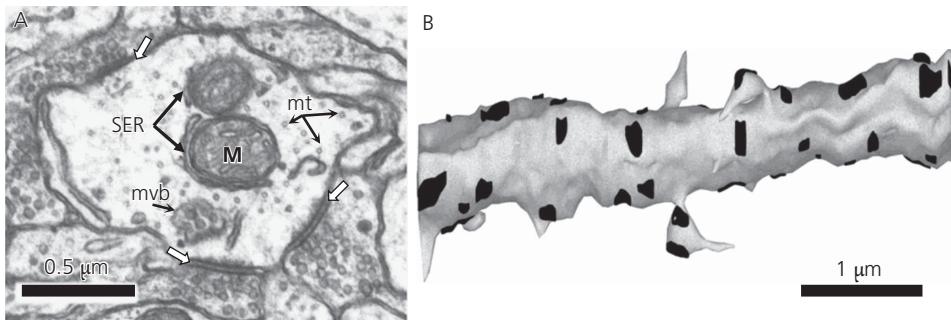


Fig. 1.9 An interneuronal dendrite from stratum radiatum of hippocampal area CA1 in a 21-day-old rat with mostly shaft synapses. (A) Electron micrograph illustrating a cross-sectional view where this dendrite hosts three excitatory synapses (white arrows) directly on the dendritic shaft. This section also illustrates that interneuronal dendrites have dispersed microtubules among a similar complement of subcellular components as the spiny dendrites including a close association between SER and mitochondria (black arrows, M), microtubules (mt), and a sorting endosome with a multivesicular body (mvb) illustrated on this section. (B) Three-dimensional reconstruction from serial section electron microscopy of a 4.6- μm segment of the dendrite reveals 45 excitatory synaptic contacts (black), 91% of which are located on the dendrite shaft and three on protrusions.

Simple dendritic spines are the most common synaptic specialization of dendrites (Fig. 1.10), being the site of more than 90% of excitatory synapses across brain regions. *Simple spines* have a *sessile* shape with no distinction between the diameter of the neck and head, or a *pedunculated* shape that has been described as thin or mushroom shaped (Fig. 1.10E). In the mature brain, 3DEM has revealed that simple spines vary greatly in size, with volumes ranging from less than $0.01 \mu\text{m}^3$ to more than $1.5 \mu\text{m}^3$ (Table 1.4). Simple spines of different sizes and shapes can be neighbors on the same parent dendrite (Harris and Stevens, 1988b, 1989) and occasionally form synapses with the same presynaptic bouton (Sorra and Harris, 1993; Sorra et al., 1998; Fiala et al., 2002a). Measuring spine neck dimensions is functionally relevant since the presence of a neck constriction can serve to isolate the spine head compartment from the dendrite (Holmes, 1990;

Table 1.4 Dimensions of simple spines on spiny neurons

Neuron	Neck diameter (μm)	Volume (μm^3)	Surface area (μm^2)	Synapse area (μm^2)	Ratio of synapse area to surface area
Cerebellar Purkinje cell	0.1–0.3	0.06–0.2	0.7–2	0.04–0.4	0.17 ± 0.09
CA1 pyramidal cell	0.04–0.5	0.004–0.6	0.1–4	0.01–0.5	0.12 ± 0.06
Visual cortex pyramidal cell	0.07–0.5	0.02–0.8	0.5–5	0.02–0.7	0.10 ± 0.04
Neostriatal spiny neuron	0.1–0.3	0.04–0.3	0.6–3	0.02–0.3	0.125
Dentate gyrus granule cell	0.05–0.5	0.003–0.2	0.1–3	0.003–0.2	—

Sources: Spacek and Hartman (1983), Wilson et al. (1983), Harris and Stevens (1988a), Harris and Stevens (1989), Trommald and Hullenburg (1997).

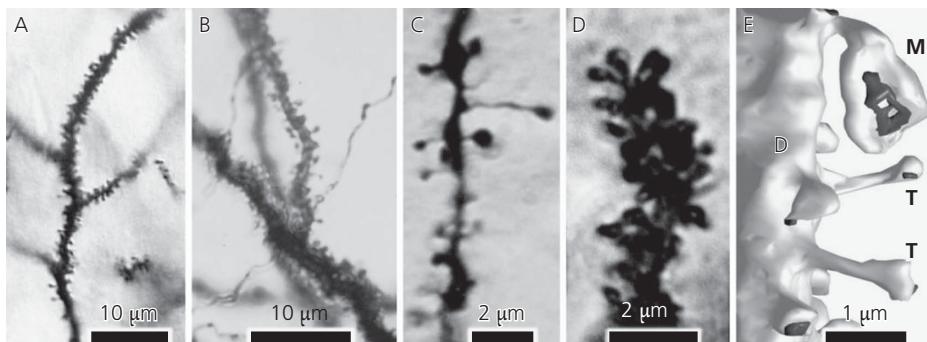


Fig. 1.10 Dendritic spines at increasing magnifications. (A) The apical dendrite of a neocortical pyramidal neuron has few spines near the soma (bottom of image) and many spines distally (Golgi; rat). (B) Spiny dendrites of a CA1 pyramidal hippocampal cell (Golgi; rat). (C) High magnification of a dendrite of a neocortical pyramidal neuron (Golgi; mouse). (D) Spiny branchlet of a cerebellar Purkinje cell (Golgi; mouse). (E) Three-dimensional reconstruction of a segment of CA1 pyramidal cell dendrite (D) showing typical shapes of thin (T) and mushroom (M) pedunculated spines. (serial section electron microscopy; rat).

Parts C and D reproduced from *Anatomy and Embryology*, 167(2), pp. 289–310, Three-dimensional analysis of dendritic spines, Josef Spacek and Miroslav Hartmann © 1983, Springer Science and Business Media. With permission from Springer Science and Business Media.

Koch and Zador, 1993; Svoboda et al., 1996; Tønnesen et al., 2014) and influence charge transfer and network properties (Yuste, 2013).

Additional types of simple spines are often found on specific neurons. One example is the bent sessile spines in the cerebellar dentate nucleus, called *crook thorns* (Chan-Palay, 1977). The granule cells of the olfactory bulb have particularly large pedunculated spines sometimes referred to as *gemmales*. These spines may be 5 μm long, with heads 1–2 μm in diameter (Cameron et al., 1991).

Branched spines are two or more simple spines that share a common stalk (Table 1.3). The individual branches exhibit the same range of morphologies as simple spines, and in a mature brain each branch synapses with a bouton from different axons (Harris and Stevens, 1988a, 1989; Trommald and Hulleberg, 1997; Sorra et al., 1998). Branching is a rare event because spine origins exhibit a non-random tendency to separate (Trommald et al., 1995; Ward et al., 1995); thus only about 2% of all dendritic protrusions on CA1 pyramidal cells (Harris et al., 1992) or dentate granule cells (Trommald and Hulleberg, 1997) are branched. Branched spines are slightly more frequent on dendrites with higher spine densities, such as Purkinje cell dendrites where approximately 6% of spines are branched (Harris and Stevens, 1988a). In addition, higher spine densities lead to larger numbers of branches per branched spine, with up to five branches having been found on the branched spine of a Purkinje cell (Harris and Stevens, 1988a), while branched spines of CA1 pyramidal cell rarely have more than two branches (Sorra et al., 1998). Exactly how simple spines become branched is unclear. Their rarity suggests an accidental proximity of synapses that emerge close enough to one another to have the same origin. Alternatively, one branch may emerge as a filopodium-like structure, a spine head protrusion, from an existing spine and make a synapse onto a different axon (Richards et al., 2005). The branched simple spines are distinguished from the thorny excrescences discussed later in that the different heads of a branched simple spine never

synapse with the same presynaptic axon; this also supports the hypothesis that they are not due to the splitting of a synapse (Fiala et al., 2002a).

Synaptic crests are specializations found occasionally on spiny neurons throughout the brain, but especially concentrated on the dendrites of the habenula, subfornical organ, and interpeduncular nucleus (Milhaud and Pappas, 1966; Akert et al., 1967; Lenn, 1976). Crest synapses are formed by two axons on either side of the thin lamellar neck of the crest. The synapses are closely apposed inside the crest and may exhibit characteristic subjunctional bodies connecting the two postsynaptic densities. In some instances a synaptic crest can contain multiple folds with many pairs of crest synapses (Lenn, 1976).

Synaptic complexes called glomeruli often occur when the terminals of large axons interact with particular dendrites. The dendrites extend multi-lobed protrusions into the glomeruli and make many synaptic contacts. A simple example is the *claw endings* of the dendrites of cerebellar granule cells (Fig. 1.1), which make several synapses with a single mossy cell axon terminal (Eccles et al., 1967). These cerebellar glomeruli also host *brush endings* of the unipolar brush cells, multi-lobed protrusions that are both presynaptic to the claw endings of the granule cells and postsynaptic to mossy fibers, which may account for their unusual appearance (Floris et al., 1994; Mugnaini et al., 1994; Mugnaini and Floris, 1994).

Thorny excrescences are another type of synaptic specialization associated with large axonal boutons. These specializations are exemplified by those found in the thalamus where, in a reconstructed glomerulus of the ventrobasal thalamic nucleus, 44 synapses were located on a single excrescence that invaginated a giant lemniscal bouton (Spacek and Lieberman, 1974). Mossy fiber axons from dentate granule cells synapsing onto CA3 pyramidal cells are another classic example (Chicurel and Harris, 1992; Hama et al., 1994). The complexity of the CA3 thorny excrescences varies, with some having as many as 16 lobes and others having just a few. Usually, all these lobes synapse with the same presynaptic bouton, but in some cases one or more lobes synapse with different axons.

Racemose appendages have a more sparsely lobed appearance and are common on neurons in the inferior olive (Ruigrok et al., 1990) and lateral reticular nucleus (Hrycyshyn and Flumerfelt, 1981a,b,c). This type of protrusion can also be found on spiny neurons such as neocortical pyramidal cells.

Coralline excrescences are found on dendrites of the small neurons of the cerebellar and vestibular nuclei. These complex dendritic varicosities exhibit numerous synaptic protrusions (Chan-Palay, 1977) and sometimes also have thin tendrils with a similar appearance to filopodia (Morest, 1969; Angaut and Sotelo, 1973; Sotelo and Angaut, 1973), leading to the suggestion that the coral-line excrescences are growth processes on adult dendrites.

The dendritic synaptic specializations outlined in Table 1.3 do not describe all the specialized synaptic shapes found in dendrites. Furthermore, individual dendritic segments exhibit a wide variety of spine types as well as other synaptic specializations. Thus, the shape of dendritic synaptic specializations is not fixed by the postsynaptic neuron but appears to reflect the source and activation history of the presynaptic partner. Furthermore, synaptic specializations can be highly dynamic structures, capable of structural change throughout life. This structural plasticity of synapses blurs morphological classifications, and many related and intermediate forms are to be expected. For example, filopodia become dendritic spines, and small spines have been shown to grow or shrink over time and with experience, as discussed later. Furthermore, the distribution of the dimensions of dendritic spines and other synaptic specializations is broad, and essentially continuous. Super-resolution microscopy and 3DEM provide a means to obtain reasonably accurate measurements; hence, future measurements will enhance understanding of the functional

consequences of changes in the shapes of dendritic specializations during development and synaptic plasticity in the mature nervous system. As discussed in the next section, the subcellular composition of spines and other synaptic specializations further distinguishes them in their capacity to undergo calcium-mediated or protein-synthesis-dependent changes in structure and molecular composition.

Composition of synaptic specializations of dendrites

The diversity of shapes of synaptic specializations is accompanied by diversity in their intracellular composition. Like the dendritic shaft, different synaptic specializations in dendrites contain different intracellular components, including various synaptic and adhesive junctions, SER, the spine apparatus (a possible Golgi outpost), rough endoplasmic reticulum and polyribosomes, mitochondria, microtubules, smooth vesicles, and organelles of the endosomal and recycling pathways. The presence or absence of perisynaptic astroglial processes influences the structure and composition of the dendritic synaptic specializations. Furthermore, the size of a synaptic specialization correlates with the composition of subcellular structures. Thus, even within and surrounding the synaptic specializations, resources are present that can respond rapidly to local changes in synaptic efficacy.

Postsynaptic densities

The synaptic specializations of dendrites receive chemical synapses consisting of apposed membranes separated by a gap called the synaptic cleft (Figs 1.11 and 1.12). Neurotransmitters released from synaptic vesicles on the presynaptic side of the cleft diffuse across the cleft to activate receptors in the postsynaptic membrane. The presynaptic element is usually a varicosity or end bulb of an axon, called a bouton. In aldehyde-fixed tissue, several different types of chemical synapses can be distinguished based on the size and shape of the presynaptic vesicles and perisynaptic structures (Colonnier, 1968; Peters and Palay, 1996). Two principal types are commonly referred

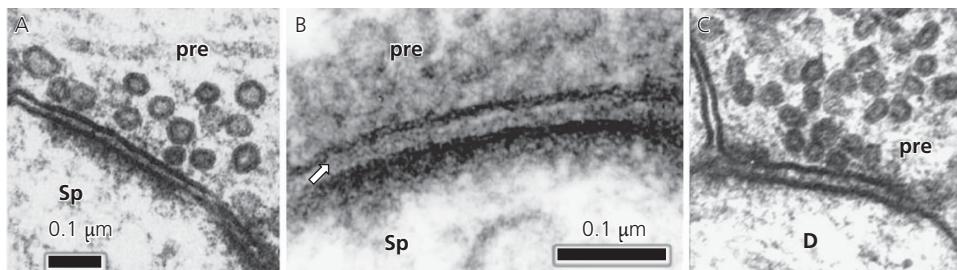


Fig. 1.11 Asymmetrical and symmetrical synapses on dendrites of spiny neurons. (A) An asymmetrical synapse located on a spine head (Sp) from the hippocampus of an adult rat illustrates the characteristic thickened postsynaptic density and round presynaptic (pre) vesicles. (B) High-magnification image of another asymmetrical synapse from mouse cerebellum shows dense material in the synaptic cleft (white arrow). (C) Symmetrical synapse on the shaft of a dendrite from rat hippocampus illustrates the thin and essentially equal densities in the presynaptic (pre) and postsynaptic (D) compartments and pleiomorphic presynaptic vesicles, which are smaller and more flattened than the vesicles of an asymmetric synapse (compare with those in A). The scale bar in part A is the same as for part C.

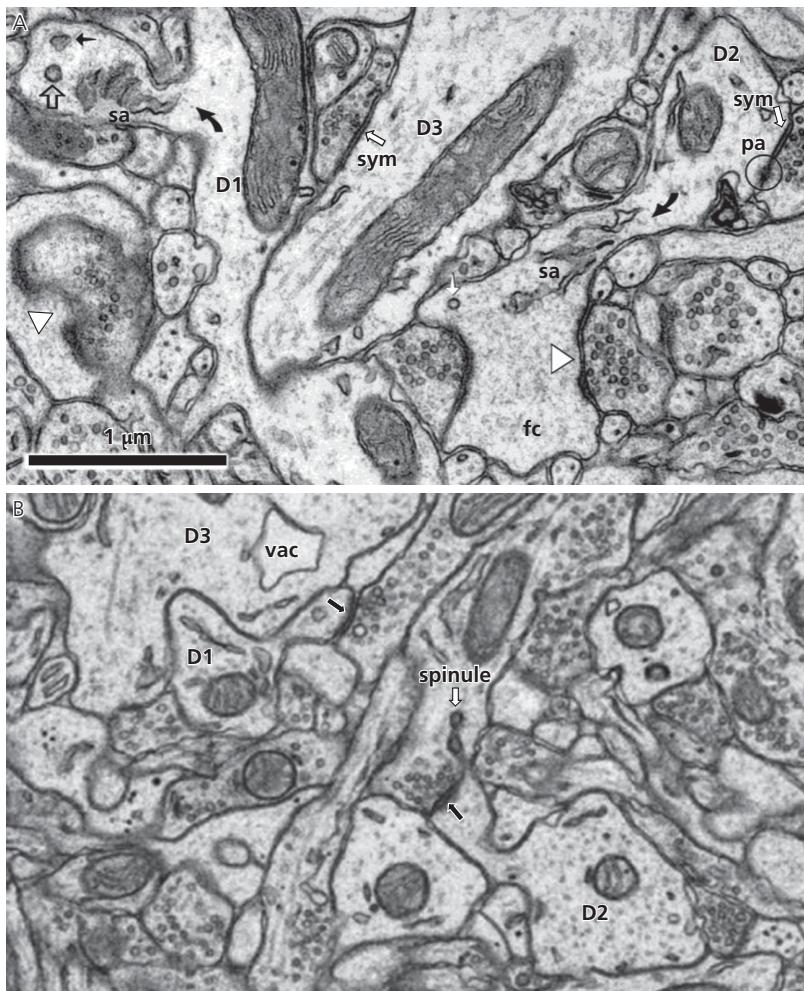


Fig. 1.12 Different dendritic spines contain unique subcellular compositions. **(A)** On this section, each of two dendrites (D1, D2) in rat CA1 stratum radiatum gives rise to large spines (curved arrows) that contain a spine apparatus (sa). The sa of the D1 spine is connected on adjacent sections to the tubule (small arrow), and this spine also contains a spherical vesicle (open arrow). The bouton presynaptic to the D2 spine wraps around the spine head so that the synapse appears on both sides in this section. The postsynaptic density is interrupted, or perforated, on one side (white arrowhead). In addition to the sa, the D2 spine contains a small clear profile (white arrow) found in series to be endosomal in nature. Both spines contain a floccular cytoplasm (fc) consistent with an actin matrix. Symmetric shaft synapses (sym, white arrows) are present on D2 and D3. Next to the D2 symmetric synapse is a punctum adhaerens junction (pa). **(B)** Two dendrites (D1, D2) give rise to small spines with macular synapses (black arrows). The spine of D2 has a spinule emerging from the edge of the synapse into the presynaptic axon. A third dendrite (D3) contains a vacuole (vac), which on serial section reconstruction is part of a macro-autophagosome. The scale bar in part A is for both A and B.

to as asymmetric and symmetric synapses. Asymmetric synapses are characterized by round presynaptic vesicles about 30–50 nm in diameter and a prominent postsynaptic density (Figs 1.11A, B and 1.12A, B). The postsynaptic density is a densely stained structure that contains numerous receptors, structural proteins, and signaling molecules that are important for synaptic transmission and plasticity (Kennedy, 2000; see also Chapter 6). The synaptic cleft also contains dense-staining material (Fig. 1.11B) probably containing the extracellular components of receptors and other signaling or adhesion molecules. Symmetric synapses have a much thinner postsynaptic density, matched by a nearly equal density on the presynaptic side, where many of the presynaptic vesicles appear flattened (Figs 1.11C and 1.12A). Asymmetric synapses are usually excitatory and use the neurotransmitter glutamate, whereas symmetric synapses use the inhibitory neurotransmitters gamma-aminobutyric acid (GABA) or glycine and the molecular composition of their receptors and downstream signaling molecules matches these structural and functional distinctions (Harris and Weinberg, 2012).

Asymmetric and symmetric synapses are differentially distributed along dendrites. By definition, simple spines have an excitatory asymmetric synapse located on the spine head. Occasionally, simple spines have more than one synapse (Spacek and Hartmann, 1983; Fiala et al., 1998; Bourne and Harris, 2011). The second synapse can be either symmetric or asymmetric (Jones and Powell, 1969). For example, in the visual cortex 84% of synapses are asymmetric and 16% symmetric (Beaulieu and Colonnier, 1985). Most of the asymmetric synapses (79%) occur on simple dendritic spines, while 21% occur on dendrite shafts and very few (0.1%) are found on cell bodies. The symmetric synapses are much less frequent and yet their positioning might effectively nullify the excitatory input because most (62%) occur directly on the dendrite shafts, 31% occur on a fraction of the dendritic spines, and 7% occur on cell bodies and axon initial segments. Symmetric synapses are only 7% of all dendritic spine synapses but 93% of all soma synapses in the visual cortex. In the neostriatum, as another example, approximately 8% of spines receive both an excitatory asymmetric and an inhibitory symmetric synapse (Wilson et al., 1983). Some striatal neurons make reciprocal connections with the substantia nigra, and in this field 39% of their spines are contacted by a different type of symmetric synapse containing large, pleiomorphic vesicles which are probably dopaminergic (Freund et al., 1984). Glomeruli often contain both excitatory and inhibitory axon terminals. Thus, it is common for dendritic synaptic specializations that project into glomeruli to receive multiple types of synaptic contacts. For example, the racemose appendages of inferior olfactory neurons receive both excitatory and inhibitory synapses (De Zeeuw et al., 1990).

Cell adhesion junctions (*puncta adhaerentia*) and nascent zones adjacent to synapses

Cell adhesion junctions, sometimes referred to as *puncta adhaerentia* (Figs 1.12A and 1.13), are characterized by pre- and postsynaptic thickenings but no presynaptic vesicles. They can be located at the edges of the postsynaptic densities of dendritic spines and other synaptic specializations (Uchida et al., 1996; Spacek and Harris, 1998; Benson and Huntley, 2012). Extensive adherent contacts form a meshwork in the glomeruli of thalamic relay nuclei (Lieberman and Spacek, 1997). Cell adhesion junctions contain a host of adhesion molecules (e.g., cadherins, neuroligins, and neurexins) with associated signaling cascades that differentiate them from the receptor-containing portions of the postsynaptic density (Fannon and Colman, 1996; Fields and Itoh, 1996; Benson and Huntley, 2012).

In addition to the *puncta adhaerentia*, we have identified the *nascent zone* (Fig. 1.13), an ultrastructurally distinct region at the edge of synapses in the intact mature hippocampus (Bell et al., 2014), which was previously described as a vesicle-free transition zone (Spacek and Harris, 1998).

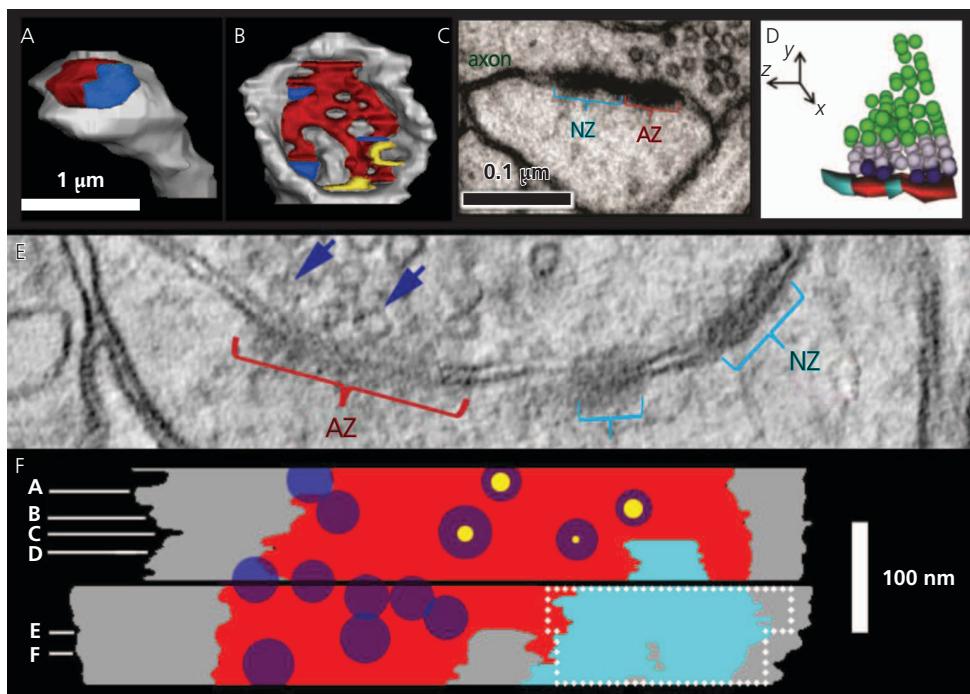


Fig. 1.13 Substructure of the postsynaptic density on dendritic spines. **(A)** 3DEM of a spine with a macular synapse on its head. The active zone (red) is adjacent to a large punctum adhaerens junction (blue). **(B)** A view of the synaptic face of a spine with a perforated postsynaptic density with a large active zone (red), three puncta adhaerens (blue), and two vesicle-free transition zones (yellow). **(C)** Nascent zones (NZ) is the revised name for vesicle-free transition zones. Nascent zones do not have any presynaptic vesicles, thus distinguishing them from the active zone (AZ). **(D)** 3DEM of the docked vesicles (dark blue) and the adjacent (light gray) and distant (green) reserve pool vesicles, viewed on edge to reveal the nascent zones (turquoise) and active zones (red). **(E)** Identification and measurement of nascent zones in virtual sections from tilt tomography revealed vesicles, with some pressed against the active zone (blue arrows), but no vesicles above the nascent zones. **(F)** Stacked projection of the axon–spine interface (gray), active zone (red), and nascent zone (teal) that were first traced through the 2–3-nm virtual sections and then displayed orthogonal to the virtual section planes with white lines illustrating the locations of the virtual sections and F equal to the virtual section in the micrograph E. Maximum diameters of docked vesicles are illustrated as dark blue circles with scaled pores (yellow circles) circumscribed in vesicles that had them. The scale bar in part F also applies to E.

Parts A and B reproduced from Three-dimensional organization of cell adhesion junctions at synapses and dendritic spines in area CA1 of the rat hippocampus, Josef Spacek and Kristen M. Harris, *Journal of Comparative Neurology*, 393(1), pp. 58–68 Copyright © 1998 Wiley-Liss, Inc. Parts C–F reproduced from Dynamics of nascent and active zone ultrastructure as synapses enlarge during long-term potentiation in mature hippocampus, Maria Elizabeth Bell, Jennifer N. Bourne, Michael A. Chirillo, John M. Mendenhall, Masaaki Kuwajima and Kristen M. Harris, *Journal of Comparative Neurology*, 522(17), pp. 3861–3884, Copyright © 2014 Wiley Periodicals, Inc.

Both nascent and active zones have a postsynaptic density, but, unlike the active zone, the presynaptic side of a nascent zone lacks the small clear synaptic vesicles that are required for the release of glutamate. Unlike the puncta adhaerentia, nascent zones have no presynaptic thickening. Synaptic edges are highly dynamic regions where alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors (AMPARs), which mediate fast excitatory transmission, diffuse laterally until they are stabilized by activity (Choquet and Triller, 2013; MacGillavry et al., 2013). However, even if AMPARs were present in a nascent zone, the absence of presynaptic vesicles could render the nascent zone functionally silent because the diffusion path from release sites is too distant (Franks et al., 2002, 2003; Raghavachari and Lisman, 2004; Christie and Jahr, 2006; MacGillavry et al., 2013; Nair et al., 2013). Their distinct locations, composition, and morphology likely serve to modulate synaptic efficacy since their disassembly and reassembly may be needed for synaptic plasticity (Luthi et al., 1994; Muller et al., 1996; Tang et al., 1998); recent 3DEM results show that they disappear early and indeed are reassembled 2 hours after the induction of long-term potentiation (Bell et al., 2014).

The postsynaptic densities and associated adhesions exhibit size-dependent variations in morphology (Spacek and Hartmann, 1983; Harris and Stevens, 1988a, 1989; Humeau et al., 2005; Nicholson and Geinisman, 2009). Most synapses have a continuous postsynaptic density when viewed in 3DEM, often called macular in shape (Figs 1.9, 1.10E (T spines), 1.12B, and 1.13A). Larger postsynaptic densities often exhibit interior regions devoid of pre- and postsynaptic density, which can be U-shaped or annular or exhibit multiple holes, and are often called perforated (Figs 1.12A and 1.13B), segmented, or multifocal synapses (Geinisman et al., 1987; Sorra et al., 1998).

Both macular and perforated synapses occur on a variety of dendritic synaptic specializations. When located on spines, the synaptic area occupies approximately 10% of the surface area of the spine head (Spacek and Hartmann, 1983; Harris and Stevens, 1988a, 1989). This relationship is consistent over different spine morphologies and neuron types (Table 1.4) and also appears to hold for more complicated synaptic specializations, such as the thorny excrescences of CA3 (Chicurel and Harris, 1992). Spine surface area, spine volume, bouton volume, and the number of synaptic vesicles correlate with synapse size in most cases (Harris and Stevens, 1988a, 1989; Lisman and Harris, 1993; Schikorski and Stevens, 2001). Thus, smaller thin spines have smaller synapses, which tend to be macular, and larger mushroom spines have larger synapses, which can be perforated (Sorra et al., 1998; Bourne and Harris, 2007). Larger synapses contain more receptors and other signaling molecules, and therefore represent more effective connections (Nusser, 2000; Matsuzaki et al., 2001; Tanaka et al., 2005; Holderith et al., 2012). Differences in synaptic efficacy have important implications for both long-term information storage and short-term neurotransmission. For example, excitatory synapses in the most distal apical dendrites of CA1 pyramidal cells are more often perforated and larger than those synapses more proximal to the cell body (Megias et al., 2001; Nicholson et al., 2006). This property may help compensate for distance-dependent attenuation of postsynaptic potentials (see Chapter 12).

Smooth endoplasmic reticulum

Some synaptic specializations also contain elements of the dendritic SER network (Figs 1.12 and 1.14). In pyramidal neurons of the cerebral cortex and hippocampus, only about 15% of dendritic spines contain SER, and this organelle is mostly absent from thin spines (Spacek and Harris, 1997). All dendritic spines of cerebellar Purkinje cells contain SER (Spacek, 1985a; Harris and Stevens, 1988a). In claw endings of cerebellar granule cells, each mitochondrion is surrounded by a single cistern of SER. Organelles derived from SER also appear subjacent to the puncta adhaerentia on both pre- and postsynaptic sides.

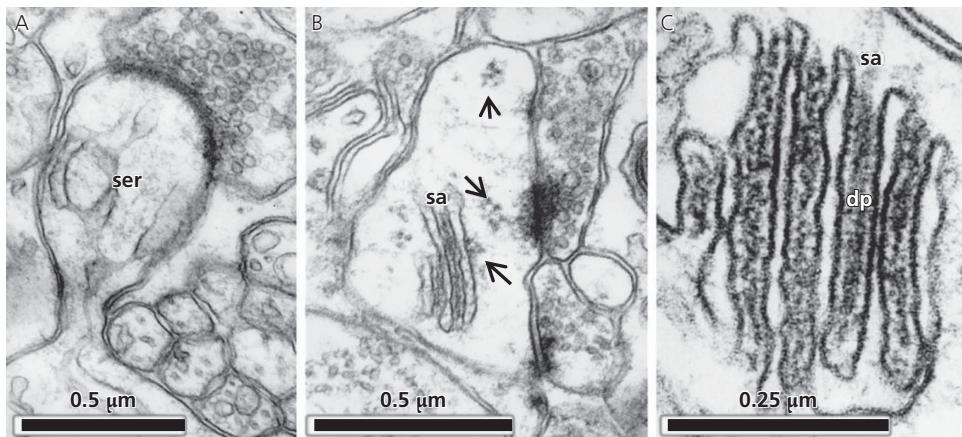


Fig. 1.14 Smooth endoplasmic reticulum in dendritic spines. (A) A cerebellar Purkinje cell spine showing the network of SER that extends into the spine from the dendrite. (B) The head of a neocortical mushroom spine containing a spine apparatus (sa) and polyribosomes (arrows). The lowest cluster of polyribosomes is located immediately adjacent to the sa, suggesting it may be rough endoplasmic reticulum. (C) High-magnification image of the spine apparatus (sa) in a mushroom spine of the mouse visual cortex shows the inner dense plates (dp) between cisternae of SER.

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The spine apparatus (possible Golgi outpost), rough endoplasmic reticulum, and ribosomes

Large spines often possess a large, perforated synapse and contain a spine apparatus (Fig. 1.12A). As mentioned above, the spine apparatus has structural and molecular features similar to the Golgi apparatus (Gray, 1959; Spacek and Harris, 1997; Pierce et al., 2000; 2001; Deller et al., 2007). The largest mushroom spines and gemmules often contain the spine apparatus, as do the thorny excrescences of hippocampal area CA3. The spine apparatus can also be found in association with polyribosomes (Fig. 1.14B). High concentrations of polyribosomes have been found in the lobes of thalamic thorny excrescences (Fig 1.15) and those of the CA3 pyramidal cells (Chicurel and Harris, 1989). Under quiescent conditions, only about 12% of simple dendritic spines contain *polyribosomes* (Steward et al., 1996; Ostroff et al., 2002). During tetanus-induced long-term potentiation, polyribosomes increase dramatically in spines (Ostroff et al., 2002; Bourne et al., 2007), contrasting with theta-burst induction of long-term potentiation where polyribosome frequency increases transiently in several spines, but then concentrates by 2 hours in those spines with the most enlarged synapses (Chirillo et al., 2015). The spine apparatus is often found to have a structural association with puncta adhaerentia and nascent zones, indicating a possible role in synthesizing or maintaining them (Spacek and Harris, 1998; Bell et al., 2014). In addition, this location could enhance signaling via metabotropic glutamate receptors, which are also concentrated at the edges of excitatory synapses (Baude et al., 1993; Kennedy, 2000).

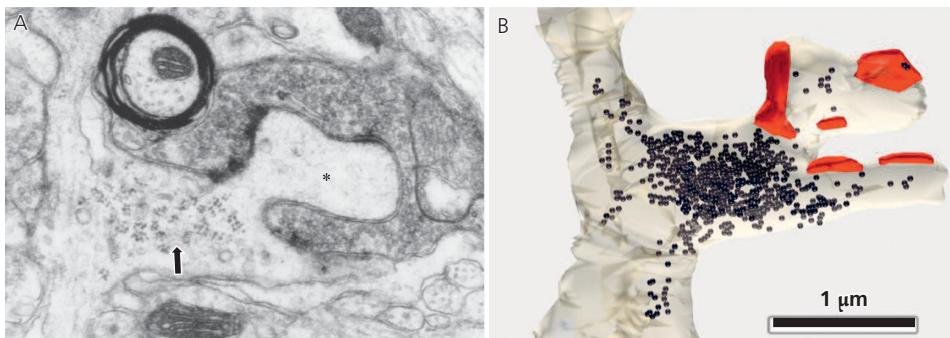


Fig. 1.15 A small thorny excrescence emerging from a thalamocortical relay neuron of the ventrobasal nucleus. (A) Electron micrograph of a section through the excrescence. Beneath the synaptic lobe (rectangle with cross) is a region (arrow) with a large number of polyribosomes. (B) 3DEM of the excrescence (gray) reveals the intense concentration of polyribosomes (black spheres) beneath the synapses (red).

Mitochondria

Small synaptic specializations such as filopodia and simple dendritic spines rarely contain mitochondria as most are too small, but larger dendritic synaptic specializations often contain mitochondria. Gemmules of olfactory bulb granule cells often have mitochondria in their head, and since these spines make reciprocal synapses on mitral cell dendrites it has been suggested that the presence of these mitochondria may be related to their presynaptic function (Cameron et al., 1991). Other specializations with both postsynaptic and presynaptic functions, such as the varicosities of AI amacrine cells and the brush endings of unipolar brush cells, likewise contain numerous mitochondria. Although the large thorny excrescences and claw endings do not have synaptic vesicles, they may contain a mitochondrion, suggesting a role beyond vesicular release. The larger synaptic specializations of the relay neurons of the lateral geniculate nucleus also contain many mitochondria but have no presynaptic function (Wilson et al., 1984). During development, mitochondria appear to visit the base of dynamic dendritic protrusions in culture (Li et al., 2004). Hence, mitochondria may provide differential support in the forms of calcium regulation, local protein synthesis, and production of ATP, depending on the function, size, and developmental status of a synaptic specialization.

Microtubules

Small dendritic protrusions such as simple spines and filopodia have cytoskeletons based primarily on actin rather than microtubules, although tubulin is ubiquitous in subcellular fractions of the postsynaptic density. Actin-based cytoskeletons are thought to facilitate rapid, calcium-induced changes in shape (Fifkova, 1985; Fischer et al., 1998; Halpain et al., 1998). Microtubules are not readily observed through serial section electron microscopy in mature hippocampal, cortical, or cerebellar dendritic spines under normal conditions, although they do occur normally in the extra large CA3 thorny excrescences (Ebner and Colonnier, 1975; Chicurel and Harris, 1992) and in hippocampal and cortical dendritic spines during development, apparently emanating from the spine apparatus (Westrum and Gray, 1977; Westrum et al., 1980). During the short period of synaptic quiescence after making a hippocampal slice, microtubules protrude into mature dendritic

spines; however, these spine microtubules can no longer be detected 30 minutes after the slice has recovered in vitro (Fiala et al., 2003). Recent work in hippocampal cultures suggests that microtubules are highly dynamic, and rapidly enter and retract from dendritic spines (Gu et al., 2008; Hu et al., 2008; Jaworski et al., 2009; Dent et al., 2011) such that at any one moment less than 1% of spines may contain microtubule. This transience, along with their sensitivity to calcium, may explain why microtubules are rarely detected in a polymerized state in mature dendritic spines of aldehyde-fixed brain in vivo.

Organelles of the endosomal and recycling pathways

Smooth and coated vesicles and elements of the endosomal pathway also occur in synaptic specializations. The discrete tubules and vesicles of the endosomal compartments are distinguished from the essentially continuous network of SER through 3DEM. In addition, recycling endosomes engulf gold particles conjugated with bovine serum albumin (BSA-gold) that had been delivered in the extracellular space of a hippocampal slice (Cooney et al., 2002). About 50% of normal hippocampal dendritic spines contain no membrane-bound organelles, whereas some spines contain endosomes and others contain SER. Only rarely does a single spine contain both organelles. An endosomal sorting complex occurs about once every 10 µm along the length of a dendrite and hence serves about 10–20 hippocampal dendritic spines (Cooney et al., 2002). Interestingly, the endocytosis-related proteins clathrin, AP-2, dynamin (Racz et al., 2004; Lu et al., 2007), and an isoform of the exocytosis-related protein syntaxin (Kennedy et al., 2010) are all localized to a putative endocytic zone at the edge of postsynaptic densities. Endosomal compartments are dynamically regulated during synaptic plasticity and may provide a local source of new membrane for spinal enlargement (Park et al., 2006). Some endosomal compartments in dendrites may also be involved in autophagocytosis, a process in which proteins and other structures are engulfed by a membrane-bound organelle and incorporated into the lysosomal pathway for subsequent degradation (Bingol and Sheng, 2011). However, the complement of organelles in each instance is unique, suggesting local regulation of subcellular functions, possibly in response to different levels of neuronal activity. Extensive tubular bodies also appear in the dendritic cytoplasm subjacent to puncta adhaerentia (Spacek and Harris, 1998).

Dendritic spines occasionally have even smaller protrusions, *spinules*, that extend from them into the interior of surrounding structures such as boutons or glia (Westrum and Blackstad, 1962; Tarrant and Routtenberg, 1977; Sorra et al., 1998; Spacek and Harris, 2004; Richards et al., 2005). These spinules are surrounded by invaginations of apposed membrane, often with a clathrin-like coat visible on the cytoplasmic side of the engulfing membrane at the tip of the invagination. Spinules in the hippocampus originate from all parts of the dendrite surface, often at the edges of synapses (Fig. 1.12B) and especially from within perforations of the postsynaptic density (Fig. 1.16). The function of spinules is not known; however, they could be involved in bulk membrane recycling or signaling by way of transendocytosis (Spacek and Harris, 2004). Similar structures are found on other types of synaptic specializations, such as on the claw endings of cerebellar granule cells (Eccles et al., 1967) and on the lobes of thorny excrescences (Chicurel and Harris, 1992). It has been suggested that perforations in the postsynaptic density may be related to synaptic plasticity and represent an intermediate stage in a process of proliferation of synapses through splitting (Jones and Harris, 1995; Luscher et al., 2000). However, dendritic spines do not split (Sorra et al., 1998; Fiala et al., 2002a). Indeed, perforations in the postsynaptic density are rather transient structures during plasticity (Applegate et al., 1987; Applegate and Landfield, 1988; Geinisman et al., 1996). Thus, the perforations in the postsynaptic density may arise as a consequence of presynaptic vesicle membrane fusion that pushes the cell adhesion molecules of the pre- and postsynaptic densities

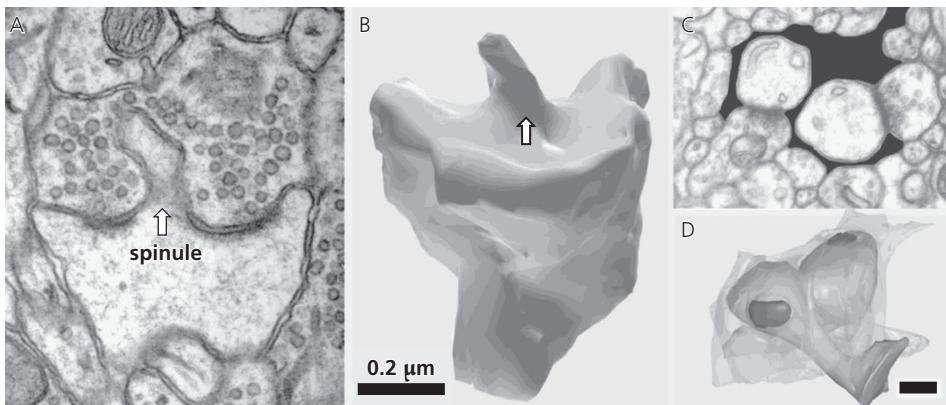


Fig. 1.16 Perisynaptic structure of dendritic spines. **(A)** A spinule (arrow) extends into the presynaptic bouton from the middle of a perforated postsynaptic density on a dendritic spine in adult rat CA1. **(B)** 3DEM of spine with the same spinule (arrow). **(C)** An astrocytic process (black) contacts the edges of synapses onto two dendritic spines in mouse cerebellar cortex. **(D)** 3DEM reveals that these two spines are completely enveloped by the astrocyte (translucent gray).

Part A reproduced from KM Harris and JK Stevens, Dendritic spines of CA1 pyramidal cells in the rat hippocampus: serial electron microscopy with reference to their biophysical characteristics, *The Journal of Neuroscience*, 9(8), pp. 2982–2997, © 1989, The Society for Neuroscience.

sideways and opens a perforation; the postsynaptic density is then knitted back together as bulk endocytosis later ingests the membrane of the postsynaptic spinule (Applegate et al., 1987; Applegate and Landfield, 1988; Geinisman et al., 1996; Spacek and Harris, 2004; Bourne and Harris, 2011, 2012).

Perisynaptic astroglia

Finally, synapses differ in the degree to which they are surrounded by glial processes. In the cerebellar cortex, nearly all spine synapses are completely ensheathed by Bergmann astroglial processes (Fig. 1.16C, D) (Spacek, 1985b). In contrast, only 58% of hippocampal synapses have even partial astroglial ensheathment (Ventura and Harris, 1999), which is comparable to cortical spine synapses (Spacek, 1985b). Thus, many but certainly not all synapses have astrocytic processes at their perimeter, whereby spillover of neurotransmitter between neighboring synapses could be detected and limited (Bergles and Jahr, 1997). Another important aspect of perisynaptic astroglia at excitatory synapses is energy metabolism. Perisynaptic astrocytes contain rich stores of glycogen that provide local energy replacement following synaptic activity (Magistretti et al., 1999). Perisynaptic astroglial processes provide crucial factors, both contact-mediated and secreted factors, for the formation and maturation as well as the maintenance and elimination of synapses (Clarke and Barres, 2013). Interestingly, localization of perisynaptic astroglia in the human hippocampus is also highly susceptible to pathology such as epilepsy (Witcher et al., 2010).

Diversity in spine and synapse density across dendritic arbors

Neurons have been generally classified as *spiny*, *sparsely spiny*, and *non-spiny* (or *smooth*) according to the density of simple dendritic spines throughout the dendritic arbors (Feldman and Peters,

1978). Dendritic spines increase the number of potential synaptic partners for a neuron by extending the reach of dendrites to a larger pool of axons while only slightly increasing brain volume (Swindale, 1981; Harris and Kater, 1994; Chklovskii et al., 2004). The ratio of actual synapses to the number of potential synapses, the so-called *filling fraction*, is estimated to be about 0.2 for cortical pyramidal cells (Stepanyants et al., 2002). Indeed, dense 3DEM reconstructions from hippocampal CA1 neuropil reveal that only 20% of axons touching a dendrite actually made a synapse with that dendrite (Mishchenko et al., 2010).

Classification of neurons by spine density is complicated by the fact that different dendrites of a given neuron may exhibit widely different spine densities. Spine densities can vary ten-fold along the length of a single dendritic segment, and nominally non-spiny dendrites often exhibit a few spines. For example, a CA1 pyramidal cell in a rat has about 30,000 spines, with 55% of them located in the apical dendritic arbor and 40% in the basilar dendritic arbor (Megias et al., 2001). The spine density correlates with dendrite caliber, ranging from less than one spine per micron at the tapered dendritic tips, to two or three spines per micron on oblique dendritic branches and ten or more spines per micron along the thick apical dendrites of hippocampal CA1 pyramidal neurons as they pass through the middle of the stratum radiatum (Harris et al., 1992; Trommald et al., 1995; Bannister and Larkman, 1995b; Megias et al., 2001). High spine densities are found on other neuron types, such as certain neostriatal neurons that have seven spines per micron (Wilson et al., 1983; Graveland et al., 1985). By comparison, pyramidal cells of visual cortex are less spiny, averaging only about one and a half spines per micron (Larkman, 1991).

The spiniest dendrites may be the spiny branchlets of cerebellar Purkinje cells, with 3DEM revealing spine densities reaching 15 spines per micron (Harris and Stevens, 1988a). Thus, a single Purkinje neuron in an adult rat may have over 160,000 spines (Napper and Harvey, 1988a,b). The giant pyramidal cells of Meynert in the visual cortex have basilar dendrites and an apical dendrite in layer 5 that are densely covered with spines, few spines along the apical dendrite in layers 3–4, with a high density of spines occurring again as the dendritic arbor reaches layer 2 (Chan-Palay et al., 1974). For most spiny neurons, the dendrites emerging from the soma receive inputs at shaft synapses; hence, like the soma, proximal dendrites are usually devoid of spines. Thus, differences in characteristic spine densities across the dendritic arbor may reflect differences in connectivity to various inputs.

Dendritic pathology

The characteristic dendritic arbors of neurons are created through a combination of intrinsic developmental programs and environmental influences, as discussed further in later chapters. The cellular environment continues to influence dendrites throughout life, and a number of structural pathologies arise from various adverse conditions (Fiala et al., 2002b; Kuwajima et al., 2013). These pathologies must be distinguished from the normal changes in dendritic and synaptic structure that accompany development, learning, memory, and a host of experiences (Bourne and Harris, 2008, 2012).

One strong influence on dendrite structure is excitatory synaptic input, as studied for many years by lesion-induced degeneration in Golgi-impregnated brain tissue (Globus, 1975). Axonal inputs are necessary for the proper development and maintenance of dendrites. For instance, when granule cells are eliminated from the developing cerebellar cortex, Purkinje cells exhibit profoundly reduced, deformed, and misoriented dendritic arbors (Altman and Bayer, 1997). Dendrite structure continues to be dependent on the preservation of axonal afferents throughout adulthood, since deafferentation is followed by atrophy of the dendritic arbor (e.g., Anderson and Flumerfelt,

1986a,b). The hippocampus offers another example, in which a lesion of the entorhinal cortex atrophies the dendritic arbors of granule cells in the dentate gyrus (Caceres and Steward, 1983): the total dendritic length is reduced to less than 2,000 μm (cf. Table 1) 10 days after the lesion. Most of this reduction occurs in the distal dendrites that normally receive the entorhinal input. Remarkably, the dendritic arbors of deafferented neurons often recover (at least partially) within a few months through reinnervation from sprouting axon collaterals.

Reductions in dendritic arborization are seen in many pathological conditions, such as mental retardation (Kaufmann and Moser, 2000), prionosis (Beck et al., 1975), and Alzheimer's disease (Scheibel, 1982), possibly due to neuronal loss and the associated deafferentation (see also Chapter 24). Even aging seems to produce a degree of dendritic atrophy (Scheibel et al., 1975; Jacobs et al., 1993; Chen and Hillman, 1999; Peters, 2002). Trophic support for the dendritic arbor may come not only from synaptic inputs but also from the synaptic connections of a neuron's axon. In the peripheral nervous system, the dendritic arbor shrinks when a neuron's axon is transected (Purves et al., 1988). In the central nervous system, however, axotomy often produces a retrograde degeneration in which the axotomized neuron dies (Ramón y Cajal, 1991). This neurodegeneration is often characterized by an accumulation in the soma and proximal dendrites of granular endoplasmic reticulum that disintegrates and leads to chromatolysis. The signs of cell death are reflected in the dendrites as shrinking and densification of the cytoplasm, a form of dendritic pathology frequently seen after traumatic injury.

The pathological effects of deafferentation or axotomy may require days to materialize. More immediate alterations in dendrites are apparent with hypoxia or ischemia, as during a stroke. Hypoxia causes a loss of energy that retards the ability of dendrites to maintain ionic polarity at the cell membrane. Abnormal dendritic varicosities are seen in brain tissue damaged by tumors (Fig. 1.17; Spacek, 1987), convulsions (Scheibel and Scheibel, 1977), and cold temperatures.

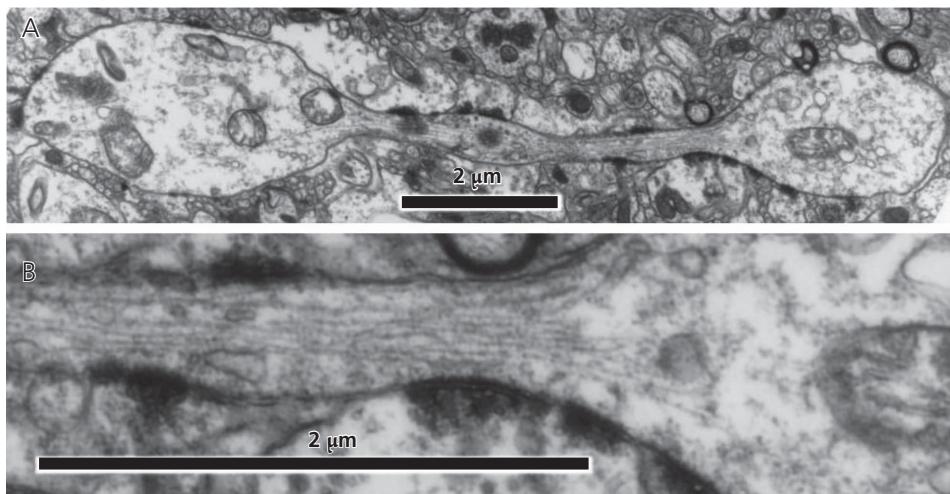


Fig. 1.17 Dendrites beaded by hydropic swelling. **(A)** Electron micrograph showing a segment of dendrite in human peritumorous neocortex in which two varicosities with watery cytoplasm are separated by a narrower region of denser cytoplasm. **(B)** Higher magnification of the intervaricosity region shows that it contains some vesicular components and microtubules and filaments that do not span the varicosities.

Dendritic swelling also affects the cytoplasmic organelles. The SER can become dilated or even swollen into large vacuoles. In Purkinje cells the dendritic SER first forms into lamellar arrays of cisternae (Banno and Kohno, 1996). Mitochondria can also be swollen in dendrites following hypoxia, and microtubules can be completely depolymerized.

A number of progressive neurodegenerative disorders are associated with other forms of dendrite pathology (Hirano, 1981). A different type of dendritic varicosity containing abnormal protein aggregations known as Lewy bodies occurs in Parkinson's disease. In Creutzfeldt-Jakob disease, a form of prionosis, vacuolar dystrophy within dendrites causes a spongiform appearance of the neuropil. Other inclusions of various metabolites may fill the dendritic cytoplasm in different enzymopathies.

Pathology of synaptic specializations of dendrites

The synaptic specializations of dendrites are also prone to structural distortions by a variety of insults and diseases (see Chapter 24). The pathologies of dendritic spines have been particularly well-studied, as summarized in Fig. 1.18 and reviewed in Fiala et al. (2002b) and Kuwajima et al. (2013). Two general categories of spine pathology are distinguished as pathologies of distribution and pathologies of ultrastructure.

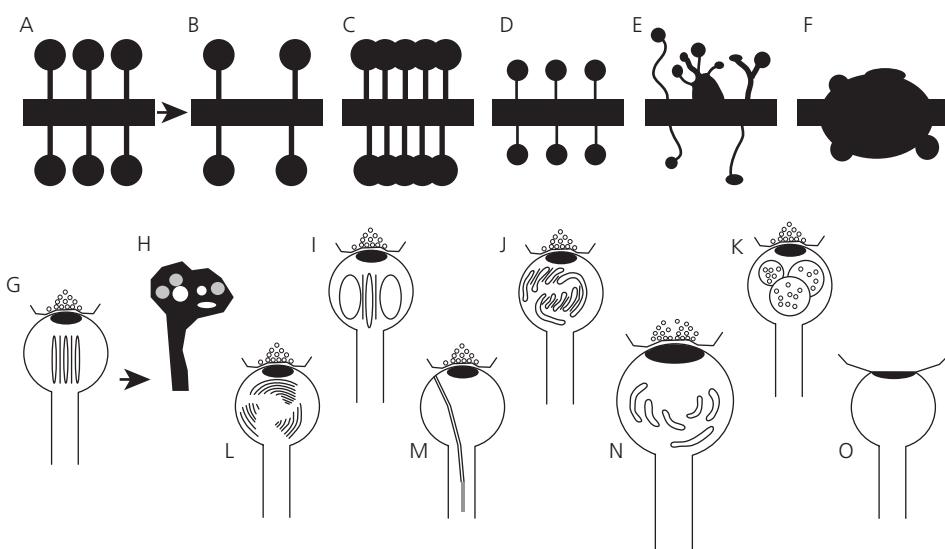


Fig. 1.18 Schematic of dendritic spine pathologies. (A–F) Pathologies of distribution as seen, for example, by light microscopy, involve differences from normal spines (A) by: (B) decreased density, (C) increased density, (D) reduction in spine size, (E) distortions of normal spine shapes, and (F) abnormally varicose dendrites that absorb spines. (G–O) Observed pathologies from normal spine ultrastructure (G) include: (H) shrunken spines with dense cytoplasm, (I) altered endoplasmic reticulum, (J) hypertrophied spine apparatus, (K) hypertrophied multivesicular bodies, (L) hypertrophied cytoskeleton, (M) spine microtubules, (N) giant spines, and (O) axonless spines with an axon-free postsynaptic density.

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Many conditions lead to changes in the number of dendritic spines along spiny dendrites. Spine loss commonly occurs within a few days of deafferentation. Permanent spine loss is evident in most forms of mental retardation, including those resulting from prenatal infection, malnutrition, and exposure to toxins or alcohol. Spine loss is also seen in epilepsy, prionosis, and various neurodegenerative disorders. Increased spine density is seen paradoxically in some types of deafferentation, such as when Purkinje cells are deprived of their climbing fiber input. Increased spine numbers have also been reported following chronic use of stimulatory drugs. In some cases, an overabundance of dendritic spines may represent a failure of normal elimination of synapses during development, as has been suggested for fragile-X syndrome (Cruz-Martin et al., 2010; Portera-Cailliau, 2012).

The absence of normal levels of presynaptic activity without the destruction of the input axons can result in a reduction in synapse and spine size, as reported in the visual cortex following visual deprivation from birth (Globus, 1975). In contrast, deafferentation often results in excessive enlargement of the remaining spines and synapses as an apparent compensatory mechanism for the decreased synaptic input. Deafferentation can also lead to lengthening of dendritic spines, and a similar distortion of the shape of dendritic spines is seen in mental retardation and other conditions. The unusually long and tortuous spines, which have no head enlargement or multiple swellings along their length, resemble in many respects the filopodia seen during developmental synaptogenesis. This resemblance suggests that synaptogenic mechanisms are active in pathological conditions and long, tortuous spines are an additional compensatory response to loss of afferents (Fiala et al., 2002b).

Another response to deafferenting conditions is the formation of axonless spines. Axonless spines exhibit an intracellular structure that looks like a postsynaptic density, but this structure does not form part of a synapse with an axon. Rather, axonless spines contact glia or the dendrites of other neurons (Spacek, 1982). Although they are observed occasionally in the normal brain, the occurrence of axonless spines increases in many pathological conditions, for example developmental agenesis of cerebellar granule cells (Altman and Bayer, 1997).

Alterations in spine organelles can occur for several reasons. One may be degeneration of the postsynaptic cell, as is often the case when the dendritic cytoplasm becomes dense and dark. Another reason may be excitotoxic injury due to excessive presynaptic release of glutamate, which characteristically leads to thickening of the postsynaptic density, a condition often seen in ischemia. Ischemia also induces postsynaptic density-like structures that lie free in the cytoplasm of the dendrite (Tao-Cheng et al., 2001).

Changes in spine endosomes, endoplasmic reticulum, and cytoskeleton have been occasionally observed in edema, for example after traumatic injury. Hydropic swelling and vacuolization of the endoplasmic reticulum of dendritic spines is frequently seen in edematous tissue. In addition to swelling, the spine apparatus can become elaborated or atrophied. Loss of ionic regulation at the plasma membrane can also lead to depolymerization of microtubules in the dendrite shaft, as already mentioned.

Concluding remarks

A hundred years after Ramón y Cajal's pioneering work, the intricacies of the relationship between structure and function in neurons are still being discovered. The pattern of dendritic arborization is clearly related to connectivity but also contributes to dendritic computation, particularly when the dendrite is endowed with active mechanisms (see Chapter 12). The synaptic specializations extended by dendrites also make a significant contribution to connectivity,

allowing thin dendrites to interdigitate in a relatively small brain volume. Their diverse structures presumably have diverse functions related to neuronal computation and learning (see Chapter 18). What these changes in role or differences in dendritic and synaptic structure confer during normal development, learning, and memory remains an area of active investigation. Assuming that form predicts function, the diversity in dendritic and synaptic structure and composition discussed here suggests that many mechanisms are available for adjusting the strength of connectivity across the brain.

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References

- Akert K, Pfenninger K, Sandri C (1967) Crest synapses with subjuncional bodies in the subfornical organ. *Brain Research* 5:118–120.
- Altman J (1971) Coated vesicles and synaptogenesis. A developmental study in the cerebellar cortex of the rat. *Brain Research* 30:311–322.
- Altman J, Bayer SA (1997) *Development of the Cerebellar System: in Relation to its Evolution, Structure, and Functions*. Boca Raton, FL: CRC Press.
- Amaral DG, Ishizuka N, Claiborne B (1990) Neurons, numbers and the hippocampal network. *Progress in Brain Research* 83:1–11.
- Amaral DG, Witter MP (1989) The three-dimensional organization of the hippocampal formation: a review of anatomical data. *Neuroscience* 31:571–591.
- Anderson WA, Flumerfelt BA (1986a) A comparison of the effects of climbing fiber deafferentation in adult and weanling rats. *Brain Research* 383:228–244.
- Anderson WA, Flumerfelt BA (1986b) Long-term effects of parallel fiber loss in the cerebellar cortex of the adult and weanling rat. *Brain Research* 383:245–261.
- Angaut P, Sotelo C (1973) The fine structure of the cerebellar central nuclei in the cat. II. Synaptic organization. *Experimental Brain Research* 16:431–454.
- Applegate MD, Landfield PW (1988) Synaptic vesicle redistribution during hippocampal frequency potentiation and depression in young and aged rats. *Journal of Neuroscience* 8:1096–1111.
- Applegate MD, Kerr DS, Landfield PW (1987) Redistribution of synaptic vesicles during long-term potentiation in the hippocampus. *Brain Research* 401:401–406.
- Ascoli GA, et al. (2008) Petilla terminology: nomenclature of features of GABAergic interneurons of the cerebral cortex. *Nature Reviews Neuroscience* 9:557–568.
- Bannister NJ, Larkman AU (1995a) Dendritic morphology of CA1 pyramidal neurones from the rat hippocampus: I. Branching patterns. *Journal of Comparative Neurology* 360:150–160.
- Bannister NJ, Larkman AU (1995b) Dendritic morphology of CA1 pyramidal neurones from the rat hippocampus: II. Spine distributions. *Journal of Comparative Neurology* 360:161–171.
- Banno T, Kohno K (1996) Conformational changes of smooth endoplasmic reticulum induced by brief anoxia in rat Purkinje cells. *Journal of Comparative Neurology* 369:462–471.
- Bassell GJ, Zhang H, Byrd AL, Femino AM, Singer RH, Taneja KL, Lifshitz LM, Herman IM, Kosik KS (1998) Sorting of beta-actin mRNA and protein to neurites and growth cones in culture. *Journal of Neuroscience* 18:251–265.

- Baude A, Nusser Z, Roberts JD, Mulvihill E, McIlhinney RA, Somogyi P (1993) The metabotropic glutamate receptor (mGluR1 alpha) is concentrated at perisynaptic membrane of neuronal subpopulations as detected by immunogold reaction. *Neuron* **11**:771–787.
- Beaulieu C, Colonnier M (1985) A laminar analysis of the number of round-asymmetrical and flat-symmetrical synapses on spines, dendritic trunks, and cell bodies in area 17 of the cat. *Journal of Comparative Neurology* **231**:180–189.
- Beck E, Bak IJ, Christ JF, Gajdusek DC, Gibbs CJ, Jr., Hassler R (1975) Experimental kuru in the spider monkey. Histopathological and ultrastructural studies of the brain during early stages of incubation. *Brain* **98**:595–612.
- Bell ME, Bourne JN, Chirillo MA, Mendenhall JM, Kuwajima M, Harris KM (2014) Dynamics of nascent and active zone ultrastructure as synapses enlarge during long-term potentiation in mature hippocampus. *Journal of Comparative Neurology* **522**:3861–3884.
- Bennett MV, Zukin RS (2004) Electrical coupling and neuronal synchronization in the mammalian brain. *Neuron* **41**:495–511.
- Benson DL, Huntley GW (2012) Building and remodeling synapses. *Hippocampus* **22**:954–968.
- Bergles DE, Jahr CE (1997) Synaptic activation of glutamate transporters in hippocampal astrocytes. *Neuron* **19**:1297–1308.
- Bingol B, Sheng M (2011) Deconstruction for reconstruction: the role of proteolysis in neural plasticity and disease. *Neuron* **69**:22–32.
- Bourne J, Harris KM (2007) Do thin spines learn to be mushroom spines that remember? *Current Opinion in Neurobiology* **17**:381–386.
- Bourne JN, Harris KM (2008) Balancing structure and function at hippocampal dendritic spines. *Annual Review of Neuroscience* **31**:47–67.
- Bourne JN, Harris KM (2011) Coordination of size and number of excitatory and inhibitory synapses results in a balanced structural plasticity along mature hippocampal CA1 dendrites during LTP. *Hippocampus* **21**:354–373.
- Bourne JN, Harris KM (2012) Nanoscale analysis of structural synaptic plasticity. *Current Opinion in Neurobiology* **22**:372–382.
- Bourne JN, Sorra KE, Hurlburt J, Harris KM (2007) Polyribosomes are increased in spines of CA1 dendrites 2 h after the induction of LTP in mature rat hippocampal slices. *Hippocampus* **17**:1–4.
- Caceres A, Steward O (1983) Dendritic reorganization in the denervated dentate gyrus of the rat following entorhinal cortical lesions: a Golgi and electron microscopic analysis. *Journal of Comparative Neurology* **214**:387–403.
- Cameron HA, Kaliszewski CK, Greer CA (1991) Organization of mitochondria in olfactory bulb granule cell dendritic spines. *Synapse* **8**:107–118.
- Cauli B, Zhou X, Tricoire L, Toussay X, Staiger JF (2014) Revisiting enigmatic cortical calretinin-expressing interneurons. *Frontiers in Neuroanatomy* **8**:52.
- Chan-Palay V (1977) *Cerebellar Dentate Nucleus: Organization, Cytology, and Transmitters*. Berlin: Springer.
- Chan-Palay V, Palay SL, Billings-Gagliardi SM (1974) Meynert cells in the primate visual cortex. *Journal of Neurocytology* **3**:631–658.
- Chang FL, Greenough WT (1982) Lateralized effects of monocular training on dendritic branching in adult split-brain rats. *Brain Research* **232**:283–292.
- Chen S, Hillman DE (1999) Dying-back of Purkinje cell dendrites with synapse loss in aging rats. *Journal of Neurocytology* **28**:187–196.
- Chicurel ME, Harris KM (1989) Serial electron microscopy of CA3 dendritic spines synapsing with mossy fibers of rat hippocampus. *Society for Neuroscience Abstracts* **15**:256.
- Chicurel ME, Harris KM (1992) Three-dimensional analysis of the structure and composition of CA3 branched dendritic spines and their synaptic relationships with mossy fiber boutons in the rat hippocampus. *Journal of Comparative Neurology* **325**:169–182.

- Chirillo MA, Bourne J, Lindsey L, Harris K** (2015) Complexity of dendritic SER increases at enlarging synapses during LTP. *bioRxiv* doi: <http://dx.doi.org/10.1101/015974>.
- Chklovskii DB, Mel BW, Svoboda K** (2004) Cortical rewiring and information storage. *Nature* **431**:782–788.
- Christie JM, Jahr CE** (2006) Multivesicular release at Schaffer collateral-CA1 hippocampal synapses. *Journal of Neuroscience* **26**:210–216.
- Claiborne BJ, Amaral DG, Cowan WM** (1990) Quantitative, three-dimensional analysis of granule cell dendrites in the rat dentate gyrus. *Journal of Comparative Neurology* **302**:206–219.
- Clarke LE, Barres BA** (2013) Emerging roles of astrocytes in neural circuit development. *Nature Reviews Neuroscience* **14**:311–321.
- Colonnier M** (1968) Synaptic patterns on different cell types in the different laminae of the cat visual cortex. An electron microscope study. *Brain Research* **9**:268–287.
- Cooney JR, Hurlburt JL, Selig DK, Harris KM, Fiala JC** (2002) Endosomal compartments serve multiple hippocampal dendritic spines from a widespread rather than a local store of recycling membrane. *Journal of Neuroscience* **22**:2215–2224.
- Cruz-Martin A, Crespo M, Portera-Cailliau C** (2010) Delayed stabilization of dendritic spines in fragile X mice. *Journal of Neuroscience* **30**:7793–7803.
- Cuello AC** (1983) Nonclassical neuronal communications. *Federation Proceedings* **42**:2912–2922.
- Cui-Wang T, Hanus C, Cui T, Helton T, Bourne J, Watson D, Harris KM, Ehlers MD** (2012) Local zones of endoplasmic reticulum complexity confine cargo in neuronal dendrites. *Cell* **148**:309–321.
- Dailey ME, Smith SJ** (1996) The dynamics of dendritic structure in developing hippocampal slices. *Journal of Neuroscience* **16**:2983–2994.
- Deller T, Orth CB, Del TD, Vlachos A, Burbach GJ, Drakew A, Chabanis S, Korte M, Schwegler H, Haas CA, Frotscher M** (2007) A role for synaptopodin and the spine apparatus in hippocampal synaptic plasticity. *Annals of Anatomy* **189**:5–16.
- Dent EW, Merriam EB, Hu X** (2011) The dynamic cytoskeleton: backbone of dendritic spine plasticity. *Current Opinion in Neurobiology* **21**:175–181.
- De Robertis ED, Bennett HS** (1955) Some features of the submicroscopic morphology of synapses in frog and earthworm. *Journal of Biophysical and Biochemical Cytology* **1**:47–58.
- De Zeeuw CI, Ruigrok TJ, Holstege JC, Jansen HG, Voogd J** (1990) Intracellular labeling of neurons in the medial accessory olive of the cat: II. Ultrastructure of dendritic spines and their GABAergic innervation. *Journal of Comparative Neurology* **300**:478–494.
- DiFiglia M, Carey J** (1986) Large neurons in the primate neostriatum examined with the combined Golgi-electron microscopic method. *Journal of Comparative Neurology* **244**:36–52.
- Dunaevsky A, Tashiro A, Majewska A, Mason C, Yuste R** (1999) Developmental regulation of spine motility in the mammalian central nervous system. *Proceedings of the National Academy of Sciences of the United States of America* **96**:13438–13443.
- Ebner FF, Colonnier M** (1975) Synaptic patterns in the visual cortex of turtle: an electron microscopic study. *Journal of Comparative Neurology* **160**:51–79.
- Eccles JC, Ito M, Szentagothai J** (1967) *The Cerebellum as a Neuronal Machine*. New York: Springer.
- Ehlers MD** (2013) Dendritic trafficking for neuronal growth and plasticity. *Biochemical Society Transactions* **41**:1365–1382.
- Ellias SA, Stevens JK** (1980) The dendritic varicosity: a mechanism for electrically isolating the dendrites of cat retinal amacrine cells? *Brain Research* **196**:365–372.
- Fannon AM, Colman DR** (1996) A model for central synaptic junctional complex formation based on the differential adhesive specificities of the cadherins. *Neuron* **17**:423–434.
- Feldman ML, Peters A** (1978) The forms of non-pyramidal neurons in the visual cortex of the rat. *Journal of Comparative Neurology* **179**:761–793.
- Fernández E, Jelinek HF** (2001) Use of fractal theory in neuroscience: methods, advantages, and potential problems. *Methods* **24**:309–321.

- Fiala JC, Feinberg M, Popov V, Harris KM (1998) Synaptogenesis via dendritic filopodia in developing hippocampal area CA1. *Journal of Neuroscience* **18**:8900–8911.
- Fiala JC, Allwardt B, Harris KM (2002a) Dendritic spines do not split during hippocampal LTP or maturation. *Nature Neuroscience* **5**:297–298.
- Fiala JC, Spacek J, Harris KM (2002b) Dendritic spine pathology: cause or consequence of neurological disorders? *Brain Research Brain Research Reviews* **39**:29–54.
- Fiala JC, Kirov SA, Feinberg MD, Petrak LJ, George P, Goddard CA, Harris KM (2003) Timing of neuronal and glial ultrastructure disruption during brain slice preparation and recovery in vitro. *Journal of Comparative Neurology* **465**:90–103.
- Fields RD, Itoh K (1996) Neural cell adhesion molecules in activity-dependent development and synaptic plasticity. *Trends in Neurosciences* **19**:473–480.
- Fifkova E (1985) A possible mechanism of morphometric changes in dendritic spines induced by stimulation. *Cellular and Molecular Neurobiology* **5**:47–63.
- Fischer M, Kaech S, Knutti D, Matus A (1998) Rapid actin-based plasticity in dendritic spines. *Neuron* **20**:847–854.
- Floris A, Dino M, Jacobowitz DM, Mugnaini E (1994) The unipolar brush cells of the rat cerebellar cortex and cochlear nucleus are calretinin-positive: a study by light and electron microscopic immunocytochemistry. *Anatomy and Embryology (Berlin)* **189**:495–520.
- Franks KM, Bartol TM, Jr., Sejnowski TJ (2002) A Monte Carlo model reveals independent signaling at central glutamatergic synapses. *Biophysical Journal* **83**:2333–2348.
- Franks KM, Stevens CF, Sejnowski TJ (2003) Independent sources of quantal variability at single glutamatergic synapses. *Journal of Neuroscience* **23**:3186–3195.
- Freund TF, Powell JF, Smith AD (1984) Tyrosine hydroxylase-immunoreactive boutons in synaptic contact with identified striatonigral neurons, with particular reference to dendritic spines. *Neuroscience* **13**:1189–1215.
- Fukuda Y, Hsiao CF, Watanabe M, Ito H (1984) Morphological correlates of physiologically identified Y-, X-, and W-cells in cat retina. *Journal of Neurophysiology* **52**:999–1013.
- Geinisman Y, Morrell F, de Toledo-Morrell L (1987) Axospinous synapses with segmented postsynaptic densities: a morphologically distinct synaptic subtype contributing to the number of profiles of “perfected” synapses visualized in random sections. *Brain Research* **423**:179–188.
- Geinisman Y, de Toledo-Morrell L, Morrell F, Persina IS, Beatty MA (1996) Synapse restructuring associated with the maintenance phase of hippocampal long-term potentiation. *Journal of Comparative Neurology* **368**:413–423.
- Globus A (1975) Brain morphology as a function of presynaptic morphology and activity. In: *The Developmental Neuropsychology of Sensory Deprivation* (Reisen H, ed.), pp. 9–91. New York: Academic Press.
- Golgi C (1908) La doctrine du neurone, théorie et faits. In: *Les Prix Nobel 1906* (Hasselberg KB, Petterson SO, Mörner KAH, Wirsén CD, Santesson MCG, eds), pp. 1–31. Stockholm: Imprimerie Royale, Norstedt & Söner.
- Graveland GA, Williams RS, DiFiglia M (1985) Evidence for degenerative and regenerative changes in neostriatal spiny neurons in Huntington’s disease. *Science* **227**:770–773.
- Gray EG (1959) Axo-somatic and axo-dendritic synapses of the cerebral cortex: an electron microscope study. *Journal of Anatomy* **93**:420–433.
- Gray EG (1982) Rehabilitating the dendritic spine. *Trends in Neurosciences* **5**:5–6.
- Greenough WT, Volkmar FR, Juraska JM (1973) Effects of rearing complexity on dendritic branching in frontolateral and temporal cortex of the rat. *Experimental Neurology* **41**:371–378.
- Grutzendler J, Kashuri N, Gan WB (2002) Long-term dendritic spine stability in the adult cortex. *Nature* **420**:812–816.
- Gu J, Firestein BL, Zheng JQ (2008) Microtubules in dendritic spine development. *Journal of Neuroscience* **28**:12120–12124.

- Guillery RW** (2005) Observations of synaptic structures: origins of the neuron doctrine and its current status. *Philosophical Transactions of the Royal Society B: Biological Sciences* **360**:1281–1307.
- Halpain S, Hipolito A, Saffer L** (1998) Regulation of F-actin stability in dendritic spines by glutamate receptors and calcineurin. *Journal of Neuroscience* **18**:9835–9844.
- Hama K, Arii T, Kosaka T** (1994) Three-dimensional organization of neuronal and glial processes: high voltage electron microscopy. *Microscopy Research and Technique* **29**:357–367.
- Harris KM** (1999) Structure, development, and plasticity of dendritic spines. *Current Opinion in Neurobiology* **9**:343–348.
- Harris KM, Kater SB** (1994) Dendritic spines: cellular specializations imparting both stability and flexibility to synaptic function. *Annual Review of Neuroscience* **17**:341–371.
- Harris KM, Landis DM** (1986) Membrane structure at synaptic junctions in area CA1 of the rat hippocampus. *Neuroscience* **19**:857–872.
- Harris KM, Stevens JK** (1988a) Dendritic spines of rat cerebellar Purkinje cells: serial electron microscopy with reference to their biophysical characteristics. *Journal of Neuroscience* **8**:4455–4469.
- Harris KM, Stevens JK** (1988b) Study of dendritic spines by serial electron microscopy and three-dimensional reconstructions. In: *Intrinsic Determinants of Neuronal Form and Function. Neurology and Neurobiology Volume 37* (Lasek RJ, Black MM, eds), pp. 179–199. New York: Alan R. Liss.
- Harris KM, Stevens JK** (1989) Dendritic spines of CA1 pyramidal cells in the rat hippocampus: serial electron microscopy with reference to their biophysical characteristics. *Journal of Neuroscience* **9**:2982–2997.
- Harris KM, Weinberg RJ** (2012) Ultrastructure of synapses in the mammalian brain. *Cold Spring Harbor Perspectives in Biology* doi:10.1101/cshperspect.a005587.
- Harris KM, Marshall PE, Landis DM** (1985) Ultrastructural study of cholecystokinin-immunoreactive cells and processes in area CA1 of the rat hippocampus. *Journal of Comparative Neurology* **233**:147–158.
- Harris KM, Jensen FE, Tsao B** (1992) Three-dimensional structure of dendritic spines and synapses in rat hippocampus (CA1) at postnatal day 15 and adult ages: implications for the maturation of synaptic physiology and long-term potentiation. *Journal of Neuroscience* **12**:2685–2705.
- Harris KM, Bourne JN, Mendenhall JM, Spacek J** (2007) Hippocampal CA1 dendrites of greater caliber have more spines and contain more microtubules as a subcellular supply route. *Society for Neuroscience Abstracts* **147**:19.
- Harvey RJ, Napper RM** (1991) Quantitative studies on the mammalian cerebellum. *Progress in Neurobiology* **36**:437–463.
- Henkart M, Landis DM, Reese TS** (1976) Similarity of junctions between plasma membranes and endoplasmic reticulum in muscle and neurons. *Journal of Cell Biology* **70**:338–347.
- Higley MJ, Sabatini BL** (2008) Calcium signaling in dendrites and spines: practical and functional considerations. *Neuron* **59**:902–913.
- Hirano A** (1981) *A Guide to Neuropathology*. Tokyo: Igaku-Shoin.
- Holderith N, Lorincz A, Katona G, Rozsa B, Kulik A, Watanabe M, Nusser Z** (2012) Release probability of hippocampal glutamatergic terminals scales with the size of the active zone. *Nature Neuroscience* **15**:988–997.
- Holmes WR** (1990) Is the function of dendritic spines to concentrate calcium? *Brain Research* **519**:338–342.
- Horton AC, Ehlers MD** (2003) Dual modes of endoplasmic reticulum-to-Golgi transport in dendrites revealed by live-cell imaging. *Journal of Neuroscience* **23**:6188–6199.
- Horton AC, Ehlers MD** (2004) Secretory trafficking in neuronal dendrites. *Nature Cell Biology* **6**:585–591.
- Horton AC, Racz B, Monson EE, Lin AL, Weinberg RJ, Ehlers MD** (2005) Polarized secretory trafficking directs cargo for asymmetric dendrite growth and morphogenesis. *Neuron* **48**:757–771.
- Hrycyshyn AW, Flumerfelt BA** (1981a) A light microscopic investigation of the afferent connections of the lateral reticular nucleus in the cat. *Journal of Comparative Neurology* **197**:477–502.
- Hrycyshyn AW, Flumerfelt BA** (1981b) An electron microscopic study of the afferent connections of the lateral reticular nucleus of the cat. *Journal of Comparative Neurology* **197**:503–516.

- Hrycyna AW, Flumerfelt BA (1981c) Cytology and synaptology of the lateral reticular nucleus of the cat. *Journal of Comparative Neurology* **197**:459–475.
- Hu X, Viessmann C, Nam S, Merriam E, Dent EW (2008) Activity-dependent dynamic microtubule invasion of dendritic spines. *Journal of Neuroscience* **28**:13094–13105.
- Humeau Y, Herry C, Kemp N, Shaban H, Fourcaudot E, Bissiere S, Luthi A (2005) Dendritic spine heterogeneity determines afferent-specific Hebbian plasticity in the amygdala. *Neuron* **45**:119–131.
- Jacobs B, Batal HA, Lynch B, Ojemann G, Ojemann LM, Scheibel AB (1993) Quantitative dendritic and spine analyses of speech cortices: a case study. *Brain and Language* **44**:239–253.
- Jaworski J, Kapitein LC, Gouveia SM, Dortland BR, Wulf PS, Grigoriev I, Camera P, Spangler SA, Di SP, Demmers J, Krugers H, Defilippi P, Akhmanova A, Hoogenraad CC (2009) Dynamic microtubules regulate dendritic spine morphology and synaptic plasticity. *Neuron* **61**:85–100.
- Jones EG (1975) Varieties and distribution of non-pyramidal cells in the somatic sensory cortex of the squirrel monkey. *Journal of Comparative Neurology* **160**:205–267.
- Jones DG, Harris RJ (1995) An analysis of contemporary morphological concepts of synaptic remodelling in the CNS: perforated synapses revisited. *Reviews in the Neurosciences* **6**:177–219.
- Jones EG, Powell TP (1969) Morphological variations in the dendritic spines of the neocortex. *Journal of Cell Science* **5**:509–529.
- Kaufmann WE, Moser HW (2000) Dendritic anomalies in disorders associated with mental retardation. *Cerebral Cortex* **10**:981–991.
- Kennedy MB (2000) Signal-processing machines at the postsynaptic density. *Science* **290**:750–754.
- Kennedy MJ, Davison IG, Robinson CG, Ehlers MD (2010) Syntaxin-4 defines a domain for activity-dependent exocytosis in dendritic spines. *Cell* **141**:524–535.
- Kirov SA, Sorra KE, Harris KM (1999) Slices have more synapses than perfusion-fixed hippocampus from both young and mature rats. *Journal of Neuroscience* **19**:2876–2886.
- Kishi K, Mori K, Tazawa Y (1982) Three-dimensional analysis of dendritic trees of mitral cells in the rabbit olfactory bulb. *Neuroscience Letters* **28**:127–132.
- Klopfenstein DR, Kappeler F, Hauri HP (1998) A novel direct interaction of endoplasmic reticulum with microtubules. *EMBO Journal* **17**:6168–6177.
- Kniffler KD, Pawlak M, Vahle-Hinz C (1994) Fractal dimensions and dendritic branching of neurons in the somatosensory thalamus. In: *Fractals in Biology and Medicine* (Nonnenmacher TF, Losa GA, Weibel ER, eds), pp. 221–229. Basel: Birkhäuser.
- Koch C, Zador A (1993) The function of dendritic spines: devices subserving biochemical rather than electrical compartmentalization. *Journal of Neuroscience* **13**:413–422.
- Kolb H, Linberg KA, Fisher SK (1992) Neurons of the human retina: a Golgi study. *Journal of Comparative Neurology* **318**:147–187.
- Kolb H, Fernández E, Schouten J, Ahnelt P, Linberg KA, Fisher SK (1994) Are there three types of horizontal cell in the human retina? *Journal of Comparative Neurology* **343**:370–386.
- Kraev IV, Godukhin OV, Patrushev IV, Davies HA, Popov VI, Stewart MG (2009) Partial kindling induces neurogenesis, activates astrocytes and alters synaptic morphology in the dentate gyrus of freely moving adult rats. *Neuroscience* **162**:254–267.
- Krichevsky AM, Kosik KS (2001) Neuronal RNA granules: a link between RNA localization and stimulation-dependent translation. *Neuron* **32**:683–696.
- Kruger L, Otis TS (2007) Whither withered Golgi? A retrospective evaluation of reticularist and synaptic constructs. *Brain Research Bulletin* **72**:201–207.
- Kuwajima M, Spacek J, Harris KM (2013) Beyond counts and shapes: studying pathology of dendritic spines in the context of the surrounding neuropil through serial section electron microscopy. *Neuroscience* **251**:75–89.
- Larkman AU (1991) Dendritic morphology of pyramidal neurones of the visual cortex of the rat: III. Spine distributions. *Journal of Comparative Neurology* **306**:332–343.

- Lenn NJ (1976) Synapses in the interpeduncular nucleus: electron microscopy of normal and habenula lesioned rats. *Journal of Comparative Neurology* **166**:77–99.
- Li Z, Okamoto K, Hayashi Y, Sheng M (2004) The importance of dendritic mitochondria in the morphogenesis and plasticity of spines and synapses. *Cell* **119**:873–887.
- Lieberman AR (1973) Neurons with presynaptic perikarya and presynaptic dendrites in the rat lateral geniculate nucleus. *Brain Research* **59**:35–59.
- Lieberman AR, Spacek J (1997) Filamentous contacts: the ultrastructure and three-dimensional organization of specialized non-synaptic interneuronal appositions in thalamic relay nuclei. *Cell and Tissue Research* **288**:43–57.
- Lisman J, Harris KM (1993) Quantal analysis and synaptic anatomy—integrating two views of hippocampal plasticity. *Trends in Neurosciences* **16**:141–147.
- Lu J, Helton TD, Blanpied TA, Racz B, Newpher TM, Weinberg RJ, Ehlers MD (2007) Postsynaptic positioning of endocytic zones and AMPA receptor cycling by physical coupling of dynamin-3 to Homer. *Neuron* **55**:874–889.
- Luscher C, Nicoll RA, Malenka RC, Muller D (2000) Synaptic plasticity and dynamic modulation of the postsynaptic membrane. *Nature Neuroscience* **3**:545–550.
- Luthi A, Laurent JP, Figurov A, Muller D, Schachner M (1994) Hippocampal long-term potentiation and neural cell adhesion molecules L1 and NCAM. *Nature* **372**:777–779.
- McBrayer M, Nixon RA (2013) Lysosome and calcium dysregulation in Alzheimer's disease: partners in crime. *Biochemical Society Transactions* **41**:1495–1502.
- MacGillavry HD, Song Y, Raghavachari S, Blanpied TA (2013) Nanoscale scaffolding domains within the postsynaptic density concentrate synaptic AMPA receptors. *Neuron* **78**:615–622.
- MacNeil MA, Masland RH (1998) Extreme diversity among amacrine cells: implications for function. *Neuron* **20**:971–982.
- McWilliams JR, Lynch G (1981) Sprouting in the hippocampus is accompanied by an increase in coated vesicles. *Brain Research* **211**:158–164.
- Magistretti PJ, Pellerin L, Rothman DL, Shulman RG (1999) Energy on demand. *Science* **283**:496–497.
- Majewski L, Kuznicki J (2015) SOCE in neurons: signaling or just refilling? *Biochimica et Biophysica Acta* doi:10.1016/j.bbamcr.2015.01.019.
- Mariani AP (1990) Amacrine cells of the rhesus monkey retina. *Journal of Comparative Neurology* **301**:382–400.
- Marrs GS, Green SH, Dailey ME (2001) Rapid formation and remodeling of postsynaptic densities in developing dendrites. *Nature Neuroscience* **4**:1006–1013.
- Martone ME, Zhang Y, Simpliciano VM, Carragher BO, Ellisman MH (1993) Three-dimensional visualization of the smooth endoplasmic reticulum in Purkinje cell dendrites. *Journal of Neuroscience* **13**:4636–4646.
- Matsuzaki M, Ellis-Davies GC, Nemoto T, Miyashita Y, Iino M, Kasai H (2001) Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons. *Nature Neuroscience* **4**:1086–1092.
- Megias M, Emri Z, Freund TF, Gulyas AI (2001) Total number and distribution of inhibitory and excitatory synapses on hippocampal CA1 pyramidal cells. *Neuroscience* **102**:527–540.
- Milhaud M, Pappas GD (1966) The fine structure of neurons and synapses of the habenula of the cat with special reference to subjunctional bodies. *Brain Research* **3**:158–173.
- Mishchenko Y, Hu T, Spacek J, Mendenhall J, Harris KM, Chklovskii DB (2010) Ultrastructural analysis of hippocampal neuropil from the connectomics perspective. *Neuron* **67**:1009–1020.
- Morest DK (1969) The differentiation of cerebral dendrites: a study of the post-migratory neuroblast in the medial nucleus of the trapezoid body. *Zeitschrift für Anatomie und Entwicklungsgeschichte* **128**:271–289.
- Mugnaini E, Floris A (1994) The unipolar brush cell: a neglected neuron of the mammalian cerebellar cortex. *Journal of Comparative Neurology* **339**:174–180.

- Mugnaini E, Floris A, Wright-Goss M (1994) Extraordinary synapses of the unipolar brush cell: an electron microscopic study in the rat cerebellum. *Synapse* **16**:284–311.
- Muller D, Wang C, Skibo G, Toni N, Cremer H, Calaora V, Rougon G, Kiss JZ (1996) PSA-NCAM is required for activity-induced synaptic plasticity. *Neuron* **17**:413–422.
- Nafstad PH, Blackstad TW (1966) Distribution of mitochondria in pyramidal cells and boutons in hippocampal cortex. *Zeitschrift für Zellforschung und Mikroskopische Anatomie* **73**:234–245.
- Nair D, Hosy E, Petersen JD, Constals A, Giannone G, Choquet D, Sibarita JB (2013) Super-resolution imaging reveals that AMPA receptors inside synapses are dynamically organized in nanodomains regulated by PSD95. *Journal of Neuroscience* **33**:13204–13224.
- Nansen F (1887) The structure and combination of the histological elements of the central nervous system. *Bergens Museums Aarsberetning for 1886* 27–215.
- Napper RM, Harvey RJ (1988a) Number of parallel fiber synapses on an individual Purkinje cell in the cerebellum of the rat. *Journal of Comparative Neurology* **274**:168–177.
- Napper RM, Harvey RJ (1988b) Quantitative study of the Purkinje cell dendritic spines in the rat cerebellum. *Journal of Comparative Neurology* **274**:158–167.
- Nicholson DA, Geinisman Y (2009) Axospinous synaptic subtype-specific differences in structure, size, ionotropic receptor expression, and connectivity in apical dendritic regions of rat hippocampal CA1 pyramidal neurons. *Journal of Comparative Neurology* **512**:399–418.
- Nicholson DA, Trana R, Katz Y, Kath WL, Spruston N, Geinisman Y (2006) Distance-dependent differences in synapse number and AMPA receptor expression in hippocampal CA1 pyramidal neurons. *Neuron* **50**:431–442.
- Nixon RA (2007) Autophagy, amyloidogenesis and Alzheimer disease. *Journal of Cell Science* **120**:4081–4091.
- Nixon RA (2013) The role of autophagy in neurodegenerative disease. *Nature Medicine* **19**:983–997.
- Nusser Z (2000) AMPA and NMDA receptors: similarities and differences in their synaptic distribution. *Current Opinion in Neurobiology* **10**:337–341.
- Ostapoff EM, Feng JJ, Morest DK (1994) A physiological and structural study of neuron types in the cochlear nucleus. II. Neuron types and their structural correlation with response properties. *Journal of Comparative Neurology* **346**:19–42.
- Ostroff LE, Fiala JC, Allwardt B, Harris KM (2002) Polyribosomes redistribute from dendritic shafts into spines with enlarged synapses during LTP in developing rat hippocampal slices. *Neuron* **35**:535–545.
- Overly CC, Rieff HI, Hollenbeck PJ (1996) Organelle motility and metabolism in axons vs dendrites of cultured hippocampal neurons. *Journal of Cell Science* **109**:971–980.
- Palade GE, Palay SL (1954) Electron microscopic observations of interneuronal and neuromuscular synapses. *Anatomical Record* **118**:335–336.
- Palay SL (1978) The Meynert Cell. In: *Architectonics of the Cerebral Cortex*. (Brazier MA, Petsche H, eds), pp 31–42. New York: Raven Press.
- Palay SL, Chan-Palay V (1974) *Cerebellar Cortex: Cytology and Organization*. New York: Springer.
- Panico J, Sterling P (1995) Retinal neurons and vessels are not fractal but space-filling. *Journal of Comparative Neurology* **361**:479–490.
- Park M, Salgado JM, Ostroff L, Helton TD, Robinson CG, Harris KM, Ehlers MD (2006) Plasticity-induced growth of dendritic spines by exocytic trafficking from recycling endosomes. *Neuron* **52**:817–830.
- Parra P, Gulyas AI, Miles R (1998) How many subtypes of inhibitory cells in the hippocampus? *Neuron* **20**:983–993.
- Peters A (2002) Structural changes in the normally aging cerebral cortex of primates. *Progress in Brain Research* **136**:455–465.
- Peters A, Kaiserman-Abramof IR (1970) The small pyramidal neuron of the rat cerebral cortex. The perikaryon, dendrites and spines. *American Journal of Anatomy* **127**:321–355.

- Peters A, Palay SL (1996) The morphology of synapses. *Journal of Neurocytology* **25**:687–700.
- Pierce JP, van Leyen K, McCarthy JB (2000) Translocation machinery for synthesis of integral membrane and secretory proteins in dendritic spines. *Nature Neuroscience* **3**:311–313.
- Pierce JP, Mayer T, McCarthy JB (2001) Evidence for a satellite secretory pathway in neuronal dendritic spines. *Current Biology* **11**:351–355.
- Popov VI, Medvedev NI, Rogachevskii VV, Ignat'ev DA, Stewart MG, Fesenko EE (2003) [Three-dimensional organization of synapses and astroglia in the hippocampus of rats and ground squirrels: new structural and functional paradigms of the synapse function]. *Biofizika* **48**:289–308 [in Russian with English abstract].
- Popov V, Medvedev NI, Davies HA, Stewart MG (2005) Mitochondria form a filamentous reticular network in hippocampal dendrites but are present as discrete bodies in axons: a three-dimensional ultrastructural study. *Journal of Comparative Neurology* **492**:50–65.
- Porter R, Ghosh S, Lange GD, Smith TG, Jr. (1991) A fractal analysis of pyramidal neurons in mammalian motor cortex. *Neuroscience Letters* **130**:112–116.
- Portera-Cailliau C (2012) Which comes first in fragile X syndrome, dendritic spine dysgenesis or defects in circuit plasticity? *Neuroscientist* **18**:28–44.
- Price JL, Powell TP (1970) The synaptology of the granule cells of the olfactory bulb. *Journal of Cell Science* **7**:125–155.
- Prieto JJ, Winer JA (1999) Layer VI in cat primary auditory cortex: Golgi study and sublaminar origins of projection neurons. *Journal of Comparative Neurology* **404**:332–358.
- Purves D, Snider WD, Voyvodic JT (1988) Trophic regulation of nerve cell morphology and innervation in the autonomic nervous system. *Nature* **336**:123–128.
- Racz B, Blanpied TA, Ehlers MD, Weinberg RJ (2004) Lateral organization of endocytic machinery in dendritic spines. *Nature Neuroscience* **7**:917–918.
- Raghavachari S, Lisman JE (2004) Properties of quantal transmission at CA1 synapses. *Journal of Neurophysiology* **92**:2456–2467.
- Ramón y Cajal S (1954) *Neuron Theory or Reticular Theory* [English translation] (Purkiss MU, Fox CA, eds). Madrid: Consejo Superior De Investigaciones Científicas.
- Ramón y Cajal S (1991) *Degeneration and Regeneration of the Nervous System*. New York: Oxford University Press.
- Ramón y Cajal S (1995) *Histology of the Nervous System of Man and Vertebrates* [English translation]. New York: Oxford University Press.
- Ramón-Moliner E (1968) The morphology of dendrites. In: *The Structure and Function of Nervous Tissue* (Bourne GH, ed.), pp. 205–267. New York: Academic Press.
- Rapp M, Segev I, Yarom Y (1994) Physiology, morphology and detailed passive models of guinea-pig cerebellar Purkinje cells. *Journal of Physiology* **474**:101–118.
- Richards DA, Mateos JM, Hugel S, De Paola V, Caroni P, Gahwiler BH, McKinney RA (2005) Glutamate induces the rapid formation of spine head protrusions in hippocampal slice cultures. *Proceedings of the National Academy of Sciences of the United States of America* **102**:6166–6171.
- Rollenhagen A, Satzler K, Rodriguez EP, Jonas P, Frotscher M, Lubke JH (2007) Structural determinants of transmission at large hippocampal mossy fiber synapses. *Journal of Neuroscience* **27**:10434–10444.
- Ruigrok TJ, De Zeeuw CI, van der Burg J, Voogd J (1990) Intracellular labeling of neurons in the medial accessory olive of the cat: I. Physiology and light microscopy. *Journal of Comparative Neurology* **300**:462–477.
- Scheibel AB (1982) Age-related changes in the human forebrain. *Neuroscience Research Program Bulletin* **20**:577–583.
- Scheibel ME, Scheibel AB (1977) Specific postnatal threats to brain development: dendritic changes. In: *Brain: Fetal and Infant. Current Research on Normal and Abnormal Development* (Berenberg SR, ed.), pp. 302–315. The Hague: Martinus Nijhoff Medical Division.

- Scheibel ME, Lindsay RD, Tomiyasu U, Scheibel AB (1975) Progressive dendritic changes in aging human cortex. *Experimental Neurology* **47**:392–403.
- Schikorski T, Stevens CF (2001) Morphological correlates of functionally defined synaptic vesicle populations. *Nature Neuroscience* **4**:391–395.
- Scorcioni R, Lazarewicz MT, Ascoli GA (2004) Quantitative morphometry of hippocampal pyramidal cells: differences between anatomical classes and reconstructing laboratories. *Journal of Comparative Neurology* **473**:177–193.
- Shepherd GM (1991) *Foundations of the Neuron Doctrine*. New York: Oxford University Press.
- Shepherd GM, Harris KM (1998) Three-dimensional structure and composition of CA3→CA1 axons in rat hippocampal slices: implications for presynaptic connectivity and compartmentalization. *Journal of Neuroscience* **18**:8300–8310.
- Sholl DA (1953) Dendritic organization in the neurons of the visual and motor cortices of the cat. *Journal of Anatomy* **87**:387–406.
- Sholl DA (1967) *The Organization of the Cerebral Cortex*. New York: Hafner Publishing Company.
- Sloper JJ, Powell TP (1978) Dendro-dendritic and reciprocal synapses in the primate motor cortex. *Proceedings of the Royal Society of London Series B: Biological Sciences* **203**:23–38.
- Smith TG Jr., Marks WB, Lange GD, Sheriff WH Jr., Neale EA (1989) A fractal analysis of cell images. *Journal of Neuroscience Methods* **27**:173–180.
- Sorra KE, Harris KM (1993) Occurrence and three-dimensional structure of multiple synapses between individual radiatum axons and their target pyramidal cells in hippocampal area CA1. *Journal of Neuroscience* **13**:3736–3748.
- Sorra KE, Fiala JC, Harris KM (1998) Critical assessment of the involvement of perforations, spinules, and spine branching in hippocampal synapse formation. *Journal of Comparative Neurology* **398**:225–240.
- Sotelo C, Angaut P (1973) The fine structure of the cerebellar central nuclei in the cat. I. Neurons and neuromegial cells. *Experimental Brain Research* **16**:410–430.
- Spacek J (1982) "Free" postsynaptic-like densities in normal adult brain: their occurrence, distribution, structure and association with subsurface cisterns. *Journal of Neurocytology* **11**:693–706.
- Spacek J (1985a) Three-dimensional analysis of dendritic spines. II. Spine apparatus and other cytoplasmic components. *Anatomy and Embryology (Berlin)* **171**:235–243.
- Spacek J (1985b) Three-dimensional analysis of dendritic spines. III. Glial sheath. *Anatomy and Embryology (Berlin)* **171**:245–252.
- Spacek J (1987) Ultrastructural pathology of dendritic spines in epitumorous human cerebral cortex. *Acta Neuropathologica* **73**:77–85.
- Spacek J, Harris KM (1997) Three-dimensional organization of smooth endoplasmic reticulum in hippocampal CA1 dendrites and dendritic spines of the immature and mature rat. *Journal of Neuroscience* **17**:190–203.
- Spacek J, Harris KM (1998) Three-dimensional organization of cell adhesion junctions at synapses and dendritic spines in area CA1 of the rat hippocampus. *Journal of Comparative Neurology* **393**:58–68.
- Spacek J, Harris KM (2004) Trans-endocytosis via spinules in adult rat hippocampus. *Journal of Neuroscience* **24**:4233–4241.
- Spacek J, Hartmann M (1983) Three-dimensional analysis of dendritic spines. I. Quantitative observations related to dendritic spine and synaptic morphology in cerebral and cerebellar cortices. *Anatomy and Embryology (Berlin)* **167**:289–310.
- Spacek J, Lieberman AR (1974) Ultrastructure and three-dimensional organization of synaptic glomeruli in rat somatosensory thalamus. *Journal of Anatomy* **117**:487–516.
- Spacek J, Lieberman AR (1980) Relationships between mitochondrial outer membranes and agranular reticulum in nervous tissue: ultrastructural observations and a new interpretation. *Journal of Cell Science* **46**:129–147.

- Stepanyants A, Hof PR, Chklovskii DB (2002) Geometry and structural plasticity of synaptic connectivity. *Neuron* **34**:275–288.
- Sterling P and Demb JB (2004) Retina. In: *The Synaptic Organization of the Brain* (Shepherd GM, ed.). New York: Oxford University Press p. 217.
- Steward O (1976) Topographic organization of the projections from the entorhinal area to the hippocampal formation of the rat. *Journal of Comparative Neurology* **167**:285–314.
- Steward O, Falk PM, Torre ER (1996) Ultrastructural basis for gene expression at the synapse: synapse-associated polyribosome complexes. *Journal of Neurocytology* **25**:717–734.
- Stoyanova I, Dandov A, Lazarov N, Chouchkov C (1998) GABA- and glutamate-immunoreactivity in sensory ganglia of cat: a quantitative analysis. *Archives of Physiology and Biochemistry* **106**:362–369.
- Svoboda K, Tank DW, Denk W (1996) Direct measurement of coupling between dendritic spines and shafts. *Science* **272**:716–719.
- Swindale NV (1981) Dendritic spines only connect. *Trends in Neurosciences* **4**:240–242.
- Takeda T, Ishikawa A, Ohtomo K, Kobayashi Y, Matsuoka T (1992) Fractal dimension of dendritic tree of cerebellar Purkinje cell during onto- and phylogenetic development. *Neuroscience Research* **13**:19–31.
- Tanaka J, Matsuzaki M, Tarusawa E, Momiyama A, Molnar E, Kasai H, Shigemoto R (2005) Number and density of AMPA receptors in single synapses in immature cerebellum. *Journal of Neuroscience* **25**:799–807.
- Tang L, Hung CP, Schuman EM (1998) A role for the cadherin family of cell adhesion molecules in hippocampal long-term potentiation. *Neuron* **20**:1165–1175.
- Tao-Cheng JH, Vinade L, Smith C, Winters CA, Ward R, Brightman MW, Reese TS, Dosemeci A (2001) Sustained elevation of calcium induces Ca(2+)/calmodulin-dependent protein kinase II clusters in hippocampal neurons. *Neuroscience* **106**:69–78.
- Tarrant SB, Routtenberg A (1977) The synaptic spinule in the dendritic spine: electron microscopic study of the hippocampal dentate gyrus. *Tissue and Cell* **9**:461–473.
- Tønnesen J, Katona G, Rozsa B, Nagerl UV (2014) Spine neck plasticity regulates compartmentalization of synapses. *Nature Neuroscience* **17**:678–685.
- Trommald M, Hulleberg G (1997) Dimensions and density of dendritic spines from rat dentate granule cells based on reconstructions from serial electron micrographs. *Journal of Comparative Neurology* **377**:15–28.
- Trommald M, Jensen V, Andersen P (1995) Analysis of dendritic spines in rat CA1 pyramidal cells intracellularly filled with a fluorescent dye. *Journal of Comparative Neurology* **353**:260–274.
- Uchida N, Honjo Y, Johnson KR, Wheelock MJ, Takeichi M (1996) The catenin/cadherin adhesion system is localized in synaptic junctions bordering transmitter release zones. *Journal of Cell Biology* **135**:767–779.
- Ulfhake B, Kellerth JO (1981) A quantitative light microscopic study of the dendrites of cat spinal alpha-motoneurons after intracellular staining with horseradish peroxidase. *Journal of Comparative Neurology* **202**:571–583.
- Uylings HB, van Pelt J (2002) Measures for quantifying dendritic arborizations. *Network* **13**:397–414.
- Vedrenne C, Klopfenstein DR, Hauri HP (2005) Phosphorylation controls CLIMP-63-mediated anchoring of the endoplasmic reticulum to microtubules. *Molecular Biology of the Cell* **16**:1928–1937.
- Ventura R, Harris KM (1999) Three-dimensional relationships between hippocampal synapses and astrocytes. *Journal of Neuroscience* **19**:6897–6906.
- Volkmar FR, Greenough WT (1972) Rearing complexity affects branching of dendrites in the visual cortex of the rat. *Science* **176**:1445–1447.
- Ward R, Moreau B, Marchand MJ, Garenc C (1995) A note on the distribution of dendritic spines. *Journal für Hirnforschung* **36**:519–522.

- Westrum LE, Blackstad TW (1962) An electron microscopic study of the stratum radiatum of the rat hippocampus (regio superior, CA 1) with particular emphasis on synaptology. *Journal of Comparative Neurology* **119**:281–309.
- Westrum LE, Gray EG (1977) Microtubules associated with postsynaptic “thickenings.” *Journal of Neurocytology* **6**:505–518.
- Westrum LE, Jones DH, Gray EG, Barron J (1980) Microtubules, dendritic spines and spine apparatuses. *Cell and Tissue Research* **208**:171–181.
- Wilson CJ, Groves PM, Kitai ST, Linder JC (1983) Three dimensional structure of dendritic spines in rat striatum. *Journal of Neuroscience* **3**:383–398.
- Wilson JR, Friedlander MJ, Sherman SM (1984) Fine structural morphology of identified X- and Y-cells in the cat’s lateral geniculate nucleus. *Proceedings of the Royal Society of London Series B: Biological Sciences* **221**:411–436.
- Wingate RJ, Fitzgibbon T, Thompson ID (1992) Lucifer yellow, retrograde tracers, and fractal analysis characterise adult ferret retinal ganglion cells. *Journal of Comparative Neurology* **323**:449–474.
- Witcher MR, Park YD, Lee MR, Sharma S, Harris KM, Kirov SA (2010) Three-dimensional relationships between perisynaptic astroglia and human hippocampal synapses. *Glia* **58**:572–587.
- Wolfe DM, Lee JH, Kumar A, Lee S, Orenstein SJ, Nixon RA (2013) Autophagy failure in Alzheimer’s disease and the role of defective lysosomal acidification. *European Journal of Neuroscience* **37**:1949–1961.
- Xu-Friedman MA, Harris KM, Regehr WG (2001) Three-dimensional comparison of ultrastructural characteristics at depressing and facilitating synapses onto cerebellar Purkinje cells. *Journal of Neuroscience* **21**:6666–6672.
- Yelnik J, Percheron G, Francois C (1984) A Golgi analysis of the primate globus pallidus. II. Quantitative morphology and spatial orientation of dendritic arborizations. *Journal of Comparative Neurology* **227**:200–213.
- Yuan A, Rao MV, Veeranna, Nixon RA (2012) Neurofilaments at a glance. *Journal of Cell Science* **125**:3257–3263.
- Yuste R (2013) Electrical compartmentalization in dendritic spines. *Annual Review of Neuroscience* **36**:429–449.

Chapter 2

Evolution and scaling of dendrites

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Summary

Dendrites, the neuronal processes that receive synaptic inputs, are found in all nervous systems. The great diversity of dendrites, both within individual species and across phylogeny, reflects their adaptation to particular functional roles. In this chapter we explore this diversity and consider dendrites from an evolutionary perspective. We review evidence that dendrites scale across phylogeny to preserve the number of synapses per neuron, as well as the architecture of local microcircuits, including the number of neurons in a single neocortical column. Dendritic spines may have originated to form and regulate connections, and later acquired the function of maximizing information storage in densely packed neuropil. These observations are consistent with the unifying principle that dendrites have adapted to keep circuit-level function the same even though program metabolic rates vary widely. Finally, we consider an emerging area of investigation in both normal function and disease: the molecular and developmental mechanisms regulating dendritic form, the targets upon which selection has acted.

Introduction

Dendrites of various neurons share basic features at the molecular, cell biological, and biophysical levels. Yet they also show a remarkable diversity in form, molecular composition, integrative properties, and the ability to conduct action potentials. This diversity, which is found both among different types of neuron and in the same type of neuron across species, suggests the possibility that dendrites have adapted during evolution to fill a wide range of functional roles.

Although evolutionary changes in dendrites have not been observed directly, inferences about their history and relatedness can be made via comparative studies of living animals. Neurons in different species or brain regions may have similar evolutionary origins (homology) or play similar functional roles (analogy). Evidence for such similarities can be found by examining a neuron's: (1) structure or composition, (2) position in neural circuitry, (3) developmental history, (4) information-processing or storage capabilities, and (5) functional role in a given neural system.

Dendrite evolution must be considered in terms of the function of the entire system, the level at which selection occurs. Thus dendrites may change as a direct result of selection pressure to optimize a specific function subject to constraints at the cellular or network level, as a byproduct of optimizing another system, or simply as a result of genetic drift. We will review dendrite evolution in terms of the observed variation in morphology, biophysics, and molecular composition. We describe possible evolutionary constraints influencing dendrites and spines, including biophysical, energetic, and metabolic limits and the functional needs of the organism. Finally, we discuss

how this diversity may have arisen, considering how genetic events, developmental programs, and homeostatic mechanisms shape the form and function of dendrites—and how these mechanisms may be compromised in disease. Overall, this framework provides a means for understanding how and why dendrites vary.

Scaling of dendritic arbors with brain size

Broadly, neurons can be categorized according to whether or not they increase in complexity in animals with larger brains or bodies. This fundamental observation was first framed by Santiago Ramón y Cajal in 1911: “From the appearance of the very first vertebrates, some individual neurons or groups of neurons have been modified more or less continuously before reaching their current state of refinement. In contrast, some neurons remain unchanged over long periods of time, seemingly impervious to all progress” (Ramón y Cajal, 1995, p. 87).

After more than 100 years of comparative studies, Ramón y Cajal’s original observation of dendritic diversity remains intact. However, greater structural complexity is found in neurons from large brains in general, and not just “refined” ones. Thus dendritic size and structural complexity scale predominantly not with cognitive complexity but rather with absolute brain (and body) size (Purves, 1988). This implies that dendritic form may be shaped largely by global tradeoffs common to all animals of a class.

Tradeoffs in the nervous system must take into account both information-processing and biological and metabolic constraints. One such constraint occurs at the level of single cells. Most organs scale with body size by using cells that are of roughly the same size across species and increasing the number of cells (Thompson, 1942). The relative invariance of cell size across species is typical of a wide variety of cell types (Savage et al., 2007), including liver cells, thyroid epithelial cells, renal epithelial cells, pancreatic acinar cells, and red blood cells (Levi, 1905; Teissier, 1939; Altman and Dittmer, 1961; Purves, 1988). Invariance is perhaps unsurprising since many cellular functions would be expected to scale up directly with cell number; for instance, doubling the number of red blood cells would be expected to double the amount of oxygen carried. In this way, a simple increase in cell number might be expected to benefit the organism in a direct and scalable manner.

However, several cell types do scale up with mammalian body size, including neurons in the central nervous system (CNS) (Savage et al., 2007) and neocortical astrocytes (Oberheim et al., 2009). In the case of neurons, scaling takes place at the level of cell bodies, axons (Wang et al., 2008), and dendritic arbors (Fig. 2.1). In addition, the per-gram rate of energy use in mammalian tissues scales as the $-1/4$ power of overall body mass. A negative allometric relationship holds in a variety of tissues, including brain tissue, which is among the most metabolically demanding of tissues. This principle might be of special importance for neocortical astrocytes which actively participate in energy delivery (Bélanger et al., 2011).

The brain constitutes an interconnected network of neurons, and thus its information-processing capabilities depend not only on the number of neural elements but also on the connections formed. The fact that most neurons and their dendrites become larger and more complex as overall brain or body size increases suggests that convergence (the number of synapses received by a neuron) and divergence (the number of neurons contacted by a neuron) make an integral contribution to the overall information-processing capability of the brain. Changes in convergence are reflected, at least to some degree, by the overall size of the dendrite, just as divergence of information is affected by axonal projection and arborization. Understanding the principles governing which types of dendrites do not scale with brain size, and which dendrites do scale, may help us to understand their different roles in information processing.

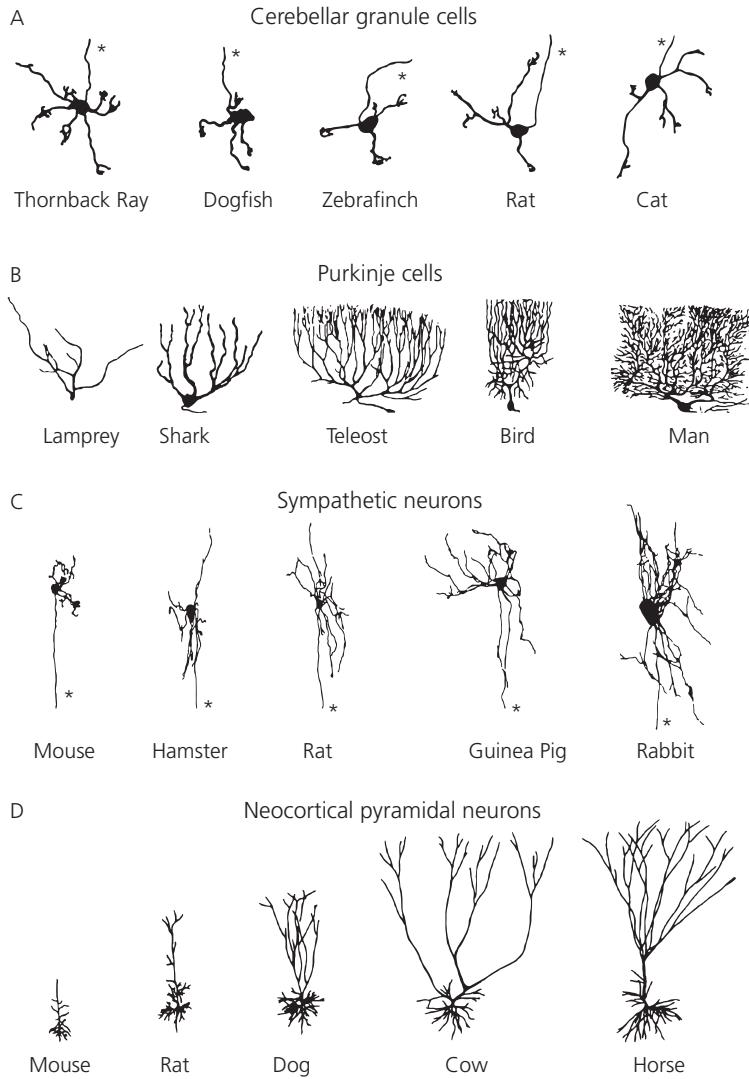


Fig. 2.1 Comparison of vertebrate dendrites across phylogeny. (A) Cerebellar granule cells. (B) Cerebellar Purkinje neurons. Neurons in A and B are scaled to fit the page. (C) Sympathetic neurons. (D) Neocortical pyramidal neurons. Neurons in C and D are scaled to reflect their true size relative to one another. Where appropriate, axons are indicated with asterisks (*).

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Neurons that stay the same

Some types of neurons vary relatively little in size or structural complexity among vertebrates. In these cases the dominant optimization principle may occur at the level of the single cell, such as the requirement for a fixed number of synaptic inputs or for temporal precision.

A prominent example of neuronal constancy is the cerebellar granule cell. Granule cells are a canonical element of the cerebellum, a major division of nearly all vertebrate brains (Eccles, 1969; Llinás, 1969; Nieuwenhuys et al., 1998). Cerebellar granule cells have a small cell body and extrude four or five short, clawed dendrites, which receive input from mossy fibers (Palay and Chan-Palay, 1974). This overall structure is universal to all cerebella, including that of one of the most primitive vertebrates, the dogfish (Alvarez and Anadon, 1987). The most salient change in the evolution of cerebellar granule cells is the appearance of a T-junction in the axon of the granule cell to form two axonal branches per parallel fiber. The constancy of dendritic structure suggests that the number of mossy fiber inputs per granule cell is central to its role in information processing (Marr, 1969; Tyrrell and Willshaw, 1992; Billings et al., 2014). This constancy of granule cell structure contrasts with the fact that their main postsynaptic targets, Purkinje cell dendrites, vary considerably across phylogeny. This scaling of Purkinje cell dendrites may be developmentally driven by the amount of trophic support provided by increasing numbers of granule cells in larger brains, a topic to which we will return.

Receptor cells are, in many cases, also unchanging across species. For instance, in the retina, rod outer segments from diurnal mammals are of fairly uniform length and diameter (Sterling, 2003), and these dimensions are similar in other warm-blooded animals such as nocturnal mammals (Sterling, 2003) and birds (Braekevelt, 1998; Rojas et al., 2004). Rods in these animals are uniformly narrow, 1 to 2 μm in diameter, ensuring a rapid response time—which is linked to the fact that mammals and birds move quickly. Temporal performance requirements may thus impose a general cell-level constraint across species (Sterling, 2003). In contrast, some cold-blooded vertebrates that move more slowly have considerably larger rods (for instance, five-fold wider in sluggish amphibians) with correspondingly slower response times (Sterling, 1997). Thus optimization of rod structure appears to respond to organismal selection expressed directly on the functional properties of single cells, without regard to network size.

Neurons that change as brains get larger

In contrast to granule cells, most other types of neurons vary in their dendritic complexity across species. As originally codified by Ramón y Cajal, in these cases the general form of the dendrite remains recognizable across species, although addition and loss of dendritic elements can occur (Dryer and Graziadei, 1994). Typically, the diameter of the cell body and dendritic arbor size both increase as a function of body size (and therefore of brain size) (Bok, 1959; Purves and Lichtman, 1985; Snider, 1987; Kötter and Feizelmeier, 1998). Scaling of dendritic size occurs throughout the peripheral and central nervous systems. Examples include sympathetic ganglion cells (Ebbesson, 1968a,b; Purves and Lichtman, 1985), olfactory mitral cells (Dryer and Graziadei, 1994), thalamocortical neurons (Ohara and Hayton, 1994), cerebellar Purkinje neurons (Smolyaninov, 1971; Harvey and Napper, 1991), substantia nigra neurons (Yelnik et al., 1987; Tepper et al., 1994; Kötter and Feizelmeier, 1998), hippocampal mossy cells (Buckmaster and Amaral, 2001), and pyramidal neurons of the hippocampus (Bekkers and Stevens, 1990; Buckmaster and Amaral, 2001), entorhinal cortex (Buckmaster et al., 2004), and neocortex (Barasa, 1960; Ramón y Cajal, 1995).

Although dendrites are often larger and more branching in large animals, their general forms stay the same. In somatosensory thalamocortical projection neurons of the rat, cat, and macaque,

Ohara and Havton (1994) found that total dendritic length per neuron varied, but branching parameters such as the degree of bifurcation and terminal branch order were conserved. Similar conservation of dendritic branching form has been found among several neuron types of the substantia nigra in rat, macaque, and human (Yelnik et al., 1987; Tepper et al., 1994; Kötter and Feizelmeier, 1998) and in frog, rat, and cat motoneurons (Dityatev et al., 2001). This conservation of branch patterning suggests that dendrites grow according to shared developmental mechanisms.

A case study: sympathetic neurons

A particularly well-studied case of dendritic diversity is that of autonomic ganglion cells. These neurons are unusual in that they have only one axonal input to an entire dendrite. Scaling principles in these neurons have been investigated extensively (Ebbesson, 1968a,b; Forehand, 1985; Purves and Lichtman, 1985; Purves et al., 1986; Snider, 1987; Ivanov and Purves, 1989).

Purves and colleagues made a number of correlative measurements in five mammals ranging in body size (and target size, in this case the eyeball) from mouse to rabbit. They found that the number of sympathetic neurons increased with body size but did not maintain proportionality. However, they also found that sympathetic neurons from larger-sized animals have more dendrites (Purves and Lichtman, 1985) and larger cell bodies (Snider, 1987). Increases in dendrite number are matched one-to-one by increases in the number of innervating axons: a neuron with no dendrites or one dendrite typically has one input, one with two dendrites two inputs, and so on (Lichtman and Purves, 1980; Lichtman et al., 1980; Purves and Hume, 1981; Hume and Purves, 1983; Forehand, 1985; Purves and Lichtman, 1985). They suggested that in the sympathetic periphery, the number of dendrites, by modulating competition among the axons innervating the same neuron, determines the number of inputs. Thus cell number and dendritic complexity may both provide ways to match target size with the total amount of central drive (Purves, 1988). The fact that scaling occurs at these different stages of processing suggests that adaptations matching a neural system to target size can be distributed over multiple steps of the neural pathway.

Scaling and the biophysics of dendrite diameter

Dendrites vary not only in form but also in diameter along their length. Variations in dendritic diameter are important determinants of both electrical signaling properties and transport properties (Hillman, 1979). These functions may therefore play a critical role in optimizing the shape of dendrites.

As dendrites become larger and more branched, voltage signals traverse a longer distance before reaching the cell body, and are therefore more affected by the filtering properties of the dendritic membrane. These properties are determined primarily by the diameter of the dendrite. In larger-caliber dendrites, passive signals are attenuated less, and both passive spread and active propagation are faster (Rall, 1977). Because of this relationship between diameter and signaling properties, the form of a dendrite might be shaped in part by its signal integration requirements (Purves and Lichtman, 1985; Bekkers and Stevens, 1990; Olsen et al., 1996).

Indeed, dendrites do show great variability in diameter scaling. At one extreme there is zero scaling, in which dendrites are of similar diameter regardless of length. This trend would be sufficient to support signaling in short dendrites, where passive losses are minimal, or in active dendrites, where active properties are tuned to minimize attenuation, assuming negligible delays in conduction. At the other extreme are dendrites whose thickness increases as the square of total dendrite length. This scaling would tend to conserve the amount of passive attenuation along the length of the dendrite. These forms of dendritic scaling, as well as intermediate cases, have been observed

across species and are discussed later. The type of scaling that occurs plays a key role in the properties of signal integration by the dendrite as a whole.

Non-scaling dendrites

Non-scaling dendrites (having a constant mean diameter across a range of brain/body size) have been observed in neurons of the pars reticulata and pars lateralis of the substantia nigra (Yelnik et al., 1987; reviewed by Kötter and Feizelmeier, 1998). In these neurons, soma diameter and dendritic length are larger in humans than in macaques, but dendrite thickness is about the same. Such a lack of scaling suggests that, in these neurons, input location does not influence integration by postsynaptic summation mechanisms (Bekkers and Stevens, 1990). This would occur if, for instance, information processing in these neurons were dominated by regenerative events in dendrites or the cell body.

Constant-diameter dendrites would also be expected in neurons that receive input from only one axon per dendrite, such as cerebellar granule cells. In these neurons, summation occurs at the cell body, and a single input may be sufficient to cause the cell to fire. In this situation small dendrites might be favored as a means of reducing capacitance and therefore the energy cost of changes in membrane voltage. This comparative measurement has not been done (though note the apparent similarity of traced dendritic thickness across species in another cell type with one input per dendrite, namely superior cervical ganglion cells; Purves and Lichtman, 1985).

Another case in which dendritic diameter is approximately constant is in dentate gyrus granule cells of the hippocampus (Bekkers and Stevens, 1990). In a comparison of human and cat, principal dendritic shafts measured at the midpoint of the arbor were of similar diameter. More detailed measurements in macaque and rat show that dentate gyrus granule cells (St. John et al., 1997) have a similar total dendritic length, somatic area, and passive electrical properties, but somewhat different action potential widths. A modest increase in the vertical extent of dendrites occurs in larger animals (St. John et al., 1997).

Isoelectrotonic scaling

At the other extreme of scaling, Bekkers and Stevens (1990) reported that dendrites might vary in an electrotonically conservative fashion, conserving passive cable properties, across species (cat versus human); they cited the example of apical dendritic shafts of CA1 pyramidal neurons. The main shafts were substantially thicker in human than in cat. They proposed that dendritic diameter scaled up as the square of dendrite length in order to preserve the electrotonic distance from the cell body to the ends of the dendrites. Their proposition would be consistent with a more passive summation process in apical dendrites. Thickening of dendrites also argues against active processes, since active amplification in a thicker process would add to the energy cost without contributing to signal integration properties.

Bekkers and Stevens measured dendrite diameter at a single site halfway up the apical dendrite. However, the scaling of apical dendrites would not necessarily be true of basal dendrites, which in rat CA1 pyramidal cells can generate local regenerative voltage events (Polsky et al., 2004). Even within the apical dendrite, regenerative spike-like events can be induced in distal dendrites (Golding et al., 2002) and plateau potentials can be induced in both distal and oblique dendrites (Cai et al., 2004). Dendritic action potentials may serve multiple functions, including the propagation of signals to guide associative plasticity (Hebb, 1949; Brown et al., 1988; Raymond et al., 1996; Malenka and Nicoll, 1999), reset excitability (Häusser et al., 2001), and even drive secretory events (Lledo et al., 1998). These possibilities suggest the need for measurements of dendritic diameter at multiple sites, and correlation with physiological observations.

Scaling of dendrite diameter with length has also been studied over the lifetime of an individual as it grows (Huxley, 1932). Isoelectrotonic growth has been observed in the medial giant interneuron of the cricket, in which passive membrane integrative properties are preserved even as the organism increases seven-fold in length and the dendrite increases 2.6-fold in length (Hill et al., 1994). Isoelectrotonic scaling is also observed in some retinal ganglion cells in the rainbow trout (Picones et al., 2003). In both of these examples, the relative efficacies of synapses on different locations of the arbor are preserved independently of the size or pattern of their dendritic arbor.

Intermediate scaling

Intermediate cases of scaling of dendritic diameter have also been observed. In type I neurons of the substantia nigra pars compacta, the maximum dendrite length is progressively longer from rat to macaque to human (Yelnik et al., 1987; Tepper et al., 1994; Kötter and Feizelmeier, 1998). However, diameters scale up only linearly (isometrically) with dendrite length, which is insufficient to preserve electrotonic properties (Kötter and Feizelmeier, 1998). In these cases conservation of electrotonic function would require additional scaling of channel density and the balance and distribution of channels (Kötter and Feizelmeier, 1998). Isometric scaling of dendrites has also been observed during growth of the lateral giant interneuron of crayfish (Edwards et al., 1994; Hill et al., 1994) and in some retinal ganglion cells in the rainbow trout (Picones et al., 2003). Isometric scaling may represent a case in which the exact integrative properties of dendrites are not critical to the neuron's function.

Other biophysical constraints

Preservation of electrotonic properties is not the only possible explanation for the scaling of dendritic diameters. Dendrites are also conduits for the transport of materials along their length. As a result they may be under pressure to move material efficiently. This is supported by the observation that when a dendrite branches, its daughter branches are smaller in diameter than the parent branch (Lux et al., 1970; Barrett and Crill, 1974a,b; Hillman, 1979). Daughter branch diameters d_1 , d_2 and the parent diameter D approximately obey the relationship $d_1^N + d_2^N = D^N$, where N is between 1.5 and 2. An exponent of $N = 2$ indicates the preservation of cross-sectional area before and after a branch point, a condition that would preserve per-area rates of transport; this has been seen in pyramidal and Purkinje cells (Hillman, 1979). An exponent of $N = 1.5$ is consistent with preservation of charge, and has been approximately observed in motoneurons (Lux et al., 1970; Barrett and Crill, 1974a,b; Hillman, 1979). In any case, this range of exponents corresponds to a fairly narrow range of branching ratios; for symmetric bifurcations, exponents between 1.5 and 2 correspond to a daughter branch being 0.63–0.71 times as wide as its parent.

These branching characteristics may contribute to the above observations of diameter scaling made by Bekkers and Stevens (1990). If in CA1 pyramidal neurons the branching depth (the number of branch points between the soma and dendritic tips) depends on the arbor diameter and the terminal tip diameter is constant, then the diameter at the midpoint of the arbor would have to be larger in human pyramidal neurons, which are more elaborate and have more branches than their counterparts in smaller-brained mammals.

Summary

To summarize, dendritic diameter scales in two ways: (1) across species, measured at a defined location, diameter is either constant or scales up as a function of arbor size, and (2) within an individual arbor diameter decreases with successive branching. Taken together, these phenomena suggest that scaling of dendrite diameter is related to dendrite-specific mechanisms for establishing

a branched architecture. If the size of the smallest dendritic structures is conserved, variations in diameter may regulate the total size of the arbor and thus the degree of synaptic convergence (Purves, 1988; Chklovskii, 2000). Dendrite diameter also affects cable and electrical signaling properties (Purves, 1988; Bekkers and Stevens, 1990) and may be optimized for efficient transport of materials (West et al., 1997; Banavar et al., 1999). The fact that a dendrite must carry out all of these functions suggests that scaling of dendritic diameter may result from the competing influence of these needs. Given that both electrical signaling and transport of materials require scaling it seems likely that, to a certain degree, dendrites conserve both electrotonic properties and transport (Cuntz et al., 2010, 2012). As we will show later, dendrites may also help optimize functional network properties.

Comparative physiology of molecules influencing dendritic computation

In addition to physical form, another feature distinguishing dendrites of different neuronal types is the expression pattern of various molecules that are important for dendrite function. In vertebrates, reception and processing of signals on dendrites begins primarily at chemical synapses. The subsequent processing of inputs is determined by the composition of passive and voltage-gated ion channels and their spatial distribution in the dendritic arbor as well as the complement of neurotransmitters, neuromodulators, postsynaptic receptors, and signal transduction machinery (see Chapter 6). The manner in which neuronal inputs are processed can change over time through synaptic plasticity, which depends on many signaling molecules, including neurotransmitter receptors, second messenger receptors, and downstream kinases, phosphatases, and other enzymes.

These molecular building blocks are found across the vertebrates, and some occur in a wider variety of organisms. Their expression among neurons can vary considerably. For example, excitatory pyramidal neurons of neocortical areas and the hippocampus have apical dendrites in which action potentials can backpropagate at least part of the way, which when paired with presynaptic activation can induce synaptic plasticity (Magee and Johnston, 1997; Markram et al., 1997). In contrast, the large tree-like Purkinje cell dendrites do not support action potential backpropagation at all, but instead generate calcium channel-based action potentials (Llinás and Sugimori, 1980). Yet, like pyramidal neuron action potentials, these calcium-based action potentials can also drive synaptic plasticity (Raymond et al., 1996).

Neurons also differ greatly in their firing properties. These properties are set in part by patterns of channel expression on the soma and dendritic arbor (Migliore and Shepherd, 2002), as well as by the interplay between these channels and dendritic morphology (Mainen & Sejnowski, 1996; Vetter et al., 2001). Additional differences exist at the level of cell biology, neurochemistry, receptor type, and plasticity transduction machinery. This great diversity suggests that different cell types have evolved either to perform different computations or under different sets of constraints. Contrasts between dendrites in different neuron classes are discussed in detail elsewhere in this book (see Chapters 1 and 12).

In recent years a large body of DNA and protein sequence information has been generated, enabling comparative sequence analysis across a variety of species. Particular insights have come from analyzing the proteins associated with synaptic function (Emes and Grant, 2012). In particular, the machinery of the postsynaptic terminal, which is composed of scaffolding molecules, receptors, and signaling enzymes, can be traced back to unicellular eukaryotes, in which the precursors of these proteins played necessary roles in intercellular communication and adaptive responses

(Emes and Grant, 2011). Postsynaptic proteome diversity does not differ greatly between mouse and human (Bayés et al., 2012).

A handful of works illustrate the potential of comparative studies to yield insights into neuronal function. In the cerebellum, Llinás and co-workers found that the climbing fiber input triggers a characteristic, dendritically generated complex spike that takes on a similar multiphasic waveform in the Purkinje neurons of mammals, alligator, frog, and an elasmobranch fish (Llinás, 1969). These spikes have been suggested to provide an instructional signal to guide plasticity (Raymond et al., 1996). Whatever their function, their role is likely to be as old as the vertebrates. As an example of dissimilarity across species, resting firing rates of sympathetic neurons increase with body size (Ivanov and Purves, 1989); both subthreshold and suprathreshold activity rates are higher in larger mammals. This variation may be driven by progressively larger amounts of converging synaptic drive, in line with anatomical findings (see the section Scaling and biophysics of dendrite diameter), and provide an example in which firing rates are not conserved across phylogeny. Finally, at the level of channels, gating parameters may be adapted across species. In a variety of mollusks, sodium channel gating parameters are correlated with locomotor speed (Gilly et al., 1997): animals with faster rates of movement have faster neuronal sodium channel gating.

The rise of dendritic spines as connection maximizers

Dendritic spines vary in size and structure (see Chapter 1 and Yuste, 2011), generally taking the form of protrusions from dendritic shafts of submicron size to as large as 3 μm . Often, spines have a narrow neck terminating in a bulbous head. As postsynaptic structures, they have specialized molecular machinery for receiving synaptic signals (see Chapter 20). The surfaces of spines are covered in neurotransmitter receptors clustered together by the scaffold of the postsynaptic density, a many-component system involved in both synaptic transmission and subsequent biochemical responses. How and why did such specialized structures arise?

Spines are thought to play two major functional roles: to regulate the processing of synaptic signals and to facilitate the formation of synaptic connections. In their first role, spines have been shown to constrain the diffusion of second messengers such as calcium ions as well as confining signaling proteins, including both receptors and supporting machinery. In their second role, spines are motile and have the ability to form and break synaptic connections, especially during development.

Although these two functional roles often coexist in mammals, we will argue that it is unlikely that they arose simultaneously in the evolutionary history of dendrites. Evidence suggests that spines (or spine-like structures) originally arose to form connections dynamically, with signal processing and information optimization arising later in their history.

The evolutionary origin of spines

Dendritic spines have been found in the brains of all vertebrates examined to date, including lampreys (Christensen, 1976). However, not all functions of spines have been present throughout this rich lineage. For example, the exuberant riot of synaptogenesis that occurs in the early postnatal life of mammals cannot be the original function of spines, since such an intensive growth period is unknown among cold-blooded vertebrates. A similar phase of synapse proliferation and elimination occurs in birds (Tzschentke and Plagemann, 2006), and this process is prominent in avian forebrain association areas (Bock and Braun, 1999b). Since spines are deeply involved in brain development in both birds and mammals, this might be an example of convergent evolution, in

which the suitability of a feature for a particular function is exploited independently in different lines of descent.

Closely related to the phenomenon of synaptogenesis is that of synapse elimination, which outstrips the rate of new synapse formation about 30 days after birth in rodent neocortex (Pan and Gan, 2008) and about 1 year after birth in humans (Huttenlocher, 1990; Huttenlocher and Dabholkar, 1997). The resulting net decrease in the number of synapses leads to a mature neocortex with a synaptic connectivity that is considerably sparser than during development.

Spines increase network storage capacity

Stepanyants and Chklovskii (2005) have calculated that the possibilities for synaptic connectivity are far greater if dendrites and axons can reach out to others via spines and boutons, compared with a situation in which axons and dendritic shafts must touch directly. They suggest that the combinatorial increase leads to a substantial increase in information storage capacity for a given amount of space.

Such optimization would be most important in densely packed neuropil, in which all signaling components—cell bodies, dendrites, axons, and synapses—are extremely closely packed. This is the case in the gray matter of the neocortex, cerebellum, and other brain structures. In these structures, spiny neurons are extremely common, as seen in Chapter 1 and in the Neurolex database (Larson and Martone, 2013). Indeed, in many instances, spiny neurons are known to have substantial structural plasticity in adulthood or development (see Chapter 19). If a principal function of spines is to expand the computational capabilities of neuropil combinatorially by expanding the potential dendrite/axon interactions, this suggests that all spiny neurons should explore possible combinations of connectivity at some point during life.

This principle may extend to invertebrates. Spiny morphologies have been described in a variety of invertebrate lineages, including flatworms (Keenan et al., 1981), cephalopods (Young, 1973), crayfish (Kondoh and Hisada, 1986), and many insects (Leiss et al., 2009). Spines are a particularly widespread feature in insect brains, in which closely packed neuropil occurs throughout, including in the antennal lobe, the lobula plate, and the mushroom bodies. The number of synapses in *Drosophila* can change in a matter of hours, linked to the sleep–wake cycle (Bushey et al., 2011), and spines in honeybees change in morphology based on cumulative nursing and foraging experiences (Coss et al., 1980). Volume plasticity has been seen in adult mushroom bodies (Fahrbach, 2006). Notably, developing dendrites in *Drosophila* share molecular mechanisms of structural plasticity with mammals (Andersen et al., 2005). Thus shared mechanisms of structural change that enable combinatorial sampling of possible connectivity patterns have arisen independently in insect and vertebrate lineages.

There is an important caveat when considering invertebrate dendrites: neurites often do not serve dedicated presynaptic or postsynaptic roles, in which case protrusions would not necessarily be referred to as spines. For example, spine-like structures on crayfish neuronal processes have been observed on a branch that is both afferent and efferent (Kondoh and Hisada, 1986). At this point the boundaries begin to blur between spines, boutons, and even varicosities. Since at least some spine proteins descend from ancestors that precede the vertebrate–invertebrate divide (Hultqvist et al., 2012), it should eventually be possible to quantify the similarity in protein composition of spines and spine-like structures.

Although spines have the capacity to form a connection where none existed before, it is an open question whether such events more often represent the formation of a new neuron–neuron connection or regulate the strength of an existing connection, which is often composed of multiple

contacts. For example, in squid, giant fiber postsynaptic collaterals bear many spine-like structures that receive input from the same presynaptic axon. The same is true in mammalian brains: many recordings from connected pairs are made from neurons that are close to one another in neuropil (Markram et al., 1997; Montgomery and Madison, 2002). However, these pairs were investigated rather to maximize the likelihood of finding a connection than to ascertain typical connection strength. In only a few pathways, including the CA3–CA1 synapse (Sorra and Harris, 1993) and the parallel fiber–Purkinje cell synapse (Harris and Stevens, 1988), does the connection between pairs of neurons consist of a single bouton-to-spine contact. In addition, synaptic contacts, especially in their early stages, may not have all the necessary molecular and structural components to generate a physiological response in the receiving neuron; such contacts are called silent synapses (Atwood and Wojtowicz, 1999). It is not at all clear whether mammalian CNS connections are typically composed of one spine or multiple spines. This question awaits the full recovery of circuits, which is now possible using exhaustive reconstruction methods (Denk et al., 2012; Chapter 22).

Finally we turn to the subject of compartmentalization. Spines, by virtue of their narrow neck, have been observed to restrict both biochemical and electrical signals (see Chapters 10, 12, and 15). This compartmentalization of signals has been suggested to be a core function of spines in the mammalian CNS (Koch and Zador, 1993; Yuste, 2011). Note that before the appearance of vertebrates, spine-like structures would not have been able to localize postsynaptic signals and signal localization is possible without spines, but may be less precise when barriers to diffusion are lacking. For example, calcium signaling can still be spatially confined in a dendrite by means of Ca^{2+} buffering and extrusion. Furthermore, before the appearance of vertebrates, spine-like structures that were not postsynaptic would, by definition, not have been able to localize postsynaptic signals. Thus spines enable neighboring synapses in a densely packed dendrite to undergo plasticity more independently, increasing the information storage capacity—and therefore the network function—of the neuron. In summary, the ability of spines to enhance compartmentalization may have taken on added significance after the advent of the vertebrates.

Scaling principles of dendritic spines: changes in size and signaling properties

Local synaptic functions have physical and chemical requirements that are likely to be universal, and therefore impose requirements that are shared across species. These include synaptic transmission, second messenger signaling, and cell biological phenomena such as vesicle trafficking and secretion. One neuronal structure at which all of these functions occur is the synapse.

As already discussed, dendritic spines have been reported to have similar dimensions across species (DeFelipe et al., 2002). In addition, postsynaptic proteome diversity (Bayés et al., 2012) does not differ greatly between rodents and humans. This raises the possibility of *synaptic constancy*: single presynaptic and postsynaptic terminals of a given type may be similar in size across species, and be optimized in a way that is independent of overall brain size. In the first minutes of synaptic plasticity, changes in strength have an all-or-none quality (O'Connor et al., 2005), suggesting that an early step of long-term potentiation (LTP) or long-term depression (LTD) might involve the efficient storage of a single bit of information. Such a capability might require a spine of some minimum necessary volume (Varshney et al., 2006).

Mouse brains have five to ten times as many neurons per unit volume as human brains (Tower, 1954; DeFelipe et al., 2002), yet several quantities seem to vary by less than two-fold: (1) synapse

density per unit volume, and (2) the number of synapses per neuron. Two other quantities may vary even less: (3) the volume of a single bouton-plus-spine, and (4) the number of neurons in a neocortical column. We refer to these as near-invariant quantities of neocortical anatomy. All four quantities are connected to dendritic function.

In a previous edition of this chapter, we wrote that in the mammalian neocortex the total density of synapses per unit volume of neocortical neuropil appeared to be relatively constant both among functional areas and species (Beaulieu and Colonnier, 1985; Cragg, 1967; O'Kusky and Colonnier, 1982). However, a subsequent report (DeFelipe et al., 2002) showed synaptic densities 1.3-fold and 2.7-fold higher in the rat and mouse, respectively, compared with humans. This measurement is consistent with the fact that metabolic rates are higher in smaller brains. Since synapses are thought to consume most of the energy involved in generating neural signals (Alle et al., 2009), one might expect larger brains to have a lower density of synapses and/or lower power consumption per synapse. Indeed, in a comparison between neocortical synapses of mouse and human (Bayés et al., 2012), mouse neocortical synapses express about two to four times more postsynaptic density proteins, including AMPA-type and NMDA-type glutamate receptors, GABA_A receptors, and calcium channels. These molecules are the main means by which ionic gradients are dissipated, so that for a given level of spike activity, a mouse synapse should use up more energy than a human synapse of the same type. In summary, neocortical synapses in larger brains appear to scale down in both their numbers of signaling molecules and their number density, features that may be compatible with the need for a lower per-gram metabolic budget.

Network-level invariant principles that depend on dendritic form

While several clear trends are seen in how dendrites scale with brain or body size, it is difficult to be certain *why* these trends are observed at the dendritic level. The fitness of a neural phenotype is likely to reflect a combination of information-processing needs in single dendrites, network-level functional requirements, and global energetic constraints. Along these lines a number of functional principles have been suggested.

Metabolic/energetic cost and wire length minimization

Neural tissue is energetically costly to operate; this creates pressure to minimize metabolic energy costs arising from neuronal activity (Attwell and Laughlin, 2001). As a result, developmental mechanisms would be expected to drive dendrites to take forms that minimize volume and/or length (Cherniak, 1994; Van Essen, 1997; Chklovskii and Stevens, 2000; Zhang and Sejnowski, 2000; Changizi, 2001). For instance, in layered structures a particular width of dendritic arbor can minimize and equalize the total volumes of axons and dendrites; the arbor width may therefore be closely related to synaptic convergence and divergence ratios (Chklovskii, 2000), and the branching patterns of arbors may have the smallest possible length to achieve a given interconnectivity (Chklovskii, 2004).

Relative invariance of network architecture and input convergence

Central neurons may also be constrained by the need to communicate with one another within the brain. Even when dendrites vary, other principles of circuit function and organization may remain constant. In the neocortex, where most of the comparative work has been done, a review of evidence suggests that two quantities stand out as varying relatively little across species: the number of neurons per neocortical column and the number of synapses per neuron.

The number of neurons per neocortical column

The density of neurons per unit surface area of neocortex has been suggested to be constant (Rockel et al., 1980; Carlo and Stevens, 2013). However, this claim conflicts with the fact that per-volume neuron density goes down fairly steeply, as the -0.32 ± 0.01 power of brain volume. Between mice and humans the difference in cell density is approximately five-fold (DeFelipe et al., 2002; reviewed in Harrison et al., 2002; Wang et al., 2008). Yet the human neocortical sheet is not five times thicker than a mouse's (Hofman, 1988). This implies that the per-area number of neurons must decline substantially in larger brains, a trend that has been observed in counts of dissociated neurons from fixed tissue (Herculano-Houzel et al., 2008). The disagreement may arise from difficulties that occur when counting methods fall short of full-volume reconstruction. Resolution may require the application of more complete structural methods (Tsai et al., 2009).

A more important functional principle may be preserved. Neocortical columns tend to be larger in larger-brained mammals (Changizi, 2003). When this scaling trend is included along with increases in neocortical thickness, the volume of a column may go up approximately as the $+1/3$ power of brain size (Harrison et al., 2002). If this is true, then the number of neurons per column may be invariant even though per-area density varies.

It should be noted that such a scaling principle is only an approximation. Within the neocortex, circuit principles are likely to vary locally according to the functions of specialized regions (Jones and Rakic, 2010). Eventually, identification of conserved principles will require analysis of homogeneous populations of neuron subtypes.

The number of synapses per neuron

Convergence can be quantified as the number of synapses per neuron. This number has variously been reported to be similar across matching regions of the neocortex or to scale up with brain size (Cragg, 1967; Beaulieu and Colonnier, 1985; DeFelipe et al., 2002). This number has been reported to be 0.6-fold greater in the monkey primary visual cortex compared with the cat (Beaulieu and Colonnier, 1985), 0.8-fold greater in the monkey visual cortex compared with the mouse (Cragg, 1967), 1.4-fold greater in the human compared with the mouse (DeFelipe et al., 2002), 1.7-fold greater in the human compared with the macaque (Elston et al., 2001), 1.7-fold greater in the human compared with the rat (DeFelipe et al., 2002), and 4.6-fold greater in the monkey motor cortex compared with the mouse (Cragg, 1967). The median ratio, 1.5 ± 0.6 , is considerably smaller than the more than 1,000-fold range in brain volumes represented by these species. Thus, on average, the number of synapses per neocortical neuron increases weakly or does not change as a function of brain size.

Invariant columns, but of varying size?

If the number of synapses per neuron and the number of neurons in a column are only weakly varying quantities, then the architecture of a neocortical column could be a constant functional entity. This principle has been suggested as an organizing principle that holds across mammalian phylogeny (Rakic, 1995). If so, this would suggest that such a principle has been roughly maintained despite variations in a column's physical width and the thickness of the cortical sheet.

How do columns fit into a neocortex-wide architecture?

Even a modest increase in the number of synapses per neuron could have substantial effects on network-wide function. In a recurrently connected network a parameter of great functional interest is the network diameter, defined as the minimum number of synapses connecting any two

neurons, on average. Maintaining the network diameter at a given value N requires the number of connections per node (i.e., synapses per neuron) to scale proportionally to the N th root of the number of neurons in the network (Changizi, 2001). For example, to maintain a network diameter of $N = 6$, a brain with 100 times as many neurons would only need to have approximately 1.7 times as many synapses per neuron.

From monkey to human, an increase in number of synapses per neuron has been suggested to support cognitive complexity (Elston et al., 2001). However, this increase may well be simply a general scaling consequence of the large absolute size of human brains. If the increase in individual neuronal complexity with respect to total brain size is simply a general scaling relationship that maintains network connectivity, then the relative cognitive complexity of some animals (e.g., chimpanzees, humans crows, and parrots) may be more appropriately thought of as a function of anomalous total brain size for a given body size (Jerison, 1973; Reader and Laland, 2002; Burish et al., 2004). It will be of interest to ascertain whether the neocortex maintains a constant network diameter across a wide range of mammals.

In non-recurrent networks such as the cerebellar cortex, which has a multilayered architecture (Marr, 1969; Albus, 1971), the optimal design of a network may require some of its elements to scale up with overall network size. As described earlier, cerebellar granule cells do not scale with cerebellum size. However, Purkinje cell arbors, which predominantly receive parallel fiber synapses from granule cells, may become larger with brain size. (Nieuwenhuys, 1967; Eccles, 1969; Smolyaninov, 1971). In a comparison of the rat and the cat (Harvey and Napper, 1991) the number of spines per Purkinje cell increased from 150,000–175,000 in the rat to 300,000–360,000 in the cat. This, and some structural data from other species (Smolyaninov, 1971; Harvey and Napper, 1991), indicates that processing in large cerebella is made most efficient in some way by scaling up the amount of parallel fiber convergence on each Purkinje cell. What is being optimized by such scaling is not known.

Network degeneracy

Neurons are adapted to the wider context of the network in which they reside. For example, in the central pattern generator controlling aerial respiration in the slug *Lymnaea stagnalis* (McComb et al., 2003), respiration frequency is higher in adults than in juveniles. However, the neuron initiating rhythrogenesis, RPeD1, scales isometrically over the animal's lifetime, showing a decrease in membrane resistance and an increase in membrane time constant. This modification alone would correspond with decreased excitability in the adult; instead a compensatory increase in synaptic connectivity works to increase rhythmic activity of the pattern generator. More complex tradeoffs can also occur at a whole-network level. For example, in the lobster stomatogastric system very different combinations of neuronal parameters can lead to nearly indistinguishable output patterns (Bucher et al., 2005). Thus, even in a simple network the large number of degrees of freedom in the system can adapt in many ways to produce a limited range of output patterns.

Brain architecture and species-specific adaptations

The finding that dendritic form scales largely as a function of brain and body size suggests that the differing capabilities of brains in different species would be more likely to be generated not by varying dendrites and other aspects of neuronal form, but rather by varying the size of a brain or brain region (Kaas, 2000). If this is the case, then individual neuronal design may be imposed by the overall layout and absolute size of a neural architecture, and added functionality may come about in large part from adding more neurons or modules of neurons. Prominent examples of enhanced

function associated with larger region size include: the correspondence between cognitive complexity and telencephalic size in mammals (Dunbar, 1992; Clark et al., 2001) and birds (Lefebvre et al., 2002; Burish et al., 2004); electrolocation/echolocation and cerebellar volume in mormyrid fishes, bats, and whales (Meek, 1992; Clark et al., 2001); and song repertoire and volume of song system nuclei in passeriform birds (DeVoogd et al., 1993).

In some cases, however, variation in dendritic form may be species-specific (Fig. 2.1B and Nieuwenhuys, 1967). Such specializations might be evident as a departure from the expected scaling relationship. These departures are of particular interest from evolutionary or ethological perspectives. Perhaps as a result, the best evidence for exceptional dendrites has been found in animals with unusual behavioral specializations.

One such type of specialization encompasses acoustic localization, echolocation, and electroreception. In all of these functions, localization of acoustic and electrical stimuli requires calculation of phase differences among signals from multiple sources. Structural adaptations in dendrites may aid in this process biophysically by making the timing of membrane electrical signals particularly precise or fast. In barn owls, which use interaural time and phase differences to locate prey by their sounds, there are more neurons in the nucleus magnocellularis than in other birds (Carr and Boudreau, 1993). In addition, neurons of the magnocellularis and laminaris that are sensitive to higher frequencies have few and short dendrites and thick axons. These differences have been suggested to have the effect of reducing capacitance and increasing the speed of responses. This could be useful for detecting fine differences in the timing of acoustic signals. As another example, mormyrid fish are capable of electroreception. In their exceptionally large “gigantocerebella” the palisade patterns of Purkinje cell dendrites may be adapted in some way for signal processing via parallel fiber pathways (Meek and Nieuwenhuys, 1991). Echolocation, an ability found in cetaceans and insectivorous bats, is accompanied by exceptionally large cerebella (Clark et al., 2001); dendrites of cerebellar neurons in these species have not been examined systematically.

Another place in which unusual dendritic specializations may occur is the neocortex of great apes, which show unusual social and cognitive complexity. These animals have several types of giant neocortical neurons, including Betz cells (Sherwood et al., 2003), Meynert cells (Sherwood et al., 2003), and spindle cells (Nimchinsky et al., 1999). These giant cells may have arisen in great apes as extreme adaptations of pyramidal neurons. The somata of primates show distinct scaling relationships relative to brain and body size. Like other neocortical pyramidal neurons (Elston et al., 2001), they may vary in dendritic extent and synapse number across species as well.

Genetic and developmental constraints influencing dendritic form

Although in animal evolution the unit of natural selection is the individual organism, the target upon which selection acts is development. Thus, to better understand the origins of diversity in dendritic structure and to get a feel for what types of changes might occur, we need to consider how changes at the level of DNA can lead to changes in the molecular mechanisms that shape dendrite structure. The evolution of single proteins is a well-known concept. Here we will emphasize a few additional points concerning how morphological changes can arise.

Gene regulatory networks are organized, at least in part, in a hierarchical manner. Genes at the top of such hierarchies may act as “master control genes” for large-scale developmental programs (Keynes and Krumlauf, 1994; Halder et al., 1995). For example, the cerebellum and several cerebellar-like structures, including the medial octavolateral nucleus, dorsal octavolateral nucleus, electrosensory lobe, and dorsal cochlear nucleus, show striking similarities at both the cellular and network level and originate from one region of the alar plate (Devor, 2000; Bell, 2002), suggesting

that these structures arise from a shared developmental program (Bell, 2002). Indeed, small, ectopic cerebellum-like structures can be induced in the forebrain or midbrain simply by placing growth factor-coated beads into developing embryos (Martinez et al., 1999). Hierarchical regulation may also regulate modular structures: new neocortical areas have been suggested to arise from the duplication of an existing area by changing a discrete developmental step (Krubitzer, 1995; Kaas, 2000). Thus, mutations of important developmental genes represent one means of achieving large-scale modifications in brain structure.

Another means of effecting changes in macroscopic and cellular form is mutation that regulates growth rates during development. These mutations could affect multiple cell types, but not necessarily so; for example, mutations in *cis*-regulatory DNA elements can have fairly specific effects and have been suggested to be a major source of organ-level morphological evolution (Stern, 2000). Application of these concepts at the level of dendritic structure suggests that, depending on the specificity of effect, mutations might be able to affect the scaling of one type of neuron or many types of neurons at once.

In the case of dendritic form and size, the targets of selection would be mechanisms regulating the growth and maturation of arbors (Jan and Jan, 2010). These mechanisms are studied in several key model systems, including cultured mammalian cells (Craig and Banker, 1994; McAllister, 2000; Scott and Luo, 2001; Whitford et al., 2002) and *in vivo* studies of identified neurons from the common fruit fly, *Drosophila melanogaster* (Gao et al., 1999; Gao and Bogert, 2003; Grueber and Jan, 2004). Thorough reviews of the literature can be found in this book (see Chapters 3 and 4).

Dendrite development can be broken down into four stages: initial dendrite outgrowth, branching, stopping, and pruning and refinement. Here we offer a brief overview of the intrinsic and extrinsic mechanisms regulating dendrite shape and size. This overview provides a means for thinking about how developmental mechanisms, shaped by natural selection, might influence dendritic form.

Dendrite outgrowth and branching

The first stage of dendrite growth is the polarization of neurons to generate distinct axonal and dendritic processes. Neurons develop axon-somato-dendritic polarity even in cell culture (for review see Craig and Banker, 1994). Even at this early developmental stage, initial process outgrowth can be greatly influenced by extrinsic factors such as growth factors and extracellular matrix proteins (Chamak and Prochiantz, 1989; Lein and Higgins, 1989; Lein et al., 1992; Osterhout et al., 1992). As development proceeds, dendrites begin to branch. Diversity of branching structures has been generated by modeling different growth rules (van Pelt et al., 1997; Cannon et al., 1999), suggesting these rules to be a target for selection. Branching patterns can be described in terms of branching probability as a function of distance from the soma, a parameter that can capture differences between complex pyramidal cells and cells of simpler dendritic structure, such as various classes of motoneurons and dentate granule cells (van Pelt et al., 1997). Two different types of branching events have been observed: splitting of growth cones to generate two new branches and interstitial branching, the emergence of a new branch from an established dendritic shaft (Bray, 1973; Dailey and Smith, 1996; Scott and Luo, 2001). The first step in interstitial branch formation is the extension of a filopodium from a shaft, which can extend, retract, or become a spine or new branch (Dailey and Smith, 1996). In this way a combination of growth and retraction results in an elaborated arbor of branches and spines.

Dendritic growth and branching is affected by both intrinsic and extrinsic factors (see Chapter 3). Branching is influenced intrinsically by cytoskeletal-associated proteins, small GTPases,

transmembrane proteins, and transcription factors (Threadgill et al., 1997; Ruchhoeft et al., 1999; Lee et al., 2000; Li et al., 2000; Hakeda-Suzuki et al., 2002; Luo, 2002; Whitford et al., 2002; Gao and Bogert, 2003; Emoto et al., 2004; Rosso et al., 2005). Extrinsic factors include a wide range of neurotrophins, which affect both cell survival and dendritic growth in a neurotrophin- and cell type-specific manner (Snider, 1988; McAllister et al., 1995; 1997). The growth of dendrites proceeds through several stages marked by the appearance of morphological features, and different factors are involved in the regulation of each stage of growth (Scott and Luo, 2001).

However, normal branching patterns cannot be entirely replicated in culture. For instance, although Purkinje neurons co-cultured with granule cells or densely plated Purkinje cells or neurotrophins (BDNF or NT-4) develop distinctive Purkinje-like arbors (Baptista et al., 1994; Morrison and Mason, 1998), they do not have the same planar arrangement and appear to branch less frequently. Likewise, cultured pyramidal neurons (Dotti et al., 1988; Collin et al., 1997) fail to form distinct apical and basal dendrites. This suggests that cultures lack other signaling factors or some other aspect of the normal three-dimensional matrix in which neurons grow.

In addition to branching patterns, another key aspect of a neuron's function is the orientation of its dendrites relative to other neural elements. As in the case of axon guidance, this orientation is established, at least in part, by chemical cues. One well-studied example is the effect of the diffusible ligand semaphorin 3A (Sem3A). Dendrites of neocortical pyramidal neurons grow toward and axons grow away from the cortical plate where Sem3A is expressed (Giger et al., 1996; Polleux et al., 1998; Scott and Luo, 2001); a similar effect of Sem3A is seen in slice cultures (Polleux et al., 1998; 2000). In larger-brained animals, the fact that this gradient should extend over a larger region in space may result in larger dendrites. Dendrite orientation is also shaped by self-avoidance mechanisms that include Dscam and Slit/Robo (Matthews et al., 2007; Gibson et al., 2014).

Stopping

Establishment of a dendritic arbor must include a mechanism for stopping growth. One well-studied example is the effect of contact inhibition on halting the growth of neurons of the same type (homotypic inhibition). A well-known example of tiling is observed in the retina, where the dendrites of some ganglion cell types appear to tile the retina with minimal overlap of neighboring dendritic fields (Wässle et al., 1975). Tiling mechanisms are beginning to be investigated in *Drosophila* (Gao et al., 2000; Grueber et al., 2002; 2003; Emoto et al., 2004) and in *Caenorhabditis elegans* (Gallegos and Bargmann, 2004). In *Drosophila*, the dendrites of larval medial dorsal (MD) neurons grow until their arbors contact one another around the dorsal midline (Gao et al., 2000). Ablation of one MD neuron results in overgrowth of the surviving contralateral neuron. In addition, the dendritic arborization (DA) neurons of the epidermis also exhibit tiling and can be classified into four neuronal subtypes based on their dendritic branching morphologies and tiling rules. Type I and II neurons show homotypic repulsion. Neurons in the third class only repel one another at terminal branches. Neurons of the fourth class show persistent repulsion at all branches (Grueber et al., 2002; 2003).

Such stopping mechanisms may provide a means of determining how some dendrites will scale relative to brain size. For example, neuron density has been observed to decrease with increasing brain size (Tower, 1954; Lange, 1975; reviewed in Harrison et al., 2002). Thus regulation of dendrite growth by intercellular mechanisms would tend to lead to larger dendritic arbors in larger brains. Conversely, because gray matter is composed of closely packed neural elements, stopping mechanisms could affect neuron density itself by regulating the amount of space occupied by dendrites.

Pruning and refinement

Another well-studied type of extrinsic regulation of dendritic form is neural activity (see Chapter 3). Neural activity has been shown to influence structural changes including the addition and retraction of spines (Engert and Bonhoeffer, 1999; Pan and Gan, 2008) as well as larger-scale effects on addition and elimination of branches (McAllister et al., 1997; Maletic-Savatic et al., 1999). In several brain regions it has been observed that synaptic density decreases with age (Purves and Lichtman, 1980; Rakic et al., 1986; Bourgeois and Rakic, 1993; Rakic et al., 1994), suggesting that overgrowth occurs during the initial development of dendrites in these brain regions. In many cases, the subsequent pruning of synapses over the course of maturation appears to be activity dependent (Bock and Braun, 1999a; Kakizawa et al., 2000). The effects of neural activity on structure at the synaptic and the dendritic level seem to be exerted by factors such as neurotrophins and/or other molecules involved in synaptic plasticity, such as NMDA receptors (Katz and Shatz, 1996; McAllister et al., 1996, 1997; Bock and Braun, 1999a).

These developmental mechanisms provide numerous possibilities for the regulation of dendritic form. The dramatic influence of extrinsic factors on dendrite development suggests that these factors may participate in driving the scaling of dendrites with brain and body size. These factors could act independently or in conjunction with neural activity to regulate growth. The role of extrinsic factors also suggests that in larger-brained animals, gradients of diffusible ligands may extend over larger spatial scales; for example, chemoattractive gradients should extend over larger spatial scales in larger brains and perhaps make dendrites grow longer. Intercellular inhibitory mechanisms will also scale if neurons of the same type (for example, hippocampal neurons in human and mouse) are spaced farther apart in larger brains. Finally, dendrite development could proceed in a programmed fashion, so that larger dendrites could be generated simply by allowing growth to proceed for a longer period of time.

Disease states

Molecular mechanisms of dendrite growth and plasticity are likely to be both targets and underlying causes of disease processes. Most diseases of the human brain are studied today using imaging methods such as structural and functional MRI. These approaches do not capture aspects of dendrite structure and function at the single-neuron level, but instead measure aggregate phenomena on the scale of millimeters and seconds. However, they may point the way toward understanding disorders at the level of circuit or single neurons, especially in conjunction with other methods that can probe dendritic function, such as morphology and gene expression in postmortem specimens, as well as physiological approaches in animal models (see also Chapter 24).

As an example, autism spectrum disorder is often accompanied by aberrant early life trajectories of gray matter growth in both the neocortex and cerebellum (Schumann et al., 2010; Wang et al., 2014). At the level of single dendrites, developmental spine pruning is reduced in the temporal lobe (Tang et al., 2014). Together, these findings suggest that normal mechanisms of dendritic plasticity may have gone off track. This could occur if the mechanisms of neural plasticity are not working properly in a particular region of the brain, or if local mechanisms are intact but incoming information is aberrant, thus driving activity-dependent plasticity mechanisms in a pathological manner (for instance, developmental diaschisis of cerebellar influence over neocortical plasticity; see Wang et al., 2014). In either case, a key challenge is reconciling macroscopic and microscopic changes in tissue with one another (Schumann and Nordahl, 2011), as well as linking these changes to heritable and environmental causes (Wang et al., 2014).

Epilogue and future directions

In reviewing variations in dendrites across species we have identified three major themes. The first theme is that many neurons and dendrites scale up in size and complexity as a function of overall brain or body size. This scaling is not homogeneous: some cell types show strong scaling and others are nearly constant in form. The second theme is the presence of constraints at the level of cellular and network function that have guided dendritic form. The third theme is the fact that this dendritic diversity has been generated by natural selection acting upon developmental mechanisms. We suggest that the study of dendritic optimization and evolution could be advanced by directed work in a number of areas: comparative investigations of scaling; links to animal behavior; and developmental mechanisms.

Comparative investigations of scaling

The bulk of work on comparing dendritic form has focused on branching patterns. Advances during the last 20 years in the study of synaptic physiology and dendritic function have established a framework within which to examine interspecies variability. Parameters that need to be characterized on a comparative basis are: morphological, such as dendrite diameter and spine number; biochemical, such as the abundance and kinetics of signaling molecules; and physiological, such as neuronal firing rates and the integrative properties of dendrites. These investigations would be directed at the questions of how parameters vary systematically across phylogeny and what neuronal functional principles are invariant. It would be most advantageous to focus on standard cell types that are already well studied in rodents at all these levels. A prominent candidate would be pyramidal neurons in either the hippocampus or neocortex. Other good choices would include Purkinje neurons and cerebellar granule cells, which are found in nearly all vertebrates and furthermore offer a contrast in scaling properties with one another.

Links to animal behavior

A significantly under-investigated area is the link between behavioral specializations and variation at a cellular level. In brain regions and cell types with known involvement in specific behaviors it should be possible to systematically connect variations in the number of neurons and their anatomical and physiological features with variations in function. One particularly promising target of study is the song system in passeriform birds, in which at a gross level the diversity of song repertoire has been demonstrated to be correlated with the size of associated nuclei such as HVC and RA (DeVoogd et al., 1993).

Developmental mechanisms

How these dendritic variations arose during evolution must ultimately be understood in terms of developmental mechanisms and how they have been selected to generate the diversity of observed forms. The study of dendrite development is an exciting area of research, but at present it is focused on specific model organisms such as *Drosophila* or the mouse. Future expansion to consider the variation of developmental mechanisms across species would be greatly illuminating. Of particular interest are the mechanisms that guide the formation of branching patterns such as extension, pruning, and stopping. It is becoming feasible to use high-throughput genomic, proteomic, and connectomic methods to perform comparative analyses, and to elucidate the role of transcriptional regulatory networks during development. Such analysis would identify general molecular

mechanisms that shape changes in dendrites, their connectivity, and brain architecture throughout evolution (Reilly et al., 2015). The future availability of comparative information will allow a better understanding of how dendrites have evolved in terms of optimization principles, systems neurobiology, and developmental mechanisms. Ultimately, such an integrated approach may reveal universal functions of dendrites that transcend their functions in any one species.

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References

- Albus JS (1971) A theory of cerebellar function. *Mathematical Biosciences* **10**:25–61.
- Alle H, Roth A, Geiger JR (2009) Energy-efficient action potentials in hippocampal mossy fibers. *Science* **325**:1405–1408.
- Altman J, Dittmer DS (1961) *Blood and Other Body Fluids*. Bethesda, MD: Federation of American Societies for Experimental Biology.
- Alvarez R, Anadon R (1987) The cerebellum of the dogfish, *Scyliorhinus canicula*: a quantitative study. *Journal für Hirnforschung* **28**:133–137.
- Andersen R, Li Y, Resseguie M, Brennan JE (2005) Calcium/calmodulin-dependent protein kinase II alters structural plasticity and cytoskeletal dynamics in *Drosophila*. *Journal of Neuroscience* **25**:8878–8888.
- Attwell D, Laughlin SB (2001) An energy budget for signaling in the grey matter of the brain. *Journal of Cerebral Blood Flow and Metabolism* **21**:1133–1145.
- Atwood HL, Wojtowicz JM (1999) Silent synapses in neural plasticity: current evidence. *Learning and Memory* **6**:542–571.
- Banavar JR, Maritan A, Rinaldo A (1999) Size and form in efficient transportation networks. *Nature* **399**:130–132.
- Baptista CA, Hatten ME, Blazquez R, Mason CA (1994) Cell–cell interactions influence survival and differentiation of purified Purkinje cells in vitro. *Neuron* **12**:243–260.
- Barasa A (1960) [Form, size, and density of the neurons in the cerebral cortex of mammals of different body sizes.]. *Zeitschrift für Zellforschung und Mikroskopische Anatomie* **53**:69–89.
- Barrett JN, Crill WE (1974a) Influence of dendritic location and membrane properties on the effectiveness of synapses on cat motoneurons. *Journal of Physiology* **239**:325–345.
- Barrett JN, Crill WE (1974b) Specific membrane properties of cat motoneurons. *Journal of Physiology* **239**:301–324.
- Bayés A, Collins MO, Croning MD, van de Lagemaat LN, Choudhary JS, Grant SGN (2012) Comparative study of human and mouse postsynaptic proteomes finds high compositional conservation and abundance differences for key synaptic proteins. *PLoS ONE* **7**:e46683.
- Beaulieu C, Colonnier M (1985) A laminar analysis of the number of round-asymmetrical and flat-symmetrical synapses on spines, dendritic trunks, and cell bodies in area 17 of the cat. *Journal of Comparative Neurology* **231**:180–189.
- Bekkers JM, Stevens CF (1990) Two different ways evolution makes neurons larger. *Progress in Brain Research* **83**:37–45.
- Bélanger M, Allaman I, Magistretti PJ (2011) Brain energy metabolism: focus on astrocyte-neuron metabolic cooperation. *Cell Metabolism* **14**:724–738.
- Bell CC (2002) Evolution of cerebellum-like structures. *Brain, Behavior and Evolution* **59**:312–326.

- Billings G, Piasini E, Lőrincz A, Nusser Z, Silver RA (2014) Network structure within the cerebellar input layer enables lossless sparse encoding. *Neuron* **83**:960–974.
- Bock J, Braun K (1999a) Blockade of N-methyl-D-aspartate receptor activation suppresses learning-induced synaptic elimination. *Proceedings of the National Academy of Sciences of the United States of America* **96**:2485–2490.
- Bock J, Braun K (1999b) Filial imprinting in domestic chicks is associated with spine pruning in the associative area, dorsocaudal neostriatum. *European Journal of Neuroscience* **11**:2566–2570.
- Bok ST (1959) *Histology of the Cerebral Cortex*. Amsterdam: Elsevier.
- Bourgeois JP, Rakic P (1993) Changes of synaptic density in the primary visual cortex of the macaque monkey from fetal to adult stage. *Journal of Neuroscience* **13**:2801–2820.
- Braekevelt CR (1998) Fine structure of the retinal photoreceptors of the emu (*Dromaius novaehollandiae*). *Tissue Cell* **30**:137–148.
- Bray D (1973) Branching patterns of individual sympathetic neurons in culture. *Journal of Cell Biology* **56**:702–712.
- Brown TH, Chapman PF, Kairiss EW, Keenan CL (1988) Long-term synaptic potentiation. *Science* **242**:724–728.
- Bucher D, Prinz AA, Marder E (2005) Animal-to-animal variability in motor pattern production in adults and during growth. *Journal of Neuroscience* **25**:1611–1619.
- Buckmaster PS, Amaral DG (2001) Intracellular recording and labeling of mossy cells and proximal CA3 pyramidal cells in macaque monkeys. *Journal of Comparative Neurology* **430**:264–281.
- Buckmaster PS, Alonso A, Canfield DR, Amaral DG (2004) Dendritic morphology, local circuitry, and intrinsic electrophysiology of principal neurons in the entorhinal cortex of macaque monkeys. *Journal of Comparative Neurology* **470**:317–329.
- Burish MJ, Kueh HY, Wang SS (2004) Brain architecture and social complexity in modern and ancient birds. *Brain, Behavior and Evolution* **63**:107–124.
- Bushey D, Tononi G, Cirelli C (2011) Sleep and synaptic homeostasis: structural evidence in *Drosophila*. *Science* **332**:1576–1581.
- Cai X, Liang CW, Muralidharan S, Kao JP, Tang CM, Thompson SM (2004) Unique roles of SK and Kv4.2 potassium channels in dendritic integration. *Neuron* **44**:351–364.
- Cannon RC, Wheal HV, Turner DA (1999) Dendrites of classes of hippocampal neurons differ in structural complexity and branching patterns. *Journal of Comparative Neurology* **413**:619–633.
- Carlo CN, Stevens CF (2013) Structural uniformity of neocortex, revisited. *Proceedings of the National Academy of Sciences of the United States of America* **110**:1488–1493.
- Carr CE, Boudreau RE (1993) Organization of the nucleus magnocellularis and the nucleus laminaris in the barn owl: encoding and measuring interaural time differences. *Journal of Comparative Neurology* **334**:337–355.
- Chamak B, Prochiantz A (1989) Influence of extracellular matrix proteins on the expression of neuronal polarity. *Development* **106**:483–491.
- Changizi MA (2001) Principles underlying mammalian neocortical scaling. *Biological Cybernetics* **84**:207–215.
- Changizi MA (2003) *The Brain from 25,000 Feet: High Level Explorations of Brain Complexity, Perception, Induction and Vagueness*. Dordrecht: Kluwer Academic.
- Cherniak C (1994) Component placement optimization in the brain. *Journal of Neuroscience* **14**:2418–2427.
- Chklovskii DB (2000) Optimal sizes of dendritic and axonal arbors in a topographic projection. *Journal of Neurophysiology* **83**:2113–2119.
- Chklovskii DB (2004) Synaptic connectivity and neuronal morphology: two sides of the same coin. *Neuron* **43**:609–617.

- Chklovskii DB, Stevens CF** (2000) Wiring optimization in the brain. In: *Advances in Neural Information Processing Systems 12* (Solla SA, Leen TK, Müller K, eds), pp. 103–107. La Jolla, CA: Neural Information Processing Systems Foundation, Inc.
- Christensen BN** (1976) Morphological correlates of synaptic transmission in lamprey spinal cord. *Journal of Neurophysiology* **39**:197–212.
- Clark DA, Mitra PP, Wang SS-H** (2001) Scalable architecture in mammalian brains. *Nature* **411**:189–193.
- Collin C, Miyaguchi K, Segal M** (1997) Dendritic spine density and LTP induction in cultured hippocampal slices. *Journal of Neurophysiology* **77**:1614–1623.
- Coss RG, Brandon JG, Globus A** (1980) Changes in morphology of dendritic spines on honeybee calycal interneurons associated with cumulative nursing and foraging experiences. *Brain Research* **192**:49–59.
- Cragg BG** (1967) The density of synapses and neurons in the motor and visual areas of the cerebral cortex. *Journal of Anatomy* **101**:639–654.
- Craig AM, Banker G** (1994) Neuronal polarity. *Annual Review of Neuroscience* **17**:267–310.
- Cuntz H, Forstner F, Borst A, Häusser M** (2010) One rule to grow them all: a general theory of neuronal branching and its practical application. *PLoS Computational Biology* **6**:e1000877.
- Cuntz H, Mathy A, Häusser M** (2012) A scaling law derived from optimal dendritic wiring. *Proceedings of the National Academy of Sciences of the United States of America* **109**:11014–11018.
- Dailey ME, Smith SJ** (1996) The dynamics of dendritic structure in developing hippocampal slices. *Journal of Neuroscience* **16**:2983–2994.
- DeFelipe J, Alonso-Nancarres L, Arellano JI** (2002) Microstructure of the neocortex: comparative aspects. *Journal of Neurocytology* **31**:299–316.
- Denk W, Briggman KL, Helmstaedter M** (2012) Structural neurobiology: missing link to a mechanistic understanding of neural computation. *Nature Reviews Neuroscience* **13**:351–358.
- DeVoogd TJ, Krebs JR, Healy SD, Purvis A** (1993) Relations between song repertoire size and the volume of brain nuclei related to song: comparative evolutionary analyses amongst oscine birds. *Proceedings of the Royal Society B: Biological Sciences* **254**:75–82.
- Devor A** (2000) Is the cerebellum like cerebellar-like structures? *Brain Research Brain Research Reviews* **34**:149–156.
- Dityatev A, Birinyi A, Puskar Z, Antal M, Clamann HP** (2001) A correlative physiological and morphological analysis of monosynaptically connected propriospinal axon-motoneuron pairs in the lumbar spinal cord of frogs. *Neuroscience* **106**:405–417.
- Dotti CG, Sullivan CA, Banker GA** (1988) The establishment of polarity by hippocampal neurons in culture. *Journal of Neuroscience* **8**:1454–1468.
- Dryer L, Graziadei PP** (1994) Mitral cell dendrites: a comparative approach. *Anatomy and Embryology (Berlin)* **189**:91–106.
- Dunbar RIM** (1992) Neocortex size as a constraint on group size in primates. *Journal of Human Evolution* **20**:469–493.
- Ebbesson SOE** (1968a) Quantitative studies of superior cervical sympathetic ganglia in variety of primates including man. II. Neuronal packing density. *Journal of Morphology* **124**:181–186.
- Ebbesson SOE** (1968b) Quantitative studies of superior cervical sympathetic ganglia in a variety of primates including man. I. The ratio of preganglionic fibers to ganglionic neurons. *Journal of Morphology* **124**:117–132.
- Eccles JC** (1969) The development of the cerebellum of vertebrates in relation to the control of movement. *Naturwissenschaften* **56**:525–534.
- Edwards DH, Yeh SR, Barnett LD, Nagappan PR** (1994) Changes in synaptic integration during the growth of the lateral giant neuron of crayfish. *Journal of Neurophysiology* **72**:899–908.
- Elston GN, Benavides-Piccione R, DeFelipe J** (2001) The pyramidal cell in cognition: a comparative study in human and monkey. *Journal of Neuroscience* **21**:RC163.

- Emes RD, Grant SGN (2011) The human postsynaptic density shares conserved elements with proteomes of unicellular eukaryotes and prokaryotes. *Frontiers in Neuroscience* 5:44.
- Emes RD, Grant SGN (2012) Evolution of synapse complexity and diversity. *Annual Review of Neuroscience* 35:111–131.
- Emoto K, He Y, Ye B, Grueber WB, Adler PN, Jan LY, Jan YN (2004) Control of dendritic branching and tiling by the tricornered-kinase/furry signaling pathway in *Drosophila* sensory neurons. *Cell* 119:245–256.
- Engert F, Bonhoeffer T (1999) Dendritic spine changes associated with hippocampal long-term synaptic plasticity. *Nature* 399:66–70.
- Fahrbach SE (2006) Structure of the mushroom bodies of the insect brain. *Annual Review of Entomology* 51:209–232.
- Forehand CJ (1985) Density of somatic innervation on mammalian autonomic ganglion cells is inversely related to dendritic complexity and preganglionic convergence. *Journal of Neuroscience* 5:3403–3408.
- Gallegos ME, Bargmann CI (2004) Mechanosensory neurite termination and tiling depend on SAX-2 and the SAX-1 kinase. *Neuron* 44:239–249.
- Gao FB, Bogert BA (2003) Genetic control of dendritic morphogenesis in *Drosophila*. *Trends in Neurosciences* 26:262–268.
- Gao FB, Brenman JE, Jan LY, Jan YN (1999) Genes regulating dendritic outgrowth, branching, and routing in *Drosophila*. *Genes and Development* 13:2549–2561.
- Gao FB, Kohwi M, Brenman JE, Jan LY, Jan YN (2000) Control of dendritic field formation in *Drosophila*: the roles of flamingo and competition between homologous neurons. *Neuron* 28:91–101.
- Gibson DA, Tymanskyj S, Yuan RC, Leung HC, Lefebvre JL, Sanes JR, Chédotal A, Ma L (2014) Dendrite self-avoidance requires cell-autonomous Slit/Robo signaling in cerebellar Purkinje cells. *Neuron* 81:1040–1056.
- Giger RJ, Wolfer DP, De Wit GM, Verhaagen J (1996) Anatomy of rat semaphorin III/collapsin-1 mRNA expression and relationship to developing nerve tracts during neuroembryogenesis. *Journal of Comparative Neurology* 375:378–392.
- Gilly WF, Gillette R, McFarlane M (1997) Fast and slow activation kinetics of voltage-gated sodium channels in molluscan neurons. *Journal of Neurophysiology* 77:2373–2384.
- Golding NL, Staff NP, Spruston N (2002) Dendritic spikes as a mechanism for cooperative long-term potentiation. *Nature* 418:326–331.
- Grueber WB, Jan YN (2004) Dendritic development: lessons from *Drosophila* and related branches. *Current Opinion in Neurobiology* 14:74–82.
- Grueber WB, Jan LY, Jan YN (2002) Tiling of the *Drosophila* epidermis by multidendritic sensory neurons. *Development* 129:2867–2878.
- Grueber WB, Ye B, Moore AW, Jan LY, Jan YN (2003) Dendrites of distinct classes of *Drosophila* sensory neurons show different capacities for homotypic repulsion. *Current Biology* 13:618–626.
- Hakeda-Suzuki S, Ng J, Tzu J, Dietzl G, Sun Y, Harms M, Nardine T, Luo L, Dickson BJ (2002) Rac function and regulation during *Drosophila* development. *Nature* 416:438–442.
- Halder G, Callaerts P, Gehring WJ (1995) Induction of ectopic eyes by targeted expression of the eyeless gene in *Drosophila*. *Science* 267:1788–1792.
- Harris KM, Stevens JK (1988) Dendritic spines of rat cerebellar Purkinje cells: serial electron microscopy with reference to their biophysical characteristics. *Journal of Neuroscience* 8:4455–4469.
- Harrison KH, Hof PR, Wang SS-H (2002) Scaling laws in the mammalian neocortex: does form provide clues to function? *Journal of Neurocytology* 31:289–298.
- Harvey RJ, Napper RMA (1991) Quantitative studies on the mammalian cerebellum. *Progress in Neurobiology* 36:437–463.
- Häusser M, Major G, Stuart GJ (2001) Differential shunting of EPSPs by action potentials. *Science* 291:138–141.

- Hebb DO (1949) *Organization of Behavior: a Neuropsychological Theory*. New York: Wiley.
- Herculano-Houzel S, Collins CE, Wong P, Kaas JH, Lent R (2008) The basic nonuniformity of cerebral cortex. *Proceedings of the National Academy of Sciences of the United States of America* **105**:12593–12598.
- Hill AAV, Edwards DH, Murphrey RK (1994) The effect of neuronal growth on synaptic integration. *Journal of Computational Neuroscience* **1**:239–254.
- Hillman DE (1979) Neuronal shape parameters and substructures as a basis of neuronal form. In: *Neurosciences: Fourth Study Program* (Adelman G, Smith BH, eds), pp. 477–498. Cambridge, MA: MIT Press.
- Hofman MA (1988) Size and shape of the cerebral cortex in mammals. I. The cortical surface. *Brain, Behavior and Evolution* **27**:28–40.
- Hultqvist G, Daza DO, Larhammar D, Killmann MW (2012) Evolution of the vertebrate paralemmin gene family: ancient origin of gene duplicates suggests distinct functions. *PLoS ONE* **7**:e41850.
- Hume RI, Purves D (1983) Apportionment of the terminals from single preganglionic axons to target neurones in the rabbit ciliary ganglion. *Journal of Physiology* **338**:259–275.
- Huttenlocher PR (1990) Morphometric study of human cerebral cortex development. *Neuropsychologia* **28**:517–527.
- Huttenlocher PR, Dabholkar AS (1997) Regional differences in synaptogenesis in human cerebral cortex. *Journal of Comparative Neurology* **387**:167–178.
- Huxley J (1932) *Problems of Relative Growth*. London: Methuen.
- Ivanov A, Purves D (1989) Ongoing electrical activity of superior cervical ganglion cells in mammals of different size. *Journal of Comparative Neurology* **284**:398–404.
- Jan Y-N, Jan L (2010) Branching out: mechanisms of dendritic arborization. *Nature Reviews Neuroscience* **11**:316–328.
- Jerison HJ (1973) *Evolution of the Brain and Intelligence*. New York: Academic Press.
- Jones EG, Rakic P (2010) Radial columns in cortical architecture: it is the composition that counts. *Cerebral Cortex* **20**:2261–2264.
- Kaas JH (2000) Why is brain size so important: design problems and solutions as neocortex gets bigger or smaller. *Brain and Mind* **1**:7–23.
- Kakizawa S, Yamasaki M, Watanabe M, Kano M (2000) Critical period for activity-dependent synapse elimination in developing cerebellum. *Journal of Neuroscience* **20**:4954–4961.
- Katz LC, Shatz CJ (1996) Synaptic activity and the construction of cortical circuits. *Science* **274**:1133–1138.
- Keenan CL, Coss R, Koopowitz H (1981) Cytoarchitecture of primitive brains: Golgi studies in flatworms. *Journal of Comparative Neurology* **195**:697–716.
- Keynes R, Krumlauf R (1994) Hox genes and regionalization of the nervous system. *Annual Review of Neuroscience* **17**:109–132.
- Koch C, Zador A (1993) The function of dendritic spines: devices subserving biochemical rather than electrical computation. *Journal of Neuroscience* **13**:413–422.
- Kondoh Y, Hisada M (1986) Regional specialization in synaptic input and output in an identified local nonspiking interneuron of the crayfish revealed by light and electron microscopy. *Journal of Comparative Neurology* **251**:334–348.
- Kötter R, Feizelmeier M (1998) Species-dependence and relationship of morphological and electrophysiological properties in nigral compacta neurons. *Progress in Neurobiology* **54**:619–632.
- Krubitzer L (1995) The organization of neocortex in mammals: are species differences really so different? *Trends in Neurosciences* **18**:408–417.
- Lange W (1975) Cell number and cell density in the cerebellar cortex of man and some other mammals. *Cell and Tissue Research* **157**:115–124.
- Larson SD, Martone ME (2013) NeuroLex.org: an online framework for neuroscience knowledge. *Frontiers in Neuroinformatics* **7**:18.

- Lee T, Winter C, Marticke SS, Lee A, Luo L (2000) Essential roles of *Drosophila* RhoA in the regulation of neuroblast proliferation and dendritic but not axonal morphogenesis. *Neuron* **25**:307–316.
- Lefebvre L, Nicolakakis N, Boire D (2002) Tools and brains in birds. *Behaviour* **135**:1077–1097.
- Lein PJ, Higgins D (1989) Laminin and a basement membrane extract have different effects on axonal and dendritic outgrowth from embryonic rat sympathetic neurons in vitro. *Developmental Biology* **136**:330–345.
- Lein PJ, Bunker GA, Higgins D (1992) Laminin selectively enhances axonal growth and accelerates the development of polarity by hippocampal neurons in culture. *Brain Research. Developmental Brain Research* **69**:191–197.
- Leiss F, Koper E, Hein I, Fouquet W, Lindner J, Sigrist S, Tavosanis G (2009) Characterization of dendritic spines in the *Drosophila* central nervous system. *Developmental Neurobiology* **69**:221–234.
- Levi G (1905) Studi sulla grandezza delle cellule. *Archivio Italiano di Anatomia e di Embriologia [Italian Journal of Anatomy and Embryology]* **5**:291–358.
- Li Z, Van Aelst L, Cline HT (2000) Rho GTPases regulate distinct aspects of dendritic arbor growth in *Xenopus* central neurons in vivo. *Nature Neuroscience* **3**:217–225.
- Lichtman JW, Purves D (1980) The elimination of redundant preganglionic innervation to hamster sympathetic ganglion cells in early post-natal life. *Journal of Physiology* **301**:213–228.
- Lichtman JW, Purves D, Yip JW (1980) Innervation of sympathetic neurones in the guinea-pig thoracic chain. *Journal of Physiology* **298**:285–299.
- Lledo PM, Zhang X, Sudhof TC, Malenka RC, Nicoll RA (1998) Postsynaptic membrane fusion and long-term potentiation. *Science* **279**:399–403.
- Llinás R (ed.) (1969) *Neurobiology of Cerebellar Evolution and Development*. Chicago, IL: American Medical Association.
- Llinás R, Sugimori M (1980) Electrophysiological properties of in vitro Purkinje cell dendrites in mammalian cerebellar slices. *Journal of Physiology* **305**:197–213.
- Luo L (2002) Actin cytoskeleton regulation in neuronal morphogenesis and structural plasticity. *Annual Review of Cell and Developmental Biology* **18**:601–635.
- Lux HD, Schubert P, Kreutzberg GW (1970) Direct matching of morphological and electrophysiological data in cat spinal motoneurons. In: *Excitatory Synaptic Mechanisms* (Anderson P, Kansen JKI, eds), Oslo: Universitetsforlaget.
- McAllister AK (2000) Cellular and molecular mechanisms of dendrite growth. *Cerebral Cortex* **10**:963–973.
- McAllister AK, Lo DC, Katz LC (1995) Neurotrophins regulate dendritic growth in developing visual cortex. *Neuron* **15**:791–803.
- McAllister AK, Katz LC, Lo DC (1996) Neurotrophin regulation of cortical dendritic growth requires activity. *Neuron* **17**:1057–1064.
- McAllister AK, Katz LC, Lo DC (1997) Opposing roles for endogenous BDNF and NT-3 in regulating cortical dendritic growth. *Neuron* **18**:767–778.
- McComb C, Meems R, Syed N, Lukowiak K (2003) Electrophysiological differences in the CpG aerial respiratory behavior between juvenile and adult *Lymnaea*. *Journal of Neurophysiology* **90**:983–992.
- Magee JC, Johnston D (1997) A synaptically controlled, associative signal for Hebbian plasticity in hippocampal neurons. *Science* **275**:209–213.
- Mainen ZF, Sejnowski TJ (1996) Influence of dendritic structure on firing pattern in model neocortical neurons. *Nature* **382**:363–366.
- Malenka RC, Nicoll RA (1999) Long-term potentiation—a decade of progress? *Science* **285**:1870–1874.
- Maletic-Savatic M, Malinow R, Svoboda K (1999) Rapid dendritic morphogenesis in CA1 hippocampal dendrites induced by synaptic activity. *Science* **283**:1923–1927.

- Markram H, Lübke J, Frotscher M, Sakmann B (1997) Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. *Science* **275**:213–215.
- Marr D (1969) A theory of cerebellar cortex. *Journal of Physiology* **202**:437–470.
- Martinez S, Crossley PH, Cobos I, Rubenstein JL, Martin GR (1999) FGF8 induces formation of an ectopic isthmic organizer and isthmocerebellar development via a repressive effect on Otx2 expression. *Development* **126**:1189–1200.
- Matthews BJ, Kim ME, Flanagan JJ, Hattori D, Clemens JC, Zipursky SL, Grueber WB (2007) Dendrite self-avoidance is controlled by Dscam. *Cell* **129**:593–604.
- Meek J (1992) Comparative aspects of cerebellar organization: from mormyrids to mammals. *European Journal of Morphology* **30**:37–51.
- Meek J, Nieuwenhuys R (1991) Palisade pattern of mormyrid Purkinje cells: a correlated light and electron microscopic study. *Journal of Comparative Neurology* **306**:156–192.
- Migliore M and Shepherd GM (2002) Emerging rules for the distributions of active dendritic conductances. *Nature Reviews Neuroscience* **3**:362–370.
- Montgomery JM, Madison DV (2002) State-dependent heterogeneity in synaptic depression between pyramidal cell pairs. *Neuron* **33**:765–777.
- Morrison ME, Mason CA (1998) Granule neuron regulation of Purkinje cell development: striking a balance between neurotrophin and glutamate signaling. *Journal of Neuroscience* **18**:3563–3573.
- Nicholson C, Llinas R, Precht W (1969) Neural elements of the cerebellum in elasmobranch fishes: Structural and functional characteristics. In: *Neurobiology of Cerebellar Evolution and Development* (Llinas R, ed.), pp. 215–243. Chicago, IL: American Medical Association.
- Nieuwenhuys R (1967) Comparative anatomy of the cerebellum. In: *Progress in Brain Research, Volume 25* (Fox CA, Snider RS, eds), pp. 1–93. Amsterdam: Elsevier.
- Nieuwenhuys R, ten Donkelaar H, Nicholson C (1998) *The Central Nervous System of Vertebrates*. Berlin: Springer.
- Nimchinsky EA, Gilissen E, Allman JM, Perl DP, Erwin JM, Hof PR (1999) A neuronal morphologic type unique to humans and great apes. *Proceedings of the National Academy of Sciences of the United States of America* **96**:5268–5273.
- Oberheim NA, Takano T, Han X, He W, Lin JH, Wang F, Xu Q, Wyatt JD, Pilcher W, Ojemann JG, Ransom BR, Goldman SA, Nedergaard M (2009) Uniquely hominid features of adult human astrocytes. *Journal of Neuroscience* **29**:3276–3287.
- O'Connor DH, Wittenberg GM, Wang SS-H (2005) Graded bidirectional synaptic plasticity is composed of switch-like unitary events. *Proceedings of the National Academy of Sciences of the United States of America* **102**:9679–9684.
- Ohara PT, Havton LA (1994) Preserved features of thalamocortical projection neuron dendritic architecture in the somatosensory thalamus of the rat, cat and macaque. *Brain Research* **648**:259–264.
- O'Kusky J, Colonier M (1982) A laminar analysis of the number of neurons, glia, and synapses in the visual cortex (area 17) of adult macaque monkeys. *Journal of Comparative Neurology* **210**:278–290.
- Olsen O, Nadim F, Hill AA, Edwards DH (1996) Uniform growth and neuronal integration. *Journal of Neurophysiology* **76**:1850–1857.
- Osterhout DJ, Frazier WA, Higgins D (1992) Thrombospondin promotes process outgrowth in neurons from the peripheral and central nervous systems. *Developmental Biology* **150**:256–265.
- Palay SL, Chan-Palay V (1974) *Cerebellar Cortex: Cytology and Organization*. Berlin: Springer.
- Pan F, Gan W-B (2008) Two-photon imaging of dendritic spine development in the mouse cortex. *Developmental Neurobiology* **68**:771–778.
- van Pelt J, Dityatev AE, Uylings HB (1997) Natural variability in the number of dendritic segments: model-based inferences about branching during neurite outgrowth. *Journal of Comparative Neurology* **387**:325–340.

- Picones A, Chung SC, Korenbrot JI (2003) Developmental maturation of passive electrical properties in retinal ganglion cells of rainbow trout. *Journal of Physiology* **548**:71–83.
- Polleux F, Giger RJ, Ginty DD, Kolodkin AL, Ghosh A (1998) Patterning of cortical efferent projections by semaphorin–neuropilin interactions. *Science* **282**:1904–1906.
- Polleux F, Morrow T, Ghosh A (2000) Semaphorin 3A is a chemoattractant for cortical apical dendrites. *Nature* **404**:567–573.
- Polksy A, Mel BW, Schiller J (2004) Computational subunits in thin dendrites of pyramidal cells. *Nature Neuroscience* **7**:621–627.
- Purves D (1988) *Body and Brain: A Trophic Theory of Neural Connections*. Cambridge, MA: Harvard University Press.
- Purves D, Hume RI (1981) The relation of postsynaptic geometry to the number of presynaptic axons that innervate autonomic ganglion cells. *Journal of Neuroscience* **1**:441–452.
- Purves D, Lichtman JW (1980) Elimination of synapses in the developing nervous system. *Science* **210**:153–157.
- Purves D, Lichtman JW (1985) Geometrical differences among homologous neurons in mammals. *Science* **228**:298–302.
- Purves D, Rubin E, Snider WD, Lichtman JW (1986) Relation of animal size to convergence, divergence and neuronal number in peripheral sympathetic pathways. *Journal of Neuroscience* **6**:158–163.
- Rakic P (1995) A small step for the cell, a giant leap for mankind: a hypothesis of neocortical expansion during evolution. *Trends in Neurosciences* **18**:383–388.
- Rakic P, Bourgeois J-P, Eckenhoff MF, Zecevic N, Goldman-Rakic P (1986) Concurrent overproduction of synapses in diverse regions of the primate cerebral cortex. *Science* **232**:232–235.
- Rakic P, Bourgeois JP, Goldman-Rakic PS (1994) Synaptic development of the cerebral cortex: implications for learning, memory, and mental illness. *Progress in Brain Research* **102**:227–243.
- Rall W (1977) Core conductor theory and cable properties of neurons. In: *Handbook of Physiology. The Nervous System. Cellular Biology of Neurons. Section 1* (Kandel ER, ed.), pp. 39–98. Bethesda, MD: American Physiological Society.
- Ramón y Cajal S (1995) *Histology of the Nervous System of Man and Vertebrates*. Oxford: Oxford University Press.
- Raymond JL, Lisberger SG, Mauk MD (1996) The cerebellum: a neuronal learning machine? *Science* **272**:1126–1131.
- Reader SM, Laland KN (2002) Social intelligence, innovation, and enhanced brain size in primates. *Proceedings of the National Academy of Sciences of the United States of America* **99**:4436–4441.
- Reilly SK, et al. (2015) Evolutionary changes in promoter and enhancer activity during human corticogenesis. *Science* **347**:1155–1159.
- Rockel AJ, Hiorns RW, Powell TP (1980) The basic uniformity in structure of the neocortex. *Brain* **103**:221–244.
- Rojas LM, Ramirez Y, McNeil R, Mitchell M, Marin G (2004) Retinal morphology and electrophysiology of two caprimulgiformes birds: the cave-living and nocturnal oilbird (*Steatornis caripensis*), and the crepuscularly and nocturnally foraging common pauraque (*Nyctidromus albicollis*). *Brain, Behavior and Evolution* **64**:19–33.
- Rosso SB, Sussman D, Wynshaw-Boris A, Salinas PC (2005) Wnt signaling through Dishevelled, Rac and JNK regulates dendritic development. *Nature Neuroscience* **8**:34–42.
- Ruchhoeft ML, Ohnuma S, McNeill L, Holt CE, Harris WA (1999) The neuronal architecture of *Xenopus* retinal ganglion cells is sculpted by rho-family GTPases in vivo. *Journal of Neuroscience* **19**:8454–8463.
- Savage VM, Allen AP, Brown JH, Gillooly JF, Herman AB, Woodruff WH, West GB (2007) Scaling of number, size, and metabolic rate of cells with body size in mammals. *Proceedings of the National Academy of Sciences of the United States of America* **104**:4718–4723.

- Schaper A (1898) The finer structure of the selachian cerebellum (*Mustelus vulgaris*) as shown by chrome-silver preparations. *Journal of Comparative Neurology* **8**:1–20.
- Schumann CM, Nordahl CW (2011) Bridging the gap between MRI and postmortem research in autism. *Brain Research* **1380**:175–186.
- Schumann CM, Bloss CS, Barnes CC, Wideman GM, Carper RA, Akshoomoff N, Pierce K, Hagler D, Schork N, Lord C, Courchesne E (2010) Longitudinal magnetic resonance imaging study of cortical development through early childhood in autism. *Journal of Neuroscience* **30**:4419–4427.
- Scott EK, Luo L (2001) How do dendrites take their shape? *Nature Neuroscience* **4**:359–365.
- Sherwood CC, Lee PW, Rivara CB, Holloway RL, Gilissen EP, Simmons RM, Hakeem A, Allman JM, Erwin JM, Hof PR (2003) Evolution of specialized pyramidal neurons in primate visual and motor cortex. *Brain, Behavior and Evolution* **61**:28–44.
- Smolyaninov VV (1971) Some special features of organization of the cerebellar cortex. In: *Models of the Structural-Functional Organization of Certain Biological Systems* (Gelfand IM, Gurfinkel VS, Fomin SV, Tsetlin ML, eds), pp. 251–325. Cambridge, MA: MIT Press.
- Snider WD (1987) The dendritic complexity and innervation of submandibular neurons in five species of mammals. *Journal of Neuroscience* **7**:1760–1768.
- Snider WD (1988) Nerve growth factor enhances dendritic arborization of sympathetic ganglion cells in developing mammals. *Journal of Neuroscience* **8**:2628–2634.
- Sorra KE, Harris KM (1993) Occurrence and three-dimensional structure of multiple synapses between individual radiatum axons and their target pyramidal cells in hippocampal area CA. *Journal of Neuroscience* **13**:3736–3748.
- St. John JL, Rosene DL, Luebke JI (1997) Morphology and electrophysiology of dentate granule cells in the rhesus monkey: comparison with the rat. *Journal of Comparative Neurology* **387**:136–147.
- Stepanyants A, Chklovskii DB (2005) Neurogeometry and potential synaptic connectivity. *Trends in Neurosciences* **28**:387–394.
- Sterling P (1997) Retina. In: *The Synaptic Organization of the Brain*, 4th edn (Shepherd GM, ed.), p. 656. New York: Oxford University Press.
- Sterling P (2003) How retinal circuits optimize the transfer of visual information. In: *The Visual Neurosciences* (Chalupa LM, Werner JS, eds), pp. 234–259. Cambridge, MA: MIT Press.
- Stern DL (2000) Evolutionary developmental biology and the problem of variation. *Evolution, the International Journal of Organic Evolution* **54**:1079–1091.
- Tang G, Gudsnuuk K, Kuo SH, Cotrina ML, Rosoklja G, Sosunov A, Sonders MS, Kanter E, Castagna C, Yamamoto A, Yue Z, Arancio O, Peterson BS, Champagne F, Dwork AJ, Goldman J, Sulzer D (2014) Loss of mTOR-dependent macroautophagy causes autistic-like synaptic pruning deficits. *Neuron* **83**:1131–1143.
- Teissier G (1939) Biometrie de la cellule. *Tabulae* **19**:1–64.
- Tepper JM, Damlama M, Trent F (1994) Postnatal changes in the distribution and morphology of rat substantia nigra dopaminergic neurons. *Neuroscience* **60**:469–477.
- Thompson DW (1942) *On Growth and Form*. Cambridge: Cambridge University Press.
- Threadgill R, Bobb K, Ghosh A (1997) Regulation of dendritic growth and remodeling by Rho, Rac, and Cdc42. *Neuron* **19**:625–634.
- Tower DB (1954) Structural and functional organization of mammalian cerebral cortex: the correlation of neurone density with brain size. *Journal of Comparative Neurology* **101**:9–52.
- Tsai PS et al. (2009) Correlations of neuronal and microvascular densities in murine cortex revealed by direct counting and colocalization of nuclei and vessels. *Journal of Neuroscience* **29**:14553–14570.
- Tyrrell T, Willshaw D (1992) Cerebellar cortex: its simulation and the relevance of Marr's theory. *Philosophical Transactions of the Royal Society B: Biological Sciences* **336**:239–257.

- Tzschentke B, Plagemann A (2006) Imprinting and critical periods in early development. *World's Poultry Science Journal* **62**:626–637.
- Van Essen DC (1997) A tension-based theory of morphogenesis and compact wiring in the central nervous system. *Nature* **385**:313–318.
- Varshney LR, Sjöström PJ, Chklovskii DB (2006) Optimal information storage in noisy synapses under resource constraints. *Neuron* **52**:409–423.
- Vetter P, Roth A, Häusser M (2001) Propagation of action potentials in dendrites depends on dendritic morphology. *Journal of Neurophysiology* **85**:926–937.
- Wang SS-H, Shultz JR, Burish MJ, Harrison KH, Hof PR, Towns LC, Wagers MW, Wyatt KD (2008) Functional trade-offs in white matter axonal scaling. *Journal of Neuroscience* **28**:4047–4056.
- Wang SS-H, Kloth AD, Badura A (2014) The cerebellum, sensitive periods, and autism. *Neuron* **83**:518–532.
- Wässle H, Levick WR, Kirk DL, Cleland BG (1975) Axonal conduction velocity and perikaryal size. *Experimental Neurology* **49**:246–251.
- West GB, Brown JH, Enquist BJ (1997) A general model for the origin of allometric scaling laws in biology. *Science* **276**:122–126.
- Whitford KL, Dijkhuizen P, Polleux F, Ghosh A (2002) Molecular control of cortical dendrite development. *Annual Review of Neuroscience* **25**:127–149.
- Yelnik JF, Francois C, Percheron G, Heyner S (1987) Golgi study of the primate substantia nigra. I. Quantitative morphology and typology of nigral neurons. *Journal of Comparative Neurology* **265**:455–472.
- Young JZ (1973) The giant fibre synapse of *Loligo*. *Brain Research* **57**:457–460.
- Yuste R (2011) *Dendritic Spines*. Cambridge, MA: MIT Press.
- Zhang K, Sejnowski TJ (2000) A universal scaling law between gray matter and white matter of cerebral cortex. *Proceedings of the National Academy of Sciences of the United States of America* **97**:5621–5626.

Chapter 3

Dendrite development

Hollis T. Cline

Summary

The structure of a dendritic arbor determines the presynaptic inputs to the neuron, the integration of input information, and the output from the cell. Consequently, mechanisms regulating the development and maintenance of the structure of dendritic arbors play a critical role in circuit function. In vivo imaging studies have identified the cellular events underlying the development of dendritic arbors. Branch additions and retractions are concurrent during dendritic arbor development, and even after arbor growth has reached a plateau. Arbor growth or retraction is determined by the relative balance of branch additions and retractions over time. Dendritic branch dynamics are in turn regulated by the formation and maintenance of excitatory and inhibitory synapses. Diseases and disease genes that affect synaptogenesis and synaptic plasticity are therefore predicted to affect the structural development and integrative function of dendrites, and, by extension, information processing and circuit functions. Homeostatic mechanisms operate to counteract the effects of disease perturbations on dendrite development and neuronal and circuit function.

Why does the structure of dendritic arbors matter?

Dendritic arbor structure is a primary determinant of the connectivity matrix within a circuit, because the spatial extent of a dendritic arbor determines the spatial extent of potential synaptic inputs onto a neuron. Consequently the structure of a dendritic arbor limits the afferent cell types as well as the density and distribution of their inputs onto the dendrites. The structure of the dendritic arbor also determines the biophysical properties of the dendritic tree, as well as the capacity of the neuron to integrate synaptic inputs across its dendritic arbor, to signal from distal dendrites to the cell body, and to drive neuronal output. Therefore, dendritic arbor structure controls the afferent input to the neuron, and, by controlling the integration of synaptic inputs, also has a powerful influence on the neuronal output from the cell, and by extension the circuit.

The context of dendritic arbor development

Dendritic arbor structure is plastic in response to different types of extrinsic signals, including synaptic input. Extrinsic signals can affect dendritic arbor structure by acting at different spatio-temporal scales, for instance by acting locally to affect the structural stability of synapses, spines, or dendritic branches, or by signaling to the nucleus to trigger transcriptional events. Neurodevelopmental disorders often affect the structure of dendritic arbors, and this may underlie the information processing deficits seen in these disorders; however, it is often difficult to ascertain the extent to which the dendritic arbor structure is aberrant because of aberrant synaptic activity or vice versa.

Considering a larger spatio-temporal scale, as seen in intact animals, the majority of dendritic arbors develop in the context of a developing circuit where synaptic inputs and other extrinsic cues, for instance from glia and the vasculature, change over time. Furthermore, circuit and brain development typically occur as an animal develops and begins to make use of the new capacities afforded by its developing brain circuitry. Consequently, dendrite development and function are dynamically influenced by signals originating from outside the local environment, such as feedback between brain regions, oscillatory brain waves, or metabolic state. A minority of neurons in the brain are generated in adult animals and are then incorporated into pre-existing circuits. It is not yet clear whether neurons born in the adult brain use similar or different mechanisms to elaborate complex dendrite arbors within a pre-existing circuit compared with neurons generated as the animal is developing.

This chapter will focus on activity-dependent or experience-dependent development of dendritic arbors, specifically evaluating the development of dendrites at a cellular level, without delving into the molecular mechanisms which are well reviewed elsewhere (Van Aelst and Cline, 2004; Jan and Jan, 2010; Puram and Bonni, 2013; and Chapter 4).

Spatio-temporal branch dynamics underlie dendritic arbor development

Classic work based on static data sets from different neurons in different experimental systems gave the impression that dendrite growth, synaptogenesis, synaptic pruning, and dendritic branch retraction occur sequentially during development of the CNS (Lazar, 1973; Cragg, 1975; Blue and Parnavelas, 1983; Rakic et al., 1986; Warton and McCart, 1989; Zecevic and Rakic, 1991; Huttenlocher and Dabholkar, 1997; Zecevic, 1998). This led to the expectation that distinct mechanisms govern these temporally distinct events (Bystron et al., 2008). The advent of *in vivo* time-lapse imaging allowed the cellular events underlying dendritic arbor development to be examined directly. *In vivo* imaging of dendritic arbor development was pioneered in the 1980s by the Purves lab. Repeated imaging of peripheral neurons in developing mice revealed considerable heterogeneity in the dynamics of dendritic arbor structure, which led these pioneering scientists to propose that dendrite structure was significantly affected by afferent inputs (Hume and Purves, 1981). More than a decade passed before methods were developed to label and image CNS neurons without damaging them (Dailey and Smith, 1996; Wu and Cline, 1998). Longitudinal analysis of developing dendritic arbors in individual neurons observed with confocal time-lapse imaging in hippocampal slices (Dailey and Smith, 1996) and *in vivo* in *Xenopus* optic tectum (Cline et al., 1997; Rajan and Cline, 1998; Wu and Cline, 1998) indicated that developing dendrites undergo extensive structural rearrangements—to a much greater extent than would be predicted if dendritic arbors grew by simply adding and maintaining dendritic branches. Together with *in vivo* imaging studies conducted in zebrafish (Hua and Smith, 2004; Niell et al., 2004), these studies demonstrated that the prevailing view of sequential stages of dendrite development was not correct. Initially, confocal time-lapse images were collected of individual optic tectal neurons in albino *Xenopus* tadpoles that were iontophoretically labeled with DiI or fluorescent dextrans (Cline et al., 1997; Rajan and Cline, 1998; Wu and Cline, 1998). Subsequently, neurons were labeled by expression of fluorescent proteins, which permitted a wide range of opportunities to image neuronal structure and subcellular components (Haas et al., 2001). When neurons were imaged infrequently, for instance at daily intervals, the dendritic arbor appeared to go through a period of rapid growth after which the net growth rate slowed down (Fig. 3.1). These data might suggest that there is a finite period of branch addition similar to the classic view of morphological development, but when neurons were imaged more frequently the greater temporal resolution revealed rapid rates of branch additions

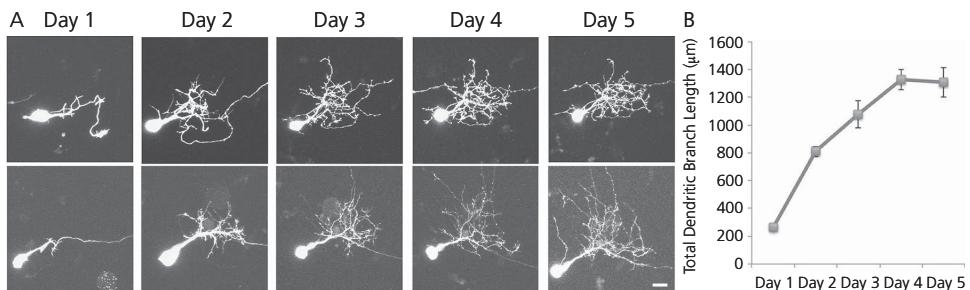


Fig. 3.1 In vivo imaging at daily intervals reveals the overall growth rate of dendritic arbors. (A) In vivo two-photon laser scanning images of GFP-expressing optic tectal neurons collected daily from *Xenopus laevis* tadpoles. On the first day of imaging, the optic tectal neurons have simple dendritic arbors and an axon, characteristic of immature neurons. The dendritic arbor becomes complex over the next days, after which the net growth rate stabilizes. (B) Plot of total dendritic branch length showing dendritic growth rate of young neurons over 5 days. Scale bar = 10 μm.

and retractions throughout the entire imaging period, even over time periods when there was no apparent net branch growth (Fig. 3.2). Further analysis indicated that dendritic arbors exhibit constant branch additions and retractions, and whether the arbor shows net growth, structural stability, or net retraction depends on the relative numbers and locations of branch additions and retractions (Sin et al., 2002; Niell et al., 2004; Haas et al., 2006; Hossain et al., 2012). These data

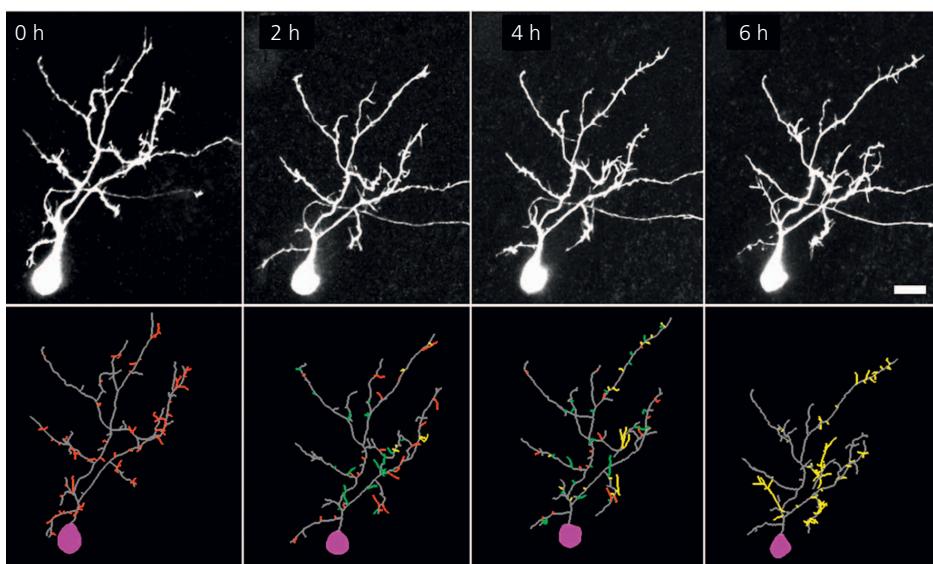


Fig. 3.2 In vivo time-lapse images at 2-hour intervals show dynamic rearrangements of dendritic branches, despite little net growth of the dendritic arbor. Top: two-photon laser scanning images of GFP-expressing optic tectal neurons collected every 2 hours over 6 hours from *Xenopus laevis* tadpoles. Bottom: reconstructions of the dendritic arbors and somata from the images in the top panel. Branches are color-coded according to their dynamics. Red branches are lost over the course of the 6-hour imaging session. Green branches are transient; they are added and lost during the imaging session. Yellow branches are added during the imaging session and maintained to the last image. Grey branches are the skeleton of the arbor which does not change over the imaging session. Scale bar = 10 μm.

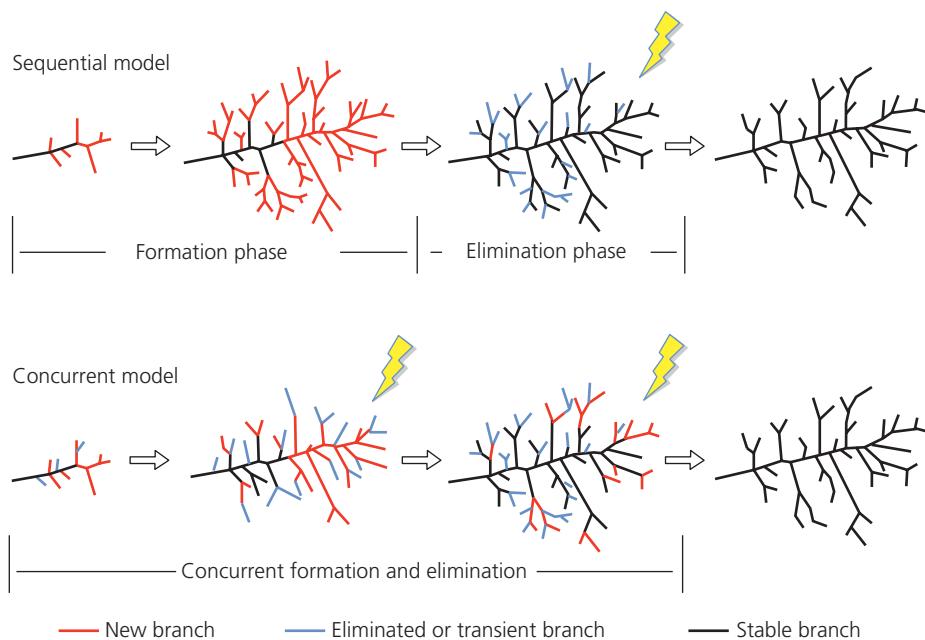


Fig. 3.3 Concurrent versus sequential models of arbor elaboration. Top: data collected at single time-points suggested that dendritic and axonal arbors undergo two phases of development, an elaboration phase during which arbors show net growth, followed by a pruning phase during which branches are lost. Bottom: longitudinal analysis afforded by time-lapse imaging data of neuronal structure shows that branch additions and eliminations are concurrent and occur over an extended period of dendritic arbor elaboration. Importantly, the endpoint dendritic arbor structures are comparable for both models, and distinguishing between the two requires time-lapse imaging and quantitative analysis of structural dynamics.

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demonstrate that dendritic arbors develop through a dynamic and iterative process of concurrent addition, retraction, and stabilization of branches (Fig. 3.3). The constant, but low, rate of branch dynamics within the relatively complex arbors of mature neurons is a likely signature of their continuing capacity for structural plasticity.

One key element of these studies is that identification of the cellular dynamics underlying dendritic (and axonal) arbor growth requires time-lapse imaging. Furthermore, longitudinal analysis of the dynamics in individual neurons helps address the heterogeneity in dendritic arbor growth rates observed within populations of neurons *in vivo*. Static images or time-lapse images collected at long intervals report outcomes or intermediate steps of arbor development, but don't necessarily provide insight into growth mechanisms. In contrast, *in vivo* time-lapse imaging conducted over a range of time intervals can help identify mechanisms of dendritic arbor development. For instance, time-lapse images collected at short intervals were used to distinguish whether lamina-specific dendritic arbor growth in the retina was regulated by local elaboration or by a general cell-wide growth signal followed by selective pruning (Choi et al., 2010). When combined with molecular

genetic manipulations of candidate regulators of dendritic arbor development, time-lapse *in vivo* imaging allows powerful quantitative analysis of the mechanisms governing dendrite development (Nedivi et al., 1998; Wu and Cline, 1998; Cantallops et al., 2000; Li et al., 2000; Niell et al., 2004; Haas et al., 2006; Bestman and Cline, 2008; Chiu et al., 2008; Schwartz et al., 2009; Chen et al., 2010; Hossain et al., 2012; Marshak et al., 2012; Ghiretti et al., 2014). Similar dendritic arbor dynamics, originally seen in optically transparent experimental systems such as *Xenopus* and zebrafish, have also been observed in rodent CNS as methods and instrumentation for *in vivo* time-lapse imaging have advanced (Lee et al., 2006).

Experience-dependent regulation of dendritic arbor growth

Compelling studies in a variety of experimental systems have demonstrated that neuronal or circuit activity regulates the structure of dendritic arbors (Katz and Shatz, 1996). Many of these studies have been carried out in sensory systems because of the relative ease with which sensory input can be controlled and experimentally modified. Dendrites extend toward some sources of afferent inputs and avoid others, as shown in the somatosensory system, where dendrites extend toward the center of cortical barrels (Greenough and Chang, 1988), and in the visual system, where dendrite morphology respects borders of ocular dominance columns (Katz and Constantine-Paton, 1988; Katz et al., 1989; Kossel et al., 1995) and laminae (Lee et al., 2006; Chen and Nedivi, 2013). Similar observations have been made in the olfactory system (Malun and Brunjes, 1996; Imamura and Greer, 2009), the spinal cord (Inglis et al., 2000), and the auditory system (Schweitzer, 1991). In the auditory brainstem of birds and mammals, afferent input regulates the developmental plasticity of synapses and dendritic arbor morphology (Sanes and Chokshi, 1992; Sanes et al., 1992; Kotak and Sanes, 1996, 1997; Sorensen and Rubel, 2006, 2011; Wang and Rubel, 2012). Sorensen and Rubel (2011) took advantage of the bitufted dendrites of neurons in chick nucleus laminaris to test the effect of local stimulation on dendritic arbor plasticity. Time-lapse imaging demonstrated that local stimulation first resulted in retraction of branches in the unstimulated dendritic tuft; then, over the next several hours, the dendrites that received local stimulation increased their elaboration.

The retina provides another excellent system in which to test for activity-dependent regulation of dendritic arbor development (Xu and Tian, 2007; Bleckert and Wong, 2011; Tian, 2011; Dunn et al., 2013; D’Orazi et al., 2014) and retinal circuitry (Rivlin-Etzion et al., 2012; Vlasits et al., 2014). Retinal ganglion cells transiently respond to light On and light Off stimuli during development, and these responses correlate with the distribution of dendritic arbors in On and Off laminae in the neuropil. Normal visual experience results in pruning of the bi-stratified arbors so that dendrites are retained in On or Off laminae, but rearing in the dark blocks the dendritic pruning so ganglion cells continue to respond to On and Off stimuli (Tian and Copenhagen, 2001, 2003). More recent work in the retina has demonstrated that sensory experience has broad effects on the development of synapses, dendritic arbor structure, neuronal connectivity, and response properties (Tian, 2011; Rivlin-Etzion et al., 2012; Dunn et al., 2013; Rosa and Feller, 2014; Vlasits et al., 2014).

Strong evidence for activity-dependent modifications of dendritic arbor structure has also come from studies of neurological diseases and addiction, as well as neurodevelopmental disorders (Kolb et al., 2003; Benavides-Piccione et al., 2004; Govek et al., 2004; Bagni and Greenough, 2005; Gonzalez et al., 2005; Morrow et al., 2005; Newey et al., 2005; Marshak et al., 2012; Faulkner et al., 2015). Together these studies have motivated a huge endeavor to identify activity-dependent mechanisms that regulate the structural plasticity of dendritic arbors and more extensive outcomes

with respect to brain function. These studies are well reviewed elsewhere (Loebrich and Nedivi, 2009; Leslie and Nedivi, 2011; Andreea and Burrone, 2014).

Interplay between synaptic and dendritic plasticity: the synaptotropic hypothesis

Experience-dependent dendritic arbor development is best understood as the coordinated regulation of synaptogenesis, synaptic strength, and dendritic arbor structure. The same cellular and molecular events and regulatory pathways often affect synaptogenesis and synapse maturation, synaptic strength, and dendritic arbor structure. Electrophysiological studies indicate that synaptic transmission at nascent glutamatergic synapses is initially mediated by NMDA receptor conductances, and AMPA-type glutamate receptors are trafficked into developing synapses *in vivo* and in slice preparations (Hestrin, 1992; Ramoa and McCormick, 1994; Wu et al., 1996; Isaac et al., 1997; Zhu et al., 2000; Ashby and Isaac, 2011). The presence of AMPAR at synapses renders the synapses functional at resting membrane potentials (Wu et al., 1996; Isaac et al., 1997), but was also postulated to stabilize the dynamic newly added dendritic branches (Cline, 2001). This suggests that synaptogenic processes, such as NMDA receptor function and AMPA receptor trafficking, play a role in experience-dependent dendritic arbor development.

The role of AMPA receptors in dendritic arbor development was clearly demonstrated by interfering with the trafficking of AMPA receptors into maturing synapses in *Xenopus* optic tectal neurons (Haas et al., 2006) and rodent spinal cord neurons (Inglis et al., 2002). In *Xenopus*, expressing a peptide corresponding to the cytoplasmic tail (called CTP) of the GluA1 or GluA2 subunits decreased the frequency of AMPA receptor-mediated spontaneous synaptic currents, indicating a decrease in synapses containing AMPA receptor. Electron microscopy analysis shows that the majority of terminal dendritic branches in optic tectal neurons are sites of synaptic contacts (Li et al., 2011), comparable to spines as a morphological signature of synapses in spiny neurons. Images of *Xenopus* optic tectal neurons collected at daily intervals over 4–5 days showed that neurons expressing GluA CTPs failed to elaborate the typical complex dendritic arbor seen in control neurons (Fig. 3.4). Rather the CTP-expressing optic tectal neurons extended long unbranched dendrites. Our previous *in vivo* imaging studies had demonstrated that arbors elaborate by the addition of many dendritic branches, but that the majority of newly added branches are rapidly retracted. The few branches that are maintained serve as a substrate for further branch addition, as the process of branch addition, retraction, and stabilization is reiterated. Failure to elaborate a complex bushy arbor suggested that newly added side branches were not stabilized in neurons expressing GluA CTPs. Indeed, images collected at 2-hourly intervals over 6 hours demonstrated that newly added branches in CTP-expressing neurons are rapidly retracted, consistent with the idea that trafficking of AMPA receptors into nascent synapses stabilizes the synapse and increases the lifetime of the newly added dendritic branches (Fig. 3.4). Together with data showing that repeated visual stimulation potentiates the strength of retinotectal synapses (Engert et al., 2002; Vislay-Meltzer et al., 2006), which occurs via an increase in AMPA receptor current (Wu et al., 1996), these studies clearly demonstrate the close relationship between functional and structural plasticity of synapses and dendrites.

Sin et al (2002) demonstrated a robust effect of visual experience on dendritic arbor development in the optic tectum of *Xenopus* tadpoles. Single optic tectal neurons were imaged before and after a 4-hour period during which the animal was maintained in the dark, and then a third image of the same neuron was collected after the animal experienced 4 hours of a simulated motion stimulus. Quantitative analysis of changes in dendritic branch tips and total dendritic

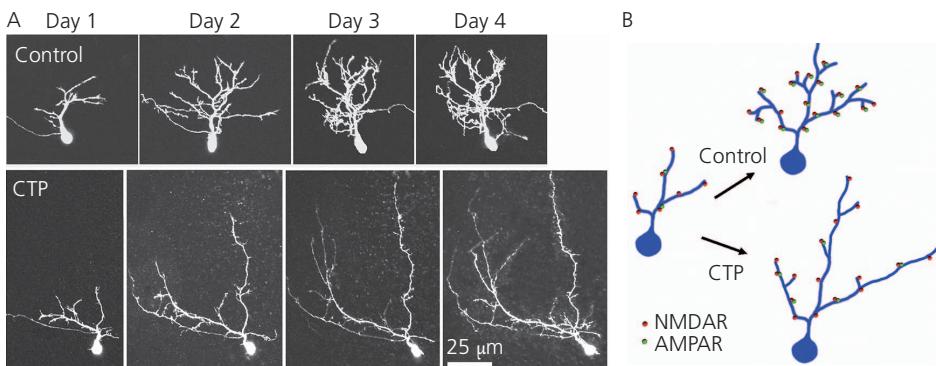


Fig. 3.4 Glutamatergic transmission through AMPA receptors is required for dendritic arbor elaboration. (A) In vivo two-photon time-lapse images of GFP-expressing optic tectal neurons of *Xenopus* tadpoles collected at daily intervals. The top panels show a control neuron. The bottom panels show a neuron expressing GFP and the C-terminal peptide (CTP) from the GluA1 receptor. CTP-expressing neurons are sparsely branched compared with controls. (B) Diagram of the effect of interfering with GluA receptor trafficking on dendritic arbor elaboration. Immature neurons have simple arbors and synapses that are dominated by GluN receptors (NMDAR, red dots). With neuronal maturation, GluA receptors (AMPAR, green dots) are incorporated into postsynaptic sites and neurons elaborate complex dendritic arbors, densely studded with synapses. CTP-expressing neurons have fewer AMPAR-containing synapses and dendritic arbors with fewer branches than controls.

branch length over the 4 hours in the dark or with visual stimulation demonstrated a consistent increase in dendritic arbor growth rate in response to the visual stimulation (Fig. 3.5). Repeated visual stimulation induces long-term potentiation of retinotectal synapses (Engert et al., 2002). NMDA receptor antagonists block the visually driven synaptic plasticity and the increase in dendritic arbor growth rate, suggesting that repeated excitatory synaptic activity and calcium influx through NMDA receptors are required for experience-dependent dendritic arbor elaboration (Sin et al., 2002) and synaptic plasticity (Engert et al., 2002). Imaging tectal neurons at a higher temporal resolution showed that the NMDA receptor activity controls the stabilization of dendritic arbor branches. NMDA receptor-mediated regulation of dendritic arbor development is seen in many experimental systems and is likely to be a highly conserved mechanism by which excitatory glutamatergic inputs regulate dendrite development and circuit connectivity (Rajan and Cline, 1998; Lee et al., 2005).

Importantly, expression of the CTPs blocked the visual stimulation-induced increase in dendritic arbor elaboration seen in control animals (Fig. 3.5). These experiments demonstrate that AMPA receptor trafficking and synapse maturation are required for visual-experience-dependent dendritic arbor elaboration *in vivo*. Because synapse maturation necessarily includes concordant maturation of presynaptic elements, these observations indicate that visual experience drives the development and plasticity of dendritic arbors and presynaptic inputs by engaging complex intercellular signaling pathways, including postsynaptic glutamatergic receptors and presynaptic machinery (Haas et al., 2006; Chen et al., 2010; Ruthazer and Aizenman, 2010).

The synaptotropic hypothesis, proposed by Vaughn, provides a useful framework in which to interpret the effects of sensory experience and synaptic activity on dendritic and axonal arbor growth (Cline and Haas, 2008). Vaughn postulated that growing axons release a transmitter that

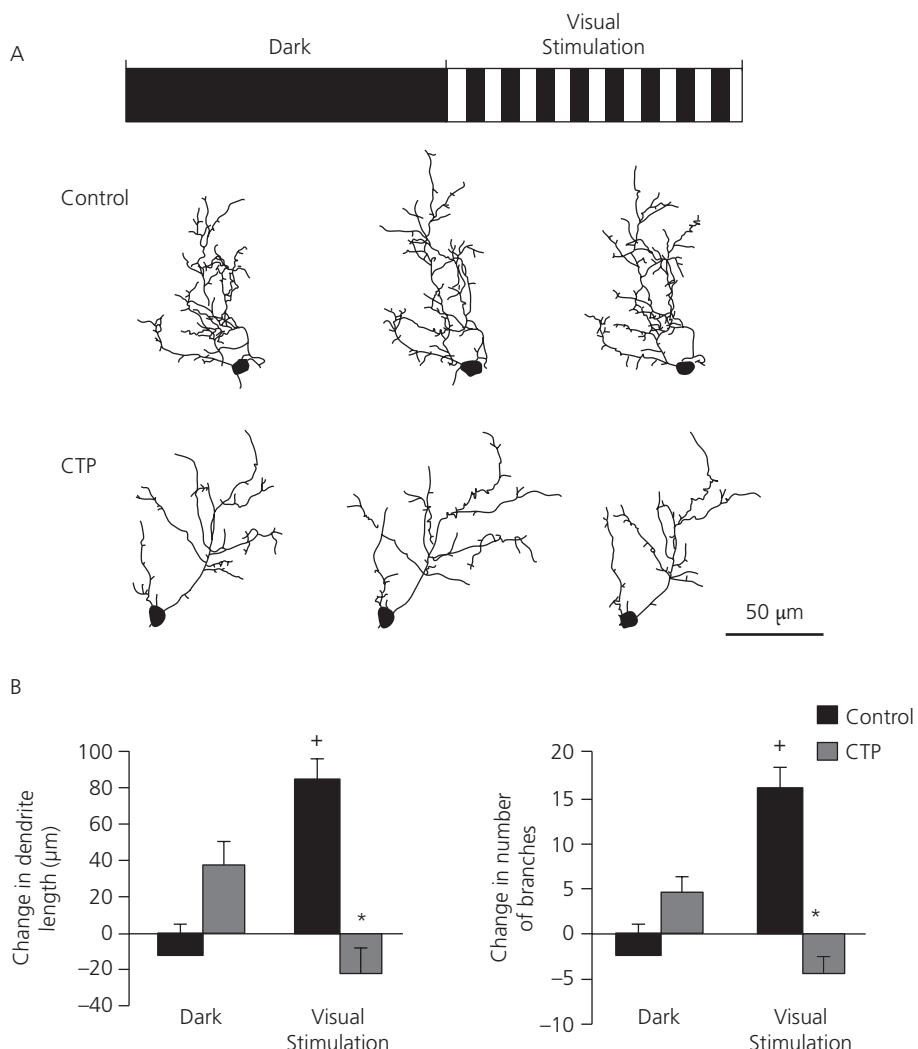


Fig. 3.5 Visual experience enhances dendritic arbor elaboration. **(A)** GFP-expressing optic tectal neurons from *Xenopus* tadpoles were exposed to 4 hours of dark followed by 4 hours of visual stimulation consisting of a simulated motion stimulus. Two-photon images were collected before and after the 4 hours in the dark and after the 4 hours of visual stimulation, as schematized in the top panel. Images of neurons expressing GFP (control) or the GluA1 C-terminal peptide (CTP) collected at the 0, 4, and 8 hour time-points are shown under the scheme of the visual experience protocol. **(B)** Quantitative analysis of the change in total dendritic branch length for the arbors (left) and change in total branch tip numbers (right). In control neurons (black) visual stimulation increases dendritic arbor elaboration whereas CTP-expressing blocks the normal increase in dendrite arbor growth.

Adapted from Kurt Haas, Jianli Li, and Hollis T. Cline, AMPA receptors regulate experience-dependent dendritic arbor growth in vivo, *Proceedings of the National Academy of Sciences of the United States of America*, 103(32), pp. 12127–12131, Figure 5a and b © 2006, by The National Academy of Sciences of the USA.

attracts growing dendrites toward them and promotes synaptogenesis. Initial synaptic transmission triggers events that either increase or decrease synapse maintenance (Vaughn et al., 1988; Vaughn, 1989). He imagined that both anterograde and retrograde signals participate in the exchange of information between pre- and postsynaptic elements that biases the outcome of synaptogenesis toward synapse stabilization or synapse elimination. Once a synapse forms and is stabilized by virtue of trans-synaptic signals, then further elaboration of the axon and/or dendrite occurs. Loss of synaptic inputs would lead to branch retraction. Longitudinal imaging has shown that branches can be added and then rapidly retracted, possibly because of failure to form synaptic contacts. Alternatively, branches can be retained for hours or days before retraction (Sin et al., 2002; Haas et al., 2006). In these cases, dendrites may form nascent synapses which fail to stabilize, leading to branch retraction (Rajan and Cline, 1998; Haas et al., 2006). Some of the most convincing evidence supporting the synaptotropic hypothesis was provided by Niell et al. (2004) who collected time-lapse images of optic tectal neurons co-expressing PSD-95–GFP fusion protein and red fluorescent protein (RFP) in zebrafish. They showed that an abundance of newly extended dendritic branches with PSD-95–GFP puncta were added to dendrites. While many of the branches and PSD-95–GFP puncta were transient, the branches that were stabilized included puncta that accumulate PSD-95–GFP, indicating that stable dendritic branches form strong synaptic connections. Together, the data from Haas et al. (2006), showing that impaired AMPA receptor trafficking into synapses decreased the stability of newly added dendritic branches and those from Niell et al (2004) showing that accumulation of PSD-95-containing puncta, and presumably other postsynaptic machinery, stabilized newly added branches, support the core element of the synaptotropic hypothesis, namely that synaptic contacts stabilize dynamic elements of the growing dendritic arbor. When these terminal branches were stabilized, they then served as a substrate for the further addition of dendritic branches. The synaptotropic hypothesis addresses the proposed function of synaptic contacts during dendrite arbor elaboration, but does not include any comment about a role for synapses in maintaining branches once the arbor is elaborated. Time-lapse imaging data demonstrate that newly added branches that are stabilized become the substrate for further branch additions. Once new branches have been added, their parent branch is maintained by the presence of the newly added terminal branches, independent of synaptic contacts onto the parent branch. Consequently, the synaptotropic hypothesis does not make any prediction that the distribution or density of synapses in complex arbors reflects the prior history of synaptogenesis and arbor elaboration (Ghiretti and Paradis, 2014).

We examined the relation between branch dynamics and synapse dynamics by combining *in vivo* two-photon time-lapse imaging and serial section transmission electron microscopy (Li et al., 2011). Single tectal neurons were transfected with a construct co-expressing GFP and membrane-targeted horseradish peroxidase (mHRP) and were imaged *in vivo* based on GFP expression at three time points, either at daily intervals or at 4-hour intervals. Dendritic branches that were either newly added (first seen at the third time point) or had been added previously and were maintained to the last time point were identified by comparing reconstructions of the dendritic arbors at the different time points. We then reconstructed the entire dendritic arbor using serial section electron microscopy, aided by expression and labeling of mHRP in the single imaged neuron, and mapped the location and maturity of each synaptic contact in the dendritic arbor. Nascent synapses are characterized by few scattered presynaptic vesicles relative to the size of the presynaptic profile, whereas mature synapses are densely packed with presynaptic vesicles (Li and Cline, 2010). This data set demonstrated that newly extended dendrites have a high density of relatively immature synapses, and that stable dendritic branches have fewer stronger synapses. Furthermore, the microcircuitry of presynaptic inputs was surprisingly complex: the

multiple presynaptic inputs that contacted the newly added dendrites originated from different presynaptic axon branches, suggesting that they represent convergent afferent inputs competing for postsynaptic partners. In addition, each of the presynaptic boutons contacting the newly added dendrites formed synapses with up to four postsynaptic partners, which all appeared to be from different postsynaptic neurons, indicating marked divergence of the inputs to multiple potential postsynaptic partners (Fig. 3.6). As dendritic branches stabilize, the synaptic configuration of the microcircuit undergoes massive pruning: the synapse density of each dendritic branch is reduced by half. Furthermore, the presynaptic boutons contacting stable dendrites have a single presynaptic release site, in contrast to the multi-synapse boutons contacting the newly added dendritic branches. It is important to point out that these major synaptic rearrangements are only visible by electron microscopy that retains high-quality intracellular organelle distribution because so far no traditional light-level microscopy can resolve these changes in pre- and postsynaptic contacts across the multiple neuronal branches in the dynamic circuit. It is interesting to note that dendrites reconstructed from animals that were visually deprived have a higher synapse density than control animals and that the synapses are less mature, consistent with the idea that visual experience increases the maturation of a select subpopulation of synapses on tectal neurons and leads to concurrent elimination of other synapses which converge onto the same dendritic branches. Exposing animals to the NMDA receptor blocker MK801 blocked both the

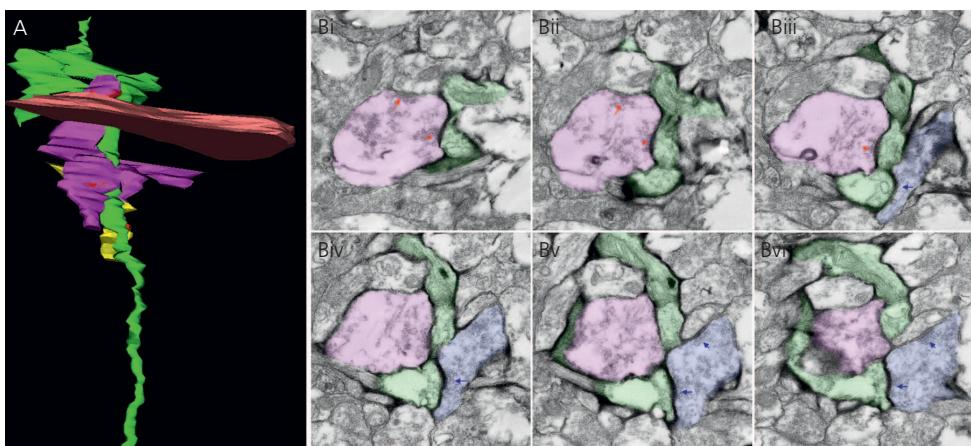


Fig. 3.6 Combined *in vivo* two-photon imaging and serial section electron microscopy reveals synaptic dynamics in developing microcircuits. Optic tectal neurons co-expressing eGFP and membrane-targeted HRP were imaged *in vivo* to identify newly added and stable dendritic branches, followed by serial section electron microscopic (EM) analysis to identify the synaptic contacts on dynamic and stable dendrites. **(A)** Reconstruction of a portion of a dendrite (green) and three converging axon branches (yellow, pink, copper). **(B)** Serial EM sections (Bi–Bvi) through a newly added dendritic branch (green) receiving multiple convergent inputs from two multi-synapse boutons (MSB; pink and blue), each of which synapses on other unlabeled dendrites, as marked by red and blue arrows within the pink and blue MSB. By contrast, stable dendritic branches tend to receive one synaptic input from single synapse boutons.

Adapted from Neuron, 69(2), Jianli Li, Alev Erisir, and Hollis Cline, *In vivo* time-lapse imaging and serial section electron microscopy reveal developmental synaptic rearrangements, pp. 273–86, Copyright 2011, Elsevier. With permission from Elsevier.

synapse maturation and concurrent synapse elimination that normally occurs with visual experience. Visual deprivation had several effects on presynaptic axons: synapses are more immature, as predicted, but, surprisingly, presynaptic boutons in visually deprived animals had significantly denser core vesicles, which were located at the perimeter of synaptic zones (Li and Cline, 2010). Dense core vesicles might carry machinery for the assembly of presynaptic specializations (Zhai et al., 2001; Bell et al., 2014) or they might release proteins, such as growth factors (Berg et al., 2000). The increase in dense core vesicles suggests that the initial phases of synapse formation may be delayed by visual deprivation, and that the cellular machinery may be poised to initiate synaptogenesis in response to an activity-dependent signal.

Together these data support a model in which activity-dependent dendrite branch dynamics, visible by time-lapse two-photon light microscopy, are accompanied by major rearrangements of their synaptic contacts within the local microcircuit, which would be impossible to resolve without high-resolution electron microscopy. The newly added dendritic branches and the fine filopodia that emerge from them appear to extend toward pre-existing presynaptic boutons and preferentially form synaptic contacts with the multi-synapse boutons, which form discreet synaptic contacts with several different postsynaptic partners. This is remarkably reminiscent of Vaughn's prediction and corroborates Kwon and Sabatini's observation that local uncaging of glutamate can increase spine-like extensions from dendrites (Kwon and Sabatini, 2011), as well as other studies documenting activity-dependent synaptogenesis (Dunn et al., 2013; Andreau and Burrone, 2014). The observations that dendritic branches emerging within the previous 4 hours *in vivo* form multiple synapses, each with multi-synapse boutons, indicates that synaptogenesis in the living tadpole tectum occurs over a timeframe of hours, as has also been documented with direct *in vivo* imaging (Ruthazer et al., 2006; Sanchez et al., 2006). In addition, we find that synapses formed on newly added dendrites are more closely spaced than synapses on more mature branches. Together these data indicate that many developing neurons are highly interconnected. The dense, but immature, interconnectivity decreases based on two strategies of synapse elimination. In axons, divergence decreases as postsynaptic contacts are lost from multi-synapse boutons, so boutons contact a single postsynaptic partner. In dendrites, convergent immature inputs are lost and the remaining sparse synapses mature. The outcome of these activity-dependent synaptic rearrangements is greater specificity of visual system connections, which would endow the circuit with greater spatial and temporal control over information processing (Ruthazer and Aizenman, 2010), for instance as shown in the retina and central visual projections (Hooks and Chen, 2006; Rivlin-Etzion et al., 2012; Rosa and Feller, 2014; Vlasits et al., 2014). Clustered synaptic inputs likely enable extended dendritic branches and filopodia to test for correlated input activity from several potential presynaptic partners (Stepanyants et al., 2002). Similarly multi-synapse boutons enable presynaptic axons to sample several postsynaptic partners. At this point it appears that there is little specificity in the process of synaptogenesis, and that the specificity of circuit connectivity arises from a selection process during synapse elimination. This generalization may apply to CNS circuits, but it is not necessarily universal: the development of different retinal synapses and circuits appears to employ activity-dependent and activity-independent mechanisms to different extents (Sanes and Zipursky, 2010; Dunn et al., 2013; Okawa et al., 2014). For instance, the development of asymmetric dendritic arbors in the retina appears to occur as a result of biased additions of dendrite branches to one side of the cell body rather than uniform addition of exploratory branches followed by spatially biased branch retractions (Mumm et al., 2006; Choi et al., 2010). These data suggest that activity-independent guidance cues may constrain the development of retinal circuitry in order to minimize variation in the sensory input (Duan et al., 2014; Wernet et al., 2014).

Inhibitory inputs regulate dendritic arbor development

Although most studies on the role of sensory experience on the development of dendritic arbors have focused on the effects mediated by excitatory synaptic input, inhibitory synaptic activity also regulates dendritic arbor development. The mechanisms by which inhibitory GABAergic or glycinergic synaptic transmission regulate dendritic arbor development depend on the expression of chloride transporters and therefore whether the transmitter depolarizes or hyperpolarizes the postsynaptic neuron. Activation of ionotropic type A GABA receptors (GABA_AR) in young neurons increases process outgrowth and synaptogenesis, possibly mediated by GABA-induced excitation (Barbin et al., 1993; Ben-Ari, 2002; Cancedda et al., 2007). Blocking inhibitory GABAergic transmission in preparations containing mature neurons also increases process outgrowth (Wayman et al., 2006), but this occurs indirectly by increasing neuronal activity. Similarly, glycinergic transmission affects dendritic arbor development, both at early stages of development, when it is depolarizing (Maric et al., 2001; Tapia et al., 2001), and later, when glycinergic transmission is inhibitory (Sanes and Chokshi, 1992; Sanes et al., 1992; Sanes and Hafidi, 1996). Blocking glycinergic input with strychnine increased dendritic arbor size, suggesting that the normal function of inhibitory input is to restrain dendrite growth. Although such experiments indicate that inhibitory transmission affects dendritic arbor development, blocking GABAergic and glycinergic transmission produces circuit-wide effects on activity levels that confound the interpretation of changes in neuronal structure (Ben-Ari et al., 1989; Chen et al., 1996; Tapia et al., 2001).

Expression of a peptide corresponding to the intracellular loop of $\gamma 2$ subunit of GABA_AR , called ICL, prevents $\gamma 2$ subunit-containing GABA_AR from anchoring at synapses (Alldred et al., 2005; Christie et al., 2006) and allows cell-autonomous manipulations of inhibitory input. Electrophysiological experiments show that ICL decreased inhibitory synaptic inputs in neurons that expressed ICL but not in untransfected neurons or those expressed a mutant ICL, called mICL. Furthermore, ICL expression increased the ratio of excitatory to inhibitory synaptic activity in ICL-expressing neurons. Time-lapse two-photon images of optic tectal neurons collected *in vivo* at daily intervals showed that ICL-expressing neurons have less elaborate dendritic arbors that span a larger area of the tectal neuropil compared with controls. Images collected at shorter intervals indicated that the decreased arbor elaboration arose from a decrease in the number of new branches added to the arbors (Shen et al., 2009), rather than an increase in branch retractions as seen when AMPA receptor trafficking into synapses was disrupted. Furthermore, decreasing inhibitory input, and thereby increasing the balance of excitation to inhibition, blocked the visual-experience-dependent increase in dendritic arbor complexity and visually guided behavior (Shen et al., 2011). These results suggest that a change in the balance of excitatory to inhibitory inputs disrupts dendritic arbor development, and this in turn impairs visual information processing and visually guided behavior. Given the current evidence that the balance of excitation to inhibition is critical for normal brain function, and that neurodevelopmental disorders such as autism spectrum disorders disrupt the balance of excitation to inhibition (Gatto and Broadie, 2010; Paluszakiewicz et al., 2011; Calfa et al., 2015), it will be of great interest to determine how changes in the relative balance of excitatory to inhibitory synaptic inputs affects the signaling pathways and cellular machinery that regulate dendritic arbor development.

Many studies have identified diverse molecular mechanisms that affect dendritic arbor development and these mechanisms have been reviewed extensively. As mentioned above, it is valuable to consider the different molecular mechanisms that impinge on dendritic arbor development within the framework of the synaptotropic hypothesis. Simply put, do the mechanisms facilitate or impair synaptogenesis, synapse stabilization, or synapse retraction? Future studies will identify nodal

points in regulatory pathways controlling synapse dynamics and structural plasticity in dendrites. By extension, these nodal points will likely ramify to affect circuit function and behavior.

Concluding comments

In this chapter we have discussed a number of experimental results in an attempt to broadly describe some examples in which synaptic activity and other types of brain activity affect the development of dendritic arbors. Because the structure of dendritic arbors has a direct impact on the biophysical properties of dendrites, and therefore their integrative properties, changes in the structure of dendrites will affect information processing and circuit input/output function. Whether such changes at the synaptic, cellular, and circuit levels culminate in changes in behavior will ultimately depend on the homeostatic resilience of the system. Failure to detect behavioral ramifications of altered dendritic arbor development, structure, and function, does not necessarily mean that these deficits are not important; rather it means that those functions are so critical that layers of compensatory mechanisms ensure that they endure.

References

- Alldred MJ, Mulder-Rosi J, Lingefelter SE, Chen G, Luscher B (2005) Distinct gamma2 subunit domains mediate clustering and synaptic function of postsynaptic GABA_A receptors and gephyrin. *Journal of Neuroscience* **25**:594–603.
- Andreae LC, Burrone J (2014) The role of neuronal activity and transmitter release on synapse formation. *Current Opinion in Neurobiology* **27**:47–52.
- Ashby MC, Isaac JT (2011) Maturation of a recurrent excitatory neocortical circuit by experience-dependent unsilencing of newly formed dendritic spines. *Neuron* **70**:510–521.
- Bagni C, Greenough WT (2005) From mRNP trafficking to spine dysmorphogenesis: the roots of fragile X syndrome. *Nature Reviews Neuroscience* **6**:376–387.
- Barbin G, Pollard H, Gaiarsa JL, Ben-Ari Y (1993) Involvement of GABA_A receptors in the outgrowth of cultured hippocampal neurons. *Neuroscience Letters* **152**:150–154.
- Bell ME, Bourne JN, Chirillo MA, Mendenhall JM, Kuwajima M, Harris KM (2014) Dynamics of nascent and active zone ultrastructure as synapses enlarge during long-term potentiation in mature hippocampus. *Journal of Comparative Neurology* **522**:3861–3884.
- Ben-Ari Y (2002) Excitatory actions of GABA during development: the nature of the nurture. *Nature Reviews Neuroscience* **3**:728–739.
- Ben-Ari Y, Cherubini E, Corradietti R, Gaiarsa JL (1989) Giant synaptic potentials in immature rat CA3 hippocampal neurones. *Journal of Physiology* **416**:303–325.
- Benavides-Piccione R, Ballesteros-Yanez I, de Lagran MM, Elston G, Estivill X, Fillat C, Defelipe J, Dierssen M (2004) On dendrites in Down syndrome and DS murine models: a spiny way to learn. *Progress in Neurobiology* **74**:111–126.
- Berg EA, Johnson RJ, Leeman SE, Boyd N, Kimerer L, Fine RE (2000) Isolation and characterization of substance P-containing dense core vesicles from rabbit optic nerve and termini. *Journal of Neuroscience Research* **62**:830–839.
- Bestman JE, Cline HT (2008) The RNA binding protein CPEB regulates dendrite morphogenesis and neuronal circuit assembly in vivo. *Proceedings of the National Academy of Sciences of the United States of America* **105**:20494–20499.
- Bleckert A, Wong RO (2011) Identifying roles for neurotransmission in circuit assembly: insights gained from multiple model systems and experimental approaches. *BioEssays* **33**:61–72.
- Blue ME, Parnavelas JG (1983) The formation and maturation of synapses in the visual cortex of the rat. II. Quantitative analysis. *Journal of Neurocytology* **12**:697–712.

- Bystron I, Blakemore C, Rakic P** (2008) Development of the human cerebral cortex: Boulder Committee revisited. *Nature Reviews Neuroscience* **9**:110–122.
- Calfa G, Li W, Rutherford JM, Pozzo-Miller L** (2015) Excitation/inhibition imbalance and impaired synaptic inhibition in hippocampal area CA3 of Mecp2 knockout mice. *Hippocampus* **25**:159–168.
- Cancedda L, Fiumelli H, Chen K, Poo MM** (2007) Excitatory GABA action is essential for morphological maturation of cortical neurons in vivo. *Journal of Neuroscience* **27**:5224–5235.
- Cantallops I, Haas K, Cline HT** (2000) Postsynaptic CPG15 promotes synaptic maturation and presynaptic axon arbor elaboration in vivo. *Nature Neuroscience* **3**:1004–1011.
- Chen JL, Nedivi E** (2013) Highly specific structural plasticity of inhibitory circuits in the adult neocortex. *The Neuroscientist* **19**:384–393.
- Chen G, Trombley PQ, van den Pol AN** (1996) Excitatory actions of GABA in developing rat hypothalamic neurones. *Journal of Physiology* **494**:451–464.
- Chen SX, Tari PK, She K, Haas K** (2010) Neurexin–neuroligin cell adhesion complexes contribute to synaptotrophic dendritogenesis via growth stabilization mechanisms in vivo. *Neuron* **67**:967–983.
- Chiu SL, Chen CM, Cline HT** (2008) Insulin receptor signaling regulates synapse number, dendritic plasticity, and circuit function in vivo. *Neuron* **58**:708–719.
- Choi JH, Law MY, Chien CB, Link BA, Wong RO** (2010) In vivo development of dendritic orientation in wild-type and mislocalized retinal ganglion cells. *Neural Development* **5**:29.
- Christie SB, Li RW, Miralles CP, Yang BY, De Blas AL** (2006) Clustered and non-clustered GABAA receptors in cultured hippocampal neurons. *Molecular and Cellular Neurosciences* **31**:1–14.
- Cline HT** (2001) Dendritic arbor development and synaptogenesis. *Current Opinion in Neurobiology* **11**:118–126.
- Cline H, Haas K** (2008) The regulation of dendritic arbor development and plasticity by glutamatergic synaptic input: a review of the synaptotrophic hypothesis. *Journal of Physiology* **586**:1509–1517.
- Cline HT, Wu G-Y, Malinow R** (1997) In vivo development of neuronal structure and function. *Cold Spring Harbor Symposia on Quantitative Biology* **61**:95–104.
- Cragg BG** (1975) The development of synapses in the visual system of the cat. *Journal of Comparative Neurology* **160**:147–166.
- Dailey ME, Smith SJ** (1996) The dynamics of dendritic structure in developing hippocampal slices. *Journal of Neuroscience* **16**:2983–2994.
- D’Orazi FD, Suzuki SC, Wong RO** (2014) Neuronal remodeling in retinal circuit assembly, disassembly, and reassembly. *Trends in Neurosciences* **37**:594–603.
- Duan X, Krishnasamy A, De la Huerta I, Sanes JR** (2014) Type II cadherins guide assembly of a direction-selective retinal circuit. *Cell* **158**:793–807.
- Dunn FA, Della Santina L, Parker ED, Wong RO** (2013) Sensory experience shapes the development of the visual system’s first synapse. *Neuron* **80**:1159–1166.
- Engert F, Tao HW, Zhang LI, Poo MM** (2002) Moving visual stimuli rapidly induce direction sensitivity of developing tectal neurons. *Nature* **419**:470–475.
- Faulkner RL, Wishard TJ, Thompson CK, Liu H-H, Cline HT** (2015) FMRP regulates neurogenesis in vivo in *Xenopus laevis* tadpoles. *eNeuro* **1**:51–60.
- Gatto CL, Broadie K** (2010) Genetic controls balancing excitatory and inhibitory synaptogenesis in neuromodulatory disorder models. *Frontiers in Synaptic Neuroscience* **2**:4.
- Ghiretti AE, Paradis S** (2014) Molecular mechanisms of activity-dependent changes in dendritic morphology: role of RKG proteins. *Trends in Neurosciences* **37**:399–407.
- Ghiretti AE, Moore AR, Brenner RG, Chen LF, West AE, Lau NC, Van Hooser SD, Paradis S** (2014) Rem2 is an activity-dependent negative regulator of dendritic complexity in vivo. *Journal of Neuroscience* **34**:392–407.
- Greenough WT, Chang FL** (1988) Dendritic pattern formation involves both oriented regression and oriented growth in the barrels of mouse somatosensory cortex. *Brain Research* **471**:148–152.

- Gonzalez CL, Gharabawie OA, Whishaw IQ, Kolb B (2005) Nicotine stimulates dendritic arborization in motor cortex and improves concurrent motor skill but impairs subsequent motor learning. *Synapse* **55**:183–191.
- Govek EE, Newey SE, Akerman CJ, Cross JR, Van der Veken L, Van Aelst L (2004) The X-linked mental retardation protein oligophrenin-1 is required for dendritic spine morphogenesis. *Nature Neuroscience* **7**:364–372.
- Haas K, Sin WC, Javaherian A, Li Z, Cline HT (2001) Single-cell electroporation for gene transfer in vivo. *Neuron* **29**:583–591.
- Haas K, Li J, Cline HT (2006) AMPA receptors regulate experience-dependent dendritic arbor growth in vivo. *Proceedings of the National Academy of Sciences of the United States of America* **103**:12127–12131.
- Hestrin S (1992) Developmental regulation of NMDA receptor-mediated synaptic currents at a central synapse. *Nature* **357**:686–689.
- Hooks BM, Chen C (2006) Distinct roles for spontaneous and visual activity in remodeling of the retino-geniculate synapse. *Neuron* **52**:281–291.
- Hossain S, Hewapathirane DS, Haas K (2012) Dynamic morphometrics reveals contributions of dendritic growth cones and filopodia to dendritogenesis in the intact and awake embryonic brain. *Developmental Neurobiology* **72**:615–627.
- Hua JY, Smith SJ (2004) Neural activity and the dynamics of central nervous system development. *Nature Neuroscience* **7**:327–332.
- Hume RI, Purves D (1981) Geometry of neonatal neurones and the regulation of synapse elimination. *Nature* **293**:469–471.
- Huttenlocher PR, Dabholkar AS (1997) Regional differences in synaptogenesis in human cerebral cortex. *Journal of Comparative Neurology* **387**:167–178.
- Imamura F, Greer CA (2009) Dendritic branching of olfactory bulb mitral and tufted cells: regulation by TrkB. *PloS ONE* **4**:e6729.
- Inglis FM, Zuckerman KE, Kalb RG (2000) Experience-dependent development of spinal motor neurons. *Neuron* **26**:299–305.
- Inglis FM, Crockett R, Korada S, Abraham WC, Hollmann M, Kalb RG (2002) The AMPA receptor sub-unit GluR1 regulates dendritic architecture of motor neurons. *Journal of Neuroscience* **22**:8042–8051.
- Isaac JT, Crair MC, Nicoll RA, Malenka RC (1997) Silent synapses during development of thalamocortical inputs. *Neuron* **18**:269–280.
- Jan YN, Jan LY (2010) Branching out: mechanisms of dendritic arborization. *Nature Reviews Neuroscience* **11**:316–328.
- Katz LC, Constantine-Paton M (1988) Relationships between segregated afferents and postsynaptic neurones in the optic tectum of three-eyed frogs. *Journal of Neuroscience* **8**:3160–3180.
- Katz LC, Shatz CJ (1996) Synaptic activity and the construction of cortical circuits. *Science* **274**:1133–1138.
- Katz LC, Gilbert CD, Wiesel TN (1989) Local circuits and ocular dominance columns in monkey striate cortex. *Journal of Neuroscience* **9**:1389–1399.
- Kolb B, Gorny G, Li Y, Samaha AN, Robinson TE (2003) Amphetamine or cocaine limits the ability of later experience to promote structural plasticity in the neocortex and nucleus accumbens. *Proceedings of the National Academy of Sciences of the United States of America* **100**:10523–10528.
- Koscel A, Lowel S, Bolz J (1995) Relationships between dendritic fields and functional architecture in striate cortex of normal and visually deprived cats. *Journal of Neuroscience* **15**:3913–3926.
- Kotak VC, Sanes DH (1996) Developmental influence of glycinergic transmission: regulation of NMDA receptor-mediated EPSPs. *Journal of Neuroscience* **16**:1836–1843.
- Kotak VC, Sanes DH (1997) Deafferentation weakens excitatory synapses in the developing central auditory system. *European Journal of Neuroscience* **9**:2340–2347.
- Kwon HB, Sabatini BL (2011) Glutamate induces de novo growth of functional spines in developing cortex. *Nature* **474**:100–104.

- Lazar G (1973) The development of the optic tectum in *Xenopus laevis*: a Golgi study. *Journal of Anatomy* **116**:347–355.
- Lee LJ, Lo FS, Erzurumlu RS (2005) NMDA receptor-dependent regulation of axonal and dendritic branching. *Journal of Neuroscience* **25**:2304–2311.
- Lee WC, Huang H, Feng G, Sanes JR, Brown EN, So PT, Nedivi E (2006) Dynamic remodeling of dendritic arbors in GABAergic interneurons of adult visual cortex. *PLoS Biology* **4**:e29.
- Leslie JH, Nedivi E (2011) Activity-regulated genes as mediators of neural circuit plasticity. *Progress in Neurobiology* **94**:223–237.
- Li J, Cline HT (2010) Visual deprivation increases accumulation of dense core vesicles in developing optic tectal synapses in *Xenopus laevis*. *Journal of Comparative Neurology* **518**:2365–2381.
- Li Z, Van Aelst L, Cline HT (2000) Rho GTPases regulate distinct aspects of dendritic arbor growth in *Xenopus* central neurons in vivo. *Nature Neuroscience* **3**:217–225.
- Li J, Erisir A, Cline H (2011) In vivo time-lapse imaging and serial section electron microscopy reveal developmental synaptic rearrangements. *Neuron* **69**:273–286.
- Loebrich S, Nedivi E (2009) The function of activity-regulated genes in the nervous system. *Physiological Reviews* **89**:1079–1103.
- Malun D, Brunjes PC (1996) Development of olfactory glomeruli: temporal and spatial interactions between olfactory receptor axons and mitral cells in opossums and rats. *Journal of Comparative Neurology* **368**:1–16.
- Maric D, Liu QY, Maric I, Chaudry S, Chang YH, Smith SV, Sieghart W, Fritschy JM, Barker JL (2001) GABA expression dominates neuronal lineage progression in the embryonic rat neocortex and facilitates neurite outgrowth via GABA(A) autoreceptor/Cl⁻ channels. *Journal of Neuroscience* **21**:2343–2360.
- Marshak S, Meynard MM, De Vries YA, Kidane AH, Cohen-Cory S (2012) Cell-autonomous alterations in dendritic arbor morphology and connectivity induced by overexpression of MeCP2 in *Xenopus* central neurons in vivo. *PLoS ONE* **7**:e33153.
- Morrow BA, Elsworth JD, Roth RH (2005) Prenatal exposure to cocaine selectively disrupts the development of parvalbumin containing local circuit neurons in the medial prefrontal cortex of the rat. *Synapse* **56**:1–11.
- Mumm JS, Williams PR, Godinho L, Koerber A, Pittman AJ, Roeser T, Chien CB, Baier H, and Wong RO (2006) In vivo imaging reveals dendritic targeting of laminated afferents by zebrafish retinal ganglion cells. *Neuron* **52**:609–621.
- Nedivi E, Wu GY, Cline HT (1998) Promotion of dendritic growth by CPG15, an activity-induced signaling molecule. *Science* **281**:1863–1866.
- Newey SE, Velamoor V, Govek EE, Van Aelst L (2005) Rho GTPases, dendritic structure, and mental retardation. *Journal of Neurobiology* **64**:58–74.
- Niell CM, Meyer MP, Smith SJ (2004) In vivo imaging of synapse formation on a growing dendritic arbor. *Nature Neuroscience* **7**:254–260.
- Okawa H, Hoon M, Yoshimatsu T, Della Santina L, Wong RO (2014) Illuminating the multifaceted roles of neurotransmission in shaping neuronal circuitry. *Neuron* **83**:1303–1318.
- Paluszewicz SM, Martin BS, Huntsman MM (2011) Fragile X syndrome: the GABAergic system and circuit dysfunction. *Developmental Neuroscience* **33**:349–364.
- Puram SV, Bonni A (2013) Cell-intrinsic drivers of dendrite morphogenesis. *Development* **140**:4657–4671.
- Rajan I, Cline HT (1998) Glutamate receptor activity is required for normal development of tectal cell dendrites in vivo. *Journal of Neuroscience* **18**:7836–7846.
- Rakic P, Bourgeois JP, Eckenhoff MF, Zecevic N, Goldman-Rakic PS (1986) Concurrent overproduction of synapses in diverse regions of the primate cerebral cortex. *Science* **232**:232–235.
- Ramoa AS, McCormick DA (1994) Enhanced activation of NMDA receptor responses at the immature retinogeniculate synapse. *Journal of Neuroscience* **14**:2098–2105.

- Rivlin-Etzion M, Wei W, Feller MB (2012) Visual stimulation reverses the directional preference of direction-selective retinal ganglion cells. *Neuron* **76**:518–525.
- Rosa JM, Feller MB (2014) Neurodevelopment: a novel role for activity in shaping retinal circuits. *Current Biology* **24**:R964–R966.
- Ruthazer ES, Aizenman CD (2010) Learning to see: patterned visual activity and the development of visual function. *Trends in Neuroscience* **33**:183–192.
- Ruthazer ES, Li J, Cline HT (2006) Stabilization of axon branch dynamics by synaptic maturation. *Journal of Neuroscience* **26**:3594–3603.
- Sanchez AL, Matthews BJ, Meynard MM, Hu B, Javed S, Cohen Cory S (2006) BDNF increases synapse density in dendrites of developing tectal neurons *in vivo*. *Development* **133**:2477–2486.
- Sanes DH, Chokshi P (1992) Glycinergic transmission influences the development of dendrite shape. *NeuroReport* **3**:323–326.
- Sanes DH, Hafidi A (1996) Glycinergic transmission regulates dendrite size in organotypic culture. *Journal of Neurobiology* **31**:503–511.
- Sanes JR, Zipursky SL (2010) Design principles of insect and vertebrate visual systems. *Neuron* **66**:15–36.
- Sanes DH, Markowitz S, Bernstein J, Wardlow J (1992) The influence of inhibitory afferents on the development of postsynaptic dendritic arbors. *Journal of Comparative Neurology* **321**:637–644.
- Schwartz N, Schohl A, Ruthazer ES (2009) Neural activity regulates synaptic properties and dendritic structure *in vivo* through calcineurin/NFAT signaling. *Neuron* **62**:655–669.
- Schweitzer L (1991) Morphometric analysis of developing neuronal geometry in the dorsal cochlear nucleus of the hamster. *Brain Research Developmental Brain Research* **59**:39–47.
- Shen W, Da Silva JS, He H, Cline HT (2009) Type A GABA-receptor-dependent synaptic transmission sculpts dendritic arbor structure in *Xenopus* tadpoles *in vivo*. *Journal of Neuroscience* **29**:5032–5043.
- Shen W, McKeown CR, Demas JA, Cline HT (2011) Inhibition to excitation ratio regulates visual system responses and behavior *in vivo*. *Journal of Neurophysiology* **106**:2285–2302.
- Sin WC, Haas K, Ruthazer ES, Cline HT (2002) Dendrite growth increased by visual activity requires NMDA receptor and Rho GTPases. *Nature* **419**:475–480.
- Sorensen SA, Rubel EW (2006) The level and integrity of synaptic input regulates dendrite structure. *Journal of Neuroscience* **26**:1539–1550.
- Sorensen SA, Rubel EW (2011) Relative input strength rapidly regulates dendritic structure of chick auditory brainstem neurons. *Journal of Comparative Neurology* **519**:2838–2851.
- Stepanyants A, Hof PR, Chklovskii DB (2002) Geometry and structural plasticity of synaptic connectivity. *Neuron* **34**:275–288.
- Tapia JC, Mentis GZ, Navarrete R, Nualart F, Figueroa E, Sanchez A, Aguayo LG (2001) Early expression of glycine and GABA(A) receptors in developing spinal cord neurons. Effects on neurite outgrowth. *Neuroscience* **108**:493–506.
- Tian N (2011) Developmental mechanisms that regulate retinal ganglion cell dendritic morphology. *Developmental Neurobiology* **71**:1297–1309.
- Tian N, Copenhagen DR (2001) Visual deprivation alters development of synaptic function in inner retina after eye opening. *Neuron* **32**:439–449.
- Tian N, Copenhagen DR (2003) Visual stimulation is required for refinement of ON and OFF pathways in postnatal retina. *Neuron* **39**:85–96.
- Van Aelst L, Cline HT (2004) Rho GTPases and activity-dependent dendrite development. *Current Opinion in Neurobiology* **14**:297–304.
- Vaughn JE (1989) Fine structure of synaptogenesis in the vertebrate central nervous system. *Synapse* **3**:255–285.
- Vaughn J, Barber R, Sims T (1988) Dendritic development and preferential growth into synaptogenic fields: a quantitative study of Golgi-impregnated spinal motor neurons. *Synapse* **2**:69–78.

- Vislay-Meltzer RL, Kampff AR, Engert F (2006) Spatiotemporal specificity of neuronal activity directs the modification of receptive fields in the developing retinotectal system. *Neuron* **50**:101–114.
- Vlasits AL, Bos R, Morrie RD, Fortuny C, Flannery JG, Feller MB, Rivlin-Etzion M (2014) Visual stimulation switches the polarity of excitatory input to starburst amacrine cells. *Neuron* **83**:1172–1184.
- Wang Y, Rubel EW (2012) In vivo reversible regulation of dendritic patterning by afferent input in bipolar auditory neurons. *Journal of Neuroscience* **32**:11495–11504.
- Warton SS, McCart R (1989) Synaptogenesis in the stratum griseum superficiale of the rat superior colliculus. *Synapse* **3**:136–148.
- Wayman GA, Impey S, Marks D, Saneyoshi T, Grant WF, Derkach V, Soderling TR (2006) Activity-dependent dendritic arborization mediated by CaM-kinase I activation and enhanced CREB-dependent transcription of Wnt-2. *Neuron* **50**:897–909.
- Wernet MF, Huberman AD, Desplan C (2014) So many pieces, one puzzle: cell type specification and visual circuitry in flies and mice. *Genes and Development* **28**:2565–2584.
- Wu GY, Cline HT (1998) Stabilization of dendritic arbor structure in vivo by CaMKII. *Science* **279**:222–226.
- Wu G, Malinow R, Cline HT (1996) Maturation of a central glutamatergic synapse. *Science* **274**:972–976.
- Xu HP, Tian N (2007) Retinal ganglion cell dendrites undergo a visual activity-dependent redistribution after eye opening. *Journal of Comparative Neurology* **503**:244–259.
- Zecevic N. (1998) Synaptogenesis in layer I of the human cerebral cortex in the first half of gestation. *Cerebral Cortex* **8**:245–252.
- Zecevic N, Rakic P (1991) Synaptogenesis in monkey somatosensory cortex. *Cerebral Cortex* **1**:510–523.
- Zhai RG, Vardinon-Friedman H, Cases-Langhoff C, Becker B, Gundelfinger ED, Ziv NE, Garner CC (2001) Assembling the presynaptic active zone: a characterization of an active one precursor vesicle. *Neuron* **29**:131–143.
- Zhu JJ, Esteban JA, Hayashi Y, Malinow R (2000) Postnatal synaptic potentiation: delivery of GluR4-containing AMPA receptors by spontaneous activity. *Nature Neuroscience* **3**:1098–1106.

Chapter 4

Molecular determinants of dendrite and spine development

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Summary

Information processing in neurons is critically dependent on dendritic morphology. The overall extent and orientation of dendrites determines the kinds of input a neuron receives. Fine dendritic appendages called spines act as subcellular compartments devoted to processing synaptic information, and the dendritic branching pattern determines the efficacy with which synaptic information is transmitted to the soma. The development of the dendritic tree is influenced by a number of factors. Studies in *Drosophila* have identified key components of the genetic program that regulates dendritic morphogenesis. Parallel studies in vertebrates have revealed that extracellular signals and neuronal activity exert a major influence on the growth and branching of dendrites and the formation of dendritic spines. The identification of genes that mediate these processes provides important insights into the molecular mechanisms of dendritic morphogenesis.

Introduction

The study of dendritic development has historically been more challenging than the study of axons, at least in part because of the complexity and diversity of dendrites (Ramón y Cajal, 1909). In addition, dendrites grow more slowly than axons *in vitro* and specific techniques to study the dynamics of dendritic development were not available until relatively recently. This has changed in the last two decades with the development of techniques to label and image dendritic dynamics with increasing resolution *in vitro* and *in vivo*, and the advent of molecular approaches to precisely manipulate gene expression in neurons. Here we discuss our current understanding of the molecular mechanisms that regulate the development of dendrites and dendritic spines. The mechanisms by which neuronal activity influences dendritic development are discussed in Chapter 19.

Stages in the development of dendrites

The differentiation of dendrites is tightly coupled to the polarization of neurons following terminal division. Migrating neurons often express dendritic markers such as microtubule associated protein-2 (MAP2; see Table 4.1) in their leading process, but these proto-dendrites do not have all the specializations that characterize mature dendrites (Fig. 4.1). Once neurons have reached their final position, they extend dendrites in particular directions to receive afferent input. This is followed by a period of dendritic growth and remodeling during which neurons achieve their characteristic morphology (Figs 4.2 and 4.3). During growth and branching, dendrites from the same

Table 4.1 Proteins, genes, and related abbreviations used in this chapter

Abbreviation	Definition
Acj6	Abnormal chemosensory jump 6; POU-domain homeobox transcription factor
ARF6	Small GTPase
ARHGEF6	GEF for Rac1 and Cdc42
APC	Anaphase-promoting complex; E3 ubiquitin ligase
BAF	Brg/Brm associated factor; chromatin remodeling complex
BAF53b	nBAF subunit
BAI1	Brain-specific angiogenesis inhibitor; GPCR
Bantam	A microRNA important for tissue growth
BAR	Bin amphiphysin rvs
Bcr	Breakpoint cluster region; Rac-GAP
BDNF	Brain-derived neurotrophic factor; ligand for TrkB receptor tyrosine kinase
bHLH	Basic helix-loop-helix; motif in a family of transcription factors
βPIX	Rho guanyl nucleotide exchange factor (same as Arhgef7)
Brg	Brahma-related gene
Brm	Brahma; part of the SWI/SNF nucleosome remodeling complex
BTB	Domain of zinc-finger protein
CaMKI	Calcium/calmodulin-dependent protein kinase 1
CaMKII	Calcium/calmodulin-dependent protein kinase 2
CaMKK	Calcium/calmodulin-dependent protein kinase kinase
Cdc20	APC coactivator
Cdc42	Cell division cycle 42; small GTPase
Cdk5	Fyn and cyclin-dependent kinase 5
Celsr1–3	Atypical seven-transmembrane cadherins 1–3 (orthologs of <i>Drosophila</i> flamingo)
Chinmo	Chronologically inappropriate morphogenesis; BTB zinc-finger protein
COE	Collier/Olf1/EBF family of HLH transcription factors
CREST	Calcium-responsive transactivator
Cul7 ^{Fbxw}	E3 ubiquitin ligase
Cut	<i>Drosophila</i> member of the homeodomain family of DNA-binding proteins
Cux1	Cut-like homeobox 1; member of the homeodomain family of DNA-binding proteins
Cux2	Cut-like homeobox 2; member of the homeodomain family of DNA-binding proteins
DCC	Deleted in colorectal carcinoma; netrin receptor (ortholog of Frazzled and UNC-40)
Dlx1/2	Distal-less homeobox 1/2 transcription factors
Drifter	A POU-homeodomain DNA-binding protein (also called ventral veins lacking)
Dscam1	Down syndrome cell adhesion molecule 1, Ig superfamily
Dsh	Dishevelled, Wnt signalling inhibitor of GSK-3β (same as Dvl)
Dvl	Dishevelled, Wnt signalling inhibitor of GSK-3β (same as Dsh)

Table 4.1 (continued) Proteins, genes, and related abbreviations used in this chapter

Abbreviation	Definition
EFA6A	Exchange factor for ARF6
EphB	Ephrin B receptor tyrosine kinase
F-BAR	FCH Bin–Amphiphysin–Rvs protein dimerization domain
FAK	Focal adhesion kinase
Fbxw8	F-box and tryptophan–aspartic acid (WD) repeat domain containing 8 protein, part of an E3 ligase complex
Fez1	Fasciculation and elongation protein zeta 1
Fezl	Forebrain embryonic zinc-finger-like protein (same as Fez, Znf312, Fezf2)
Fezf2	Forebrain embryonic zinc-finger-like protein (same as Fez, Znf312, Fezl)
Flamingo	Atypical seven transmembrane cadherin (orthologs of Celsr1–3)
Frazzled	Netrin receptor (ortholog of DCC, UNC-40)
Fry	Furry protein
Fyn	Member of the Src family of non-receptor tyrosine kinases
GAP	GTPase activating (or accelerating) protein
GEF	Guanine nucleotide exchange factor
Gorasp1	Golgi reassembly stacking protein 1 (same as Grasp65)
Grasp65	Golgi reassembly stacking protein 65 (same as Gorasp1)
GSK3-β	Glycogen synthase kinase 3-β
ham	Hamlet; multi-domain zinc-finger protein
HDAC6	Histone deacetylase 6
HDAC11	Histone deacetylase 11
HLH	Helix-loop-helix
ICAM5	Intracellular adhesion molecule 5
Id1	Inhibitor of DNA binding 1; centrosomal helix-loop-helix protein
Jnk	Jun-terminal kinase
Kalirin-7	A RhoGEF with exchange activity for Rac1
LIM	A zinc-coordinating domain; found in Lin1, Isl-1, Mec-3 transcription factors
Lim1	A LIM homeodomain transcription factor
LKB1	Liver kinase B1; a serine/threonine kinase (same as PAR-4, STK11)
MAP2	Microtubule-associated protein-2
MAPK	Mitogen-activated protein kinase
MARCKS	Myristoylated alanine-rich C-kinase substrate
MEF2A	Myocyte enhancer factor 2A
MEGAP	Slit-Robo Rho-GAP (same as SRGAP3)
nBAF	Neuronal BAF
Nedd4-1	Neural precursor cell expressed, developmentally downregulated 4; E3 ubiquitin Ligase

Table 4.1 (continued) Proteins, genes, and related abbreviations used in this chapter

Abbreviation	Definition
Netrin	Secreted tropic cue for axon guidance and cell migration
Neuropilin-1	Receptor for semaphorin
Ngn2	Neurogenin2; bHLH protein
NGF	Nerve growth factor; ligand for Trk receptor tyrosine kinase
Notch	Single-pass transmembrane signaling receptor
npBAF	Neural progenitor BAF
NT-3	Neurotrophin 3; ligand for TrkC receptor tyrosine kinase
NT-4	Neurotrophin 4; ligand for TrkB receptor tyrosine kinase
Nur77	Nuclear receptor transcription factor
PAK1	p21 protein (Cdc42/Rac)-activated serine/threonine kinase 1
PAK3	p21 protein (Cdc42/Rac)-activated serine/threonine kinase 3
Par-3	Partitioning-defective gene 3
Par-6	Partitioning-defective gene 6
Pcdh	Protocadherin; clustered Pcdhs include α (Pcdha), β (Pcdhb), and γ (Pcdhg)
PDE4	Phosphodiesterase E4
PI3 K	Phosphatidylinositol 3-kinase
PKA	Protein kinase A
PKB	Protein kinase B (same as Akt)
PKC	Protein kinase C
PKG	cGMP-dependent protein kinase
PLC	Phospholipase C
PLC γ 1	Phospholipase C γ 1
PLD1	Phospholipase D1
POU	Pit-1, Oct-1/2, Unc-86; domain of DNA-binding transcription factors
PSD95	Postsynaptic density protein 95
Rab5	Rab-related GTPase important for endosome biogenesis
Rac1	Ras-related C3 botulinum toxin substrate 1; Rho family small GTP-binding protein
Rap2	Small GTPase Ras-related protein 2
Rho	Family of GTPases in the Ras gene superfamily
RhoA	Small GTPase, Ras homolog gene family, member A
Robo1	Roundabout 1; receptor for Slit
Robo2	Roundabout 2; receptor for Slit
ROCK	Rho-dependent kinase
Sema3A	Class 3 semaphorin 3A
Sequoia	Zinc-finger, nucleic acid-binding protein
SH3	Src Homology 3 domain

Table 4.1 (continued) Proteins, genes, and related abbreviations used in this chapter

Abbreviation	Definition
Slit1	Ligand for Robo receptor 1
SPAR	Rap-specific GTPase activating protein
Src	Family of non-receptor tyrosine kinases
SRGAP2A	Slit-Robo Rho-GAP 2A
SRGAP2C	Human-specific paralog and truncated form of SRGAP2A
SRGAP3	Slit-Robo Rho-GAP 3 (same as MEGAP)
STK11	Serine/threonine kinase 11 (same as LKB1)
Su(H)	Suppressor-of-hairless
SWI/SNF2	Mating type switch/sucrose non-fermenting nucleosome remodeling complex
TGF- β	Transforming growth factor β
Tiam1	T-lymphoma invasion and metastasis-inducing protein 1; Rac1-GEF
TLN	Telencephalin (same as ICAM5)
TNIK	Traf2 and Nck-interacting kinase
TbR2	Type II receptor for TGF- β
Trc	Tricornered; kinase
TrkB	Neurotrophin receptor tyrosine kinase B
Ube3a	Ubiquitin-protein ligase E3a
UNC-5	Repulsive netrin receptor
UNC-6	Axon guidance protein (<i>C. elegans</i> ortholog of netrins)
UNC-40	Netrin receptor (<i>C. elegans</i> ortholog of DCC)
Wnt	Family of secreted proteins
Wnt7b	Member of Wnt family
Znf312	Zinc-finger protein 312 (same as Fezf2, Fezl, Fez)

cell may avoid fasciculation or crossing and thus maximize the coverage of territory (self-avoidance). In some neurons the growth of dendrites is also arrested at defined borders as a mechanism of preventing redundant coverage (tiling). Finally, most neurons extend small, specialized protrusions, called spines, along the length of the dendrite; these are the sites of major excitatory synapses in the mammalian brain. As we will discuss, these transitions in neuronal morphology are regulated by a complex interplay between intrinsic and extrinsic cellular mechanisms.

Intrinsic versus extrinsic control of dendritic morphology

Is the dendritic morphology of a neuron genetically specified or is it regulated by extracellular signals? The emerging view is that dendrite development is influenced by extracellular signals, but that the response of the neuron to these external cues is likely to depend on genetically specified programs. The evidence that extracellular signals influence dendritic development comes from dissociated cell culture experiments *in vitro* as well as studies that disrupt the cellular environment

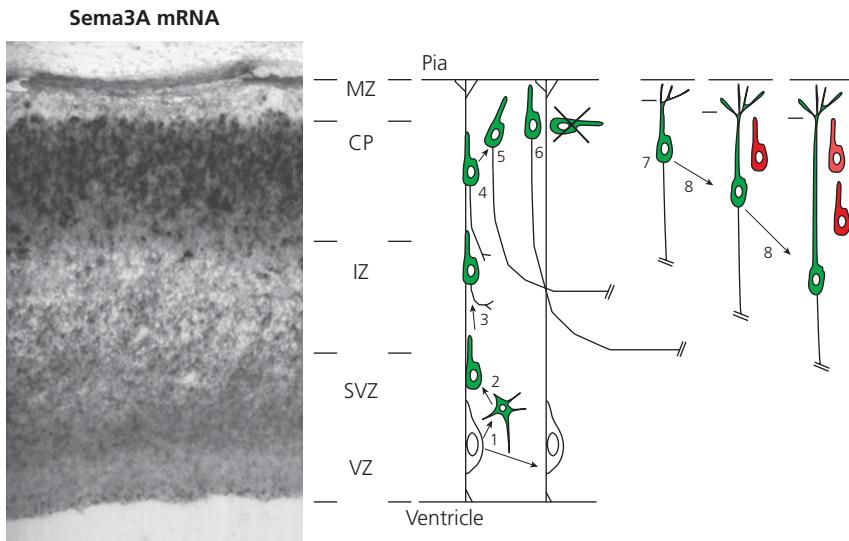
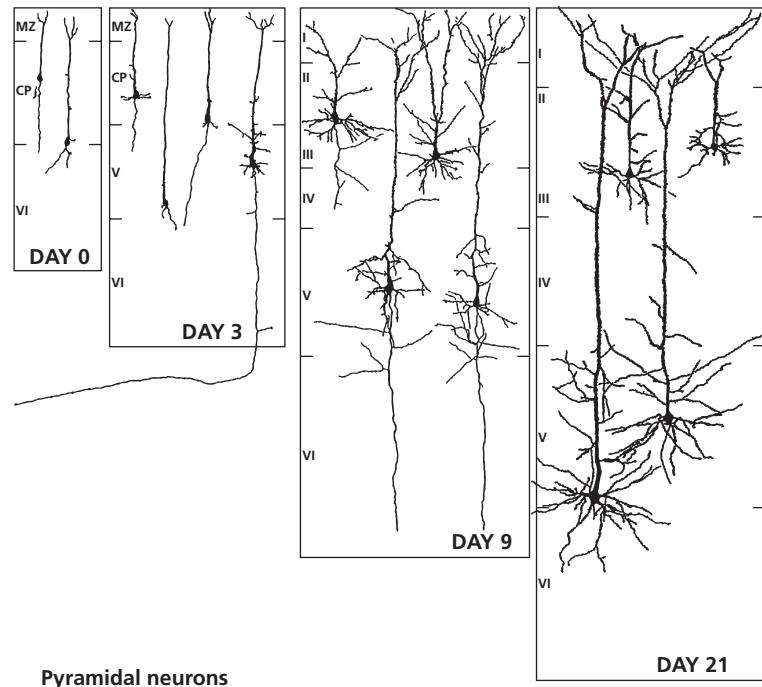


Fig. 4.1 Development of the dendritic morphology of cortical pyramidal neurons. Pyramidal neurons are generated from radial glial precursors in the dorsal telencephalon during embryonic development (see text for details). Upon cell cycle exit from the ventricular zone (VZ), young post-mitotic neurons migrate along the radial glial scaffold and display a polarized morphology with a leading process directed toward the pial surface and sometimes a trailing process directed toward the ventricle. The leading process later becomes the apical dendrite. The trailing process of some neurons (but not all) develops into an axon that grows toward the intermediate zone (IZ; the future white matter) once cells reach the cortical plate (CP). Upon reaching the top of the cortical plate, post-mitotic neurons detach from the radial glial processes and have to maintain their apical dendrite orientation toward the pial surface and axon outgrowth orientation toward the ventricle. This is likely achieved by Sema3A–Neuropilin-1 and Slit1–Robo signaling pathways.

Data from *Nature*, 404(6778), Franck Polleux, Theresa Morrow and Anirvan Ghosh, Semaphorin 3A is a chemoattractant for cortical apical dendrites, pp. 567–573, 2000 and *Neuron*, 33(1), Kristin L. Whitford, Valérie Marillat, Elke Stein, Corey S. Goodman, Marc Tessier-Lavigne, Alain Chédotal, and Anirvan Ghosh, Regulation of cortical dendrite development by Slit–Robo interactions, pp. 47–61, 2002.

of post-mitotic neurons (Pinto Lord and Caviness, 1979; Jensen and Killackey, 1984). These early studies suggested that there were pronounced effects on the dendritic morphology of pyramidal neurons in the cortex when they were ectopically positioned in the white matter. For example, some neurons lost their pyramidal morphology and started to show multipolar morphologies (Pinto Lord and Caviness, 1979; Jensen and Killackey, 1984). Certain features of dendritic morphology, however, seem to be preserved in novel environments. For instance, cerebellar Purkinje neurons maintain some aspects of their dendritic morphology even in the absence of any relevant cell–cell interactions *in vitro* (Baptista et al., 1994; Morrison and Mason, 1998). These neurons display polarized dendritic outgrowths and extend cell type-specific primary branches *in vitro*, but their terminal branches do not develop *in vitro* unless cell–cell contacts with granule cells are restored (Morrison and Mason, 1998).



Pyramidal neurons

Non-Pyramidal neurons

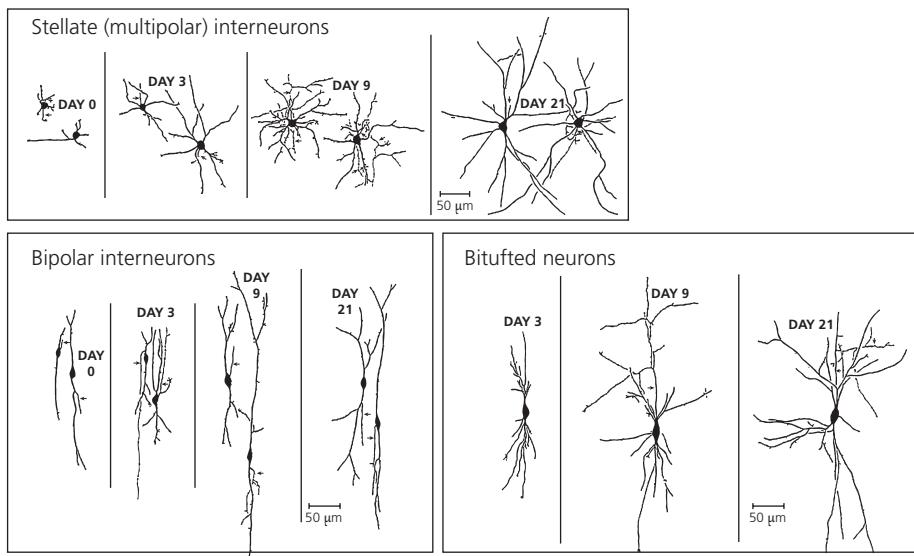


Fig. 4.2 Development of the dendritic morphology of pyramidal (top) and non-pyramidal neurons (bottom) during the postnatal development of the rodent neocortex. Note the extensive level of layer-specific dendritic branching typical of L2/3 and L5 pyramidal neurons. Interestingly, non-pyramidal neurons of distinct classes (multipolar basket cells, bitufted and bipolar interneurons) display characteristic dendritic morphologies from the onset of their differentiation (postnatal day 0).

Adapted from *Journal of Neurocytology*, 10(5), pp. 859–878, Maturation of rat visual cortex. I. A quantitative study of Golgi-impregnated pyramidal neurons, Michael Miller, © 1981, Springer Science and Business Media. With permission from Springer Science and Business Media.

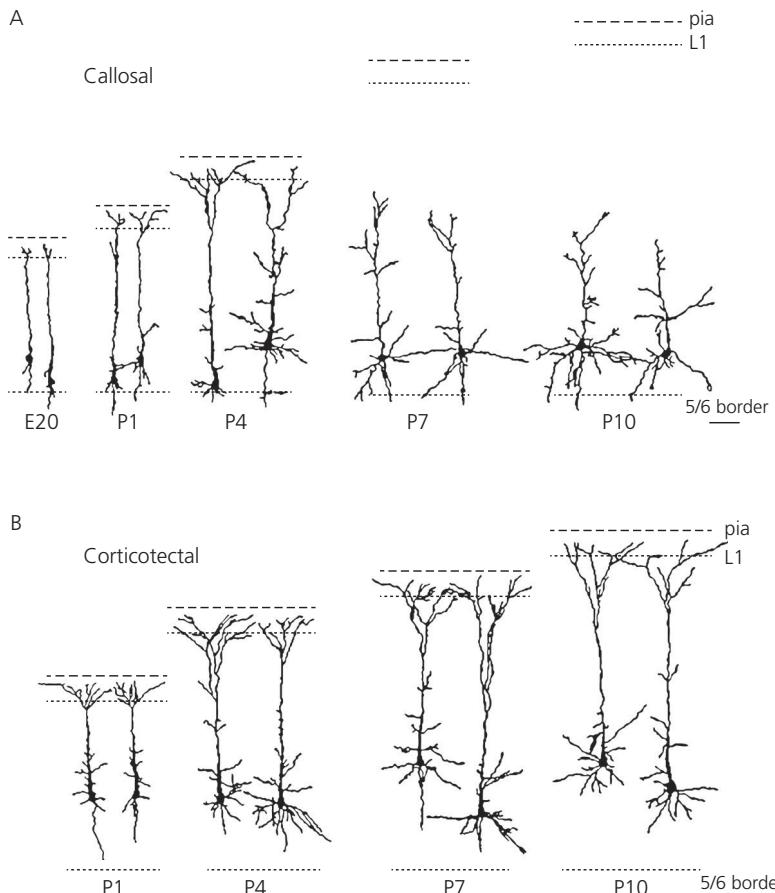


Fig. 4.3 Differential dendritic remodeling of callosal (A) and subcortically projecting (B) neurons. The dendritic morphology of L5 pyramidal neurons was reconstructed from retrogradely labeled neurons projecting to the contralateral hemisphere (callosal neurons) or the tectum (corticotectal neurons) in the rat visual cortex. Initially both classes of pyramidal neurons have an apical dendrite which reaches L1, but during early postnatal development, callosal neurons retract their apical dendrite down to the interface of L3 and L4.

Reproduced from SE Koester and DD O'Leary, Functional classes of cortical projection neurons develop dendritic distinctions by class-specific sculpting of an early common pattern, *The Journal of Neuroscience*, 12(4), pp. 1382–1393, Figure 7, © 1992, The Society for Neuroscience.

Molecular specification of dendritic shape: lessons from *Drosophila*

Invertebrate systems permit molecular exploration of dendrite morphogenesis using unbiased genetic screen approaches. One of the first forward genetic screens aimed at identifying mutations that affect dendrites was performed in *Drosophila* and led to the identification of several genes involved in dendritic development (Gao et al., 1999). Some of the first set of genes that were characterized, including *sequoia*, *flamingo*, and *hamlet*, have been cloned and have provided

important insights into genetic mechanisms of dendrite specification (Gao et al., 2000; Brenman et al., 2001). Later screens probed development at the level of single cells and have identified factors that differentially control dendrite versus axon development (Grueber et al., 2007; Ye et al., 2007) and factors that control branch positioning (Zheng et al., 2008). A recent series of screens done using the highly branched PVD neurons in *Caenorhabditis elegans* have likewise identified numerous genes that are required for the characteristic dendrite morphology of body wall sensory neurons, including genes encoding transcription factors, components of cell adhesion complexes, and guidance factors (Smith et al., 2010; Aguirre-Chen et al., 2011; Dong et al., 2013; Salzberg et al., 2013). Pathways that mediate the characteristic patterning of PVD dendrites are beginning to be delineated.

Transcriptional specification of dendritic shape

Studies of the peripheral nervous system (PNS) of *Drosophila* indicate that dendrite identity is specified by transcription factors that are expressed before and/or after neuronal birth. The gene *hamlet* (*ham*) encodes a multidomain zinc-finger protein that controls the morphological difference between two lineally related neuron types: one with a single-dendrite morphology (that of the external sensory, or “es,” neuron) and the other with a multiple dendrite morphology (the “md” neuron). In the developing nervous system, *ham* expression is limited to the es precursor and newly born es neuron (Moore et al., 2002). If es neuron precursors are made mutant for *ham*, their progeny acquire an md-like arbor. These transformed md neurons also acquire other characteristics of md fate, including the expression of reporters for an md-specific gene; however, when *ham* is driven ectopically in post-mitotic md neurons, branching is reduced, with no obvious change in the abundance of an md-specific reporter (Moore et al., 2002). Thus, *ham* plays a key role in the proper acquisition of cell identity in es precursors, and subsequently influences dendritic morphology in newborn neurons.

The homeodomain-containing protein Cut, which is expressed in many different *Drosophila* tissues, is another important regulator of subtype-specific dendrite morphology in the PNS (Grueber et al., 2003). In an analysis of es organ specification by Cut, Blochlinger et al. (1990) identified variable levels of expression in different md neurons. The DA (dendritic arborization) neurons are the most abundant group of md neurons, and have been subdivided into classes I–IV, in order of increasing dendritic branching complexity and territory size (Grueber et al., 2002). Levels of Cut-immunoreactivity correlate with dendrite morphology, with simpler neurons expressing little or no Cut, the most complex class IV neurons showing intermediate levels of Cut, and class III neurons, with extensive actin-based protrusions along their dendrites, showing the highest levels of Cut (Grueber et al., 2003). Over-expression of Cut in low-level neurons causes dendritic territory to expand and arbors to acquire branching properties that are characteristic of neurons expressing higher levels of Cut. Conversely, higher-level neurons that are made mutant for *cut* acquire simplified dendritic branching morphologies. The Abrupt transcription factor is expressed in the simpler Cut-negative neurons, and inhibits dendrite branching in these cells (Li et al., 2004; Sugimura et al., 2004). The helix-loop-helix (HLH) transcription factor Knot, a member of the COE family, is expressed selectively in class IV neurons and promotes complex growth and branching in collaboration with Cut (Hattori et al., 2007; Jinushi-Nakao et al., 2007; Crozatier and Vincent, 2008). Thus, in this system, dendrite diversity is promoted by subtype-specific levels of transcription factor expression and the combinatorial action of co-expressed transcription factors.

Interestingly, expression of a human Cut homolog also enhances the growth and branching of DA neurons that normally do not express Cut, raising the possibility of conserved roles for Cut-family transcription factors in cell morphogenesis (Grueber et al., 2003).

Dynein motor proteins, Golgi outposts, and dendritic branching

One fundamental distinction between dendrites and axons is the polarity of microtubule networks. In axons, microtubules are arranged with growing plus (+) ends distal, and capped minus (-) ends proximal. By contrast, dendrites show mixed microtubule polarity, such that (-) end distal microtubules mix with (+) end distal microtubules. This difference in microtubule polarity is significant because (+) and (-) end-directed trafficking occurs via distinct mechanisms, with kinesins functioning as (+) end-directed motors and dyneins as (-) end-directed motors. Genetic screens in *Drosophila* identified a major role for motor proteins of the dynein family in dendritic branch positioning (Satoh et al., 2008; Zheng et al., 2008). Normally, dendritic branches are enriched at the distal margins of dendritic fields. However, in the absence of dynein function, branches are enriched at more proximal sites and are largely absent distally. Thus, dynein is required for trafficking dendritic branching machinery toward the tips of arbors and, as a result, the spatial patterning of branch points. Two likely cargoes were identified as Rab5 early endosomes and satellite fragments of the Golgi apparatus, called Golgi outposts. Golgi outposts have conserved roles in dendritic branching in both vertebrate and invertebrate dendrites (Horton et al., 2005; Ye et al., 2007). In *Drosophila* sensory neurons, dendritic Golgi outposts mediate morphogenesis by providing sites for acentrosomal microtubule nucleation, which mediates the addition and extension of dendritic branches (Ori-McKenney et al., 2012).

Control of dendritic self-avoidance

In many parts of the nervous system dendritic trees extend non-overlapping arbors to provide complete, non-redundant coverage of the receptive field. Mutual exclusion of arbors from the same cell is referred to as “self-avoidance” and exclusion of dendritic trees from different neurons is termed “tiling.”

Self-avoidance relies on recognition and repulsion signals communicated between isoneuronal, or sister, dendrites. Dendrite self-recognition in *Drosophila* requires homophilic recognition molecules encoded by the *Dscam1* locus (Fig. 4.4), which can generate over 38,000 distinct isoforms (over 19,000 distinct homophilic recognition molecules), through extensive alternative splicing of four exon cassettes (Schmucker et al., 2000). Three of these exon cassettes encode extracellular immunoglobulin domains that mediate homophilic interactions (Wojtowicz et al., 2007). Neurons express multiple *Dscam1* isoforms in a probabilistic fashion, supporting the notion that each neuron could have a unique surface recognition identity contributed by the particular *Dscam1* isoform repertoire that is expressed (Neves and Chess, 2004; Zhan et al., 2004; Miura et al., 2013). Consistent with this model, null mutations in *Dscam1* cause cell-autonomous crossing and bundling of dendrites (Zhu et al., 2006a; Hughes et al., 2007; Matthews et al., 2007; Soba et al., 2007). This phenotype can be rescued by providing a single arbitrary isoform to mutant neurons, indicating that molecular diversity of *Dscam1* is not essential for self-avoidance. By contrast, if cells of different types (whose dendrites normally overlap) are forced to express the same *Dscam1* isoform their dendrites segregate as though they recognize different cells as “self.” Thousands of *Dscam1* isoforms are normally required *in vivo* to prevent aberrant non-self recognition between dendrites (Hattori et al., 2009). Thus, *Dscam1* diversity endows different cells with distinct recognition identities enabling dendrites to avoid sister dendrites but co-exist with dendrites of different cells. Self-avoidance may be particularly important as a counter to attractive guidance cues (discussed later) and cues that promote self-fasciculation to ensure that complex dendrites spread evenly over their territory without clumping (Fuerst et al., 2009; Matthews and Grueber, 2011).

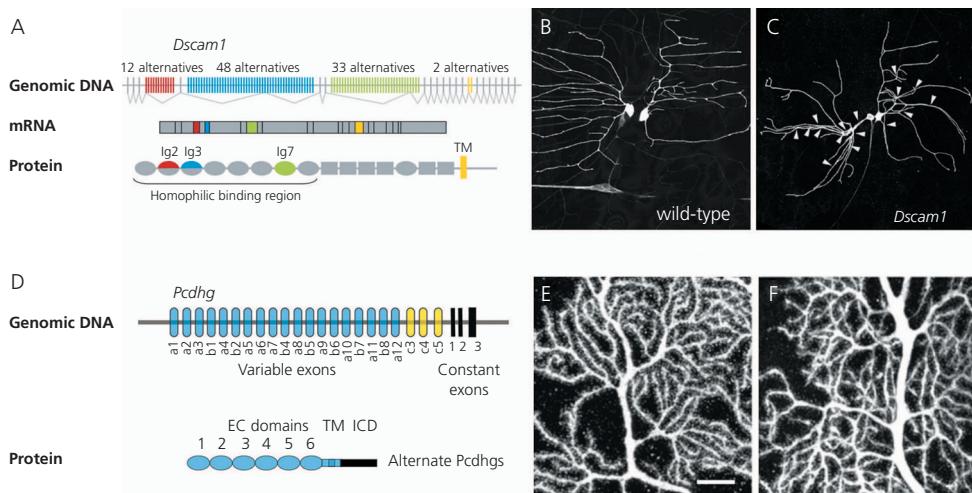


Fig. 4.4 Control of self-avoidance by Dscam1 and clustered protocadherins. **(A)** The Dscam1 locus generates 38,000 distinct isoforms that differ in their extracellular and transmembrane domains. Alternative splicing of three exon cassettes generates extensive diversity in the homophilic binding region of the extracellular domain. **(B)** Wild-type dendritic arborization neurons showing self-avoidance of branches. **(C)** In Dscam1 mutant animals, sister dendrites show fasciculation and overlap, indicating defective self-recognition and repulsion. **(D)** The gamma protocadherin (Pcdhg) locus (alpha and beta clusters not shown) has 19 variable and 3 C-type exons, which are individually spliced to three constant exons that make up the cytoplasmic tail. **(E)** Control Purkinje cell showing self-avoidance among branches. **(F)** Purkinje cell lacking Pcdhg shows extensive self-crossing of dendrites.

Part A reproduced with permission of Annual Reviews: *Annual Review of Cell and Developmental Biology*, 24(1), Daisuke Hattori, S. Sean Millard, Woj M. Wojtowicz, and S. Lawrence Zipursky, Dscam-mediated cell recognition regulates neural circuit formation, pp. 597–620, Figure 2, © 2008, Annual Reviews, <http://www.annualreviews.org>. Parts B and C reprinted from *Cell*, 129(3), Benjamin J. Matthews, Michelle E. Kim, John J. Flanagan, Daisuke Hattori, James C. Clemens, S. Lawrence Zipursky, and Wesley B. Grueber, Dendrite Self-Avoidance Is Controlled by Dscam, pp. 593–604 Figures 1e and f, Copyright 2007, Elsevier. With permission from Elsevier. Part D reprinted from *Cell*, 158(5), Chan Aye Thu, Weisheng V. Chen, Rotem Rubinstein, Maxime Chevée, Holly N. Wolcott, Klara O. Felsovalyi, Juan Carlos Tapia, Lawrence Shapiro, Barry Honig, and Tom Maniatis Single-cell identity generated by combinatorial homophilic interactions between α , β , and γ protocadherins, pp. 1045–59, Figure 1a and b, Copyright 2014, Elsevier. With permission from Elsevier. Parts E and F adapted with permission from Macmillan Publishers Ltd: *Nature*, 488(7412), Julie L. Lefebvre, Dimitar Kostadinov, Weisheng V. Chen, Tom Maniatis, Joshua R. Sanes, Protocadherins mediate dendritic self-avoidance in the mammalian nervous system, pp. 517–521, Copyright 2012, Nature Publishing Group.

Studies of PVD sensory neurons have revealed a role for Netrin/UNC-6, UNC-5, and DCC/UNC-40 in self-avoidance. In these cells, UNC-40 binds and presents UNC-6 to the UNC-5 receptor, which mediates repulsion between branches (Smith et al., 2012).

Control of dendritic tiling

Neurons of a particular functional or morphological type, such as retinal ganglion cell (RGC) and amacrine cell subtypes (Wassle et al., 1981; MacNeil and Masland, 1998) and insect dendritic (da)

neurons, are often arranged in an even-spaced mosaic across a receptive territory (reviewed in Jan and Jan, 2003; Grueber and Jan, 2004). Dendrites of neighboring neurons fill the area between cell bodies and therefore overlap minimally with each other, in a process called tiling. At least two parallel processes are important for tiling in DA neurons: a pathway for direct dendrite–dendrite repulsion and a pathway for substrate attachment that restricts arbors to a two-dimensional (2D) plane. Direct dendrite–dendrite repulsion is indicated by results from laser ablation studies in which neighboring arbors grow to fill in territories vacated by the ablation (Grueber et al., 2003; Sugimura et al., 2004). The molecular mechanisms of recognition and repulsion that underlie dendritic tiling are still poorly understood. The first mutants to be discovered that affect tiling in DA neurons were two evolutionarily conserved proteins, Trc kinase and Fry (Emoto et al., 2004). Although the mechanism of action of Trc and Fry is not yet clear, recent studies indicate interactions with pathways that promote 2D positioning of dendrites (Han et al., 2012). Sensory dendrites may either elaborate across the basal surface of the epidermis or become embedded within epidermal cell invaginations in a 3D arrangement (Han et al., 2012; Kim et al., 2012b). Interactions between dendrites and the extracellular matrix (ECM) through integrin receptors are essential for 2D restriction (Han et al., 2012; Kim et al., 2012b). In the absence of integrins, dendrites show excessive non-contacting crossing in three dimensions, whereas overexpression of integrins forces dendrites to a predominately 2D plane. Notably, dendritic overlap phenotypes can be rescued if *trc* mutant neurons are forced to express integrins, arguing that repulsion is intact, but 2D restriction is compromised by loss of Trc (Han et al., 2012).

Control of dendritic targeting

Dendrites often extend to specific target areas for proper wiring of neural circuits. Dendritic targeting is an active process, and studies in *Drosophila* have identified some of the principles and molecules that are responsible. One remarkable example of dendritic targeting specificity is observed in the *Drosophila* olfactory system. Studies over the last decade have identified a plethora of cues that interact to achieve precise wiring patterns in this system. The first highly stereotypic relay in the olfactory system occurs at the antennal lobe, where between 150 and 200 identified projection neurons (PNs) arising from three major lineages project dendrites to approximately 50 defined glomeruli. Precise dendritic targeting is essential as it determines the input that each PN receives from innervating primary olfactory receptor neurons (ORNs). The PN dendritic map is specified by at least four major mechanisms: lineage, birth order, responses to guidance cue gradients, and local interactions between processes.

Lineage-specific targeting of PN dendrites is under the control of combinatorial action of transcriptional regulators, including Cut, the POU-domain proteins Drifter and Acj6, and the LIM homeodomain transcription factors Islet and Lim1 (Komiyama et al., 2003; Komiyama and Luo, 2007). These factors control both global targeting to different regions of the antennal lobe and local targeting to defined glomeruli within these regions. Birth order also influences targeting of at least a subset of PNs via the BTB zinc-finger protein Chinmo. Levels of Chinmo are highest early in the lineage, when it functions in early born neurons to prevent adoption of projection patterns characteristic of later-born PNs (Zhu et al., 2006b). Opposing gradients of guidance cues present in the antennal lobe also direct dendrite targeting. Initial studies by the Luo group identified a gradient of the transmembrane semaphorin Sema-1a expressed along a defined axis of the antennal lobe, and showed that PN dendrites were the source of this gradient (Komiyama et al., 2007). Loss of Sema-1a from PNs caused a shift in dendrite positioning toward regions of lower Sema-1a. Given that this effect of Sema-1a was cell autonomous, the molecule appears to be functioning as a receptor.

More recently, gradients of two secreted semaphorins, Sema-2a and Sema-2b, were shown to oppose the Sema-1a gradient and repel PNs expressing high levels of Sema-1a (Sweeney et al., 2011). Finally, local dendro-dendritic interactions mediated by cadherins maintain dendritic coherence within a single glomerulus and prevent spillover to neighboring glomeruli (Zhu and Luo, 2004).

In addition to semaphorins, targeting of dendritic trees is influenced by several other chemo-tropic cues that also guide axons. Several examples of this general concept have been described in *Drosophila*. Chiba and colleagues analyzed the role of Netrin–Frazzled signaling in axonal and dendritic development (Furrer et al., 2003). Netrin-A and Netrin-B, two netrin-family proteins in *Drosophila*, are diffusible glycoproteins produced by specialized midline cells. The activation of Frazzled (known in vertebrates as deleted in colorectal cancer, DCC), a cell-surface receptor for netrins, causes chemoattraction and midline crossing of axons from neurons located near the midline (Yu and Bargmann, 2001; Huber et al., 2003). Motoneuron dendrites require Frazzled for guidance decisions at the midline (Furrer et al., 2003). For example, the RP3 motoneuron normally extends axons across the midline and elaborates dendrites on both sides the midline. However, in both *frazzled*-null and *netrinA/netrinB* double-null mutants, the RP3 neuron fails to direct its axon or dendrite toward the midline in a majority of the cases examined, suggesting that the midline-directed outgrowth of the RP3 axon and dendrite requires Netrin–Frazzled signaling (Furrer et al., 2003). Roles for chemotropic cues in dendrite targeting have also been analyzed in the context of motor circuit assembly and targeting of DA neurons (Brierley et al., 2009; Mauss et al., 2009; Matthews and Grueber, 2011).

Control of dendritic scaling

As dendritic arbors mature they must develop in synchrony with changes occurring in their immediate environment. A compelling example is the phenomenon of dendritic scaling, in which arbor growth is coordinated with substrate growth to maintain full coverage. The molecular basis of dendritic scaling is only beginning to be investigated. The body size of larval *Drosophila* expands by approximately three-fold between 48 and 120 hours after egg laying, yet class IV arbors maintain complete tiling of the body wall throughout this period, suggesting a mechanism to regulate scaling growth. Mutant and gene expression analysis identified an important role for epidermal expression of the microRNA bantam in restricting dendrite growth. Bantam regulates dendrite scaling by restricting PKB (Akt) expression in overlying sensory neurons (Parrish et al., 2009) and by modulating epidermal–substrate adhesion (Jiang et al., 2014).

Molecular control of dendritic development in vertebrates

Regulation of dendrite orientation

Most cortical neurons are generated from precursors proliferating in the germinal zones lining the ventricle. Dendritic differentiation, as determined by expression of dendrite-specific genes such as *MAP2*, does not begin until the cells have completed their migration. During radial migration, pyramidal neurons extend a trailing process toward the ventricle and a leading process toward the pial surface (Noctor et al., 2001; Barnes et al., 2007; Barnes and Polleux, 2009). The leading process will become the apical dendrite whereas the trailing process extends rapidly and becomes the axon (reviewed in Barnes and Polleux, 2009). Several extracellular cues have been identified to play a crucial role during neuronal polarization. To test the role of the local extracellular cues present in the cortical microenvironment in directing the growth of nascent axons and dendrites, Polleux et al. (1998) developed an *in vitro* assay in which dissociated neurons from a donor cortex

were labeled and plated onto cortical slices in organotypic cultures. Just 2–3 hours after plating, the vast majority of neurons extended an axon directed toward the ventricle. This demonstrated the existence of extracellular cues that were sufficient to instruct the direction of axon outgrowth during neuron specification (Polleux et al., 1998). Several factors can induce axon outgrowth, including brain-derived neurotrophic factor (BDNF) and insulin-like growth factor 1 (IGF-1), but in the absence of these factors, neurons are still capable of extending axons, indicating that other factors are involved *in vivo* (Barnes and Polleux, 2009). Recently, transforming growth factor beta (TGF- β ; acting through its type II receptor, TbR2), was shown to be required for axon specification *in vitro* and *in vivo*. TbR2 functions by phosphorylating the conserved polarity protein, Par6, at a critical serine residue (S345) and recruiting an E3 ubiquitin ligase called Smurf1 which promotes local degradation of RhoA (Yi et al., 2010). Notably, Par6 was previously demonstrated to promote axon specification (Shi et al., 2003).

The development of the dendrite does not appear to simply be a default state in the absence of cues for axon initiation. The chemotropic signal semaphorin 3A (Sema3A) can function to polarize cultured hippocampal neurons by promoting dendrite formation and growth and suppressing axon formation and extension (Shelly et al., 2011). These effects are mediated by an opposing effect of Sema3A on cyclic nucleotide signaling, increasing cGMP levels (Polleux et al., 2000) and reducing cAMP and protein kinase A (PKA) activity via PKG-mediated activation of the phosphodiesterase PDE4. PKA activity is required for a key phosphorylation event on the STK11 (LKB1) protein, which is necessary and sufficient for axon formation (Barnes et al., 2007; Shelly et al., 2007). In this way, a single signal has opposite effects on axon and dendrite initiation and ensures their patterned growth in opposite directions within the cortex.

The non-receptor tyrosine kinases Fyn and Cyclin-dependent kinase 5 (Cdk5) also play important roles in mediating the effects of Sema3A on the orientation of cortical dendrites (Sasaki et al., 2002). Fyn is a member of the Src family of non-receptor tyrosine kinases. Cdk5 is a member of the serine/threonine kinase Cdk family, and has enzymatic activity only in post-mitotic neurons due to a neuron-specific expression of the regulatory subunit p35 (Lew and Wang, 1995). Cdk5 and p35 play critical roles in the laminar organization of the cerebral cortex by regulating neuronal migration (Ohshima et al., 1996; Chae et al., 1997). Interestingly, the orientation of apical dendrites of L5 and L2/3 pyramidal neurons is not different from wild-type controls in Sema3A(+/−) and Fyn(+/−) single heterozygous mice but is significantly impaired in Sema3A(+/−)/Fyn(+/−) double heterozygous mice (Sasaki et al., 2002). These results provide genetic evidence that Fyn acts downstream of Sema3A to regulate the orientation and branching of the apical dendrite in pyramidal cortical neurons.

Regulation of dendritic growth and branching

Transcriptional regulation

Transcriptional regulation strongly influences dendrite development in mammalian neurons, from determining the timing, direction, and extent of outgrowth, to transducing activity-dependent signals, often in a cell-type-specific manner (Polleux et al., 2007; de la Torre-Ubieta and Bonni, 2011). One problem that must be solved at the earliest stages of dendritic outgrowth is to coordinate migration and dendritic outgrowth such that the two processes occur in temporal succession rather than concurrently. Very early in dendritic development the switch between migration and outgrowth of dendritic processes in GABAergic interneurons is mediated by the Dlx1/2 transcription factors. Repression of p21 protein-activated kinase 3 (PAK3) by Dlx1/2 promotes migration and represses premature process outgrowth. Loss of Dlx1/2 disrupted laminar positioning in the

cortex and caused increased axonal and dendritic outgrowth (Cobos et al., 2007). The basic helix-loop-helix (bHLH) protein Neurogenin2 (Ngn2) also coordinates morphogenesis and migration, but in quite a different manner. Neurons generated by asymmetric divisions of radial glial cells in the ventricular and subventricular zones initially have a multipolar morphology, but the cells must transition to a unipolar morphology prior to radial migration. This transition is mediated by Ngn2, which, when phosphorylated appears to interact with a transcriptional co-regulator to effect the expression of a suite of genes that promote unipolar dendritic morphology and radial migration, including signaling via regulators of the small GTPase RhoA (Hand et al., 2005; Ge et al., 2006; Heng et al., 2008). Transcriptional regulation may provide a common strategy for temporal coordination of successive events during morphogenesis.

Transcription factors can also regulate cell-specific postsynaptic structures. Granule neurons in the cerebellar cortex develop unique postsynaptic structures, termed dendritic claws, to receive input from mossy fiber terminals and Golgi cells. As the name suggests, claws form a cuplike structure at the end of dendrites. Shalizi et al. (2006) discovered a role for the transcription factor myocyte enhancer factor 2A (MEF2A) in the morphogenesis of dendritic claws. Knockdown of MEF2A by short hairpin RNA (shRNA) caused a strong reduction in the number of claws but not the total number of dendrites, indicating a selective role in claw morphogenesis. The function of MEF2A is linked to neuronal activity-induced dephosphorylation of a critical inhibitory serine residue via calcineurin. Activity-dependent phosphorylation promotes a switch from sumoylation (a transcriptional repressor form that promotes claw formation) to acetylation of MEF2A. The action of MEF2A on the formation of dendritic claws appears to be mediated by another transcription factor Nur77 that must be maintained in a repressed state for claw formation to proceed normally (Shalizi et al., 2006).

As in *Drosophila*, transcriptional regulation underlies cell-type-specific diversity in dendritic morphology in vertebrates. Different cortical layers show distinctions in both the dendrite morphology and axonal projections of pyramidal neurons, and these differences may arise in part due to differential transcriptional regulation. Murine Cut homologs Cux1 and Cux2 are expressed selectively in cortical layers II–III and were recently shown to promote dendrite morphogenesis and spine development (Cubelos et al., 2010). Cux1 and Cux2 function appears to be non-redundant, with Cux1 primarily impacting basal dendrites and Cux2 influencing apical dendrites (Cubelos et al., 2015). These data indicate that Cut/Cux family transcription factors have broadly evolutionarily conserved roles in dendrite morphogenesis. The zinc-finger transcription factor Fezf2, also called Zinc-finger protein 312 (Znf312), or Fezl, is expressed in subcortical projection neurons of L5 and L6. Reduction of Fezf2 levels disrupted subcortical axon projections, stunted basal dendritic elaboration, and disrupted apical dendrite orientation. Upper layer cortical neurons develop normally without Fezf2, but ectopic expression in these cells is sufficient to induce subcortical axonal projections (Chen et al., 2005a,b). These studies indicate a significant post-mitotic role for layer-specific transcriptional regulation in building cortical dendritic architecture.

Transcriptional control of dendritic morphogenesis is also mediated at the level of chromatin accessibility. DNA is condensed and packaged, together with proteins such as histones, into compact chromatin. Numerous molecular complexes, collectively referred to as chromatin remodeling complexes, are capable of altering the accessibility of chromatin to transcription factors, and in this way can broadly affect gene transcription. SWI/SNF2-like ATPases use the energy of ATP hydrolysis to control nucleosome–DNA interactions. Brg and Brm are two SWI/SNF2 subunits that comprise part of a large ten-subunit Brg/Brm associated factor (BAF) chromatin remodeling complex. Remarkably, the composition of the BAF complex undergoes a specific subunit switch during neuronal development from a neural progenitor BAF (npBAF) to a neuronal BAF (nBAF)

(Wu et al., 2007). npBAF subunits enhance proliferation of neural progenitors and the nBAF subunit exchange is critical for proper neural differentiation, since artificially extending the expression of npBAF subunits impairs differentiation of specific cell classes in the chick neural tube (Wu et al., 2007). Additional analysis of the role of nBAFs identified a critical requirement for the nBAF subunit BAF53b in activity-dependent dendritic growth (Wu et al., 2007). To explore the mechanistic basis for this function, Wu and colleagues took a proteomics approach and found that nBAF forms a complex with the Ca^{2+} -responsive transcriptional co-activator CREST, previously identified as important for activity-dependent dendritic growth (Aizawa et al., 2004; Wu et al., 2007). BAF53b was found to recruit nBAF and CREST to promoters of target genes that promote outgrowth of neurite processes, including numerous regulators of Rho-family GTPases (Wu et al., 2007). These studies underscore the emerging role for chromatin modifications in the control of dendrite morphogenesis.

Neurotrophins

Neurotrophic factors play an important role in regulating dendritic growth and branching in cortical neurons. Neurotrophins (NGF, BDNF, NT-3, and NT-4) exert their effects through the Trk family of receptor tyrosine kinases. Experiments in which the effects of neurotrophins on control of dendritic growth have been examined in slice cultures indicate that, in general, neurotrophins increase the dendritic complexity of pyramidal neurons by increasing total dendrite length, the number of branch points, and/or the number of primary dendrites (McAllister et al., 1995; Baker et al., 1998; Niblock et al., 2000). The response is rapid, and an increase in dendritic complexity is readily apparent within 24 hours of exposure to neurotrophin. There is a clear specificity in the short-term response of the pyramidal neurons of different cortical layers to each of the neurotrophins. For instance, NT-3 strongly increases dendritic complexity in layer-4 (L4) neurons but has no apparent effect on L5 neurons. In addition, basal dendrites in specific layers respond most strongly to single neurotrophins, whereas apical dendritic growth is increased by a wider array of neurotrophins. Live imaging of L2/3 neurons expressing BDNF shows a high level of dendrite dynamics. Both dendritic branches and spines are rapidly lost and gained in BDNF-transfected neurons (McAllister et al., 1995; Baker et al., 1998; Niblock et al., 2000). BDNF overexpression favors the addition of primary dendrites and proximal branches at the expense of more distal segments. Similarly, overexpression of TrkB in L6 pyramidal neurons results in a predominance of short proximal basal dendrites (Yacoubian and Lo, 2000). The effects of BDNF on primary dendrite formation are mediated by MAPK and PI3 K signaling (Dijkhuizen and Ghosh, 2005).

Notch signaling

The diversity of signals that can influence dendritic morphology is underscored by a series of studies on the role of mammalian Notch proteins in regulating dendritic growth and branching. Originally cloned in *Drosophila* and *C. elegans*, Notch is a type-I cell-surface protein that functions as a receptor. Proteolytic processing of full-length Notch generates two fragments that associate at the plasma membrane to form a receptor complex. The mechanism of activation of Notch receptors involves cleavage and nuclear translocation of the intracellular domain of the receptor (reviewed in Weinmaster, 2000). The intracellular domain of Notch enters the nucleus and binds the transcription factor Suppressor of Hairless (Su(H)), activating gene transcription. The possibility that Notch might play a role in regulating dendritic patterning was suggested by immunohistochemical localization studies which showed that mammalian Notch1 is expressed by dividing cells in the ventricular zone (VZ) and by post-mitotic neurons in the cortical plate (CP) (Sestan et al., 1999; Redmond et al., 2000). Several observations suggest that Notch inhibits neurite outgrowth

(Berezovska et al., 1999; Franklin et al., 1999; Sestan et al., 1999). In addition to restricting length, Notch signaling in cortical neurons was shown to promote dendritic branching (Redmond et al., 2000). Taken together these experiments reveal a positive role for Notch in dendrite branching and a negative effect on dendrite and total neurite length.

Slit/Robo family signaling

In a search for other extracellular cues that regulate dendrite development, Whitford et al. (2002) discovered that Slit proteins simultaneously repel pyramidal neuron axons and stimulate dendrite growth and branching. The Slits are a well-studied family of multifunctional guidance cues which have been shown to repel axons and migrating cells as well as promoting elongation and branching of developing sensory axons (reviewed in Huber et al., 2003). Generally, Slits exert their effects through binding to specific members of the Roundabout, or Robo, family of receptors (Huber et al., 2003). Whitford et al. (2002) demonstrated that one of the three vertebrate Slits, Slit1, and two of the three Robo receptors, Robo1 and Robo2, are expressed in the developing cortex during the time of initial differentiation of axons and dendrites and demonstrated that Slit1 is a chemorepellant for cortical axons. Interestingly, in addition to repelling cortical axons, Slit1 also potently increases dendritic growth and branching of both pyramidal and non-pyramidal cortical neurons, paralleling a similar role for Slit proteins in the regulation of axonal branching. These effects of Slit1 are mediated by the Robo1 and Robo2 receptors, since transfection of neurons with dominant-negative forms of these receptors in dissociated cultures and slices decreases dendritic branching. Slit and Robo likewise promote branching of *Drosophila* sensory neurons (Dimitrova et al., 2008).

Recent studies have shown that the Robo family member Robo2 and the secreted molecule Slit2 mediate dendritic self-avoidance in Purkinje cells, underscoring the diverse roles for these families in dendritic field formation (Gibson et al., 2014). Both Robo2 and Slit2 are expressed in Purkinje cells, but Robo2 is dispensable for several aspects of dendritic morphology, including branching, branch extension, and dendritic field size. By contrast, deletion of Robo2 or Slit2 leads to excessive overlap of sister dendrites, indicating defective self-recognition or repulsion. Notably, Slit2 acts cell autonomously in Purkinje cells and experiments using co-cultures of cerebella and Slit1/2-expressing COS cells revealed that Slits elicit a repulsive response in Purkinje cell dendrites. Together, these results suggest an autocrine role for Slits in generating dendritic self-repulsion via the Robo2 receptor.

Wnt signaling

Another illustration of the ability of individual signals to control diverse biological responses, including the control of axonal and dendritic development, is provided by the Wnt family of secreted proteins. The Wnts represent a large family of extracellular cues initially identified as potent morphogens involved in patterning organ development in both invertebrates and vertebrates. Wnts have also been shown to regulate cell proliferation, migration, and survival, and the neuronal cytoskeleton (Ciani and Salinas, 2005). Wnt proteins can function as axon-guidance molecules and as target-derived signals that regulate axonal remodeling and synapse formation (Hall et al., 2000; Krylova et al., 2002). Wnt proteins signal through at least three different pathways. The binding of Wnt proteins to Frizzled receptors results in the activation of the scaffolding protein D旌shevelled (Dsh or Dvl). In the so-called “canonical pathway,” Wnt proteins signal through Dsh to inhibit GSK3- β a serine/threonine kinase. Inhibition of GSK3- β , in turn, activates β -catenin T-cell-specific transcription factor-mediated transcription. Wnt proteins can also signal through Dsh to regulate Rho GTPases during convergent extension movements and tissue polarity during

early development. Finally, Wnt proteins can activate a Ca^{2+} -dependent pathway, again through Dsh. Wnt proteins induce axonal remodeling through the activation of Dvl and the subsequent inhibition of GSK3- β (Hall et al., 2000). Dsh has been shown to act locally to regulate microtubule stability by inhibiting a pool of GSK3- β through a β -catenin- and transcriptional-independent pathway (Ciani et al., 2004).

Wnt proteins have been implicated in the control of dendritic arborization of hippocampal neurons during development (Rosso et al., 2005). *Wnt7b* is expressed in the mouse hippocampus and induces dendritic arborization of hippocampal neurons during development. This effect is mimicked by the expression of Dsh. Importantly, analyses of the *Dvl1* mutant mouse revealed that Dvl1 is crucial for dendrite development, but acts in a non-canonical pathway (Rosso et al., 2005). In this case Wnt7B and Dvl signal through the small GTPase Rac (Rosso et al., 2005). In the same study, the authors also reported that Wnt7B and Dsh activate Jnk kinase, a downstream effector of Rac. It remains to be determined whether Jnk acts downstream of Rac, or whether the Wnt-Dsh pathway regulates Rac and Jnk independently. The findings reported by Rosso et al. (2005) demonstrate that Dsh functions as a link between extracellular Wnt factors and Rho GTPases in dendrites, and also reveal a novel role for Jnk in dendrite development.

The cadherin superfamily and β -catenin

One of the central challenges in the study of dendritic development is to understand how extracellular cues that regulate dendritic branching are integrated with Ca^{2+} activity-dependent signals. A potential clue comes from results exploring the role of β -catenin and cadherins in dendritic branching. Overexpression of β -catenin (and other members of the cadherin/catenin complex) enhances dendritic arborization, whereas sequestering endogenous β -catenin causes a decrease in dendritic branching (Yu and Malenka, 2003). Importantly, blocking β -catenin prevents the enhancement of dendritic morphogenesis caused by neuronal depolarization. Release of secreted Wnt, which occurs during normal neuronal development, is enhanced by manipulations that mimic increased activity, and Wnts contribute to the effects of neural activity on dendritic arborization. These results demonstrate that β -catenin is an important mediator of dendritic morphogenesis and that Wnt/ β -catenin signaling is likely to be important during critical stages of dendritic development (Yu and Malenka, 2003).

The cadherin superfamily consists of two subfamilies—the classical cadherins and protocadherins. Classical cadherins consist of five extracellular cadherin repeats EC1–EC5, a transmembrane region, and a characteristic intracellular domain that interacts with catenin family members. Protocadherins show a longer chain of extracellular cadherin repeats, have variable intracellular domains (Suzuki, 1996), and do not signal through catenins. The atypical seven-transmembrane cadherins (*Celsr1–3*) regulate dendritic branching of cortical pyramidal neurons (Shima et al., 2004). These atypical cadherins have been identified in vertebrates as orthologs of *flamingo*, a gene previously implicated in the control of dendritic development in *Drosophila* (Gao et al., 2000). Shima et al. (2004) combined loss-of-function techniques including RNAi-mediated gene silencing in single neurons (using biolistic-delivery in P8 organotypic slice cultures) to demonstrate that knocking-down *Celsr2* expression in both L5 pyramidal neurons and Purkinje cerebellar neurons significantly reduces dendritic branching (Shima et al., 2004). By contrast, *Celsr3* knockdown causes increased dendritic branching, revealing opposing effects for these two closely related cadherins (Shima et al., 2007). The different effects of *Celsr2* and *Celsr3* on dendrite elaboration were traced to a single amino acid difference in the intracellular domain, which may influence how effectively these molecules promote second messenger cascades (Shima et al., 2007). These results indicate that cadherins play an important role in the control of dendritic complexity.

The protocadherins comprise the largest subfamily of the cadherin superfamily of cell adhesion molecules and one particularly notable type of protocadherin, the clustered protocadherins (Pcdhs), have recently been implicated in diverse aspects of dendritic morphogenesis. The clustered Pcdhs are organized in three clusters, termed α (Pcdha, 14 isoforms), β (Pcdhb, 22 isoforms), and γ (Pcdhg, 22 isoforms). Isoform diversity is generated by alternative promoter choice, with variable extracellular domains spliced to a common cytoplasmic tail (an exception is the encoding of entire Pcdhs by single exons in the Pcdhb cluster). Multiple Pcdhs are expressed in individual cells in a stochastic fashion and show isoform-specific homophilic binding (Fig. 4.4). These properties of the clustered Pcdhs bear a striking resemblance to the molecular diversity generated at the Dscam1 locus of *Drosophila*, which functions in dendritic self-avoidance. Indeed, Lefebvre and colleagues recently showed that Pcdhg controls self-avoidance in mammalian starburst amacrine cells (SACs) and cerebellar Purkinje cells (Lefebvre et al., 2012). Pcdhg knock-outs cause SAC and Purkinje cell dendrites to show extensive self-crossing and bundling (Fig. 4.4). These defects can be rescued by a single isoform, and expression of a single Pcdhg isoform in multiple neighboring cells limits co-existence of dendrites. These studies reveal a striking case of convergence in insects and mammals in which different genomic loci (Dscam1 and the Pcdhs, respectively) contribute to self-avoidance via a similar underlying mechanism involving extensive molecular diversity, non-deterministic isoform expression, and homophilic interactions that lead to repulsive signaling (Zipursky and Grueber, 2013).

Pcdhs appear to have diverse roles in dendritic development in other regions of the vertebrate nervous system. Garrett and colleagues used a conditional Pcdhg knock-out to examine roles in the cerebral cortex. Mutant cortices showed a reduction in L1 thickness but no significant reduction in cell number (Garrett et al., 2012). Given that L1 comprises primarily apical dendrite tufts of deep-layer neurons, they investigated the role of Pcdhg in dendrite arborization. Indeed, cortical dendrites show reduced complexity, lacking Pcdhgs, and these defects arose from disruptions in branch elaboration rather than maintenance. Signaling pathways downstream of Pcdhs are poorly understood. By looking for alterations in the phosphorylation status of several candidate signaling pathways in Pcdhg mutant cortex, the authors identified increases in phosphorylated MARCKS, a substrate for protein kinase C (PKC) and a known regulator of dendrite branching (Li et al., 2008). Notably, activity of PKC was also increased in mutant tissue, as was activity of a brain isoform of phospholipase C, PLC γ 1, an effect likely mediated via interaction between Pcdhg and focal adhesion kinase (FAK). Consistent with a role for this pathway in dendrite development, pharmacological inhibition of PKC and FAK rescued dendrite phenotypes caused by Pcdhg mutation, as did transfection of neurons with either wild-type or non-phosphorylatable, but not pseudophosphorylated, MARCKS. Together these data pinpoint a role for Pcdhg in inhibition of FAK and reduction in PLC, which maintains MARCKS in a non-phosphorylated state in association with actin and the plasma membrane to promote dendrite complexity (Garrett et al., 2012).

Ubiquitin-proteasome system

Protein ubiquitination is critical for a number of developmental processes. Ubiquitination is promoted by sequential steps involving E1 ubiquitin-activating enzymes, E2 ubiquitin-conjugating enzymes, and the E3 ubiquitin ligases, which determine substrate specificity (Puram and Bonni, 2011). This series of interactions lead to protein degradation or functional modification. Several E3 ligases have been implicated in dendritic morphogenesis in vertebrates, including the anaphase-promoting complex (APC), the neuronal precursor cell expressed and developmentally downregulated protein (Nedd4-1), ubiquitin-protein ligase E3a (Ube3a), and Cul7^{Fbxw8} (Kim

et al., 2009; Kawabe et al., 2010; Litterman et al., 2011; Miao et al., 2013; Watanabe et al., 2014). APC is an E3 ligase that acts together with the APC coactivators Cdc20 and Cdh1 to regulate neuronal morphogenesis. While Cdh1-APC specifically promotes axon growth, mutant analysis has shown that Cdc20-APC promotes dendritic growth in cerebellar granule neurons (Puram and Bonni, 2013). Cdc20-APC is localized to the neuronal cytoplasm and becomes enriched at the centrosome, a site for microtubule nucleation, via a centrosome localization motif present in Cdc20. This motif, and centrosome localization, is essential for dendrite growth. The centrosomal histone deacetylase HDAC6 activates Cdc20-APC and is required for dendrite growth (Kim et al., 2009). HDAC11 colocalizes with HDAC6 at the centrosome and is likewise important for dendrite development through interactions with both HDAC6 and Cdc20 (Watanabe et al., 2014). Several functionally important targets of Cdc20-APC ubiquitination have been identified, including the centrosomal HLH protein Id1, and the fasciculation and elongation protein zeta 1 (Fez1) (Kim et al., 2009; Watanabe et al., 2014). Downregulation of Id1 increases the length of dendrites in granule neurons, whereas reduction of FEZ1 by shRNA promotes increased soma size and increased dendrite length and complexity (Kang et al., 2011; Watanabe et al., 2014). Thus, one mechanism to promote dendrite elaboration is through ubiquitination and downregulation of growth inhibitors.

The neuronal precursor cell expressed and developmentally downregulated protein (Nedd4-1) is another E3 ligase implicated in dendrite development (Hamilton and Zito, 2013; Kawabe et al., 2010). *Nedd4-1* knock-out neurons show normal polarity, axon number, and numbers of primary dendrites, but reduced dendrite complexity (Kawabe et al., 2010). This effect is rescued by introduction of recombinant enzymatically active Nedd4-1 into mutant cells. Affinity chromatography identified a number of proteins bound to Nedd4-1, including Traf2 and Nck-interacting kinase (TNIK), which regulates actin dynamics together with the small GTPase Rap2 (Taira et al., 2004). These proteins were found in a ternary complex, and this complex is necessary for Nedd4-1-dependent mono-ubiquitination of Rap2. Notably, Rap2 does not appear to be ubiquitinated in such a way as to cause proteasome-dependent degradation. Instead, ubiquitination interferes with the ability of Rap2 to interact with effector proteins (Kawabe et al., 2010). Thus, Nedd4-1-dependent ubiquitination negatively regulates the small GTPase Rap2 to promote dendrite arborization (Kawabe et al., 2010).

Two other E3 ligases, Ube3a and the Cul7^{Fbxw8} complex, control dendrite morphogenesis by regulating the morphology and distribution of the Golgi apparatus. As described earlier, Golgi function is important for protein trafficking and dendrite growth. In vertebrate neurons, asymmetric Golgi distribution is required for polarized growth of pyramidal neuron apical dendrites (Horton et al., 2005). Ube3a is essential for polarized distribution of the Golgi to the apical dendrite and apical dendrite growth (Miao et al., 2013). The F-box protein Fbxw8, which associates with the scaffold protein Cul7 to generate Cul7^{Fbxw8}, colocalizes with Golgi markers in both granule neurons and hippocampal neurons (Litterman et al., 2011). *Fbxw8* knockdown in neurons caused frequent dispersion of the normally continuous Golgi, reduced the number of Golgi outposts, and impaired dendrite growth in granule neurons, cortical neurons, and hippocampal neurons (Litterman et al., 2011). Furthermore, Cul7^{Fbxw8} interacts with the Golgi stacking protein Grasp65 (Gorasp1), which induces polyubiquitination of Grasp65 and knockdown of *Grasp65* enhanced dendrite elaboration (Litterman et al., 2011). Moreover, knockdown of *Grasp65* suppressed the effects of *Fbxw8* knockdown. These studies point to diverse roles for ubiquitination in regulating the abundance and activity of proteins that affect neuronal morphogenesis.

Rho GTPases as central effectors of dendritic development

The effects of extracellular signals on dendritic morphology appear to be mediated by the Rho family of GTPases. Rho proteins are regulators of multiple biological processes in cells including actin dynamics, and act as molecular switches. They cycle between an active GTP-bound state and an inactive GDP-bound state. In their GTP-bound state they are able to bind and activate downstream effector proteins. The transition from an inactive to an active state is mediated by guanosine nucleotide exchange factors (GEFs), and their return to the inactive, GDP-bound state is catalyzed by GTPase-activating proteins (GAPs). The Rho family of GTPases consists of ten members, and the best-studied members are RhoA, Rac1, and Cdc42. Several studies have demonstrated a central role for these proteins in mediating dendrite growth and remodeling (Threadgill et al., 1997; Ruchhoeft et al., 1999; Lee et al., 2000; Li et al., 2000; Nakayama et al., 2000).

Experiments in both vertebrates and invertebrates indicate that RhoA influences the growth of dendritic arbors. Expression of a constitutively active form of RhoA in fly mushroom body neurons (Lee et al., 2000), *Xenopus* and chick retinal ganglion cells (Ruchhoeft et al., 1999; Wong et al., 2000), and *Xenopus* tectal neurons (Li et al., 2000) and hippocampal neurons (Nakayama et al., 2000) generally leads to a decrease in dendritic growth. Active RhoA not only prevents the formation of new dendrites, but also seems to induce retraction of existing branches. Conversely, blocking RhoA function in these systems promotes the growth of dendritic segments. This is nicely demonstrated in *Drosophila*, where selective removal of RhoA in individual fly mushroom body neurons leads to overextension of dendrites into areas not normally occupied by these neurons (Lee et al., 2000). However, in most other systems, blocking RhoA function results in only a mild phenotypic defect. Perhaps the RhoA pathway is normally inactive to allow dendrite extension, and is only activated locally when dendrite arbor growth needs to be restricted. Rac1, and to a lesser extent Cdc42, appear to control dendritic branching and remodeling. The most striking phenotype of Rac1 activation in several systems is the selective increase in dendrite branch additions and retractions (Li et al., 2000; Wong et al., 2000). This restructuring induced by Rac1 over-expression is rapid, and while Rac1 has been reported to increase branching complexity, the overall dendritic morphology is not greatly affected (Threadgill et al., 1997; Ruchhoeft et al., 1999; Lee et al., 2000; Li et al., 2000; Nakayama et al., 2000).

The effects of RhoA on dendritic morphology appear to be mediated by several pathways, including Rho-associated kinase (ROCK). Blocking ROCK activation prevents RhoA-induced dendritic simplification of hippocampal neurons, while expression of activated ROCK mimics the effect of RhoA (Nakayama et al., 2000). ROCK has been shown to activate actomyosin-based contractility and to suppress microtubule assembly in neuroblastoma cells (Hirose et al., 1998), indicating a possible mechanism by which ROCK mediates dendritic retraction. A recent study indicates that inhibition of branching by RhoA is mediated by lipid signaling. Phospholipase D1 (PLD1) generates phosphatidic acid (PA) and choline from phosphatidylcholine (PC). Increases in PLD1 expression cause dendritic simplification, and RhoA-induced simplification is partially rescued by lowering PLD1 levels (Zhu et al., 2012). These effects appear to be mediated by alterations in the levels of PA (Zhu et al., 2012). Less is known about downstream effectors mediating the effects of Rac1 and Cdc42 on dendrite remodeling. Given the similarity of the morphological changes induced by activated Rac1 and Cdc42, it is likely that they signal through a common effector protein. A well-known effector that can be activated by both Rac1 and Cdc42 is PAK1. Activation of PAK1 has been shown to induce neurite formation in PC12 cells (Daniels et al., 1998) as well as in cortical neurons (Hayashi et al., 2002).

Rho GTPases, dendritic development, and mental retardation

As described above, Rho GTPases play a central role in dendritic development. The correct temporal and spatial activity of Rho GTPases is accomplished by tight regulation of the active GTP-bound and inactive GDP state. This situation is achieved by the complementary function of GEF and GAP proteins, respectively. Recent reviews show that the human genome contains approximately 70 genes encoding Rho-GEFs and 100 genes encoding Rho-GAPs (reviewed in Bernards and Settleman, 2004; Rossman et al., 2005). Most of these GEFs and GAPs show exquisite spatial and temporal expression patterns during brain development (see, e.g., Yoshizawa et al., 2003). This strongly suggests a very tight region-specific as well as cell type-specific control of the dynamics of Rho GTPase activity during brain development.

Interestingly, several mutations in genes encoding regulators of small GTPase activity have been identified in patients with several forms of mental retardation (Newey et al., 2005; Penzes and Rafalovich, 2012). In fact, dendritic abnormalities including branching defects and spine dysgenesis are the most consistent anatomical correlates of syndromic and non-syndromic forms of mental retardation (reviewed by Kaufmann and Moser, 2000). For example, ARHGEF6 represents a GEF for Rac1 and Cdc42 and was identified by the molecular analysis of an X;21 reciprocal translocation in patients with mental retardation (Kutsche et al., 2000). In a similar way, oligophrenin1, a GAP for RhoA, was found to be interrupted in a female patient carrying a balanced X;12 translocation associated with mental retardation (Bienvenu et al., 1997; Billuart et al., 1998). Finally, another gene encoding a Rho-GAP (MEGAP or SRGAP3) has recently been shown to be disrupted and functionally inactivated by a translocation breakpoint in a patient who shares some characteristic clinical features, such as hypotonia and severe mental retardation, with the 3p⁻ syndrome (Endris et al., 2002). Further analysis of other patients presenting a 3p⁻ syndrome reveals a frequent occurrence of mutations in MEGAP/SRGAP3 often found on only one allele. These data suggest that haploinsufficiency of MEGAP/SRGAP3 leads to the abnormal development of neuronal structures that are important for normal cognitive function (Endris et al., 2002). Slit-Robo Rho-GTPase Activating Protein 3 (SRGAP3) has recently been involved in dendrite morphogenesis and dendritic spine formation through its F-BAR (FCH Bin, Amphiphysin, Rvs) domain which can coordinate membrane deformation with F-actin polymerization (Soderling et al., 2007; Kim et al., 2012a). A closely related gene (Wong et al., 2001), Slit-Robo Rho-GTPase activating protein 2 (SRGAP2A), encodes a highly conserved protein containing three functional domains: an N-terminal F-BAR domain, a central Rho-GAP (Rho-GTPase-Activating Protein) domain specific for the small GTPase Rac1, and a C-terminal SH3 (Src Homology 3) domain (Guerrier et al., 2009). SRGAP2A is expressed in the neocortex by pyramidal neurons during synaptogenesis and accumulates postsynaptically, where it regulates the development of dendritic spines (Charrier et al., 2012). SRGAP2A limits dendritic spine density and promotes excitatory synaptic maturation (Charrier et al., 2012). Interestingly, SRGAP2A has undergone two major human-specific partial duplications after the separation of our common ancestor from our most closely related primates (chimpanzees/bonobos). At the protein level, SRGAP2C is a truncated form of SRGAP2A corresponding to the F-BAR domain lacking its last 49 amino acids (Charrier et al., 2012). SRGAP2C physically interacts with SRGAP2A and inhibits its function. Inactivation of SRGAP2A, as well as expression of SRGAP2C in mouse cortical pyramidal neurons *in vivo*, induces the emergence of human-specific traits, such as significantly prolonged synaptic maturation (neoteny) of excitatory synapses and higher dendritic complexity due to increased spine density and longer spine necks (Charrier et al., 2012). These results suggest that emergence of the human-specific paralog SRGAP2C partially inhibits all functions of

SRGAP2A during excitatory synaptic maturation, which might have contributed to the evolution of cortical circuits characterizing the human lineage.

Regulation of dendritic spine development

The final step in the acquisition of a mature dendritic morphology is the development of dendritic spines. These small dendritic protrusions harbor the vast majority of excitatory synapses and contain receptors and other proteins necessary for synaptic transmission (see Chapter 1; Kennedy, 2000). The development of spines closely parallels the period of synaptogenesis (see Chapter 19). According to the Golgi studies of Miller (1981) on the development of L2/3 and L5 cells in rat visual cortex, there are very few spines present along the apical shaft during the first week after birth. At the end of the first week, the number of spines increases dramatically, especially between postnatal day 6 (P6) and P9. A second increase in spine density occurs between P12 and P15. The appearance of spines at P6–9 and P12–15 correlates with the arrival of geniculate axons in the cortical plate and eye opening, respectively, suggesting that activity may regulate some aspects of dendritic spine development.

The appearance of spines is preceded by the presence of thin dendritic filopodia along dendrites, which has led to speculation that dendritic filopodia might play a key role in synapse formation and may develop into spines. The filopodia are long and thin, lacking the bulbous head characteristic of spines. Time-lapse experiments in which DiI-labeled slices of hippocampal neurons were imaged (Dailey and Smith, 1996) revealed that dendritic filopodia are highly dynamic projections, rapidly changing their length and shape but lasting for only short periods of time. These filopodia would either disappear or become more stable protospines or spines. This suggested a model in which highly protrusive filopodia might be exploring the local environment, perhaps making contact with an axon and guiding it back to the dendrite to form a spine (Yuste and Bonhoeffer, 2001, 2004). To determine whether the filopodia can directly transition into a spine, or whether a synapse must first form to induce spine formation, particle-mediated gene delivery was used to transfect early postnatal hippocampal slices with a cDNA construct encoding PSD95 (a component of the postsynaptic density, PSD) tagged with enhanced green fluorescent protein (EGFP). This protein localized to PSDs, which could then be visualized. PSDs were found to be highly dynamic, and were able to appear, move, and disappear in a matter of minutes. These PSD95–GFP clusters largely colocalized with synapsin-I, a marker of presynaptic terminals, and were found in mature spines. Transient filopodia were observed; often they would regress spontaneously, but in those cases where a PSD95–GFP cluster developed, the structure would stabilize into a protospine or spine (Marrs et al., 2001). This suggests that the cluster formed because of a synaptic contact, and that this was responsible for transforming the filopodia into a spine. Additionally, some spines were formed directly by extension from the shaft, suggesting that shaft synapses can form spines directly. A recent study identified a role for Telencephalin (TLN/intercellular adhesion molecule-5, ICAM5) in the filopodia-to-spine transition (Raemaekers et al., 2012). TLN promotes the formation and maintenance of filopodia and slows spine morphogenesis, and the transition from filopodia to spine involves mobilization of TLN from the filopodial surface. Removal of TLN depends on activity of the small GTPase ARF6. Notably, ARF6 and exchange factor for ARF6 (EFA6A), promote spine formation and maintenance in a Rac1-dependent manner (Choi et al., 2006) and Rac1-mediated actin remodeling is required for internalization of TLN (Raemaekers et al., 2012). Thus, an ARF6–Rac1 pathway promotes spine formation by trafficking negative regulators of spine maturation from filopodial precursors.

Activity-dependent regulation of dendritic spines

The development of spines appears to be influenced by sensory experience (see Chapter 19). Valverde (1967) raised mice in the dark for the first three postnatal weeks and then examined the distribution of spines on apical dendrites of L5 pyramidal cells. He found a significant and specific reduction in the density of spines in the visual cortex, suggesting that the decrease in spine density was due to the reduction in visual input. Riccio and Matthews (1985) further examined this phenomenon by injecting tetrodotoxin (TTX) into one eye to abolish action potentials, thereby completely silencing the input from the injected eye for 3 weeks. Spine densities in L5 pyramidal cells in sham-injected animals and internal controls (corresponding to the non-injected eye) were very similar, but TTX treatment resulted in a 26% reduction in spines, supporting a role for activity in spine formation or maintenance. One should note, however, that changes in spine density have not been seen in every study. Vees et al. (1998) studied the somatosensory cortex of rats following whisker plucking between 1 and 2 months of age. The spines of spiny stellate neurons in L4 were examined following serial EM reconstruction, revealing no change in spine density following whisker plucking. However, spines contralateral to the deprived side tended to have decreased volume and surface area of the spine head and increased length of the spine neck.

Pioneering studies examined spine dynamics *in vivo* by using two-photon microscopy to study spines on L2/3 neurons of the barrel cortices of P8–18 postnatal rats expressing EGFP (Lendvai et al., 2000). Their imaging demonstrated that both filopodia and spines are highly motile *in vivo*. Since filopodial motility had previously been linked to changes in synapse formation, Lendvai et al. (2000) deprived the barrel cortex of activity by trimming the rat's whiskers 1–3 days before imaging. During a brief period, P11–13, spine motility was reduced by this deprivation. This time period corresponds to a period of intense synaptogenesis, when rats first start using their whiskers to explore their environment. Other studies demonstrated that formation of new spines can occur in response to the induction of long-term potentiation (LTP) by neighboring axons (Engert and Bonhoeffer, 1999). In fact, localized glutamate release using two-photon glutamate uncaging is sufficient to induce the emergence of a new dendritic spine within minutes (Kwon and Sabatini, 2011), suggesting that local spontaneous release of glutamate from an axon could initiate filopodial dynamics and spine consolidation both during development and during synaptic plasticity in the adult brain.

NMDA receptor signaling appears to be important for the emergence of dendritic spines. The formation of new spines can be triggered *in vitro* by stimuli that induce LTP, suggesting that NMDA receptor activation may trigger cytoskeletal changes that underlie spine formation (Engert and Bonhoeffer, 1999; Maletic-Savatic et al., 1999). Several proteins have been implicated in mediating the effects of NMDA receptor activation on spine morphogenesis. SPAR, a Rap-specific GTPase-activating protein, forms a complex with NMDA receptors and PSD95 and causes an enlargement of spine size in transfected neurons (Pak et al., 2001). NMDA receptor activation also leads to recruitment of cortactin (an actin-binding protein) to the synapse. Overexpression of cortactin leads to spine elongation, and knockdown of cortactin leads to spine depletion, indicating that cortactin may be an important mediator of activity-dependent spine morphogenesis (Hering and Sheng, 2003).

Small GTPases also exert a considerable influence on dendritic spines (see Chapter 20). Transfection of Rac1 and RhoA constructs into cultured mouse cortical slices perturbs spine formation (Tashiro et al., 2000). Constitutively active Rac1 promotes spine formation, while constitutively active RhoA reduces the number of spines and the length of their necks. Similar observations have been made in Purkinje cells and hippocampal neurons (Luo et al., 1996; Nakayama et al.,

2000; Tashiro et al., 2000). Spines are actin-based structures, which explains the strong influence of Rac1 on spine morphology. A number of regulators of the RhoGTPases, the GEFs and GAPs, are also important for spine morphology (reviewed in Penzes, 2012). Kalirin-7, a RhoGEF with exchange activity for Rac1, is targeted to postsynaptic densities in spines through its association with PSD95 (Penzes et al., 2003). In cultured cortical neurons transfection of Kalirin-7 results in the production of spine-like protrusions of various morphologies reminiscent of Rac activation. NMDA receptor stimulation causes phosphorylation of Kalirin-7 by calcium/calmodulin-dependent protein kinase 2 (CaMKII), which promotes the GEF activity of Kalirin-7, leading to Rac1 activation and spine enlargement (Xie et al., 2007). Notably, a Kalirin-7–Rac1 pathway is also linked to N-cadherin-dependent spine morphogenesis (Xie et al., 2008).

Tiam1, a Rac1-GEF, plays a crucial role in coupling NMDA receptors to Rho family proteins to regulate dendritic growth and spine formation (Tolias et al., 2005). Tiam1 co-precipitates with NMDA receptors and is activated by phosphorylation upon NMDA receptor-mediated calcium influx, causing activation of Rac1. Inhibiting Tiam1 by RNAi or dominant negative proteins results in decreased dendritic branching and spine density, revealing a requirement for Tiam1 in activity-dependent dendritic development. AP5 withdrawal experiments show that NMDAR-dependent spine formation induced by this paradigm also requires Tiam1 (Tolias et al., 2005). Tiam1-dependent Rac1 activation could provide a mechanism for local dendritic stability in response to NMDA receptor activation. Interactions between Tiam1 and several other proteins determine its localization and function in spines. Partitioning-defective gene 3 (Par-3), initially identified as important for polarity of the *C. elegans* zygote (Kemphues et al., 1988), is localized to spines in cultured hippocampal neurons and via interactions with a synaptic adhesion G-protein coupled receptor (GPCR), brain-specific angiogenesis inhibitor (BAI1) (Duman et al., 2013). Par-3, in turn, binds and recruits Tiam1. Silencing of *Tiam1* causes a reduction in spine density; however, coincident knockdown of *Par-3* and *Tiam1* rescues spine density, leading to the conclusion that balanced abundance of Par-3 and Tiam1 is important for spine formation (Zhang and Macara, 2006). Par-3 thus appears to function by targeting Tiam1 to spines, leading to spatially restricted formation of Rac-GTP (Zhang and Macara, 2006). Interactions between the EphB receptor tyrosine kinase (EphB) and Tiam1 are also important for spine morphogenesis. EphB mediates spine morphogenesis (Ethell et al., 2001) and forms a complex with NMDA receptors upon activation by ephrinB ligands (Dalva et al., 2000). Tiam1 is phosphorylated by activated EphB, which causes recruitment to EphB–NMDA receptor complexes and spine development (Tolias et al., 2007). Recently, it was shown that Bcr, a Rac-GAP, forms a GEF/GAP complex with Tiam1 that mediates EphB-dependent spine development. This complex appears to be necessary to achieve the tight balance of Rac1 activity optimal for the formation of spines and synapses (Um et al., 2014). Studies of Tiam1 reveal how specific localization and tight regulation of small GTPase activity can be achieved to regulate spine morphogenesis.

In addition to Tiam1, the Rac1/Cdc42 GEF, β PIX, also couples NMDA receptor activation to Rac and spine morphogenesis. β PIX functions as part of a complex that includes the GPCR kinase-interacting protein 1 (GIT1), PAK, and Rac (Zhang et al., 2005). A proteomics approach identified an interaction between members of the CaMK cascade and β PIX (Saneyoshi et al., 2008). Given that calcium entry through NMDA receptors can stimulate calmodulin-dependent kinase kinase (CaMKK) and CaMKI, this finding provided a possible route through which Ca^{2+} regulates spine morphogenesis upstream of β PIX. Consistent with this, CaMKI was shown to increase phosphorylation of serine 516 on β PIX in hippocampal neurons. This specific phosphorylation enhances the interaction between β PIX and Rac1, and enhances its Rac-GEF activity. Importantly, knockdown of β PIX using siRNA inhibited spine morphogenesis, and this effect was rescued by wild-type

siRNA-resistant β PIX, but not a S516A β PIX mutant (Saneyoshi et al., 2008). These studies identify a pathway from NMDA receptor activation to activation of Rac1 that controls spine formation with spatial precision.

Conclusions

The study of molecular control of dendritic development has rapidly evolved during the past decade. Genetic experiments in *Drosophila* have led to the identification of key components of molecular programs that regulate dendritic growth and self-avoidance. These have been complemented by experiments in vertebrate systems that have provided insight into the dynamics and extracellular control of dendritic growth and remodeling. A key insight from the imaging experiments has been the recognition that dendrite extension and retraction is a central feature of dendritic development. Extracellular signals that regulate different aspects of dendritic development have been identified, suggesting that differential responsiveness to extracellular cues may play an important role in the generation of morphological diversity in the nervous system. The development of dendritic spines, specializations that are critical for excitatory synaptic transmission, has been a particularly active area of investigation, and has revealed the key role of Rho family GTPases and F-actin dynamics in regulating spine morphogenesis. These discoveries provide a molecular framework for understanding the control of dendritic development and should greatly facilitate investigations of the relationship between neuronal structure and function.

References

- Aguirre-Chen C, Bulow HE, Kaprielian Z (2011) *C. elegans* bcd-1, homolog of the *Drosophila* dynein accessory factor Bicaudal D, regulates the branching of PVD sensory neuron dendrites. *Development* **138**:507–518.
- Aizawa H, Hu SC, Bobb K, Balakrishnan K, Ince G, Gurevich I, Cowan M, Ghosh A (2004) Dendrite development regulated by CREST, a calcium-regulated transcriptional activator. *Science* **303**:197–202.
- Baker RE, Dijkhuizen PA, Van Pelt J, Verhaagen J (1998) Growth of pyramidal, but not non-pyramidal, dendrites in long-term organotypic explants of neonatal rat neocortex chronically exposed to neurotrophin-3. *European Journal of Neuroscience* **10**:1037–1044.
- Baptista CA, Hatten ME, Blazquez R, Mason CA (1994) Cell-cell interactions influence survival and differentiation of purified Purkinje cells in vitro. *Neuron* **12**:243–260.
- Barnes AP, Polleux F (2009) Establishment of axon-dendrite polarity in developing neurons. *Annual Review of Neuroscience* **32**:347–381.
- Barnes AP, Lilley BN, Pan YA, Plummer LJ, Powell AW, Raines AN, Sanes JR, Polleux F (2007) LKB1 and SAD kinases define a pathway required for the polarization of cortical neurons. *Cell* **129**:549–563.
- Berezovska O, McLean P, Knowles R, Frosh M, Lu FM, Lux SE, Hyman BT (1999) Notch1 inhibits neurite outgrowth in postmitotic primary neurons. *Neuroscience* **93**:433–439.
- Bernards A, Settleman J (2004) GAP control: regulating the regulators of small GTPases. *Trends in Cell Biology* **14**:377–385.
- Bienvenu T, et al. (1997) Mapping of the X-breakpoint involved in a balanced X;12 translocation in a female with mild mental retardation. *European Journal of Human Genetics* **5**:105–109.
- Billuart P, et al. (1998) Oligophrenin 1 encodes a rho-GAP protein involved in X-linked mental retardation. *Pathologie-biologie* **46**:678.
- Blochlinger K, Bodmer R, Jan LY, Jan YN (1990) Patterns of expression of cut, a protein required for external sensory organ development in wild-type and cut mutant *Drosophila* embryos. *Genes and Development* **4**:1322–1331.

- Brennan JE, Gao FB, Jan LY, Jan YN (2001) Sequoia, a tramtrack-related zinc finger protein, functions as a pan-neuronal regulator for dendrite and axon morphogenesis in *Drosophila*. *Developmental Cell* 1:667–677.
- Brierley DJ, Blanc E, Reddy OV, Vijayraghavan K, Williams DW (2009) Dendritic targeting in the leg neuropil of *Drosophila*: the role of midline signalling molecules in generating a myotopic map. *PLoS Biology* 7:e1000199.
- Chae T, Kwon YT, Bronson R, Dikkes P, Li E, Tsai LH (1997) Mice lacking p35, a neuronal specific activator of Cdk5, display cortical lamination defects, seizures, and adult lethality. *Neuron* 18:29–42.
- Charrier C, et al. (2012) Inhibition of SRGAP2 function by its human-specific paralogs induces neoteny during spine maturation. *Cell* 149:923–935.
- Chen B, Schaevitz LR, McConnell SK (2005a) Fezl regulates the differentiation and axon targeting of layer 5 subcortical projection neurons in cerebral cortex. *Proceedings of the National Academy of Sciences of the United States of America* 102:17184–17189.
- Chen JG, Rasin MR, Kwan KY, Sestan N (2005b) Zfp312 is required for subcortical axonal projections and dendritic morphology of deep-layer pyramidal neurons of the cerebral cortex. *Proceedings of the National Academy of Sciences of the United States of America* 102:17792–17797.
- Choi S, Ko J, Lee JR, Lee HW, Kim K, Chung HS, Kim H, Kim E (2006) ARF6 and EFA6A regulate the development and maintenance of dendritic spines. *Journal of Neuroscience* 26:4811–4819.
- Ciani L, Salinas PC (2005) WNTs in the vertebrate nervous system: from patterning to neuronal connectivity. *Nature Reviews Neuroscience* 6:351–362.
- Ciani L, Krylova O, Smalley MJ, Dale TC, Salinas PC (2004) A divergent canonical WNT-signaling pathway regulates microtubule dynamics: dishevelled signals locally to stabilize microtubules. *Journal of Cell Biology* 164:243–253.
- Cobos I, Borello U, Rubenstein JL (2007) Dlx transcription factors promote migration through repression of axon and dendrite growth. *Neuron* 54:873–888.
- Crozier M, Vincent A (2008) Control of multidendritic neuron differentiation in *Drosophila*: the role of Collier. *Developmental Biology* 315:232–242.
- Cubelos B, et al. (2010) Cux1 and Cux2 regulate dendritic branching, spine morphology, and synapses of the upper layer neurons of the cortex. *Neuron* 66:523–535.
- Cubelos B, Briz CG, Esteban-Ortega GM, Nieto M (2015) Cux1 and Cux2 selectively target basal and apical dendritic compartments of layer II-III cortical neurons. *Developmental Neurobiology* 75:163–172.
- Dailey ME, Smith SJ (1996) The dynamics of dendritic structure in developing hippocampal slices. *Journal of Neuroscience* 16:2983–2994.
- Dalva MB, Takasu MA, Lin MZ, Shamah SM, Hu L, Gale NW, Greenberg ME (2000) EphB receptors interact with NMDA receptors and regulate excitatory synapse formation. *Cell* 103:945–956.
- Daniels RH, Hall PS, Bokoch GM (1998) Membrane targeting of p21-activated kinase 1 (PAK1) induces neurite outgrowth from PC12 cells. *EMBO Journal* 17:754–764.
- Dijkhuizen PA, Ghosh A (2005) BDNF regulates primary dendrite formation in cortical neurons via the PI3-kinase and MAP kinase signaling pathways. *Journal of Neurobiology* 62:278–288.
- Dimitrova S, Reissaus A, Tavosanis G (2008) Slit and Robo regulate dendrite branching and elongation of space-filling neurons in *Drosophila*. *Developmental Biology* 324:18–30.
- Dong X, Liu OW, Howell AS, Shen K (2013) An extracellular adhesion molecule complex patterns dendrite branching and morphogenesis. *Cell* 155:296–307.
- Duman JG, Tzeng CP, Tu YK, Munjal T, Schwechter B, Ho TS, Tolias KF (2013) The adhesion-GPCR BAI1 regulates synaptogenesis by controlling the recruitment of the Par3/Tiam1 polarity complex to synaptic sites. *Journal of Neuroscience* 33:6964–6978.
- Emoto K, He Y, Ye B, Grueber WB, Adler PN, Jan LY, Jan YN (2004) Control of dendrite branching and tiling by the Tricornered-kinase/Furry signaling pathway in *Drosophila* sensory neurons. *Cell* 119:245–256.

- Endris V, et al. (2002) The novel Rho-GTPase activating gene MEGAP/srGAP3 has a putative role in severe mental retardation. *Proceedings of the National Academy of Sciences of the United States of America* **99**:11754–11759.
- Engert F, Bonhoeffer T (1999) Dendritic spine changes associated with hippocampal long-term synaptic plasticity. *Nature* **399**:66–70.
- Ethell IM, Irie F, Kalo MS, Couchman JR, Pasquale EB, Yamaguchi Y (2001) EphB/syndecan-2 signaling in dendritic spine morphogenesis. *Neuron* **31**:1001–1013.
- Franklin JL, Berechid BE, Cutting FB, Presente A, Chambers CB, Foltz DR, Ferreira A, Nye JS (1999) Autonomous and non-autonomous regulation of mammalian neurite development by Notch1 and Delta1. *Current Biology* **9**:1448–1457.
- Fuerst PG, Bruce F, Tian M, Wei W, Elstrott J, Feller MB, Erskine L, Singer JH, Burgess RW (2009) DSCAM and DSCAML1 function in self-avoidance in multiple cell types in the developing mouse retina. *Neuron* **64**:484–497.
- Furrer MP, Kim S, Wolf B, Chiba A (2003) Robo and Frazzled/DCC mediate dendritic guidance at the CNS midline. *Nature Neuroscience* **6**:223–230.
- Gao FB, Brenman JE, Jan LY, Jan YN (1999) Genes regulating dendritic outgrowth, branching, and routing in Drosophila. *Genes and Development* **13**:2549–2561.
- Gao FB, Kohwi M, Brenman JE, Jan LY, Jan YN (2000) Control of dendritic field formation in Drosophila: the roles of flamingo and competition between homologous neurons. *Neuron* **28**:91–101.
- Garrett AM, Schreiner D, Lobas MA, Weiner JA (2012) gamma-protocadherins control cortical dendrite arborization by regulating the activity of a FAK/PKC/MARCKS signaling pathway. *Neuron* **74**:269–276.
- Ge W, et al. (2006) Coupling of cell migration with neurogenesis by proneural bHLH factors. *Proceedings of the National Academy of Sciences of the United States of America* **103**:1319–1324.
- Gibson DA, Tymanskyj S, Yuan RC, Leung HC, Lefebvre JL, Sanes JR, Chedotal A, Ma L (2014) Dendrite self-avoidance requires cell-autonomous slit/robo signaling in cerebellar Purkinje cells. *Neuron* **81**:1040–1056.
- Grueber WB, Jan YN (2004) Dendritic development: lessons from Drosophila and related branches. *Current Opinion in Neurobiology* **14**:74–82.
- Grueber WB, Jan LY, Jan YN (2002) Tiling of the Drosophila epidermis by multidendritic sensory neurons. *Development* **129**:2867–2878.
- Grueber WB, Jan LY, Jan YN (2003) Different levels of the homeodomain protein cut regulate distinct dendrite branching patterns of Drosophila multidendritic neurons. *Cell* **112**:805–818.
- Grueber WB, Ye B, Yang CH, Younger S, Borden K, Jan LY, Jan YN (2007) Projections of Drosophila multidendritic neurons in the central nervous system: links with peripheral dendrite morphology. *Development* **134**:55–64.
- Guerrier S, Coutinho-Budd J, Sassa T, Gresset A, Jordan NV, Chen K, Jin WL, Frost A, Polleux F (2009) The F-BAR domain of srGAP2 induces membrane protrusions required for neuronal migration and morphogenesis. *Cell* **138**:990–1004.
- Hall AC, Lucas FR, Salinas PC (2000) Axonal remodeling and synaptic differentiation in the cerebellum is regulated by WNT-7a signaling. *Cell* **100**:525–535.
- Hamilton AM, Zito K (2013) Breaking it down: the ubiquitin proteasome system in neuronal morphogenesis. *Neural Plasticity* **2013**:196848.
- Han C, Wang D, Soba P, Zhu S, Lin X, Jan LY, Jan YN (2012) Integrins regulate repulsion-mediated dendrite patterning of Drosophila sensory neurons by restricting dendrites in a 2D space. *Neuron* **73**:64–78.
- Hand R, et al. (2005) Phosphorylation of Neurogenin2 specifies the migration properties and the dendrite morphology of pyramidal neurons in the neocortex. *Neuron* **48**:45–62.
- Hattori Y, Sugimura K, Uemura T (2007) Selective expression of Knot/Collier, a transcriptional regulator of the EBF/Olf-1 family, endows the Drosophila sensory system with neuronal class-specific elaborated dendrite patterns. *Genes to Cells* **12**:1011–1022.

- Hattori D, Millard SS, Wojtowicz WM, Zipursky SL (2008) Dscam-mediated cell recognition regulates neural circuit formation. *Annual Review of Cell and Developmental Biology* **24**:597–620.
- Hattori D, Chen Y, Matthews BJ, Salwinski L, Sabatti C, Grueber WB, Zipursky SL (2009) Robust discrimination between self and non-self neurites requires thousands of Dscam1 isoforms. *Nature* **461**:644–648.
- Hayashi K, Ohshima T, Mikoshiba K (2002) Pak1 is involved in dendrite initiation as a downstream effector of Rac1 in cortical neurons. *Molecular and Cellular Neurosciences* **20**:579–594.
- Heng JI, et al. (2008) Neurogenin 2 controls cortical neuron migration through regulation of Rnd2. *Nature* **455**:114–118.
- Hering H, Sheng M (2003) Activity-dependent redistribution and essential role of cortactin in dendritic spine morphogenesis. *Journal of Neuroscience* **23**:11759–11769.
- Hirose M, Ishizaki T, Watanabe N, Uehata M, Kranenburg O, Moolenaar WH, Matsumura F, Maekawa M, Bito H, Narumiya S (1998) Molecular dissection of the Rho-associated protein kinase (p160ROCK)-regulated neurite remodeling in neuroblastoma N1E-115 cells. *Journal of Cell Biology* **141**:1625–1636.
- Horton AC, Racz B, Monson EE, Lin AL, Weinberg RJ, Ehlers MD (2005) Polarized secretory trafficking directs cargo for asymmetric dendrite growth and morphogenesis. *Neuron* **48**:757–771.
- Huber AB, Kolodkin AL, Ginty DD, Cloutier JF (2003) Signaling at the growth cone: ligand-receptor complexes and the control of axon growth and guidance. *Annual Review of Neuroscience* **26**:509–563.
- Hughes ME, Bortnick R, Tsubouchi A, Baumer P, Kondo M, Uemura T, Schmucker D (2007) Homophilic Dscam interactions control complex dendrite morphogenesis. *Neuron* **54**:417–427.
- Jan YN, Jan LY (2003) The control of dendrite development. *Neuron* **40**:229–242.
- Jensen KF, Killackey HP (1984) Subcortical projections from ectopic neocortical neurons. *Proceedings of the National Academy of Sciences of the United States of America* **81**:964–968.
- Jiang N, Soba P, Parker E, Kim CC, Parrish JZ (2014) The microRNA bantam regulates a developmental transition in epithelial cells that restricts sensory dendrite growth. *Development* **141**:2657–2668.
- Jinushi-Nakao S, Arvind R, Amikura R, Kinameri E, Liu AW, Moore AW (2007) Knot/Collier and cut control different aspects of dendrite cytoskeleton and synergize to define final arbor shape. *Neuron* **56**:963–978.
- Kang E, et al. (2011) Interaction between FEZ1 and DISC1 in regulation of neuronal development and risk for schizophrenia. *Neuron* **72**:559–571.
- Kaufmann WE, Moser HW (2000) Dendritic anomalies in disorders associated with mental retardation. *Cerebral Cortex* **10**:981–991.
- Kawabe H, et al. (2010) Regulation of Rap2A by the ubiquitin ligase Nedd4-1 controls neurite development. *Neuron* **65**:358–372.
- Kemphues KJ, Priess JR, Morton DG, Cheng NS (1988) Identification of genes required for cytoplasmic localization in early *C. elegans* embryos. *Cell* **52**:311–320.
- Kennedy MB (2000) Signal-processing machines at the postsynaptic density. *Science* **290**:750–754.
- Kim AH, Puram SV, Bilimoria PM, Ikeuchi Y, Keough S, Wong M, Rowitch D, Bonni A (2009) A centrosomal Cdc20-APC pathway controls dendrite morphogenesis in postmitotic neurons. *Cell* **136**:322–336.
- Kim IH, Carlson BR, Heindel CC, Kim H, Soderling SH (2012a) Disruption of wave-associated Rac GTPase-activating protein (Wrp) leads to abnormal adult neural progenitor migration associated with hydrocephalus. *Journal of Biological Chemistry* **287**:39263–39274.
- Kim ME, Shrestha BR, Blazeski R, Mason CA, Grueber WB (2012b) Integrins establish dendrite-substrate relationships that promote dendritic self-avoidance and patterning in drosophila sensory neurons. *Neuron* **73**:79–91.
- Komiyama T, Luo L (2007) Intrinsic control of precise dendrite targeting by an ensemble of transcription factors. *Current Biology* **17**:278–285.
- Komiyama T, Johnson WA, Luo L, Jefferis GS (2003) From lineage to wiring specificity. POU domain transcription factors control precise connections of Drosophila olfactory projection neurons. *Cell* **112**:157–167.

- Komiya T, Sweeney LB, Schuldiner O, Garcia KC, Luo L (2007) Graded expression of semaphorin-1a cell-autonomously directs dendritic targeting of olfactory projection neurons. *Cell* **128**:399–410.
- Krylova O, Herreros J, Cleverley KE, Ehler E, Henriquez JP, Hughes SM, Salinas PC (2002) WNT-3, expressed by motoneurons, regulates terminal arborization of neurotrophin-3-responsive spinal sensory neurons. *Neuron* **35**:1043–1056.
- Kutsche K, et al. (2000) Mutations in ARHGEF6, encoding a guanine nucleotide exchange factor for Rho GTPases, in patients with X-linked mental retardation. *Nature Genetics* **26**:247–250.
- Kwon HB, Sabatini BL (2011) Glutamate induces de novo growth of functional spines in developing cortex. *Nature* **474**:100–104.
- Lee T, Winter C, Marticke SS, Lee A, Luo L (2000) Essential roles of Drosophila RhoA in the regulation of neuroblast proliferation and dendritic but not axonal morphogenesis. *Neuron* **25**:307–316.
- Lefebvre JL, Kostadinov D, Chen WV, Maniatis T, Sanes JR (2012) Protocadherins mediate dendritic self-avoidance in the mammalian nervous system. *Nature* **488**:517–521.
- Lendvai B, Stern EA, Chen B, Svoboda K (2000) Experience-dependent plasticity of dendritic spines in the developing rat barrel cortex *in vivo*. *Nature* **404**:876–881.
- Lew J, Wang JH (1995) Neuronal cdc2-like kinase. *Trends in Biochemical Sciences* **20**:33–37.
- Li Z, Van Aelst L, Cline HT (2000) Rho GTPases regulate distinct aspects of dendritic arbor growth in Xenopus central neurons *in vivo*. *Nature Neuroscience* **3**:217–225.
- Li W, Wang F, Menut L, Gao FB (2004) BTB/POZ-zinc finger protein abrupt suppresses dendritic branching in a neuronal subtype-specific and dosage-dependent manner. *Neuron* **43**:823–834.
- Li H, Chen G, Zhou B, Duan S (2008) Actin filament assembly by myristoylated alanine-rich C kinase substrate-phosphatidylinositol-4,5-diphosphate signaling is critical for dendrite branching. *Molecular Biology of the Cell* **19**:4804–4813.
- Litterman N, Ikeuchi Y, Gallardo G, O'Connell BC, Sowa ME, Gygi SP, Harper JW, Bonni A (2011) An ODSL1-Cul7Fbxw8 ubiquitin ligase signaling mechanism regulates Golgi morphology and dendrite patterning. *PLoS Biology* **9**:e1001060.
- Luo L, Hensch TK, Ackerman L, Barbel S, Jan LY, Jan YN (1996) Differential effects of the Rac GTPase on Purkinje cell axons and dendritic trunks and spines. *Nature* **379**:837–840.
- McAllister AK, Lo DC, Katz LC (1995) Neurotrophins regulate dendritic growth in developing visual cortex. *Neuron* **15**:791–803.
- MacNeil MA, Masland RH (1998) Extreme diversity among amacrine cells: implications for function. *Neuron* **20**:971–982.
- Maletic-Savatic M, Malinow R, Svoboda K (1999) Rapid dendritic morphogenesis in CA1 hippocampal dendrites induced by synaptic activity. *Science* **283**:1923–1927.
- Marrs GS, Green SH, Dailey ME (2001) Rapid formation and remodeling of postsynaptic densities in developing dendrites. *Nature Neuroscience* **4**:1006–1013.
- Matthews BJ, Grueber WB (2011) Dscam1-mediated self-avoidance counters netrin-dependent targeting of dendrites in Drosophila. *Current Biology* **21**:1480–1487.
- Matthews BJ, Kim ME, Flanagan JJ, Hattori D, Clemens JC, Zipursky SL, Grueber WB (2007) Dendrite self-avoidance is controlled by Dscam. *Cell* **129**:593–604.
- Mauss A, Tripodi M, Evers JF, Landgraf M (2009) Midline signalling systems direct the formation of a neural map by dendritic targeting in the Drosophila motor system. *PLoS Biology* **7**:e1000200.
- Miao S, Chen R, Ye J, Tan GH, Li S, Zhang J, Jiang YH, Xiong ZQ (2013) The Angelman syndrome protein Ube3a is required for polarized dendrite morphogenesis in pyramidal neurons. *Journal of Neuroscience* **33**:327–333.
- Miller M (1981) Maturation of rat visual cortex. I. A quantitative study of Golgi-impregnated pyramidal neurons. *Journal of Neurocytology* **10**:859–878.
- Miura SK, Martins A, Zhang KX, Graveley BR, Zipursky SL (2013) Probabilistic splicing of Dscam1 establishes identity at the level of single neurons. *Cell* **155**:1166–1177.

- Moore AW, Jan LY, Jan YN (2002) hamlet, a binary genetic switch between single- and multiple-dendrite neuron morphology. *Science* **297**:1355–1358.
- Morrison ME, Mason CA (1998) Granule neuron regulation of Purkinje cell development: striking a balance between neurotrophin and glutamate signaling. *Journal of Neuroscience* **18**:3563–3573.
- Nakayama AY, Harms MB, Luo L (2000) Small GTPases Rac and Rho in the maintenance of dendritic spines and branches in hippocampal pyramidal neurons. *Journal of Neuroscience* **20**:5329–5338.
- Neves G, Chess A (2004) Dscam-mediated self- versus non-self-recognition by individual neurons. *Cold Spring Harbor Symposia on Quantitative Biology* **69**:485–488.
- Newey SE, Velamoor V, Govek EE, Van Aelst L (2005) Rho GTPases, dendritic structure, and mental retardation. *Journal of Neurobiology* **64**:58–74.
- Niblock MM, Brunso-Bechtold JK, Riddle DR (2000) Insulin-like growth factor I stimulates dendritic growth in primary somatosensory cortex. *Journal of Neuroscience* **20**:4165–4176.
- Noctor SC, Flint AC, Weissman TA, Dammerman RS, Kriegstein AR (2001) Neurons derived from radial glial cells establish radial units in neocortex. *Nature* **409**:714–720.
- Ohshima T, Ward JM, Huh CG, Longenecker G, Veeranna, Pant HC, Brady RO, Martin LJ, Kulkarni AB (1996) Targeted disruption of the cyclin-dependent kinase 5 gene results in abnormal corticogenesis, neuronal pathology and perinatal death. *Proceedings of the National Academy of Sciences of the United States of America* **93**:11173–11178.
- Ori-McKenney KM, Jan LY, Jan YN (2012) Golgi outposts shape dendrite morphology by functioning as sites of acentrosomal microtubule nucleation in neurons. *Neuron* **76**:921–930.
- Pak DT, Yang S, Rudolph-Correia S, Kim E, Sheng M (2001) Regulation of dendritic spine morphology by SPAR, a PSD-95-associated RapGAP. *Neuron* **31**:289–303.
- Parrish JZ, Xu P, Kim CC, Jan LY, Jan YN (2009) The microRNA bantam functions in epithelial cells to regulate scaling growth of dendrite arbors in drosophila sensory neurons. *Neuron* **63**:788–802.
- Penzes P, Rafalovich I (2012) Regulation of the actin cytoskeleton in dendritic spines. *Advances in Experimental Medicine and Biology* **970**:81–95.
- Penzes P, Beeser A, Chernoff J, Schiller MR, Eipper BA, Mains RE, Huganir RL (2003) Rapid induction of dendritic spine morphogenesis by trans-synaptic ephrinB-EphB receptor activation of the Rho-GEF kalirin. *Neuron* **37**:263–274.
- Pinto Lord MC, Caviness VS, Jr. (1979) Determinants of cell shape and orientation: a comparative Golgi analysis of cell-axon interrelationships in the developing neocortex of normal and reeler mice. *Journal of Comparative Neurology* **187**:49–69.
- Polleux F, Giger RJ, Ginty DD, Kolodkin AL, Ghosh A (1998) Patterning of cortical efferent projections by semaphorin-neuropilin interactions. *Science* **282**:1904–1906.
- Polleux F, Morrow T, Ghosh A (2000) Semaphorin 3A is a chemoattractant for cortical apical dendrites. *Nature* **404**:567–573.
- Polleux F, Ince-Dunn G, Ghosh A (2007) Transcriptional regulation of vertebrate axon guidance and synapse formation. *Nature Reviews Neuroscience* **8**:331–340.
- Puram SV, Bonni A (2011) Novel functions for the anaphase-promoting complex in neurobiology. *Seminars in Cell and Developmental Biology* **22**:586–594.
- Puram SV, Bonni A (2013) Cell-intrinsic drivers of dendrite morphogenesis. *Development* **140**:4657–4671.
- Raemaekers T, Peric A, Baatsen P, Sannerud R, Declerck I, Baert V, Michiels C, Annaert W (2012) ARF6-mediated endosomal transport of Telencephalin affects dendritic filopodia-to-spine maturation. *EMBO Journal* **31**:3252–3269.
- Ramón y Cajal S (1909) *Histologie du Système Nerveux de l'Homme et des Vertébrés*. Paris: Maloine. Available at: <https://archive.org/details/histologiedusyst01ram>
- Redmond L, Oh SR, Hicks C, Weinmaster G, Ghosh A (2000) Nuclear Notch1 signaling and the regulation of dendritic development. *Nature Neuroscience* **3**:30–40.

- Riccio RV, Matthews MA (1985) Effects of intraocular tetrodotoxin on dendritic spines in the developing rat visual cortex: a Golgi analysis. *Brain Research* **351**:173–182.
- Roszman KL, Der CJ, Sondek J (2005) GEF means go: turning on RHO GTPases with guanine nucleotide-exchange factors. *Nature Reviews Molecular Cell Biology* **6**:167–180.
- Rosso SB, Sussman D, Wynshaw-Boris A, Salinas PC (2005) Wnt signaling through Dishevelled, Rac and JNK regulates dendritic development. *Nature Neuroscience* **8**:34–42.
- Ruchhoeft ML, Ohnuma S, McNeill L, Holt CE, Harris WA (1999) The neuronal architecture of Xenopus retinal ganglion cells is sculpted by rho-family GTPases in vivo. *Journal of Neuroscience* **19**:8454–8463.
- Salzberg Y, Diaz-Balzac CA, Ramirez-Suarez NJ, Attreed M, Tecle E, Desbois M, Kaprielian Z, Bulow HE (2013) Skin-derived cues control arborization of sensory dendrites in *Caenorhabditis elegans*. *Cell* **155**:308–320.
- Saneyoshi T, Wayman G, Fortin D, Davare M, Hoshi N, Nozaki N, Natsume T, Soderling TR (2008) Activity-dependent synaptogenesis: regulation by a CaM-kinase kinase/CaM-kinase I/betaPIX signaling complex. *Neuron* **57**:94–107.
- Sasaki Y, et al. (2002) Fyn and Cdk5 mediate semaphorin-3A signaling, which is involved in regulation of dendrite orientation in cerebral cortex. *Neuron* **35**:907–920.
- Satoh D, Sato D, Tsuyama T, Saito M, Ohkura H, Rolls MM, Ishikawa F, Uemura T (2008) Spatial control of branching within dendritic arbors by dynein-dependent transport of Rab5-endosomes. *Nature Cell Biology* **10**:1164–1171.
- Schmucker D, Clemens JC, Shu H, Worby CA, Xiao J, Muda M, Dixon JE, Zipursky SL (2000) Drosophila Dscam is an axon guidance receptor exhibiting extraordinary molecular diversity. *Cell* **101**:671–684.
- Sestan N, Artavanis-Tsakonas S, Rakic P (1999) Contact-dependent inhibition of cortical neurite growth mediated by notch signaling. *Science* **286**:741–746.
- Shalizi A, Gaudilliere B, Yuan Z, Stegmuller J, Shirogane T, Ge Q, Tan Y, Schulman B, Harper JW, Bonni A (2006) A calcium-regulated MEF2 sumoylation switch controls postsynaptic differentiation. *Science* **311**:1012–1017.
- Shelly M, Cancedda L, Heilshorn S, Sumbre G, Poo MM (2007) LKB1/STRAD promotes axon initiation during neuronal polarization. *Cell* **129**:565–577.
- Shelly M, Cancedda L, Lim BK, Popescu AT, Cheng PL, Gao H, Poo MM (2011) Semaphorin3A regulates neuronal polarization by suppressing axon formation and promoting dendrite growth. *Neuron* **71**:433–446.
- Shi SH, Jan LY, Jan YN (2003) Hippocampal neuronal polarity specified by spatially localized mPar3/mPar and PI 3-kinase activity. *Cell* **112**:63–75.
- Shima Y, Kengaku M, Hirano T, Takeichi M, Uemura T (2004) Regulation of dendritic maintenance and growth by a mammalian 7-pass transmembrane cadherin. *Developmental Cell* **7**:205–216.
- Shima Y, Kawaguchi SY, Kosaka K, Nakayama M, Hoshino M, Nabeshima Y, Hirano T, Uemura T (2007) Opposing roles in neurite growth control by two seven-pass transmembrane cadherins. *Nature Neuroscience* **10**:963–969.
- Smith CJ, Watson JD, Spencer WC, O'Brien T, Cha B, Albeg A, Treinin M, Miller DM, 3rd (2010) Time-lapse imaging and cell-specific expression profiling reveal dynamic branching and molecular determinants of a multi-dendritic nociceptor in *C. elegans*. *Developmental Biology* **345**:18–33.
- Smith CJ, Watson JD, VanHoven MK, Colon-Ramos DA, Miller DM, 3rd (2012) Netrin (UNC-6) mediates dendritic self-avoidance. *Nature Neuroscience* **15**:731–737.
- Soba P, Zhu S, Emoto K, Younger S, Yang SJ, Yu HH, Lee T, Jan LY, Jan YN (2007) Drosophila sensory neurons require Dscam for dendritic self-avoidance and proper dendritic field organization. *Neuron* **54**:403–416.
- Soderling SH, Guire ES, Kaech S, White J, Zhang F, Schutz K, Langeberg LK, Banker G, Raber J, Scott JD (2007) A WAVE-1 and WRP signaling complex regulates spine density, synaptic plasticity, and memory. *Journal of Neuroscience* **27**:355–365.

- Sugimura K, Satoh D, Estes P, Crews S, Uemura T (2004) Development of morphological diversity of dendrites in *Drosophila* by the BTB-zinc finger protein abrupt. *Neuron* **43**:809–822.
- Suzuki ST (1996) Structural and functional diversity of cadherin superfamily: are new members of cadherin superfamily involved in signal transduction pathway? *Journal of Cellular Biochemistry* **61**:531–542.
- Sweeney LB, Chou YH, Wu Z, Joo W, Komiyama T, Potter CJ, Kolodkin AL, Garcia KC, Luo L (2011) Secreted semaphorins from degenerating larval ORN axons direct adult projection neuron dendrite targeting. *Neuron* **72**:734–747.
- Taira K, Umikawa M, Takei K, Myagmar BE, Shinzato M, Machida N, Uezato H, Nonaka S, Kariya K (2004) The Traf2- and Nck-interacting kinase as a putative effector of Rap2 to regulate actin cytoskeleton. *Journal of Biological Chemistry* **279**:49488–49496.
- Tashiro A, Minden A, Yuste R (2000) Regulation of dendritic spine morphology by the rho family of small GTPases: antagonistic roles of Rac and Rho. *Cerebral Cortex* **10**:927–938.
- Threadgill R, Bobb K, Ghosh A (1997) Regulation of dendritic growth and remodeling by Rho, Rac, and Cdc42. *Neuron* **19**:625–634.
- Thu CA, Chen WV, Rubinstein R, Chevee M, Wolcott HN, Felsövályi KO, Tapia JC, Shapiro L, Honig B, Maniatis T (2014) Single-cell identity generated by combinatorial homophilic interactions between alpha, beta, and gamma protocadherins. *Cell* **158**:1045–1059.
- Tolias KF, Bikoff JB, Burette A, Paradis S, Harrar D, Tavazoie S, Weinberg RJ, Greenberg ME (2005) The Rac1-GEF Tiam1 couples the NMDA receptor to the activity-dependent development of dendritic arbor and spines. *Neuron* **45**:525–538.
- Tolias KF, Bikoff JB, Kane CG, Tolias CS, Hu L, Greenberg ME (2007) The Rac1 guanine nucleotide exchange factor Tiam1 mediates EphB receptor-dependent dendritic spine development. *Proceedings of the National Academy of Sciences of the United States of America* **104**:7265–7270.
- de la Torre-Ubieta L, Bonni A (2011) Transcriptional regulation of neuronal polarity and morphogenesis in the mammalian brain. *Neuron* **72**:22–40.
- Um K, et al. (2014) Dynamic control of excitatory synapse development by a Rac1 GEF/GAP regulatory complex. *Developmental Cell* **29**:701–715.
- Valverde F (1967) Apical dendritic spines of the visual cortex and light deprivation in the mouse. *Experimental Brain Research* **3**:337–352.
- Vees AM, Micheva KD, Beaulieu C, Descarpentries L (1998) Increased number and size of dendritic spines in ipsilateral barrel field cortex following unilateral whisker trimming in postnatal rat. *Journal of Comparative Neurology* **400**:110–124.
- Wassle H, Peichl L, Boycott BB (1981) Dendritic territories of cat retinal ganglion cells. *Nature* **292**:344–345.
- Watanabe Y, Khodosevich K, Monyer H (2014) Dendrite development regulated by the schizophrenia-associated gene FEZ1 involves the ubiquitin proteasome system. *Cell Reports* **7**:552–564.
- Weinmaster G (2000) Notch signal transduction: a real rip and more. *Current Opinion in Genetics and Development* **10**:363–369.
- Whitford KL, Marillat V, Stein E, Goodman CS, Tessier-Lavigne M, Chedotal A, Ghosh A (2002) Regulation of cortical dendrite development by Slit-Robo interactions. *Neuron* **33**:47–61.
- Wojtowicz WM, Wu W, Andre I, Qian B, Baker D, Zipursky SL (2007) A vast repertoire of Dscam binding specificities arises from modular interactions of variable Ig domains. *Cell* **130**:1134–1145.
- Wong WT, Faulkner-Jones BE, Sanes JR, Wong RO (2000) Rapid dendritic remodeling in the developing retina: dependence on neurotransmission and reciprocal regulation by Rac and Rho. *Journal of Neuroscience* **20**:5024–5036.
- Wong K, et al. (2001) Signal transduction in neuronal migration: roles of GTPase activating proteins and the small GTPase Cdc42 in the Slit–Robo pathway. *Cell* **107**:209–221.
- Wu JI, Lessard J, Olave IA, Qiu Z, Ghosh A, Graef IA, Crabtree GR (2007) Regulation of dendritic development by neuron-specific chromatin remodeling complexes. *Neuron* **56**:94–108.

- Xie Z, Srivastava DP, Photowala H, Kai L, Cahill ME, Woolfrey KM, Shum CY, Surmeier DJ, Penzes P (2007) Kalirin-7 controls activity-dependent structural and functional plasticity of dendritic spines. *Neuron* **56**:640–656.
- Xie Z, Photowala H, Cahill ME, Srivastava DP, Woolfrey KM, Shum CY, Huganir RL, Penzes P (2008) Coordination of synaptic adhesion with dendritic spine remodeling by AF-6 and kalirin-7. *Journal of Neuroscience* **28**:6079–6091.
- Yacoubian TA, Lo DC (2000) Truncated and full-length TrkB receptors regulate distinct modes of dendritic growth. *Nature Neuroscience* **3**:342–349.
- Ye B, Zhang Y, Song W, Younger SH, Jan LY, Jan YN (2007) Growing dendrites and axons differ in their reliance on the secretory pathway. *Cell* **130**:717–729.
- Yi JJ, Barnes AP, Hand R, Polleux F, Ehlers MD (2010) TGF-beta signaling specifies axons during brain development. *Cell* **142**:144–157.
- Yoshizawa M, Sone M, Matsuo N, Nagase T, Ohara O, Nabeshima Y, Hoshino M (2003) Dynamic and coordinated expression profile of dbl-family guanine nucleotide exchange factors in the developing mouse brain. *Gene Expression Patterns* **3**:375–381.
- Yu TW, Bargmann CI (2001) Dynamic regulation of axon guidance. *Nature Neuroscience* **4**:1169–1176.
- Yu X, Malenka RC (2003) Beta-catenin is critical for dendritic morphogenesis. *Nature Neuroscience* **6**:1169–1177.
- Yuste R, Bonhoeffer T (2001) Morphological changes in dendritic spines associated with long-term synaptic plasticity. *Annual Review of Neuroscience* **24**:1071–1089.
- Yuste R, Bonhoeffer T (2004) Genesis of dendritic spines: insights from ultrastructural and imaging studies. *Nature Reviews Neuroscience* **5**:24–34.
- Zhan XL, Clemens JC, Neves G, Hattori D, Flanagan JJ, Hummel T, Vasconcelos ML, Chess A, Zipursky SL (2004) Analysis of Dscam diversity in regulating axon guidance in Drosophila mushroom bodies. *Neuron* **43**:673–686.
- Zhang H, Macara IG (2006) The polarity protein PAR-3 and TIAM1 cooperate in dendritic spine morphogenesis. *Nature Cell Biology* **8**:227–237.
- Zhang H, Webb DJ,asmussen H, Niu S, Horwitz AF (2005) A GIT1/PIX/Rac/PAK signaling module regulates spine morphogenesis and synapse formation through MLC. *Journal of Neuroscience* **25**:3379–3388.
- Zheng Y, Wildonger J, Ye B, Zhang Y, Kita A, Younger SH, Zimmerman S, Jan LY, Jan YN (2008) Dynein is required for polarized dendritic transport and uniform microtubule orientation in axons. *Nature Cell Biology* **10**:1172–1180.
- Zhu H, Luo L (2004) Diverse functions of N-cadherin in dendritic and axonal terminal arborization of olfactory projection neurons. *Neuron* **42**:63–75.
- Zhu H, Hummel T, Clemens JC, Berdnik D, Zipursky SL, Luo L (2006a) Dendritic patterning by Dscam and synaptic partner matching in the Drosophila antennal lobe. *Nature Neuroscience* **9**:349–355.
- Zhu S, Lin S, Kao CF, Awasaki T, Chiang AS, Lee T (2006b) Gradients of the Drosophila Chinmo BTB-zinc finger protein govern neuronal temporal identity. *Cell* **127**:409–422.
- Zhu YB, Kang K, Zhang Y, Qi C, Li G, Yin DM, Wang Y (2012) PLD1 negatively regulates dendritic branching. *Journal of Neuroscience* **32**:7960–7969.
- Zipursky SL, Grueber WB (2013) The molecular basis of self-avoidance. *Annual Review of Neuroscience* **36**:547–568.

Chapter 5

Local translation in dendrites

Irena Vlatkovic and Erin M. Schuman

Summary

Neurons are unique among cells with their highly polarized and elaborate dendritic and axonal arbors. Synapses, the sites of communication, are located from microns up to a meter from the cell body and rely on proteins for communication and plasticity. In order to acquire proteins to replace those that turn-over and to modify synapses, neurons use mRNAs and local protein synthesis. Local translation from dendritically localized mRNAs provides the possibility for rapid and localized response of the neuron to outside stimuli. In this chapter we review and discuss the evidence for local translation in dendrites, its regulation, roles in synaptic plasticity, learning, and memory as well as how it is dysregulated in neurodegenerative and neurodevelopmental disorders. We also present a number of technologies that are used, or could be used, to deepen our understanding of the contribution of local translation to neuronal function.

Introduction

Neurons consist of a soma and axons and dendrites that can extend for large distances (up to 1 m) and are used to form networks. The direct exchange of information between neurons occurs at synapses, inhabited by thousands of proteins that turn over with characteristic half-lives ranging from minutes to days. In the face of this turn-over, synapses manage to process local synaptic inputs and to initiate and maintain plasticity. Dendritic arbors of pyramidal neurons, for example, possess a volume of about $5,500 \mu\text{m}^3$ as well as a length of more than 50,000 μm (Hanus and Schuman, 2013). The complexity and substantial volume of dendrites, that may be further subdivided into a number of functional micro- or nanodomains, combined with their function in the receipt, signaling, and potential amplification of signals, presumably led to the need for localized and regulated protein synthesis (Hanus and Schuman, 2013; Holt and Schuman, 2013).

Local translation, the synthesis of protein from localized mRNAs, takes place in various cells where compartments are specialized to perform specific functions and temporal control is needed. In *Drosophila* oocytes, for example, the maternal mRNAs (e.g., *bicoid*, *oscar*) are localized and translated at the cellular poles and mediate the correct embryonic patterning crucial for development of the organism (for review see Johnstone and Lasko, 2001). Furthermore, it has been shown that more than 70% of mRNAs in *Drosophila* embryos are distributed according to patterns that may have functional roles (Lecuyer et al., 2007). Similarly, in *Xenopus* oocytes, the mRNA coding for VegT transcription factor is localized to the vegetal pole of the cell and determines cell fate in the embryo (King et al., 2005). In *Saccharomyces cerevisiae*, *ASH1* mRNA is one of more than 30 bud-localized mRNAs and plays a role in the determination of the cellular

mating type (for review see Paquin and Chartrand, 2008). In chick embryonic fibroblasts, the local translation of β -actin mRNA is required for directional motility (Kislauskis et al., 1997; Condeelis and Singer, 2005).

Local translation in the nervous system has diverse roles, for example in neuronal differentiation, oligodendrocyte function, axonal growth cone navigation, and in dendrites where it supplies synapses with the proteins required for basal function and plasticity. The idea that local translation may take place in axons began in the 1970s with the detection of ribosomes in young axons (Tennyson, 1970; Bunge, 1973). There is still an ongoing debate about the role and scope of local translation in axons since ribosomes are rarely observed in adult axons. Additionally, it was recently shown that Schwann cells, from which ribosomal proteins may be translocated, can be the source of some ribosomes in young axons (Court et al., 2011). One of the explanations for the apparent lack of ribosomes in old axons may be the specific organization of translational machinery in axons, where ribosomal subunits may associate with transmembrane receptors in the plasma membrane allowing translation in the vicinity of the membrane (Tcherkezian et al., 2010). For example, deleted in colorectal carcinoma (DCC) transmembrane receptors in axons have been reported to form a complex with the translation machinery. When the extracellular ligand netrin binds to the DCC transmembrane receptor, however, the translation machinery components dissociate from DCC, promoting formation of actively elongating polyribosomes and thus locally enhancing translation. This is an example of spatially specific regulation of translation by extracellular signals in both axons and dendrites (Tcherkezian et al., 2010). In axons, one of the best examples of a functional role for local translation is in the regulation of growth cone navigation. When protein synthesis inhibitors are applied in isolated retinal axons of *Xenopus*, axons lose their ability to turn toward a specific chemotropic gradient (Campbell and Holt, 2001). From these and number of other experiments it is clear that diverse growth factors or guidance cues may lead to protein translation in axons, causing repulsive or attractive turning of growth cones (Lin and Holt, 2007). After axons arrive at a specific position, local translation regulates the rapid assembly of functional synaptic boutons (Taylor et al., 2013). Thus, when axons are growing, local synthesis of proteins is crucial for both the extension and the response to specific guidance cues, leading to the correct navigation of the growth cone as well as the formation of synapses.

Key evidence for local translation in dendrites

Local translation in dendrites could not take place without the machinery required to synthesize or translate proteins. The first evidence that translational machinery is present in dendrites came from electron microscopy studies where ribosomes positioned in proximal dendrites of spinal cord motoneurons were observed (Fig. 5.1) (Bodian, 1965). Almost 20 years later, another study examined polysome positions in dendrites more quantitatively and found that about 10% of polyribosomes are positioned under the dendritic spine neck–dendritic shaft intersections and about 70% beneath the dendritic mounds that resemble the base of spines in the distal dendrites of dentate granule neurons (Steward and Levy, 1982). In addition, polyribosomes are positively correlated with enlargement of synapses, suggesting the involvement of local translation in the modification of synapses (Ostroff et al., 2002).

In the early 1990s, a number of groups used synaptosomes to test whether synapses can produce proteins locally. Synaptosomes are biochemical preparations of pinched-off synaptic spines and presynaptic terminals obtained by the homogenization of neuronal tissue and density gradient centrifugation. Synaptosomes are biochemically dissected but retain a resting



Fig. 5.1 Translational machinery is present in dendrites. Electron micrographs of dendritic spines. The black arrows point to polyribosomes present in spines. Scale bar = 200 nm.

Modified from Synapse Web, Kristen M. Harris, PI, <http://synapses.clm.utexas.edu/>

membrane potential, can still release or take up neurotransmitters, and can respond to electrical stimulation (De Belleroche and Bradford, 1972; Blaustein and Goldring, 1975). The first studies using synaptosomes showed that radiolabeled amino acids can be incorporated into protein, providing the first piece of evidence that local translation takes place under “basal” conditions in synapses (Rao and Steward, 1991; Weiler and Greenough, 1991). Furthermore, using a two-surface cell culture system to separate dendrites/axons from somata together with pulse-labeling using ^3H -leucine, Torre and Steward (1992) observed strong autoradiographical signals in dendrites, providing the first demonstration of dendritic protein synthesis *in situ*. Soon after, Feig and Lipton (1993) questioned whether dendritic protein synthesis could be affected by afferent stimulation in hippocampal slices. They applied 10-Hz electrical stimulation to hippocampal slices in conjunction with the acetylcholine agonist carbachol and detected an increase in translation evident in the dendrites after 3 min, as measured by ^3H -leucine incorporation, although this protocol had no long-lasting effect on synaptic transmission.

The first evidence that local translation in dendrites is important for synaptic enhancement came from experiments in which growth factors, brain-derived neurotrophic factor (BDNF) or neurotrophin-3 (NT-3), were applied to hippocampal slices, causing a rapid enhancement of synaptic transmission, measured with extracellular field potentials in the neuropil hundreds of microns from the cell body layer (Fig. 5.2) (Kang and Schuman, 1995, 1996). Co-application of a protein synthesis inhibitor blocked the early potentiation elicited by the growth factor, indicating that the source of protein synthesis was local. The local nature of protein synthesis was addressed by recording from hippocampal slices in which the synaptic neuropil was physically isolated from the cell body layer (Kang and Schuman, 1996). With this study it became clear that local translation plays a functional role in synaptic plasticity. But can local translation allow synapses to respond individually to specific inputs? Martin et al. (1997) used *Aplysia* sensory-motor neuronal cultures (consisting of SN-MN synapses) to address this question. It was already known that when serotonin is repeatedly applied to SN-MN synapses during a defined

time window, long-term facilitation (LTF) occurs (Sherff and Carew, 2002). Martin et al. (1997) used the SN–MN system to show that local perfusion of serotonin at specific synapses leads to the development of LTF specifically at the perfused synapses. Inclusion of a protein synthesis inhibitor in the local perfusion medium blocked the effect of serotonin at the perfused neurite, showing that local protein synthesis is required for the induction of LTF and that this process can be neurite-specific. A role for local translation in late-phase LTP was also shown via local perfusion of protein synthesis inhibitors in synaptic regions in hippocampal slices (Bradshaw et al., 2003; Vickers et al., 2005). Local translation is also required for metabotropic glutamate receptor (mGluR)-dependent long-term depression (LTD) (Huber et al., 2000). Bath application of protein synthesis inhibitors blocked mGluR-dependent LTD in hippocampal slices in which the neuropil was isolated from the somatic region. Moreover, local protein synthesis plays a role in activating silent synapses upon dopamine activation through enhanced translation of

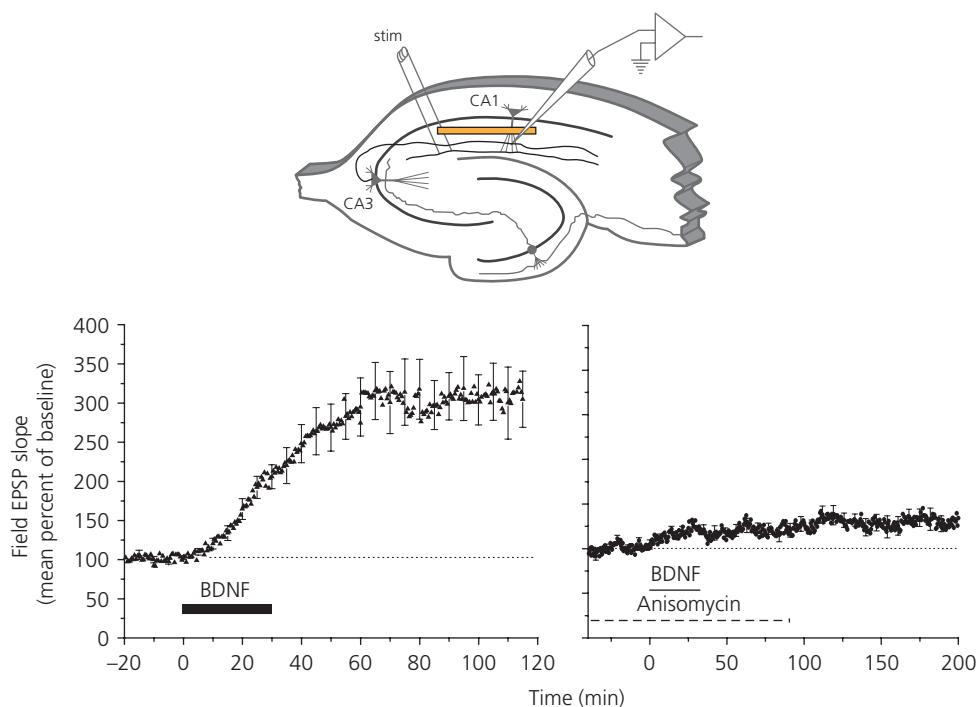


Fig. 5.2 Local translation plays a functional role in synaptic enhancement. Upper panel: schematic of a hippocampal slice with a microlesion (orange) to dissociate the cell bodies (stratum pyramidale) of CA1 neurons from the neuropil (stratum radiatum) where stimulation and electrophysiological recordings of excitatory postsynaptic potentials (EPSPs) were performed. Lower panel: slope of field EPSPs before and after application of BDNF or BDNF and the protein synthesis inhibitor anisomycin. The duration of application of each drug is shown by full or dashed lines below the treatment.

Adapted from Hyejin Kang and Erin M. Schuman, A requirement for local protein synthesis in neurotrophin-induced hippocampal synaptic plasticity, *Science*, 273(5280), pp. 1402–1406, © 1996, The American Association for the Advancement of Science. Reprinted with permission from AAAS.

the GluR1 subunit of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, as well as increased incorporation of surface GluR1 at synapses (Smith et al., 2005). All of these studies demonstrate that local protein synthesis is required for some forms of synaptic plasticity.

Does local translation in dendrites also take place under basal, unstimulated, conditions? To address this question Sutton et al. (2004) focused on examining the regulation of local translation by spontaneous excitatory synaptic events (miniature excitatory postsynaptic potentials or currents, also known as “minis”). They blocked action potentials or blocked both action potentials and minis in cultured hippocampal neurons and measured local translation using a GFP-based reporter and time-lapse microscopy. Interestingly, blocking minis led to an enhancement in local protein synthesis, indicating that under normal conditions minis repress local translation. Subsequently the authors examined the mechanism for this and showed that *N*-methyl-D-aspartic acid receptor (NMDAR) signaling, mediated by minis, leads to suppression of dendritic protein synthesis and stabilizes synaptic function in hippocampal neurons (Sutton et al., 2006). Blocking minis leads to a protein synthesis-dependent scaling up of synaptic transmission, resulting in enhanced surface expression of GluA1.

So far we have discussed the key evidence that local translation plays a role in synaptic plasticity, thought to be the molecular substrate of some forms of learning and memory. But what is the direct evidence that local translation participates in behavioral memories? Miller et al. (2002) tested if the lack of dendritic calcium/calmodulin-dependent protein kinase 2 alpha (CAMK2a) mRNA in mouse may lead to changes in memory by mutating the endogenous CAMK2a and disrupting signal coding for dendritic localization in its 3' untranslated regions (UTRs). The authors discovered that mice that lacked CAMK2a mRNA in dendrites exhibited reduced learning in both the Morris water maze and contextual fear conditioning, as well as impairments in spatial and object recognition memory. In *Drosophila*, Keleman et al. (2007) used courtship behavior to monitor long-term behavioral memory in mushroom body neurons in control versus cytoplasmic polyadenylation binding protein (CPEB) ortholog Orb2 mutants. Previously, a number of studies showed that CPEB proteins are regulators of local translation (for review see Darnell and Richter, 2012). The authors found that the *Drosophila* CPEB homolog Orb2 is acutely required for formation of long-term memory of courtship behavior in *Drosophila*.

These experiments indicate that translational machinery locally present in dendrites (e.g., polysomes) is required for protein synthesis. Localized mRNAs are the second key element that allows local translation to take place. Bagni et al. (2000) showed that specific mRNAs (CAMK2a, InsP3R1, and Arc) associate with polysomes in synaptosome fractions from mouse neocortex. Additionally, in the same study the authors showed that membrane depolarization leads to an increase in the association of CAMK2a mRNA with polysomes as well as an increase in CAMK2a protein. This study was followed by a number of others (Yin et al., 2002; Dong et al., 2003; Di Nardo et al., 2007; Troca-Marin et al., 2010; Dziembowska et al., 2012; Lin et al., 2012) showing translation of specific mRNAs locally in synaptic fractions or dendrites, resulting in cumulative evidence for the translation of about a dozen locally translated mRNAs. Recently, new approaches on a genome-wide level that have examined Purkinje cell compartments have demonstrated that many more mRNAs than previously thought are locally translated in dendrites (Kratz et al., 2014). Nevertheless, future studies are needed to fully understand the full scope and dynamics of the dendritic translatome under basal conditions, as well as after different pharmacological treatments or learning paradigms. In Table 5.1, we present a short historical overview of the key evidence supporting local translation in dendrites.

Table 5.1 Historical overview of key experiments demonstrating local translation in dendrites

Key evidence supporting local dendritic translation	Methods and technology	Major findings	References
Existence of translational machinery	Electron microscopy	Ribosomes present in proximal dendrites	Bodian (1965)
	Electron microscopy	Polyribosomes under basis of spines	Steward and Levy (1982)
Demonstration of large mRNA populations in dendrites	<i>In situ</i> hybridization	>50 mRNAs localized to dendrites/axons	Lein et al., 2007
	Microarrays	About 285 mRNAs localized to dendrite-enriched samples	Poon et al., 2006; Zhong et al., 2006
	Deep RNA sequencing	Dramatic expansion of local transcriptome: \approx 2,550 mRNAs localized to dendrites/axons	Cajigas et al. (2012)
Synthesis of proteins in synapses and dendrites	Radiolabeling of amino acids	Proteins are synthesized in synaptosomes	Rao and Steward (1991), Weiler and Greenough (1991)
	Synaptosome preparations	Synthesis of proteins in dendrites <i>in situ</i>	Torre and Steward (1992)
	Two-surface cell culture pulse-radiolabeling		
Local translation detected <i>in situ</i>	Electrical and chemical stimulation, pulse-radiolabeling	Translation increased upon carbachol stimulation after 3 min of labeling	Feig and Lipton (1993)
Local translation visualized; used for synaptic plasticity	Stimulation by neurotrophins	L-LTP in severed dendrites upon stimulation, abolished by protein inhibitors	Kang and Schuman (1996)
	Dendrites dissociated from soma		
	Electrophysiology		
Aplysia SN–MN culture		LTF only in stimulated neuronal branch, abolished by protein inhibitors	Martin et al. (1997)
	Stimulation with serotonin		
	Electrophysiology		
Metabolic labeling with ^{35}S -methionine			
Application of mGluR agonist on severed dendrites		LTD induction within minutes upon mGluR activation, only in the absence of protein inhibitors	Huber et al. (2000)
	Electrophysiology		

Table 5.1 (continued) Historical overview of key experiments demonstrating local translation in dendrites

Key evidence supporting local dendritic translation	Methods and technology	Major findings	References
Local translation regulates homeostasis	Pharmacological block of action potential and/or minis	Minis inhibit local translation through NMDAR signaling	Sutton et al. (2004)
	GFP-based reporter time-lapse microscopy		Sutton et al. (2006)
Local translation as a basis of learning and memory	Mouse knock-out of dendritic form of CAMK2a	Low performance in the Morris water maze and contextual fear conditioning	Miller et al. (2002)
	Behavioral studies	Impairments in spatial and object recognition memory	
Specific mRNAs are locally translated in dendrites	<i>Drosophila</i> mutants of a regulator of local translation	Regulation of local translation is crucial for the formation of long-term memory of courtship behavior	Keleman et al. (2007)
	Behavioral studies		
	Synaptosome fractionation	CAMK2a, InsP3R1, and Arc mRNAs associate with polysomes in synapses	Bagni et al. (2000)
	Polysome profiling		
	TRAP, nanoCAGE	Large number of mRNAs are translated in Purkinje dendrites; 20 of these show enriched translation in dendrites versus somata	Kratz et al. (2014)

mRNAs localized to dendrites: the potential for local translation

A remarkable feature of RNA is its ability to be transported and localized at specific domains in cells. Once localized, the expression of the gene from which RNA was transcribed may be regulated both spatially and temporally (for reviews see Martin and Ephrussi, 2009; Holt and Schuman, 2013). To mediate localization to a specific compartment, most RNAs contain *cis*-acting elements, often called “zipcodes” (typically in 3' UTRs, but sometimes also in 5' UTRs or in the coding sequence) that are recognized by specific RNA-binding proteins (RBPs) (Martin and Ephrussi, 2009). The RBPs together with the targeted mRNA build ribonucleoprotein (RNP) particles (Knowles et al., 1996; Krichevsky and Kosik, 2001). These RNPs can be assembled into granules and are thought to be transported to specific dendritic sites where they may be anchored and their translation is repressed until needed (Besse and Ephrussi, 2008; Martin and Ephrussi, 2009; Martin, 2010). A recent study provided concrete evidence for this idea (Buxbaum et al., 2014). Singer and colleagues used high-resolution imaging of β -actin mRNA particles in combination with imaging of ribosomes and ribosomal RNA. They developed single-molecule assays that allowed absolute quantification of mRNA and in addition were able to detect ribosomes and translational events in dendrites. The authors showed that β -actin mRNA and ribosomes are masked by the proteins building RNPs and then unmasked when exposed to neural activity. The released, unmasked mRNAs are available for translation and the authors showed that these mRNAs are indeed locally translated in dendrites.

How many mRNA are present in dendrites? For many years, dendritic localization was clear for only a handful of mRNAs [e.g., CAMK2a alpha and beta subunits, Shank family mRNAs, microtubule-associated protein 2 (MAP2), β -actin] detected by *in situ* hybridization technologies (Garner et al., 1988; Burgin et al., 1990; Tiruchinapalli et al., 2003; Bockers et al., 2004). Using the same technology, but in a high-throughput manner, the Allen Brain Atlas detected more than 50 mRNAs localized to hippocampal neuropil, as well as in the dendritic/axonal part of the cerebellar molecular layer (Lein et al., 2007).

With the advance of genomic technologies it has become possible to test if larger sets of mRNAs are localized in dendrites. Poon et al. (2006) used primary cell cultures grown on polycarbonate filters containing pores and microarray technology. The 3- μ m pores allowed separation of cellular somata, which remained on one side of the filter, and the dendrites/axons that grew through the pores. After separating the cultured dendrites/axons/glial processes, the authors isolated RNA and performed a microarray analysis, identifying more than 100 mRNAs in the dendritic/axonal fraction. In another study, Zhong et al. (2006) used microdissection of acute hippocampal slices in combination with microarrays. Using microdissection, the authors isolated the stratum pyramidale (containing mostly pyramidal neuron cell somata) and the stratum radiatum and lacunosum moleculare (containing mostly axons and dendrites, also known as “neuropil”), performed microarray analysis and detected 154 neuropil-localized mRNAs. Together these two studies expanded the number of mRNAs localized to dendrite-enriched samples from a dozen to about 285. Recently, using high-throughput RNA-sequencing Cajigas et al. (2012) showed that the total number of mRNAs present in the neuropil layer of area CA1 in the hippocampus is more than 8,300. In this study, the authors also microdissected acute hippocampal slices and separately analyzed the somatic and neuropil fractions. In order to determine whether the approximately 8,300 mRNAs detected arise from dendrites or axons, the authors filtered out glial, interneuronal, endothelial, nuclear, and mitochondrially expressed mRNAs, resulting in a conservative estimate of 2,550 mRNAs present in dendrites/axons. Among these transcripts, glutamate receptors, diverse voltage-gated ion channels, a number of postsynaptic density scaffolding as well as calcium signaling molecules were detected (Fig. 5.3). The study uncovered a previously unappreciated potential for local translation in dendrites/axons of hippocampal neurons *in vivo*.

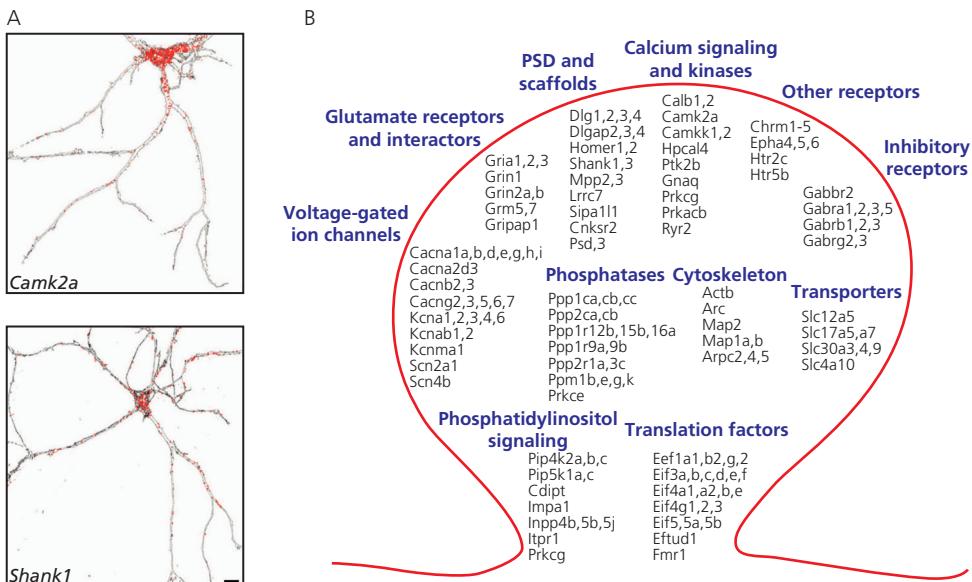


Fig. 5.3 mRNAs are localized in dendrites. **(A)** *In situ* hybridization of calcium/calmodulin-dependent protein kinase 2 alpha (CAMK2a) and SH3 and multiple ankyrin repeat domains 1 (Shank1) mRNAs (red particles) in cultured hippocampal neurons shows the localization of these mRNAs in dendrites. The neurons were immunostained with an anti-microtubule-associated protein 2 (MAP2) antibody (gray outline). **(B)** A conservative estimate of mRNA families present in the postsynaptic compartment.

Reprinted from *Neuron*, 74(3), Iván J. Cajigas, Georgi Tushev, Tristan J. Will, Susanne tom Dieck, Nicole Fuerst, Erin M. Schuman, The local transcriptome in the synaptic neuropil revealed by deep sequencing and high-resolution imaging, pp. 453–66, Figures 6a and 5c, Copyright 2012, Elsevier. With permission from Elsevier.

Technologies for studying local translation: from single mRNAs to the translatome

To study mRNA localization or local translation in dendrites, it is desirable to use a model system that will allow the following: (1) the possibility to distinguish dendrites from axons and glia; (2) the separation of dendrites from somata; (3) the possibility to manipulate the system pharmacologically (e.g., by growth factors or pharmacological treatments); (4) the possibility to test mRNA localization or local translation and their effects at the cellular, circuit, or organism levels.

To study the local translation of specific transcripts, translation reporters consisting of fluorescent proteins combined with UTRs harboring zipcodes that will target the reporter to the dendrite were used to track the dynamics of protein synthesis in living cells (for review see Chao et al., 2012) (Fig. 5.4A). For example, Schuman and co-workers designed a reporter, consisting of GFP flanked with the 5' and 3' UTRs of CAMK2a [previously found to harbor dendritically targeting zipcodes and cytoplasmic polyadenylation elements (CPEs) regulating translation; Mayford et al., 1996; Wu et al., 1998; Mori et al., 2000], as well as a myristoylation sequence to slow down diffusion. Time-lapse imaging of dendrites from neurons transfected with the above construct demonstrated an increase in fluorescence in the dendrites after treatment with BDNF (Aakalu et al., 2001). Neither transection of the dendrites nor repeated photobleaching of the soma abolished the fluorescence

increase—indicating that it originated in the dendrites. As such, this study was one of the first examples of the successful use of a dendritically targeted reporter in a study of local translation. Fluorescent reporters are under constant development, and today new photoconvertible or photoswitchable reporters can be used, allowing the detection of newly synthesized proteins in live cells with high spatial and temporal resolution. For example, Wang et al. (2009) used a photoconvertible Dendra2 reporter flanked by 5' and 3' UTRs of *sensorin* mRNA (to localize the reporter to synapses) to show that serotonin stimulates local translation in *Aplysia* SN–MN cultures. After illumination with blue light, Dendra2 is irreversibly converted from green to red fluorescence (Gurskaya et al., 2006) and this feature can be used to establish a baseline prior to stimulation such that all newly synthesized proteins will be green (Fig. 5.4B). Fluorescent reporters are clearly a great tool, but they have some disadvantages: (1) they can diffuse from the site of synthesis (this can be improved by using myristylation; Aakalu et al., 2001); (2) fluorescent proteins are not immediately observable after their synthesis since they must undergo obligatory post-translational modifications (this can be improved by using TurboGFP that has faster protein folding kinetics and chromophore maturation; Evdokimov et al., 2006); and (3) phototoxicity can occur if photoconversion or photobleaching are used. In order to circumvent some of these problems, the TimeSTAMP (TS) technology was developed (Lin et al., 2008). TS uses a time-specific tag that detects the age of a protein of interest and provides good temporal resolution for the detection of newly synthesized proteins in primary cultures and whole *Drosophila* brain. The method is based on the use of a sequence-specific protease that will remove epitope tags from the protein of interest unless a protease inhibitor is added. Practically, the protein of interest is fused to a protease flanked by protease sites and a tag [e.g., hemagglutinin (HA) that will later be recognized by immunocytochemistry] (Fig. 5.4C). The protease cleaves the tag when protease inhibitor is not present, thus the HA tag is not visible. All proteins synthesized after the addition of the protease inhibitor will retain their tags, allowing for the detection of newly synthesized proteins (Lin et al., 2008). However, this method still does not allow for live imaging of the dynamics of local translation. An updated version of TS, using fluorescent tags or photo-oxidizable tags, was developed to allow live tracking of protein dynamics or the detection of newly synthesized proteins by electron microscopy, respectively (Butko et al., 2012). Application of this technology revealed plasticity-induced local translation of the scaffolding molecule PSD95 in stimulated synapses of rat neurons (Butko et al., 2012).

There are several techniques available to study local translation in dendrites at a global level: (1) detection of local translation directly by measuring or identifying the mRNAs bound to ribosomes; (2) determination of active translation sites by detecting fluorescence resonance energy transfer (FRET) from labeled tRNAs or by puromycin-based methods; (3) direct detection of newly synthesized proteins using non-canonical amino acids (BONCAT, bio-orthogonal non-canonical amino acid tagging, and FUNCAT, fluorescent non-canonical amino acid tagging).

One technology that allows for the detection of ribosome-bound mRNAs is polysome profiling (Fig. 5.5), a technique that uses sucrose density gradients to separate different fractions of mRNAs bound to single ribosomes (monosomes) or polysomes. Fractionation can be followed by isolation of mRNAs and the preparation of cDNA libraries that can be further processed by whole-genome expression detection technologies (e.g., microarrays, RNA-sequencing) (Johannes et al., 1999; Zong et al., 1999; Schratt et al., 2004; Gandin et al., 2014). Polysome profiling is based on the assumption that all mRNAs found in polysomal fractions containing more than three ribosomes (“heavy polysomes”) are in the process of active translation (Gandin et al., 2014; Piccirillo et al., 2014), although there is still no clear consensus if mRNAs bound by one or two ribosomes are poised to be translated, stalled on the ribosome, or actually translated. While it is possible to

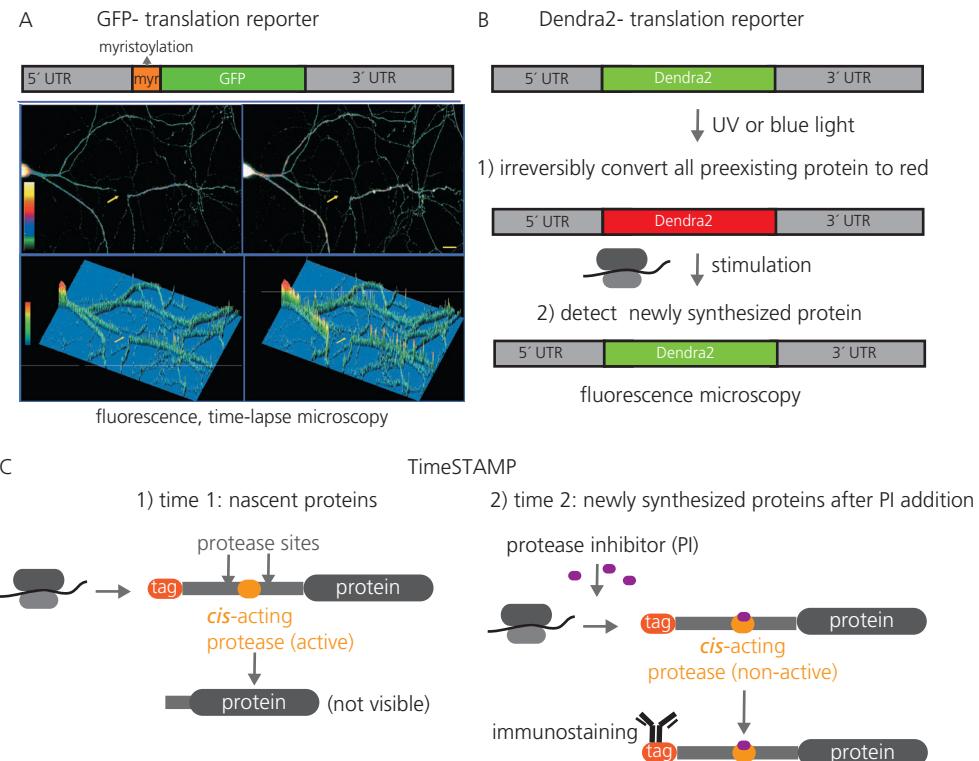


Fig. 5.4 Technologies to study local translation in a transcript-specific manner. (A) Top: schematic of a GFP-translation reporter containing the 5' and 3' untranslated regions (UTRs) harboring dendritic targeting elements (zip codes), a myristylation sequence, and the coding sequence for green fluorescent protein (GFP). Upper panel: images of transected neuron (the yellow arrow represents the transection point) before (left) and after (right) treatment with brain-derived neurotrophic factor (BDNF). Scale bar = 15 μ m. Lower panel: X-Y-Z plot of neurons from the upper panel showing color and height changes of fluorescent pixels. (B) Schematic of the Dendra2-translation reporter system consisting of 5' and 3' UTRs and the photoconvertible Dendra2 protein. Upon application of UV or blue light pre-existing Dendra2 is converted from green to red. Upon stimulation newly synthesized protein appears as green, allowing detection of newly synthesized proteins by fluorescence microscopy. (C) TimeSTAMP technology is based on the fusion of a protein of interest to a tag and a protease, flanked by protease sites. After the engineered protein has attached to the ribosome and translation starts, the protease cleaves the tag and the protein is still not visible. When protease inhibitor (PI) is added the protease is not active and the tag stays on the newly synthesized protein which can be visualized by immunostaining.

Part A reprinted from *Neuron*, 30(2), Girish Aakalu, W. Bryan Smith, Nhien Nguyen, Changan Jiang, and Erin M. Schuman, Dynamic visualization of local protein synthesis in hippocampal neurons, pp. 489–502, Figure 4a and b, Copyright 2001, Elsevier. With permission from Elsevier.

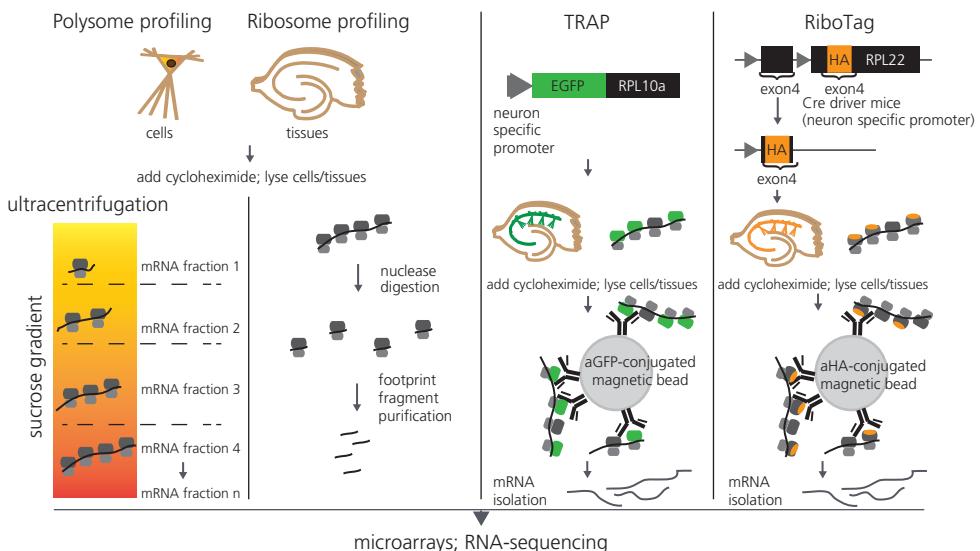


Fig. 5.5 Technologies for studying local translation by detection of mRNAs bound to ribosomes. Four technologies, polysome or ribosome profiling; translating ribosome affinity purification (TRAP) and RiboTag, to measure mRNAs bound to ribosomes are depicted from right to left, respectively. In the first step, the mRNA “freezes” on the ribosome upon addition of cycloheximide and cells are lysed. Polysome profiling is based on the ultracentrifugation of lysed cells through a sucrose gradient and separation of fractions containing a defined numbers of ribosomes and mRNAs bound to them. Nuclease digestion of unprotected mRNA and purification of undigested ribosome-protected mRNA fragments are central in ribosome profiling, allowing precise detection of the number of ribosomes bound to mRNA. The TRAP and RiboTag technologies use transgenic mice harboring a fusion of the ribosomal subunit protein, RPL10a or RPL22 with EGFP or a HA tag, respectively. TRAP allows purification of ribosome-bound mRNAs from neurons since expression of the EGFP-RPL10a fusion can be driven by a neuronal cell-type-specific promoter, whereas HA-RPL22 can be crossed with various Cre driver mice to achieve cell-type specificity. Both technologies are based on affinity purification by magnetic beads using specific antibodies (anti-GFP or anti-HA), followed by isolation of mRNA. In the last step, all four technologies allow for the genome-wide detection of ribosome-bound mRNAs, for example by whole-genome microarrays or RNA-seq.

distinguish fractions of one, two, or three ribosomes, polysome profiling does not allow the full quantitative detection of ribosome number per fraction.

Ribosome profiling was developed to further quantitatively dissect translation on a genome-wide level (Fig. 5.5) (Ingolia et al., 2009). Ribosome profiling uses cycloheximide treatment to block translation elongation, thus providing a snapshot of ribosome positions on the mRNA. Ribosomes are then purified together with the bound mRNA and the specific regions that are being translated can be determined as they are protected from nuclease digestion (Ingolia et al., 2009). Ribosome profiling allows for the annotation of translated “known” mRNAs, but also reveals novel variants of known proteins by detecting new translation initiation sites as well as novel translated sequences. Ribosome profiling also allows for the detection of the number and position of ribosomes on the mRNA, allowing one to quantify the extent of translation of a particular

mRNA (Ingolia et al., 2012, 2013). Further, ribosome profiling allows one to study translational regulation. For example, two studies showed that all mRNA targets of rapamycin [the mammalian target of rapamycin, (mTOR) kinase pathway] contain variants of 5' terminal oligopyrimidine (5' TOP) sequence motifs that may be the basis for translational regulation of these transcripts (Hsieh et al., 2012; Thoreen et al., 2012).

What is missing in polysome and ribosome profiling studies is the ability to discover the translatome of a specific cell-type. The TRAP (translating ribosome affinity purification) (Heiman et al., 2008) and RiboTag technologies (Sanz et al., 2009) add this feature (Fig. 5.5). TRAP technology is based on the development of BacTRAP mice that express enhanced green fluorescent protein (EGFP)-tagged ribosomal protein RPL10a under the control of a cell-type specific promoter (e.g., Pcp2, Drd1, Cort) (Doyle et al., 2008). GFP antibodies bound to beads are used to immunopurify the cycloheximide-stalled ribosomes along with the associated mRNA molecules. In the next step, the mRNA is purified and then whole-genome microarrays or RNA-sequencing (RNA-seq) may be applied. In addition to mice, TRAP has also been used to dissect the translatomes of specific cell types in other model organisms such as *Drosophila*, *Xenopus* and zebrafish (Housley et al., 2014; Huang et al., 2013; Watson et al., 2012). One group recently used TRAP in mouse cerebellar slices that were microdissected to separate the somata and dendrites and later RNA-sequenced after nanoCAGE (cap analysis of gene expression) library preparation (Kratz et al., 2014). By using a cell-type-specific promoter, the local translatome of Purkinje dendrites was analyzed under basal conditions and 20 genes whose translation was enriched in dendrites compared with soma were discovered (Kratz et al., 2014).

In the second approach, a RiboTag mouse has been developed in which HA tags are fused, in a Cre-recombinase-dependent manner, to ribosomal protein Rpl22 (Sanz et al., 2009). Thus, after crossing these mice with various Cre recombinase lines, tagged Rpl22 is only expressed in those cells expressing Cre. The tagged Rpl22 can be immunopurified using anti-HA antibodies followed by mRNA isolation and genome-wide expression detection technologies. The ability to cross the RiboTag mice with number of neuronal-specific Cre recombinase-expressing mice as well as the near wild-type levels of tagged ribosomal subunits are some of advantages of RiboTag technology over BacTRAP technology (Kapeli and Yeo, 2012). The technology has not yet been used to examine the local translation in dendrites. A related technique, phosphorylated ribosome capture, uses natural phosphorylation of ribosomal protein S6 to enrich for mRNAs expressed in activated cells using immunoprecipitation with anti-phosphoS6 antibodies followed by RNA-seq (Knight et al., 2012). This technology allows for the detection of mRNAs bound to ribosomes phosphorylated upon activation of phosphoinositide 3-kinase/mTOR, mitogen-activated protein kinase (MAPK), and protein kinase A signaling pathways. TRAP, RiboTag, and phosphorylated ribosome capture are all based on the assumption that any mRNA bound to a tagged ribosome is translated; none of these techniques provide quantitative information about how many ribosomes are bound to mRNA. Thus, it is possible that mRNAs that interact with a single ribosome will also be purified and indicated as translationally active. Nevertheless, these are clearly valuable techniques that may allow whole-translatome detection in a specific neuronal cell type or only in activated neurons and thus will be useful for examining local translation in dendrites *in vivo*.

An additional method to detect local translation by examining sites of active translation is fluorescent tRNA for translation monitoring (FtTM) and involves the use of FRET and fluorescently labeled tRNAs (Barhoom et al., 2011) (Fig. 5.6A). For example, stimulated astrocytes were cotransfected with Cy3-tRNA and a Rho110-tRNA that allowed detection of the FRET signal when both tRNAs were loaded into adjacent positions on the translating ribosome. This technique allows

one to detect the sites of protein synthesis in living cells with high sensitivity, while with lower sensitivity it also allows for the quantification of translation events (Barhoom et al., 2011). Another group of methods that labels sites of translation is based on the use of puromycin, a bacterial product that enters the ribosome at the acceptor site and leads to premature termination of protein synthesis (Fig. 5.6B). Puromycin can be used in the ribopuromycylation method (RPM) where ribosomes actively translating novel peptide chains are visualized by a puromycin-specific monoclonal antibody in a quantitative fashion, allowing clear spatial resolution (David et al., 2011). Fluorescently labeled puromycin (fluorescein-dC-puromycin) has also been used in hippocampal neurons to visualize protein synthesis in dendrites, where an increase in local protein synthesis is detected upon application of a dopamine receptor agonist (Smith et al., 2005). In addition, click-chemistry has been combined with the ability of puromycin to detect newly synthesized proteins, using O-propargyl-puromycin (OP-puro) (Liu et al., 2012). OP-puro can be injected into mouse tissues of interest, fixed and then “clicked” with tetramethylrhodamine-azide in order to visualize local translation in vivo.

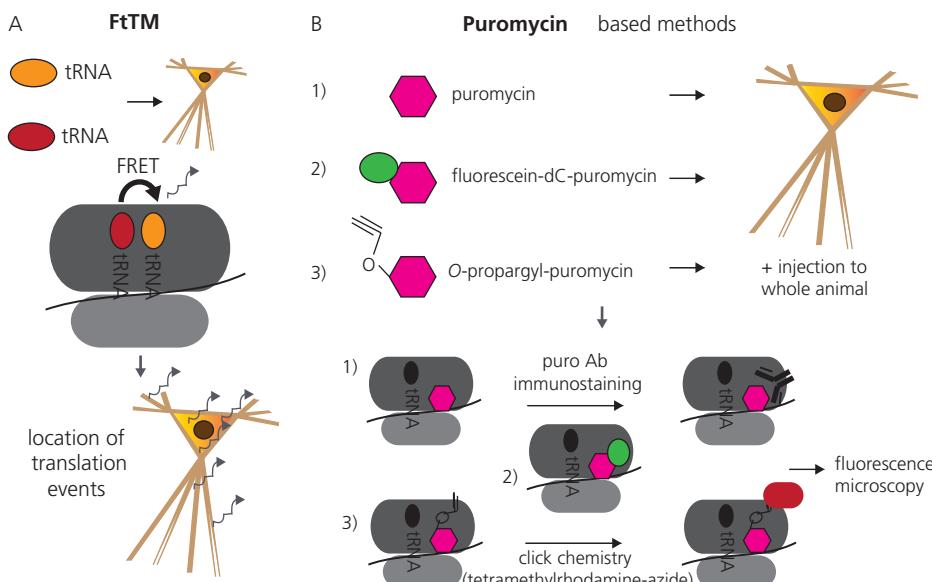


Fig. 5.6 Technologies to detect sites of active translation. **(A)** Fluorescent tRNA for translation monitoring (FtTM) involves labeling of tRNAs with two fluorescent dyes (shown as orange and red ellipses), delivery of labeled tRNAs to the cells, and detection of a fluorescence resonance energy transfer (FRET) signal, representing tRNAs loaded to adjacent positions on the ribosome. The signal allows for the detection of location of translation events. **(B)** Schematic diagram of an overview of puromycin-based methods for the detection of translation events by fluorescence microscopy. Puromycin (depicted as a purple hexagon) binds to an acceptor site on the ribosome and terminates translation. In the first method, antibodies against puromycin are used to immunostain translation sites in the cell. In the second approach puromycin is labeled by fluorophore (green ellipse = fluorescein) and directly visualized by fluorescent microscopy. The third approach uses modified puromycin (o-propargyl-puromycin) that can be injected into an animal and after tissue fixation clicked by tetramethylrhodamine-azide to allow the visualization of local translation sites by fluorescence microscopy.

Non-canonical amino acids represent a promising technique to label endogenous full-length, newly synthesized proteins. Two techniques, BONCAT and FUNCAT (Dieterich et al., 2006, 2007, 2010; Szychowski et al., 2010) allow one to label newly synthesized proteins and quantify/identify them with mass spectrometry (BONCAT) or visualize them *in situ* (FUNCAT) (Fig. 5.7). In both methods, the methionine analogs azidohomoalanine (AHA) or homopropargylglycine (HPG) that bear azide and alkyne groups, respectively, are charged onto methionine-tRNAs by endogenous methionyl-tRNA synthetases (MetRS) and incorporated into newly synthesized proteins. They can be further detected using “click chemistry” where Cu(I)-catalyzed reaction affinity-tags or fluorescent-tags can be added. Thus, in BONCAT, affinity purification and subsequent mass spectrometry-based methods can be used to detect and/or quantify newly synthesized proteome and how it changes in different conditions, while FUNCAT allows for the visualization of a full set of newly synthesized proteins *in situ* in single cells or tissues using microscopy. Hodas et al. (2012) applied BONCAT in a study of local translation in pharmacologically treated hippocampal neurons. They treated acute hippocampal slices with a dopamine receptor agonist and at the

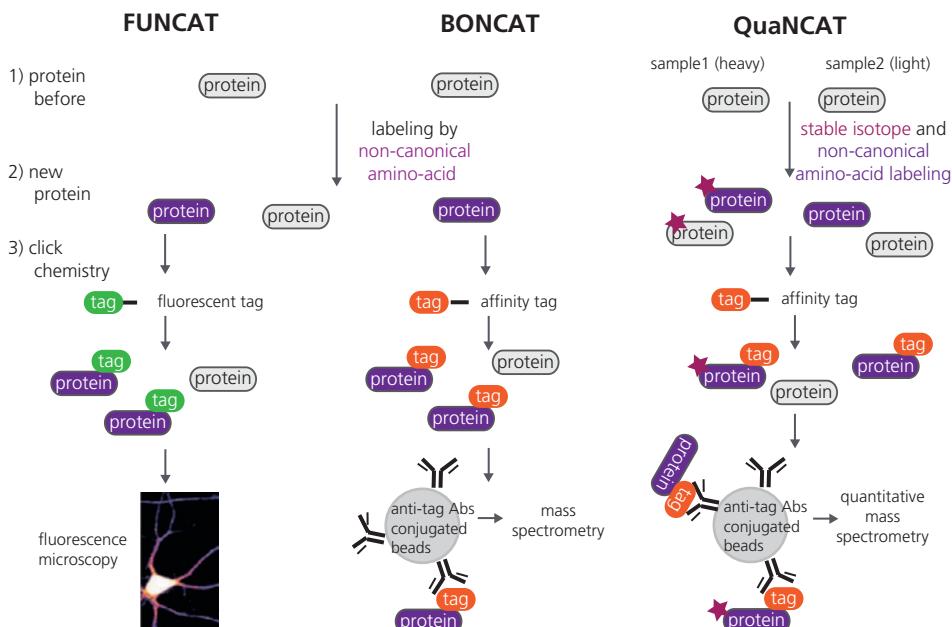


Fig. 5.7 Technologies for detecting newly synthesized proteins. The schematic represents a comparison of three technologies for the detection of newly synthesized proteins by fluorescent microscopy [fluorescent non-canonical amino acid tagging (FUNCAT)] or mass spectrometry [bio-orthogonal non-canonical amino acid tagging, (BONCAT) and quantitative non-canonical amino acid tagging (QuaNCAT)]. While the first step in FUNCAT and BONCAT is non-canonical amino acid labeling, in QuaNCAT both stable isotopes and non-canonical amino acid labeling are used. The fluorescent tags (green, in FUNCAT) or affinity tags (red, in BONCAT and QuaNCAT) are added to newly synthesized proteins labeled with non-canonical amino acids using a click chemistry reaction. This allows for the visualization of newly synthesized proteins by fluorescent microscopy in FUNCAT or affinity purification using anti-tag conjugated beads followed by mass spectrometry in BONCAT and QuaNCAT.

same time used AHA to label newly synthesized proteins. Moreover, they microdissected slices to isolate somata and neuropil and affinity-purified proteins. These were subsequently analyzed by mass spectrometry and about 300 newly synthesized proteins were identified in the dendrites and axons. Additionally, they detected a neuropil-specific increase in local protein synthesis upon D1/D5 receptor agonist treatment (Hodas et al., 2012). Recently, SILAC (stable isotope labeling by amino acids in cell culture) and BONCAT have been combined to produce QuaNCAT (quantitative non-canonical amino acid tagging) leading to substantial improvements in BONCAT data quantification (Howden et al., 2013). In this study both stable-isotope amino acid labeling and non-canonical amino acid labeling was used in primary cells of the immune system, but application to the nervous system should not be difficult. In these techniques, the direct examination of newly synthesized proteins circumvents some of the problems associated with the translational profiling data. Nevertheless, there are issues inherent to mass spectrometry (e.g., low-abundance proteins are typically not detectable) but the rapid improvement in mass spectrometry machines and analyses will hopefully increase sensitivity in the coming years.

To address the visualization and dynamics of newly synthesized proteins in hippocampal neurons, Dieterich et al. (2010) applied FUNCAT to both primary neurons and organotypic slices. After inhibiting protein synthesis by local perfusion of anisomycin to neuronal somata, protein synthesis in the dendrites was observed using FUNCAT. This synthesis was enhanced after the application of BDNF. In the same study the authors used FUNCAT to label newly synthesized neurons in living cells. They incubated neurons with AHA and then covalently coupled its azide group with a difluorinated cyclooctyne (DIFO) (membrane impermeable) bearing a biotin group (for clicking). In the second step they labeled the living cells with streptavidin-conjugated quantum dots and characterized the dynamics of newly synthesized quantum-dot labeled proteins that were present on the surface of neurons (Dieterich et al., 2010). Recently, FUNCAT was also used in vivo in larval zebrafish where an increase in protein synthesis upon application of a gamma-aminobutyric acid (GABA) antagonist was observed (Hinz et al., 2012). To study local translation in *Xenopus* retinal ganglion cell axons and its change upon axon guidance cue stimulation, FUNCAT was combined with the two-dimensional (2D)-difference gel electrophoresis (DIGE) technology (Yoon et al., 2012). In particular, AHA was incorporated into newly synthesized proteins and then, in a click chemistry reaction, fluorescently labeled with tetramethylrhodamine (TAMRA). In the second step these locally synthesized labeled axonal peptides were mixed with CyDye-labeled or -unlabeled lysates and run on a 2D gel from which specific spots were taken for matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS), which led to the discovery of specific newly synthesized axonal proteins highly enriched or depleted upon stimulation. This technique showed great sensitivity in detecting the dynamics of the axonal proteome. As an interesting possibility for the future, an engineered MetRS enzyme that recognizes the non-canonical amino acid azidonorleucine can be used in combination with both BONCAT and FUNCAT to achieve cell-type-specific labeling of newly synthesized proteins in vivo (Ngo et al., 2012).

This large spectrum of technologies has already proved useful or may be useful in future studies of local translation. Each of these technologies has both advantages and disadvantages when used with specific neuronal model systems providing a range of spatial, temporal, and molecular resolutions.

Regulated local translation in dendrites

Local protein synthesis in dendrites allows for the rapid and specific response of a neuron to external stimuli. This highly orchestrated response is regulated on many levels, some of which we

describe here: (1) control of the availability of mRNA at the dendrite; (2) regulation at the level of translational initiation; (3) regulation at the level of translational elongation; (4) regulation dependent on the existence of an internal ribosomal entry site (IRES); and (5) regulation by cytoplasmic polyadenylation.

Dendritic mRNAs that are to be translated upon stimulation, at a specific time, at a specific place, are first transported and targeted to their site in a state of translational repression and are then de-repressed to be available for translation. Steward et al. (1998) found that newly synthesized mRNA of the immediate early gene *Arc* specifically targets to active parts of dendrites in a protein synthesis-independent manner. Moreover, they observed that the Arc protein was increased only in the activated parts of dendrites, indicating that it is locally translated. As already discussed, hundreds (Poon et al., 2006; Zhong et al., 2006) and even thousands of mRNAs (Cajigas et al., 2012) are trafficked and could be available for translation in dendrites/axons under basal (non-stimulated) conditions. How different types and patterns of external stimuli (neurotransmitters, neuromodulators, etc.) affect the availability of mRNA and local translation remains to be determined.

What determines if and when the particular mRNA will be de-repressed for translation? mRNAs are transported in complexes with RNA-binding proteins (RBPs) that are thought to physically repress translation; as such, the initiation of mRNA translation can be achieved by de-repression of mRNA translation at a specific time (Sutton and Schuman, 2005; Wang et al., 2010). Local translation can be regulated at the level of initiation by (1) controlling whether a ribosome will be recruited to the 5' end of the mRNA, typically through phosphorylation of eukaryotic initiation factor 2 alpha (eIF2 α), eIF4E-binding proteins (4E-BPs), or p70 S6 kinases (S6K) or (2) proteins that bind to the 3' end of the mRNA (Sutton and Schuman, 2005; Buffington et al., 2014). In mammals, initiation of translation is a complex process where a number of core eIFs and auxiliary factors work together to promote the assembly of ribosomes on a mRNA (Jackson et al., 2010). It starts with formation of the 43S pre-initiation complex that binds to the 5' end of capped mRNA. During formation of the 43S complex, one of the crucial steps is the binding of a ternary complex consisting of a GTP-bound eIF2 and the initiator Met-tRNA to the 40S ribosomal subunit. This step can be regulated by phosphorylation of the alpha subunit of eIF2 (eIF2 α). If eIF2 α is phosphorylated at Ser51, the activity of the factor leading to exchange of GDP to GTP bound to eIF2 (eIF2B) will be blocked, causing decreased translation. Takei et al. (2001) found that a basal level of phosphorylation of eIF2 α can be detected in cultured neurons under normal conditions and that this level can be reduced with treatments that stimulate translation (e.g., BDNF). Later, a number of studies showed that dephosphorylation of eIF2 α is an important part of translational regulation that, if disturbed, can lead to impairments in L-LTP and long-term memory (LTM) storage (Costa-Mattioli et al., 2009; Jiang et al., 2010; Sidrauski et al., 2013). Recently, evidence has emerged that regulation of eIF2 α phosphorylation can have different roles in glutamatergic versus GABAergic neurons. In glutamatergic neurons dephosphorylation of eIF2 α can lead to cAMP response element-binding protein (CREB)-mediated transcription and enhancement of L-LTP and LTM, whereas in GABAergic neurons it can promote release of GABA and inhibition of L-LTP and LTM (Costa-Mattioli et al., 2005, 2007; Zhu et al., 2011).

A second well-characterized pathway through which 5' end recruitment of ribosomes and initiation of translation can be regulated involves the phosphorylation of 4E-BPs or S6K that occurs downstream of mammalian target of rapamycin complex 1 (mTORC1) signaling (Hay and Sonenberg, 2004). In order to activate the mRNA and bring it to a form that can be recognized by the 43S pre-initiation complex, the eIF4F complex is formed and binds to mRNA. The eIF4F complex consists of eIF4E, eIF4G, and eIF4A factors that, with help of eIF4B and poly(A)-binding protein (PABP), lead to an activated mRNA conformation. The formation of the eIF4F complex

stimulates translation initiation and is regulated by phosphorylation of 4E-BP proteins. If 4E-BPs are phosphorylated their binding affinity for eIF4E is reduced, making it free for assembly in the eIF4F complex. The importance of the mTORC1-mediated regulation of translation initiation in neurons was shown in a set of studies where diverse stimuli inducing L-LTP or LTD led to increase in mTORC1 phosphorylation activity; additionally inhibition of mTORC1 by rapamycin blocked L-LTP and LTM formation (Tang et al., 2002; Cammalleri et al., 2003; Hou and Klann, 2004; Blundell et al., 2008; Stoica et al., 2011). Initiation of local translation can be also regulated by PABP-interacting protein 2 (Paip2) that may influence binding of PABP to the 3' end of mRNA. Paip2 controls the translation of a set of mRNAs by changing the affinity of PABPs for their poly(A) tails as well as by competing with the eIF4G part of the eIF4F complex for PABP binding (Karim et al., 2006; Khaleghpour et al., 2001). Khoutorsky et al. (2013) recently found that Paip2 has an important regulatory role in neurons. In this study, Paip2 regulated the activity-dependent translation of CAMK2a mRNA; CAMK2a translation was enhanced upon training in a contextual fear-conditioning task in Paip2a^{-/-} mice compared with wild-type mice. Additionally, to test the effect on local dendritic translation of Camk2a, the authors induced Camk2a translation in acute hippocampal slices using high-frequency stimulation (Ouyang et al., 1999), and observed an increase in the level of CaMKIIα protein in the dendrites of slices from Paip2a^{-/-} mice but not in wild-type slices (Khoutorsky et al., 2013).

In addition to its role in the initiation of translation, phosphorylation of S6K by mTORC1 can also influence translational elongation through regulation of eukaryotic translation elongation factor 2 (eEF2) kinase activity (Wang et al., 2001). eEF2 kinase is an atypical calcium-calmodulin-dependent protein kinase that in neurons phosphorylates eEF2 in an activity-dependent manner. This phosphorylation inhibits activity of eEF2 in translocating ribosomes, and thus impairs the elongation step of translation. In neurons, activation of key receptors (e.g., AMPAR, NMDAR, and mGluR1/5) leads to an increase in eEF2 phosphorylation (Taha et al., 2013). For example, mGluR activation leads to eEF2 phosphorylation and controls dendritic BDNF synthesis, influencing spine plasticity and maturation (Verpelli et al., 2010). Interestingly, Sutton et al. (2007) found local dendritic regulation of eEF2 phosphorylation by miniature synaptic events. When eEF2 was dephosphorylated locally, translation at the same specific location was increased. Similarly, in *Aplysia* sensory neurons it was shown that eEF2 phosphorylation can be differentially regulated in different cellular compartments (Weatherill et al., 2011). Thus, these studies provide a basis for spatially restricted regulation of local translation.

Translational regulation of certain mRNAs can be also dependent on an IRES. In mRNAs that have an IRES, translation can be initiated by recruiting the 40S ribosomal subunit to the IRES sequence, independent of the formation of eIF4F complex that binds to the cap and leads to the formation of an activated mRNA conformation. Interestingly, some of the key synaptic mRNAs [e.g., Arc, CAMK2a, dendrin, neurogranin, MAP2, fragile X mental retardation protein (FMRP)] as well as other important neuronally expressed mRNAs [e.g., amyloid precursor protein (APP), Sp1 transcription factor, fibroblast growth factor 2 (FGF-2)] have IRES sequences (Audigier et al., 2008; Chiang et al., 2001; Pinkstaff et al., 2001; Yeh et al., 2011). Still, it is not completely understood which signals or cellular events can favor or switch between cap-dependent and IRES-dependent translation and whether IRES-dependent translation occurs in dendrites.

Local dendritic translation of mRNAs harboring a CPE in their 3' UTRs can be controlled through cytoplasmic polyadenylation (Darnell and Richter, 2012). CPEB1 is a member of the CPEB protein family that has been detected in dendrites and enriched in postsynaptic densities (Atkins et al., 2004; Wu et al., 1998). CPEB1 binds to the CPE while the pre-mRNA is in the nucleus and following its export to the dendrites it recruits a number of factors and enzymes (e.g.,

PARN, a poly(A) ribonuclease) that will shorten the poly(A) tail of the mRNA leading to a decrease in translation (Lin et al., 2010; Udagawa et al., 2012). Additionally, CPEB1 can repress translation initiation by competing with eIF4G for the recruitment of proteins (e.g., 4E-BP or neuroguidin), and thus interfering with mRNA activation preceding translation initiation (Stebbins-Boaz et al., 1999; Jung et al., 2006). Interestingly, the dendritic CPEB-associated polyadenylation apparatus (consisting of CPEB1, Gld2, PARN, and neuroguidin) is regulated by neuronal activity (Udagawa et al., 2012). For example, the CAMK2a 3' UTR harbors two CPE elements that can be regulated by signaling beginning with NMDA activation, followed by Aurora protein kinase activity that phosphorylates CPEB1, resulting in increased CAMK2a translation at synapses (Mendez et al., 2000a,b; Wu et al., 1998). Moreover, CAMK2a can autoregulate its synthesis in synapses by phosphorylating CPEB1 (Atkins et al., 2004). In addition, knock-down of CPEB1, Gld2, or Ngd leads to an impairment in some forms of LTP (Alarcon et al., 2004; Udagawa et al., 2012). In *Aplysia*, the CPEB homolog ApCPEB is required for LTF and can form prion-like particles that are hypothesized to be a form of long-term memory storage (Darnell, 2003; Si et al., 2010). In *Drosophila*, the Orb2 protein, a homolog of CPEB2–4, also can form prion-like particles specifically in neurons where it is expressed and is necessary for LTM (Keleman et al., 2007; Kruttner et al., 2012).

Role of local dendritic translation in neuronal disorders

Local dendritic translation is a highly regulated process that plays a role in basic neuronal mechanisms and functions including synaptic plasticity and memory. It is therefore not surprising that neurodegenerative and neurodevelopmental diseases are likely linked to the dysregulation of local translation (Liu-Yesucevitz et al., 2011; Darnell and Klann, 2013; Buffington et al., 2014). Chang et al. (2002), for example, found increased phosphorylation of eIF2 α in brains from patients with Alzheimer's disease (AD). eIF2 α phosphorylation leads to an increase in beta-secretase 1 (BACE1) translation (O'Connor et al., 2008) and promotes cleavage of APP and thus amyloidogenesis (Vassar et al., 1999). In addition, protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK) and PKR kinases, which are up-regulated in brains of AD patients, phosphorylate eIF2 α (Chang et al., 2002; Onuki et al., 2004; Sanderson et al., 2010; Trinh et al., 2012), thus revealing these kinases as potential therapeutic targets for treatment of AD. That this dysregulation may indeed be linked to the learning and memory deficits of AD patients has been demonstrated in AD mouse models. For example, upon PERK deletion in APP/presenilin1 (PS1) mice not only are normal levels of eIF2 α phosphorylation restored, but also deficits in synaptic plasticity and spatial memory are rescued (Ma et al., 2013). Dysregulation of protein synthesis has also been detected in other neurodegenerative disorders, for example prion disease where understanding of disease mechanisms have provided the first steps toward finding therapies. Recently, Moreno et al. (2013) tested a specific inhibitor of PERK kinase that resulted in neuroprotection in a mouse model of neurodegenerative prion disease; the protection by the PERK kinase inhibitor was associated with a rescue of translational repression of specific mRNAs.

Regulation of translation has been directly implicated in fragile X syndrome (FXS) (Darnell and Klann, 2013). The disease is caused by CGG triplet expansion in the 5' UTR of the *Fmr1* gene that codes for the RNA-binding protein FMRP, a presumed translational repressor. The CGG repeat results in decreased FMRP expression—leading to reduced translational repression. *Fmr1*-null *Drosophila* have defects in long-term memory that can be rescued by application of protein synthesis inhibitors, suggesting that the excessive translation, when *Fmr1* is not present, leads to impairments of cognition (Bolduc et al., 2008). It is still under debate if FMRP preferentially represses translation of its specific mRNA targets through translational initiation or through

elongation—there is evidence to support both models. For example, FMRP can repress translation initiation by binding to CYFIP1, protein that binds to eIF4E and thus interferes with formation of the eIF4F complex necessary to activate mRNA just before translation is initiated (Napoli et al., 2008). FMRP can also repress translation elongation by stalling ribosomes on their mRNA targets (Darnell et al., 2011). Interestingly, Gene Ontology analysis of FMRP targets showed a significant enrichment in synaptic transmission, neuronal projection, and synapse terms and approximately one-third of FMRP targets are postsynaptic proteins (e.g., NMDA receptor subunits, mGluR5 receptor, PSD-95, Homer1) (Darnell et al., 2011), suggesting that FMRP might regulate translation locally in dendrites. As it is clear that translation elongation might be a valuable therapeutic target for FXS, some compounds such as minocycline, a tetracycline antibiotic that targets p38 MAPK, iNOS, caspases, and matrix metalloproteinase-9 (MMP9), were tested and showed promising results (e.g., rescue of aberrant spine morphology as well as of impaired behaviors) in *Fmr1* knock-out models in mice and flies (Bilousova et al., 2009; Siller and Broadie, 2011). Moreover, minocycline treatment of FXS patients resulted in improved behaviors in two human clinical studies (Paribello et al., 2010; Leigh et al., 2013). In addition to important synaptic targets, some of the FMRP targets are candidate genes for autism spectrum disorder (ASD) (e.g. *NLGN3*, *NRXN1*, *PTEN*), potentially explaining about 5% of ASD patients where the disease appears to be caused by a loss of FMRP function (Darnell et al., 2011).

ASDs are highly heterogeneous group of neurodevelopmental disorders that share symptoms including communication and social deficits, repetitive behaviors, and, sometimes, intellectual disability. Similar to FXS, ASDs are thought to be caused by “runaway translation,” the exaggerated translation of certain mRNAs (Darnell and Richter, 2012; Santini et al., 2013). We have discussed the function of the mTORC1 signaling pathway in regulating translation initiation and its importance for activity-dependent signaling, synaptic plasticity, L-LTP, and LTM. Thus it is not surprising that a number of mutations in regulators (e.g., *PTEN*) of the mTORC1 pathway are associated with some ASD patients. Indeed, mTORC1 is elevated in some ASD patients and this overexpression is predicted to promote the formation of the eIF4F initiation complex leading to enhancement of cap-dependent translation. For example Santini et al. (2013) recently found that genetically encoded increases in the eIF4E protein (a component of eIF4F complex) in mice leads to exaggerated translation, pathological synaptic changes, and autistic-like behaviors. Moreover, these impairments can be corrected by application of an inhibitor of 4EGI-1 protein synthesis. In another study, Gkogkas et al. (2013) showed that mice lacking 4E-BP exhibit increased translation of neuroligins, proteins previously implicated in ASDs, and these mice also exhibit autistic-like behaviors, representing one more example of how dysregulation of translation may lead to disease phenotypes. A better understanding of the variety and dynamics of dysregulation of protein synthesis, its potential causation, and its impact on the onset or progression of neurodegenerative and neurodevelopmental disorders may lead to better strategies for therapy, as well as further elucidating the role of local dendritic translation in neurological disease.

Concluding remarks and future directions

In this chapter we have summarized the evidence that local dendritic translation plays an important role in neuronal function, including synaptic transmission, synaptic plasticity, and learning and memory. Additionally, the mechanisms that regulate local translation are often targets for pathological changes in neurological disorders. Thus, understanding the mechanisms that regulate local translation has the potential to inform current and future therapies. There is still plenty to do to fully understand the mechanisms and consequences of local dendritic translation. We need a

better understanding of the regulatory mechanisms that allow rapid cellular responses to different forms of activity. Does this regulation operate mainly at the level of basic signal transduction pathways or there are some novel types of regulation, as already known for some non-coding RNAs (e.g., miRNAs and BC1 ncRNA)? How independent is local translation in dendrites from nuclear control? Can we more quantitatively understand local translation, for example how many mRNAs produce how many proteins and how many polyribosomes serve single synapses, or groups of synapses on adjacent parts of the dendritic branch? The rapid development of genome-wide technologies will enable a systems biology approach to these questions.

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References

- Aakalu G, Smith WB, Nguyen N, Jiang C, Schuman EM (2001) Dynamic visualization of local protein synthesis in hippocampal neurons. *Neuron* **30**:489–502.
- Alarcon JM, Hodgman R, Theis M, Huang YS, Kandel ER, Richter JD (2004) Selective modulation of some forms of Schaffer collateral-CA1 synaptic plasticity in mice with a disruption of the CPEB-1 gene. *Learning and Memory* **11**:318–327.
- Atkins CM, Nozaki N, Shigeri Y, Soderling TR (2004) Cytoplasmic polyadenylation element binding protein-dependent protein synthesis is regulated by calcium/calmodulin-dependent protein kinase II. *Journal of Neuroscience* **24**:5193–5201.
- Audigier S, Guiramand J, Prado-Loureiro L, Conte C, Gonzalez-Herrera IG, Cohen-Solal C, Recasens M, Prats AC (2008) Potent activation of FGF-2 IRES-dependent mechanism of translation during brain development. *RNA* **14**:1852–1864.
- Bagni C, Mannucci L, Dotti CG, Amaldi F (2000) Chemical stimulation of synaptosomes modulates alpha-Ca²⁺/calmodulin-dependent protein kinase II mRNA association to polysomes. *Journal of Neuroscience* **20**:RC76.
- Barhoom S, Kaur J, Cooperman BS, Smorodinsky NI, Smilansky Z, Ehrlich M, Elroy-Stein O (2011) Quantitative single cell monitoring of protein synthesis at subcellular resolution using fluorescently labeled tRNA. *Nucleic Acids Research* **39**:e129.
- Besse F, Ephrussi A (2008) Translational control of localized mRNAs: restricting protein synthesis in space and time. *Nature Reviews Molecular Cell Biology* **9**:971–980.
- Bilousova TV, Dansie L, Ngo M, Aye J, Charles JR, Ethell DW, Ethell IM (2009) Minocycline promotes dendritic spine maturation and improves behavioural performance in the fragile X mouse model. *Journal of Medical Genetics* **46**:94–102.
- Blaustein MP, Goldring JM (1975) Membrane potentials in pinched-off presynaptic nerve terminals monitored with a fluorescent probe: evidence that synaptosomes have potassium diffusion potentials. *Journal of Physiology* **247**:589–615.
- Blundell J, Kouzer M, Powell CM (2008) Systemic inhibition of mammalian target of rapamycin inhibits fear memory reconsolidation. *Neurobiology of Learning and Memory* **90**:28–35.
- Bockers TM, Segger-Junius M, Iglauder P, Bockmann J, Gundelfinger ED, Kreutz MR, Richter D, Kindler S, Kreienkamp HJ (2004) Differential expression and dendritic transcript localization of Shank family members: identification of a dendritic targeting element in the 3' untranslated region of Shank1 mRNA. *Molecular and Cellular Neurosciences* **26**:182–190.

- Bodian D (1965) A suggestive relationship of nerve cell RNA with specific synaptic sites. *Proceedings of the National Academy of Sciences of the United States of America* **53**:418–425.
- Bolduc FV, Bell K, Cox H, Broadie KS, Tully T (2008) Excess protein synthesis in *Drosophila* fragile X mutants impairs long-term memory. *Nature Neuroscience* **11**:1143–1145.
- Bradshaw KD, Emptage NJ, Bliss TV (2003) A role for dendritic protein synthesis in hippocampal late LTP. *European Journal of Neuroscience* **18**:3150–3152.
- Buffington SA, Huang W, Costa-Mattioli M (2014) Translational control in synaptic plasticity and cognitive dysfunction. *Annual Review of Neuroscience* **37**:17–38.
- Bunge MB (1973) Fine structure of nerve fibers and growth cones of isolated sympathetic neurons in culture. *Journal of Cell Biology* **56**:713–735.
- Burgin KE, Waxham MN, Rickling S, Westgate SA, Mobley WC, Kelly PT (1990) In situ hybridization histochemistry of Ca^{2+} /calmodulin-dependent protein kinase in developing rat brain. *Journal of Neuroscience* **10**:1788–1798.
- Butko MT, Yang J, Geng Y, Kim HJ, Jeon NL, Shu X, Mackey MR, Ellisman MH, Tsien RY, Lin MZ (2012) Fluorescent and photo-oxidizing TimeSTAMP tags track protein fates in light and electron microscopy. *Nature Neuroscience* **15**:1742–1751.
- Buxbaum AR, Wu B, Singer RH (2014) Single beta-actin mRNA detection in neurons reveals a mechanism for regulating its translatability. *Science* **343**:419–422.
- Cajigas IJ, Tushev G, Will TJ, tom Dieck S, Fuerst N, Schuman EM (2012) The local transcriptome in the synaptic neuropil revealed by deep sequencing and high-resolution imaging. *Neuron* **74**:453–466.
- Cammalleri M, Lutjens R, Berton F, King AR, Simpson C, Francesconi W, Sanna PP (2003) Time-restricted role for dendritic activation of the mTOR-p70S6K pathway in the induction of late-phase long-term potentiation in the CA1. *Proceedings of the National Academy of Sciences of the United States of America* **100**:14368–14373.
- Campbell DS, Holt CE (2001) Chemotropic responses of retinal growth cones mediated by rapid local protein synthesis and degradation. *Neuron* **32**:1013–1026.
- Chang RC, Wong AK, Ng HK, Hugon J (2002) Phosphorylation of eukaryotic initiation factor-2alpha (eIF-2alpha) is associated with neuronal degeneration in Alzheimer's disease. *NeuroReport* **13**:2429–2432.
- Chao JA, Yoon YJ, Singer RH (2012) Imaging translation in single cells using fluorescent microscopy. *Cold Spring Harbor Perspectives in Biology* **4**(11): doi: 10.1101/cshperspect.a012310.
- Chiang PW, Carpenter LE, Hagerman PJ (2001) The 5'-untranslated region of the FMR1 message facilitates translation by internal ribosome entry. *Journal of Biological Chemistry* **276**:37916–37921.
- Condeelis J, Singer RH (2005) How and why does beta-actin mRNA target? *Biology of the Cell* **97**:97–110.
- Costa-Mattioli M, et al. (2005) Translational control of hippocampal synaptic plasticity and memory by the eIF2alpha kinase GCN2. *Nature* **436**:1166–1173.
- Costa-Mattioli M, et al. (2007) eIF2alpha phosphorylation bidirectionally regulates the switch from short- to long-term synaptic plasticity and memory. *Cell* **129**:195–206.
- Costa-Mattioli M, Sossin WS, Klann E, Sonnenberg N (2009) Translational control of long-lasting synaptic plasticity and memory. *Neuron* **61**:10–26.
- Court FA, Midha R, Cisterna BA, Grochmal J, Shakhabzau A, Hendriks WT, Van Minnen J (2011) Morphological evidence for a transport of ribosomes from Schwann cells to regenerating axons. *Glia* **59**:1529–1539.
- Darnell RB (2003) Memory, synaptic translation, and . . . prions? *Cell* **115**:767–768.
- Darnell JC, Klann E (2013) The translation of translational control by FMRP: therapeutic targets for FXS. *Nature Neuroscience* **16**:1530–1536.
- Darnell JC, Richter JD (2012) Cytoplasmic RNA-binding proteins and the control of complex brain function. *Cold Spring Harbor Perspectives in Biology* **4**(8): doi: 10.1101/cshperspect.a012344.
- Darnell JC, et al. (2011) FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism. *Cell* **146**:247–261.

- David A, Netzer N, Strader MB, Das SR, Chen CY, Gibbs J, Pierre P, Bennink JR, Yewdell JW (2011) RNA binding targets aminoacyl-tRNA synthetases to translating ribosomes. *Journal of Biological Chemistry* **286**:20688–20700.
- De Belleruche JS, Bradford HF (1972) Metabolism of beds of mammalian cortical synaptosomes: response to depolarizing influences. *Journal of Neurochemistry* **19**:585–602.
- Di Nardo AA, Nedelec S, Trembleau A, Volovitch M, Prochiantz A, Montesinos ML (2007) Dendritic localization and activity-dependent translation of Engrailed1 transcription factor. *Molecular and Cellular Neurosciences* **35**:230–236.
- Dieterich DC, Link AJ, Graumann J, Tirrell DA, Schuman EM (2006) Selective identification of newly synthesized proteins in mammalian cells using bioorthogonal noncanonical amino acid tagging (BONCAT). *Proceedings of the National Academy of Sciences of the United States of America* **103**:9482–9487.
- Dieterich DC, Lee JJ, Link AJ, Graumann J, Tirrell DA, Schuman EM (2007) Labeling, detection and identification of newly synthesized proteomes with bioorthogonal non-canonical amino-acid tagging. *Nature Protocols* **2**:532–540.
- Dieterich DC, Hodas JJ, Gouzer G, Shadrin IY, Ngo JT, Triller A, Tirrell DA, Schuman EM (2010) In situ visualization and dynamics of newly synthesized proteins in rat hippocampal neurons. *Nature Neuroscience* **13**:897–905.
- Dong E, Caruncho H, Liu WS, Smalheiser NR, Grayson DR, Costa E, Guidotti A (2003) A reelin-integrin receptor interaction regulates Arc mRNA translation in synaptoneuroosomes. *Proceedings of the National Academy of Sciences of the United States of America* **100**:5479–5484.
- Doyle JP, et al. (2008) Application of a translational profiling approach for the comparative analysis of CNS cell types. *Cell* **135**:749–762.
- Dziembowska M, Milek J, Janusz A, Rejmak E, Romanowska E, Gorkiewicz T, Tiron A, Bramham CR, Kaczmarek L (2012) Activity-dependent local translation of matrix metalloproteinase-9. *Journal of Neuroscience* **32**:14538–14547.
- Evdokimov AG, Pokross ME, Egorov NS, Zaraisky AG, Yampolsky IV, Merzlyak EM, Shkoporov AN, Sander I, Lukyanov KA, Chudakov DM (2006) Structural basis for the fast maturation of Arthropoda green fluorescent protein. *EMBO Reports* **7**:1006–1012.
- Feig S, Lipton P (1993) Pairing the cholinergic agonist carbachol with patterned Schaffer collateral stimulation initiates protein synthesis in hippocampal CA1 pyramidal cell dendrites via a muscarinic, NMDA-dependent mechanism. *Journal of Neuroscience* **13**:1010–1021.
- Gandin V, Sikstrom K, Alain T, Morita M, McLaughlan S, Larsson O, Topisirovic I (2014) Polysome fractionation and analysis of mammalian translatomes on a genome-wide scale. *Journal of Visualized Experiments* doi: 10.3791/51455.
- Garner CC, Tucker RP, Matus A (1988) Selective localization of messenger RNA for cytoskeletal protein MAP2 in dendrites. *Nature* **336**:674–677.
- Gkogkas CG, et al. (2013) Autism-related deficits via dysregulated eIF4E-dependent translational control. *Nature* **493**:371–377.
- Gurskaya NG, Verkhusha VV, Shcheglov AS, Staroverov DB, Chepurnykh TV, Fradkov AF, Lukyanov S, Lukyanov KA (2006) Engineering of a monomeric green-to-red photoactivatable fluorescent protein induced by blue light. *Nature Biotechnology* **24**:461–465.
- Hanus C, Schuman EM (2013) Proteostasis in complex dendrites. *Nature Reviews Neuroscience* **14**:638–648.
- Hay N, Sonenberg N (2004) Upstream and downstream of mTOR. *Genes and Development* **18**:1926–1945.
- Heiman M, et al. (2008) A translational profiling approach for the molecular characterization of CNS cell types. *Cell* **135**:738–748.
- Hinz FI, Dieterich DC, Tirrell DA, Schuman EM (2012) Non-canonical amino acid labeling in vivo to visualize and affinity purify newly synthesized proteins in larval zebrafish. *ACS Chemical Neuroscience* **3**:40–49.

- Hodas JJ, Nehring A, Hoche N, Sweredoski MJ, Pielot R, Hess S, Tirrell DA, Dieterich DC, Schuman EM (2012) Dopaminergic modulation of the hippocampal neuropil proteome identified by bioorthogonal noncanonical amino acid tagging (BONCAT). *Proteomics* **12**:2464–2476.
- Holt CE, Schuman EM (2013) The central dogma decentralized: new perspectives on RNA function and local translation in neurons. *Neuron* **80**:648–657.
- Hou L, Klann E (2004) Activation of the phosphoinositide 3-kinase-Akt-mammalian target of rapamycin signaling pathway is required for metabotropic glutamate receptor-dependent long-term depression. *Journal of Neuroscience* **24**:6352–6361.
- Housley MP, Reischauer S, Dieu M, Raes M, Stainier DY, Vanhollebeke B (2014) Translational profiling through biotinylation of tagged ribosomes in zebrafish. *Development* **141**:3988–3993.
- Howden AJ, et al. (2013) QuaNCAT: quantitating proteome dynamics in primary cells. *Nature Methods* **10**:343–346.
- Hsieh AC, et al. (2012) The translational landscape of mTOR signalling steers cancer initiation and metastasis. *Nature* **485**:55–61.
- Huang Y, Ainsley JA, Reijmers LG, Jackson FR (2013) Translational profiling of clock cells reveals circadianly synchronized protein synthesis. *PLoS Biology* **11**:e1001703.
- Huber KM, Kayser MS, Bear MF (2000) Role for rapid dendritic protein synthesis in hippocampal mGluR-dependent long-term depression. *Science* **288**:1254–1257.
- Ingolia NT, Ghaemmaghami S, Newman JR, Weissman JS (2009) Genome-wide analysis in vivo of translation with nucleotide resolution using ribosome profiling. *Science* **324**:218–223.
- Ingolia NT, Brar GA, Rouskin S, McGeachy AM, Weissman JS (2012) The ribosome profiling strategy for monitoring translation in vivo by deep sequencing of ribosome-protected mRNA fragments. *Nature Protocols* **7**:1534–1550.
- Ingolia NT, Brar GA, Rouskin S, McGeachy AM, Weissman JS (2013) Genome-wide annotation and quantitation of translation by ribosome profiling. *Current Protocols in Molecular Biology* **103:II:4.18**:4.18.1–4.18.19.
- Jackson RJ, Hellen CU, Pestova TV (2010) The mechanism of eukaryotic translation initiation and principles of its regulation. *Nature Reviews Molecular Cell Biology* **11**:113–127.
- Jiang Z, Belforte JE, Lu Y, Yabe Y, Pickel J, Smith CB, Je HS, Lu B, Nakazawa K (2010) eIF2alpha Phosphorylation-dependent translation in CA1 pyramidal cells impairs hippocampal memory consolidation without affecting general translation. *Journal of Neuroscience* **30**:2582–2594.
- Johannes G, Carter MS, Eisen MB, Brown PO, Sarnow P (1999) Identification of eukaryotic mRNAs that are translated at reduced cap binding complex eIF4F concentrations using a cDNA microarray. *Proceedings of the National Academy of Sciences of the United States of America* **96**:13118–13123.
- Johnstone O, Lasko P (2001) Translational regulation and RNA localization in Drosophila oocytes and embryos. *Annual Review of Genetics* **35**:365–406.
- Jung MY, Lorenz L, Richter JD (2006) Translational control by neuroguidin, a eukaryotic initiation factor 4E and CPEB binding protein. *Molecular and Cellular Biology* **26**:4277–4287.
- Kang H, Schuman EM (1995) Long-lasting neurotrophin-induced enhancement of synaptic transmission in the adult hippocampus. *Science* **267**:1658–1662.
- Kang H, Schuman EM (1996) A requirement for local protein synthesis in neurotrophin-induced hippocampal synaptic plasticity. *Science* **273**:1402–1406.
- Kapeli K, Yeo GW (2012) Genome-wide approaches to dissect the roles of RNA binding proteins in translational control: implications for neurological diseases. *Frontiers in Neuroscience* **6**:144 doi: 10.3389/fnins.2012.00144
- Karim MM, Svitkin YV, Kahvejian A, De Crescenzo G, Costa-Mattioli M, Sonenberg N (2006) A mechanism of translational repression by competition of Paip2 with eIF4G for poly(A) binding protein (PABP) binding. *Proceedings of the National Academy of Sciences of the United States of America* **103**:9494–9499.

- Keleman K, Kruttner S, Alenius M, Dickson BJ (2007) Function of the *Drosophila* CPEB protein Orb2 in long-term courtship memory. *Nature Neuroscience* **10**:1587–1593.
- Khaleghpour K, Svitkin YV, Craig AW, DeMaria CT, Deo RC, Burley SK, Sonnenberg N (2001) Translational repression by a novel partner of human poly(A) binding protein, Paip2. *Molecular Cell* **7**:205–216.
- Khoutorsky A, et al. (2013) Control of synaptic plasticity and memory via suppression of poly(A)-binding protein. *Neuron* **78**:298–311.
- King ML, Messitt TJ, Mowry KL (2005) Putting RNAs in the right place at the right time: RNA localization in the frog oocyte. *Biology of the Cell* **97**:19–33.
- Kislaukis EH, Zhu X, Singer RH (1997) Beta-actin messenger RNA localization and protein synthesis augment cell motility. *Journal of Cell Biology* **136**:1263–1270.
- Knight ZA, Tan K, Birsoy K, Schmidt S, Garrison JL, Wysocki RW, Emiliano A, Ekstrand MI, Friedman JM (2012) Molecular profiling of activated neurons by phosphorylated ribosome capture. *Cell* **151**:1126–1137.
- Knowles RB, Sabry JH, Martone ME, Deerinck TJ, Ellisman MH, Bassell GJ, Kosik KS (1996) Translocation of RNA granules in living neurons. *Journal of Neuroscience* **16**:7812–7820.
- Kratz A, et al. (2014) Digital expression profiling of the compartmentalized translatome of Purkinje neurons. *Genome Research* **24**:1396–1410.
- Krichevsky AM, Kosik KS (2001) Neuronal RNA granules: a link between RNA localization and stimulation-dependent translation. *Neuron* **32**:683–696.
- Kruttner S, Stepien B, Noordermeer JN, Mommaas MA, Mechtler K, Dickson BJ, Keleman K (2012) *Drosophila* CPEB Orb2A mediates memory independent of its RNA-binding domain. *Neuron* **76**:383–395.
- Lecuyer E, Yoshida H, Parthasarathy N, Alm C, Babak T, Cerovina T, Hughes TR, Tomancak P, Krause HM (2007) Global analysis of mRNA localization reveals a prominent role in organizing cellular architecture and function. *Cell* **131**:174–187.
- Leigh MJ, et al. (2013) A randomized double-blind, placebo-controlled trial of minocycline in children and adolescents with fragile X syndrome. *Journal of Developmental and Behavioral Pediatrics* **34**:147–155.
- Lein ES, et al. (2007) Genome-wide atlas of gene expression in the adult mouse brain. *Nature* **445**:168–176.
- Lin AC, Holt CE (2007) Local translation and directional steering in axons. *EMBO Journal* **26**:3729–3736.
- Lin MZ, Glenn JS, Tsien RY (2008) A drug-controllable tag for visualizing newly synthesized proteins in cells and whole animals. *Proceedings of the National Academy of Sciences of the United States of America* **105**:7744–7749.
- Lin CL, Evans V, Shen S, Xing Y, Richter JD (2010) The nuclear experience of CPEB: implications for RNA processing and translational control. *RNA* **16**:338–348.
- Lin YT, Huang CC, Hsu KS (2012) Oxytocin promotes long-term potentiation by enhancing epidermal growth factor receptor-mediated local translation of protein kinase Mzeta. *Journal of Neuroscience* **32**:15476–15488.
- Liu J, Xu Y, Stoleru D, Salic A (2012) Imaging protein synthesis in cells and tissues with an alkyne analog of puromycin. *Proceedings of the National Academy of Sciences of the United States of America* **109**:413–418.
- Liu-Yesucevitz L, Bassell GJ, Gitler AD, Hart AC, Klann E, Richter JD, Warren ST, Wolozin B (2011) Local RNA translation at the synapse and in disease. *Journal of Neuroscience* **31**:16086–16093.
- Ma T, Trinh MA, Wexler AJ, Bourbon C, Gatti E, Pierre P, Cavener DR, Klann E (2013) Suppression of eIF2alpha kinases alleviates Alzheimer's disease-related plasticity and memory deficits. *Nature Neuroscience* **16**:1299–1305.
- Martin KC (2010) Anchoring local translation in neurons. *Cell* **141**:566–568.
- Martin KC, Ephrussi A (2009) mRNA localization: gene expression in the spatial dimension. *Cell* **136**:719–730.

- Martin KC, Casadio A, Zhu H, Yaping E, Rose JC, Chen M, Bailey CH, Kandel ER (1997) Synapse-specific, long-term facilitation of aplysia sensory to motor synapses: a function for local protein synthesis in memory storage. *Cell* **91**:927–938.
- Mayford M, Baranes D, Podsypanina K, Kandel ER (1996) The 3'-untranslated region of CaMKII alpha is a cis-acting signal for the localization and translation of mRNA in dendrites. *Proceedings of the National Academy of Sciences of the United States of America* **93**:13250–13255.
- Mendez R, Hake LE, Andresson T, Littlepage LE, Ruderman JV, Richter JD (2000a) Phosphorylation of CPE binding factor by Eg2 regulates translation of c-mos mRNA. *Nature* **404**:302–307.
- Mendez R, Murthy KG, Ryan K, Manley JL, Richter JD (2000b) Phosphorylation of CPEB by Eg2 mediates the recruitment of CPSF into an active cytoplasmic polyadenylation complex. *Molecular Cell* **6**:1253–1259.
- Miller S, Yasuda M, Coats JK, Jones Y, Martone ME, Mayford M (2002) Disruption of dendritic translation of CaMKIIalpha impairs stabilization of synaptic plasticity and memory consolidation. *Neuron* **36**:507–519.
- Moreno JA, et al. (2013) Oral treatment targeting the unfolded protein response prevents neurodegeneration and clinical disease in prion-infected mice. *Science Translational Medicine* **5**:206ra138.
- Mori Y, Imaizumi K, Katayama T, Yoneda T, Tohyama M (2000) Two cis-acting elements in the 3' untranslated region of alpha-CaMKII regulate its dendritic targeting. *Nature Neuroscience* **3**:1079–1084.
- Napoli I, et al. (2008) The fragile X syndrome protein represses activity-dependent translation through CYFIP1, a new 4E-BP. *Cell* **134**:1042–1054.
- Ngo JT, Babin BM, Champion JA, Schuman EM, Tirrell DA (2012) State-selective metabolic labeling of cellular proteins. *ACS Chemical Biology* **7**:1326–1330.
- O'Connor T, et al. (2008) Phosphorylation of the translation initiation factor eIF2alpha increases BACE1 levels and promotes amyloidogenesis. *Neuron* **60**:988–1009.
- Onuki R, Bando Y, Suyama E, Katayama T, Kawasaki H, Baba T, Tohyama M, Taira K (2004) An RNA-dependent protein kinase is involved in tunicamycin-induced apoptosis and Alzheimer's disease. *EMBO Journal* **23**:959–968.
- Ostroff LE, Fiala JC, Allwardt B, Harris KM (2002) Polyribosomes redistribute from dendritic shafts into spines with enlarged synapses during LTP in developing rat hippocampal slices. *Neuron* **35**:535–545.
- Ouyang Y, Rosenstein A, Kreiman G, Schuman EM, Kennedy MB (1999) Tetanic stimulation leads to increased accumulation of Ca(2+)/calmodulin-dependent protein kinase II via dendritic protein synthesis in hippocampal neurons. *Journal of Neuroscience* **19**, 7823–7833.
- Paquin N, Chartrand P (2008) Local regulation of mRNA translation: new insights from the bud. *Trends in Cell Biology* **18**:105–111.
- Paribello C, Tao L, Folino A, Berry-Kravis E, Tranfaglia M, Ethell IM, Ethell DW (2010) Open-label add-on treatment trial of minocycline in fragile X syndrome. *BMC Neurology* **10**:91 doi: 10.1186/1471-2377-10-91.
- Piccirillo CA, Bjur E, Topisirovic I, Sonenberg N, Larsson O (2014) Translational control of immune responses: from transcripts to translomes. *Nature Immunology* **15**:503–511.
- Pinkstaff JK, Chappell SA, Mauro VP, Edelman GM, Krushel LA (2001) Internal initiation of translation of five dendritically localized neuronal mRNAs. *Proceedings of the National Academy of Sciences of the United States of America* **98**:2770–2775.
- Poon MM, Choi SH, Jamieson CA, Geschwind DH, Martin KC (2006) Identification of process-localized mRNAs from cultured rodent hippocampal neurons. *Journal of Neuroscience* **26**:13390–13399.
- Rao A, Steward O (1991) Evidence that protein constituents of postsynaptic membrane specializations are locally synthesized: analysis of proteins synthesized within synaptosomes. *Journal of Neuroscience* **11**:2881–2895.
- Sanderson TH, Deogracias MP, Nangia KK, Wang J, Krause GS, Kumar R (2010) PKR-like endoplasmic reticulum kinase (PERK) activation following brain ischemia is independent of unfolded nascent proteins. *Neuroscience* **169**:1307–1314.

- Santini E, Huynh TN, MacAskill AF, Carter AG, Pierre P, Ruggero D, Kaphzan H, Klann E (2013) Exaggerated translation causes synaptic and behavioural aberrations associated with autism. *Nature* **493**:411–415.
- Sanz E, Yang L, Su T, Morris DR, McKnight GS, Amieux PS (2009) Cell-type-specific isolation of ribosome-associated mRNA from complex tissues. *Proceedings of the National Academy of Sciences of the United States of America* **106**:13939–13944.
- Schratt GM, Nigh EA, Chen WG, Hu L, Greenberg ME (2004) BDNF regulates the translation of a select group of mRNAs by a mammalian target of rapamycin-phosphatidylinositol 3-kinase-dependent pathway during neuronal development. *Journal of Neuroscience* **24**:7366–7377.
- Sherff CM, Carew TJ (2002) Coincident induction of long-term facilitation at sensory-motor synapses in *Aplysia*: presynaptic and postsynaptic factors. *Neurobiology of Learning and Memory* **78**:498–507.
- Si K, Choi YB, White-Grindley E, Majumdar A, Kandel ER (2010) *Aplysia* CPEB can form prion-like multimers in sensory neurons that contribute to long-term facilitation. *Cell* **140**:421–435.
- Sidrauski C, et al. (2013) Pharmacological brake-release of mRNA translation enhances cognitive memory. *eLife* **2**:e00498.
- Siller SS, Broadie K (2011) Neural circuit architecture defects in a Drosophila model of Fragile X syndrome are alleviated by minocycline treatment and genetic removal of matrix metalloproteinase. *Disease Models and Mechanisms* **4**:673–685.
- Smith WB, Starck SR, Roberts RW, Schuman EM (2005) Dopaminergic stimulation of local protein synthesis enhances surface expression of GluR1 and synaptic transmission in hippocampal neurons. *Neuron* **45**:765–779.
- Stebbins-Boaz B, Cao Q, de Moor CH, Mendez R, Richter JD (1999) Maskin is a CPEB-associated factor that transiently interacts with eIF-4E. *Molecular Cell* **4**:1017–1027.
- Steward O, Levy WB (1982) Preferential localization of polyribosomes under the base of dendritic spines in granule cells of the dentate gyrus. *Journal of Neuroscience* **2**:284–291.
- Steward O, Wallace CS, Lyford GL, Worley PF (1998) Synaptic activation causes the mRNA for the IEG Arc to localize selectively near activated postsynaptic sites on dendrites. *Neuron* **21**:741–751.
- Stoica L, Zhu PJ, Huang W, Zhou H, Kozma SC, Costa-Mattioli M (2011) Selective pharmacogenetic inhibition of mammalian target of Rapamycin complex I (mTORC1) blocks long-term synaptic plasticity and memory storage. *Proceedings of the National Academy of Sciences of the United States of America* **108**:3791–3796.
- Sutton MA, Schuman EM (2005) Local translational control in dendrites and its role in long-term synaptic plasticity. *Journal of Neurobiology* **64**:116–131.
- Sutton MA, Wall NR, Aakalu GN, Schuman EM (2004) Regulation of dendritic protein synthesis by miniature synaptic events. *Science* **304**:1979–1983.
- Sutton MA, Ito HT, Cressy P, Kempf C, Woo JC, Schuman EM (2006) Miniature neurotransmission stabilizes synaptic function via tonic suppression of local dendritic protein synthesis. *Cell* **125**:785–799.
- Sutton MA, Taylor AM, Ito HT, Pham A, Schuman EM (2007) Postsynaptic decoding of neural activity: eEF2 as a biochemical sensor coupling miniature synaptic transmission to local protein synthesis. *Neuron* **55**:648–661.
- Szychowski J, Mahdavi A, Hodas JJ, Bagert JD, Ngo JT, Landgraf P, Dieterich DC, Schuman EM, Tirrell DA (2010) Cleavable biotin probes for labeling of biomolecules via azide-alkyne cycloaddition. *Journal of the American Chemical Society* **132**:18351–18360.
- Taha E, Gildish I, Gal-Ben-Ari S, Rosenblum K (2013) The role of eEF2 pathway in learning and synaptic plasticity. *Neurobiology of Learning and Memory* **105**:100–106.
- Takei N, Kawamura M, Hara K, Yonezawa K, Nawa H (2001) Brain-derived neurotrophic factor enhances neuronal translation by activating multiple initiation processes: comparison with the effects of insulin. *Journal of Biological Chemistry* **276**:42818–42825.

- Tang SJ, Reis G, Kang H, Gingras AC, Sonnenberg N, Schuman EM (2002) A rapamycin-sensitive signaling pathway contributes to long-term synaptic plasticity in the hippocampus. *Proceedings of the National Academy of Sciences of the United States of America* **99**:467–472.
- Taylor AM, Wu J, Tai HC, Schuman EM (2013) Axonal translation of beta-catenin regulates synaptic vesicle dynamics. *Journal of Neuroscience* **33**:5584–5589.
- Tcherkezian J, Brittis PA, Thomas F, Roux PP, Flanagan JG (2010) Transmembrane receptor DCC associates with protein synthesis machinery and regulates translation. *Cell* **141**:632–644.
- Tennyson VM (1970) The fine structure of the axon and growth cone of the dorsal root neuroblast of the rabbit embryo. *Journal of Cell Biology* **44**:62–79.
- Thoreen CC, Chantranupong L, Keys HR, Wang T, Gray NS, Sabatini DM (2012) A unifying model for mTORC1-mediated regulation of mRNA translation. *Nature* **485**:109–113.
- Tiruchinapalli DM, Oleynikov Y, Kelic S, Shenoy SM, Hartley A, Stanton PK, Singer RH, Bassell GJ (2003) Activity-dependent trafficking and dynamic localization of zipcode binding protein 1 and beta-actin mRNA in dendrites and spines of hippocampal neurons. *Journal of Neuroscience* **23**:3251–3261.
- Torre ER, Steward O (1992) Demonstration of local protein synthesis within dendrites using a new cell culture system that permits the isolation of living axons and dendrites from their cell bodies. *Journal of Neuroscience* **12**:762–772.
- Trinh MA, Kaphzan H, Wek RC, Pierre P, Cavener DR, Klann E (2012) Brain-specific disruption of the eIF2alpha kinase PERK decreases ATF4 expression and impairs behavioral flexibility. *Cell Reports* **1**:676–688.
- Troca-Marin JA, Alves-Sampaio A, Tejedor FJ, Montesinos ML (2010) Local translation of dendritic RhoA revealed by an improved synaptoneurosome preparation. *Molecular and Cellular Neurosciences* **43**:308–314.
- Udagawa T, Swanger SA, Takeuchi K, Kim JH, Nalavadi V, Shin J, Lorenz LJ, Zukin RS, Bassell GJ, Richter JD (2012) Bidirectional control of mRNA translation and synaptic plasticity by the cytoplasmic polyadenylation complex. *Molecular Cell* **47**:253–266.
- Vassar R, et al. (1999) Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science* **286**:735–741.
- Verpelli C, Piccoli G, Zibetti C, Zanchi A, Gardoni F, Huang K, Brambilla D, Di Luca M, Battaglioli E, Sala C (2010) Synaptic activity controls dendritic spine morphology by modulating eEF2-dependent BDNF synthesis. *Journal of Neuroscience* **30**:5830–5842.
- Vickers CA, Dickson KS, Wyllie DJ (2005) Induction and maintenance of late-phase long-term potentiation in isolated dendrites of rat hippocampal CA1 pyramidal neurones. *Journal of Physiology* **568**:803–813.
- Wang X, Li W, Williams M, Terada N, Alessi DR, Proud CG (2001) Regulation of elongation factor 2 kinase by p90(RSK1) and p70 S6 kinase. *EMBO Journal* **20**:4370–4379.
- Wang DO, Kim SM, Zhao Y, Hwang H, Miura SK, Sossin WS, Martin KC (2009) Synapse- and stimulus-specific local translation during long-term neuronal plasticity. *Science* **324**:1536–1540.
- Wang DO, Martin KC, Zukin RS (2010) Spatially restricting gene expression by local translation at synapses. *Trends in Neurosciences* **33**:173–182.
- Watson FL, Mills EA, Wang X, Guo C, Chen DF, Marsh-Armstrong N (2012) Cell type-specific translational profiling in the *Xenopus laevis* retina. *Developmental Dynamics* **241**:1960–1972.
- Weatherill DB, McCamphill PK, Pethoukov E, Dunn TW, Fan X, Sossin WS (2011) Compartment-specific, differential regulation of eukaryotic elongation factor 2 and its kinase within *Aplysia* sensory neurons. *Journal of Neurochemistry* **117**:841–855.
- Weiler IJ, Greenough WT (1991) Potassium ion stimulation triggers protein translation in synaptoneuroosomal polyribosomes. *Molecular and Cellular Neurosciences* **2**:305–314.
- Wu L, Wells D, Tay J, Mendis D, Abbott MA, Barnitt A, Quinlan E, Heynen A, Fallon JR, Richter JD (1998) CPEB-mediated cytoplasmic polyadenylation and the regulation of experience-dependent translation of alpha-CaMKII mRNA at synapses. *Neuron* **21**:1129–1139.

- Yeh SH, Yang WB, Gean PW, Hsu CY, Tseng JT, Su TP, Chang WC, Hung JJ** (2011) Translational and transcriptional control of Sp1 against ischaemia through a hydrogen peroxide-activated internal ribosomal entry site pathway. *Nucleic Acids Research* **39**:5412–5423.
- Yin Y, Edelman GM, Vanderklish PW** (2002) The brain-derived neurotrophic factor enhances synthesis of Arc in synaptoneuroosomes. *Proceedings of the National Academy of Sciences of the United States of America* **99**:2368–2373.
- Yoon BC, Jung H, Dwivedy A, O'Hare CM, Zivraj KH, Holt CE** (2012) Local translation of extranuclear lamin B promotes axon maintenance. *Cell* **148**:752–764.
- Zhong J, Zhang T, Bloch LM** (2006) Dendritic mRNAs encode diversified functionalities in hippocampal pyramidal neurons. *BMC Neuroscience* **7**:17.
- Zhu PJ, et al.** (2011) Suppression of PKR promotes network excitability and enhanced cognition by interferon-gamma-mediated disinhibition. *Cell* **147**:1384–1396.
- Zong Q, Schummer M, Hood L, Morris DR** (1999) Messenger RNA translation state: the second dimension of high-throughput expression screening. *Proceedings of the National Academy of Sciences of the United States of America* **96**:10632–10636.

Chapter 6

Structure and molecular organization of the postsynaptic density

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Summary

The morphology and molecular composition of synapses provides the basis of communication between neurons. Synapses are highly ordered, having developed structural and molecular specializations at their pre- and postsynaptic sites. At excitatory synapses, presynaptic sites of communication are faithfully juxtaposed to the postsynaptic density (PSD), an organized micro-compartment housed beneath the postsynaptic membrane. This largely invariant association suggests that the PSD is tightly coupled to synaptic function, allowing the pre-and postsynapse to form a cohesive unit. The PSD comprises several hundred proteins, including receptors, cytoskeletal, scaffolding, and signal transduction components. The organization of the PSD plays a central role in linking the molecular machinery responsible for neurotransmission to synaptic plasticity. This chapter details the structural organization of the PSD, with a particular emphasis on how its molecular constituents influence the structure and function of glutamatergic synapses.

Introduction

Dendritic spines are one of the most organized and best understood components of a neuron. They are highly specialized structures that are central to communication between neurons. Dendritic spines are small protrusions found along the shaft of dendrites and emanate from several different types of central nervous system (CNS) neurons. First noted by Ramón y Cajal (1888), dendritic spines were initially dismissed by many nineteenth-century scientists as an artifact of the Golgi stain method (Yuste, 2010). Determined that spines were real structures, Ramón y Cajal further suggested that they constituted the primary site of contact between dendrites and axons and were involved in learning and memory (Ramón y Cajal, 1891, 1893). More than half a century passed before electron microscopic (EM) studies definitively demonstrated that dendritic spines are sites of synaptic contact (De Robertis and Bennett, 1954; Palade and Palay, 1954; Palay, 1958; Gray, 1959).

Early EM studies of synapses identified “localised regions of thickening and increased density” within both the pre- and postsynaptic membranes (Palade and Palay, 1954; Palay, 1958; Gray, 1959). The presynaptic electron-dense region is the “active zone,” constituting a dense network of macromolecular complexes involved in the exocytosis of synaptic vesicles (Heuser and Reese, 1977; Landis et al., 1988). The relatively thin presynaptic active zone lies directly apposed to a thicker electron-dense region within the postsynaptic membrane (Palade and Palay, 1954; Palay, 1958). The prominence of the electron-dense region tethered to the postsynaptic membrane, termed the postsynaptic density (PSD), was used by Gray (1959) to classify synapses into

two principal categories: type 1, or asymmetric, having a pronounced postsynaptic membrane thickening (later identified as glutamatergic and excitatory), and type 2, or symmetric, synapses lacking a pronounced PSD (shown to be GABAergic and glycinergic inhibitory synapses) (Peters et al., 1991). Asymmetric, glutamatergic synapses can form on dendritic shafts or on dendritic spines, while symmetric, GABAergic synapses form predominantly on dendritic shafts. Excitatory glutamatergic synapses on interneurons form along the dendritic shaft (axo-dendritic synapses), while on principal cortical (Spacek and Hartmann, 1983) and hippocampal (Harris and Stevens, 1989) pyramidal neurons, and cerebellar Purkinje neurons (Harris and Stevens, 1988), the majority of glutamatergic synapses are formed on the dendritic spines (axo-spinous synapses). Despite the stark difference in synaptic structure among these neuronal types, the basic architecture of the PSD is maintained regardless of whether a glutamatergic synapse is formed on the dendritic shaft or spine. Most of the molecular components of the PSD at shaft and spine synapses are also the same, but spine synapses through their unique structure create a compartmentalized chemical environment in which signaling pathways can be limited to individual synapses.

Cytoarchitecture and laminar construction of the PSD

Attempts to understand how PSD structure might be organized to unify pre- and postsynaptic function has been a central focus in neurobiology for several decades. The classic approach to address this question involves coupling microscopy and biochemistry to dissect and identify each of the molecular constituents of the PSD. The PSD is a highly abundant neuronal structure made from a dense and relatively stable network of interacting proteins, allowing for this structure to survive biochemical fractionation as well as reaction with the heavy metal stains used for EM (Carlin et al., 1980). Together, these properties have allowed for examination of the cytoarchitecture of the PSD and its molecular composition. Differential centrifugation of brain tissue and subsequent sucrose gradient sedimentation leads to enrichment of synaptosomes (a mixture of isolated and intact pre- and postsynaptic terminals). A final extraction of synaptic membranes with non-ionic detergents results in purified PSDs (Carlin et al., 1980). More recently, quantitative fluorescence imaging methods and comprehensive proteomics analyses of primarily excitatory synapses have suggested that a single PSD comprises several hundred to a thousand proteins (Satoh et al., 2002; Jordan et al., 2004; Li et al., 2004; Peng et al., 2004; Yoshimura et al., 2004; Sugiyama et al., 2005). The molecular mass of a single PSD is estimated to be approximately 1 gigadalton (i.e., the equivalent of roughly 10,000 molecules if each weighed 100 kilodaltons) (Chen et al., 2005; Sugiyama et al., 2005; Sheng and Hoogenraad, 2007).

A typical PSD appears on EM as a density extending approximately 30–50 nm from the plasma membrane into the cytoplasm, with a diameter ranging between 200 and 500 nm (Carlin et al., 1980; Gulley and Reese, 1981). The opacity of PSDs visualized by EM represents proteins organized into a molecular complex that is specialized for postsynaptic signaling and plasticity. Recent advances in super-resolution microscopy have revealed the lateral distribution of synaptic proteins across single PSDs (Betzig et al., 2006; Hess et al., 2006). These studies demonstrate that within the surface of the synaptic membrane, proteins actually cluster into nanodomains, or distinct regions enriched in receptors and scaffolding proteins separated from each other by narrow gaps (Hoze et al., 2012; MacGillavry et al., 2013; Nair et al., 2013). Filamentous structures attached to or projecting from the PSD may limit receptor mobility within these PSD nanodomains, or anchor the PSD to the underlying spine cytoskeleton. Immediately below the PSD, many spines contain smooth endoplasmic reticulum (SER) (Spacek and Hartmann, 1983; Spacek and Harris, 1997) which may appear as amorphous tubulovesicular structures or as a spine apparatus (a stack of SER

cisternae intercalated by dense plates) (Gray, 1959). Such proximate localization of SER to the PSD suggests that local stores of intracellular calcium and sources for local vesicular cycling/delivery of PSD components (e.g., lipids and transmembrane receptor proteins) are provided to maintain the structure and function of the PSD within the spine (Fig. 6.1).

Using serial EM to reconstruct the surface of a synapse, individual PSDs can be classified as being either disk-like continuous “macular” structures, or irregularly shaped PSDs having “perforated” segments, or gaps devoid of PSD material; perforated PSD segments may or may not be interconnected within a single spine (Geinisman et al., 1987a,b; Peters et al., 1991; Nicholson et al., 2004). Representative examples of perforated and non-perforated axo-spinous synapses are shown in Fig. 6.2. While thin spines primarily house macular PSDs, it is estimated that more than 80% of large-volume mushroom-shaped spines harbor perforated PSDs (Harris et al., 1992). Whether or not specific PSD structures have a distinct function remains unclear. However, it is conceivable that the preponderance of perforated PSDs in larger spines reflects mechanisms of rapid PSD growth, rather than indicating a functional difference between macular and perforated types of PSDs. For instance, if PSDs grow in size by the addition of molecular constituents along their edges, then perforations within a PSD would provide increased surface availability for

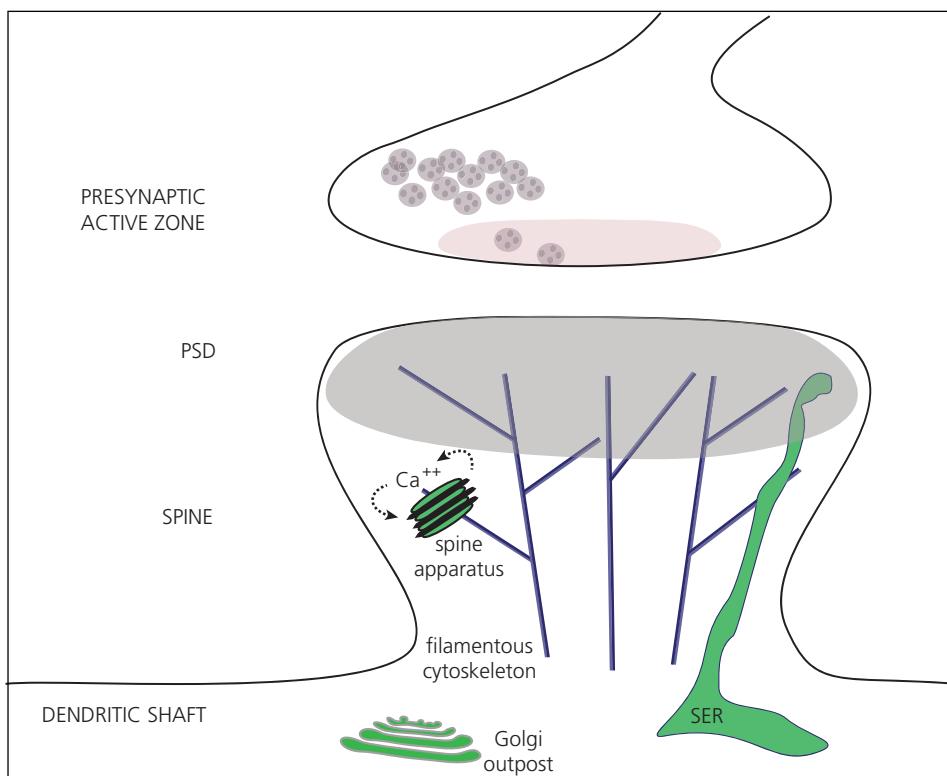


Fig. 6.1 Simplified model of the overall structure of a synapse. The presynaptic active zone aligns in close apposition to the postsynaptic density (PSD) within a spine emanating from the dendritic shaft. The dendritic spine harbors the molecular components of the PSD, cytoskeleton, and smooth endoplasmic reticulum (SER).

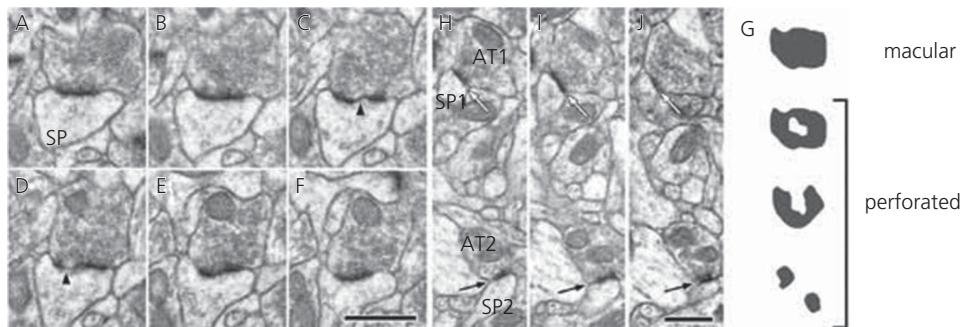


Fig. 6.2 Subtypes of hippocampal axospinous synapses from the rat CA1 stratum radiatum that are distinguished by their postsynaptic density (PSD) configuration. Electron micrographs of consecutive ultrathin sections demonstrate, respectively, a synapse with a perforated PSD (**A–F**) and two synapses (indicated by black or white arrows) with a non-perforated PSD (**H–J**). The synapse illustrated in parts **A–F** belongs to the perforated subtype because it exhibits a discontinuity or perforation in PSD sectional profiles (arrowheads). In contrast, the synapses observed in parts **H–J** have continuous, or macular PSD profiles in all consecutive sections. The presynaptic and postsynaptic elements of each synapse are labeled in **A** and **H** by AT (axon terminal) and SP (spine head). Two-dimensional reconstructions of PSD plates (**G**) reveal their macular or perforated configuration (perforated PSDs having a donut or horseshoe shape or consisting of separate segments). Scale bars = 0.5 μ m.

Adapted from Daniel A. Nicholson, Rie Yoshida, Robert W. Berry, Michela Gallagher, and Yuri Geinisman, Reduction in size of perforated postsynaptic densities in hippocampal axospinous synapses and age-related spatial learning impairments, *The Journal of Neuroscience*, 24(35), pp. 24–35, © 2004, The Society for Neuroscience.

incorporation of additional material. The absolute size and type of a PSD has been shown to vary widely, not only between distinct classes of neurons (e.g., Purkinje versus granule cell versus CA1 synapses) but even within a single neuron (Spacek and Hartmann, 1983; Harris and Stevens, 1988). This degree of variability suggests that distinct properties intrinsic to cell type, as well as the individual history of activity for a given synapse, are relevant to shaping PSD structure. Despite this variability, the surface area of the PSD is tightly correlated with spine head volume, the number of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamatergic receptors, and the total number of presynaptic vesicles within the active zone (Harris and Stevens, 1989; Harris and Sultan, 1995; Nusser et al., 1998; Schikorski and Stevens, 1999; Matsuzaki et al., 2001; Katz et al., 2009; Menon et al., 2013). This complex association suggests that the specific characteristics of PSD structure could inform overall synaptic function. Supporting this notion are observations that activity-dependent enhancement of synaptic transmission in several models of learning and memory increases the size and number of spines with perforated PSDs (Desmond and Levy, 1986; Geinisman et al., 1991, 1992; Harris et al., 1992; Popov et al., 2004).

Despite the remarkable density of proteins within the PSD, recent advances in high resolution microscopy coupled with specific antibody labeling have revealed that the three-dimensional disposition of these molecules is extremely ordered. The molecular constituents of the PSD are broadly organized into three laminar groups along the axo-dendritic axis (i.e., parallel to the plane of synaptic membrane) (Fig. 6.3). In the first PSD layer molecules are embedded within the exterior face of the PSD and plasma membrane, which primarily includes neurotransmitter

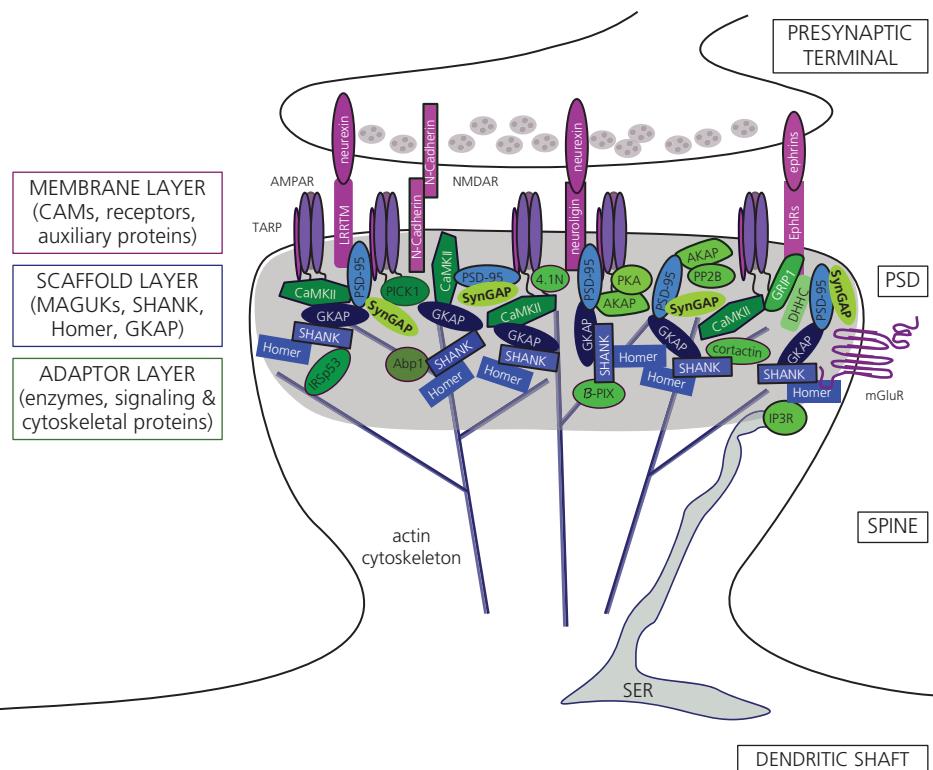


Fig. 6.3 Schematic representation of the laminar organization of a PSD, where some of the central molecular constituents and their interactions are indicated by overlap/contact between proteins.

receptors, ion channels, and trans-synaptic adhesion molecules. Approximately 10–20 nm below the plasma membrane is the second PSD layer which is enriched in scaffolding proteins, arranged orthogonal to the PSD, that are closely coupled to the membrane receptors and ion channels above. The third layer comprises adaptor or signaling proteins, many of which function to bridge the PSD with the actin cytoskeleton of the dendritic spine (Landis and Reese, 1983; Valschanoff and Weinberg, 2001; Petralia et al., 2005; Dani et al., 2010).

Molecular constituents of the PSD

Layer 1: membrane-associated PSD components

Receptors

Of the hundreds of proteins comprising the PSD, none are more definitive to synaptic function than the receptors concentrated within the postsynaptic membrane. At glutamatergic synapses, ionotropic and G-protein-coupled metabotropic glutamate receptors mediate the response to synaptic release of glutamate (for review see Chapter 7). The structure of the PSD positions glutamate receptors directly across from presynaptic sites of glutamate release, and links the receptors to intracellular signaling cascades that ultimately modulate synaptic function.

The majority of excitatory neurotransmission in the mammalian CNS is mediated by the ionotropic family of glutamate-gated receptors. This group includes the AMPA receptor (AMPAR), the *N*-methyl-d-aspartate receptor (NMDAR), and kainate receptors (KAR) which were named according to the pharmacology of channel activation by different synthetic agonists. The ionotropic receptors are ligand-gated ion channels, each formed by a tetrameric assembly of similar subunits that allow the influx of cations upon glutamate binding (for review see Traynelis et al., 2010). NMDARs are additionally voltage-dependent, such that their activation requires ligand binding as well as postsynaptic depolarization to remove a blockade by magnesium in order for channel conductance to occur.

Each of the four subunits that comprise an ionotropic channel share a similar structure of well-conserved domains: the extracellular amino-terminal (ATD), ligand-binding (LBD), transmembrane (TMD), and intracellular carboxy-terminal (CTD) domains. In general, binding of an agonist to the LBD of receptors within the PSD causes a conformational shift that alters the angle of the TMD regions. The resultant shift opens a pore in the membrane and allows for influx of sodium, potassium, and/or calcium into the spine. Although the ionotropic receptors have similar membrane topologies, they differ significantly in their electrophysiological properties, calcium permeability, intracellular trafficking, regulation, and anchoring within the PSD (Shepherd and Huganir, 2007; Traynelis et al., 2010; Pahl et al., 2014). The functional complexity of these channels is augmented by the fact that the relative synaptic abundance of a receptor's subunits is developmentally regulated, and their four subunits can combine in different stoichiometries to confer distinct kinetic properties to each channel (Monyer et al., 1994; Wenzel et al., 1997; Lilliu et al., 2001).

The regulation of calcium influx mediated by NMDARs is important for modulation of synaptic plasticity. Robust activation of NMDARs initiates signaling cascades that recruit AMPARs into the PSD and result in an overall strengthening of the synapse—a form of synaptic plasticity called long-term potentiation (LTP). Conversely, prolonged but weak activation of NMDARs triggers postsynaptic removal of AMPARs and a weakening of the synapse, known as long-term depression (LTD) (Huganir and Nicoll, 2013). While both AMPA and NMDA receptors can be mobile, within the PSD, nanodomains enriched in NMDARs appear to remain considerably less dynamic than those with AMPARs (Choquet and Triller, 2013; MacGillavry et al., 2013; Nair et al., 2013).

Similar to the NMDA and AMPA ionotropic glutamate receptors, KAR channels comprise subunit tetramers (Contractor et al., 2011; Sihra et al., 2014). There are five different KAR subunits, grouped on the basis of their pharmacological affinity to kainate: the low-affinity group (including GluK1–3, formerly known as GluR5–7, respectively) and the high-affinity group (including GluK4 and GluK5, formerly known as A1 and KA2, respectively) (Collingridge et al., 2009). The low-affinity subunits, GluK1–3, can form functional homomeric channels, whereas the high-affinity subunits GluK4/5 require low-affinity subunits to form functional heteromeric channels (Contractor et al., 2011; Sihra et al., 2014). Although the molecular mechanisms governing the cellular distribution of KARs between different types of synapses, neurons, and across brain regions remains unclear (Palacios-Filardo et al., 2014), KARs modulate the release of neurotransmitters and mediate synaptic transmission at some synapses (Bortolotto et al., 1999; Contractor et al., 2001; Lauri et al., 2001; Schmitz et al., 2001; Kidd et al., 2002).

Because AMPARs mediate most of the fast excitatory signal transmissions in the brain, their relative abundance within the postsynaptic membrane ultimately determines the strength of these synapses. Therefore, determining the physical and molecular factors that regulate AMPAR trafficking is a critical step toward elucidating the mechanisms involved in learning and memory formation, such as LTP and LTD (Huganir and Nicoll, 2013).

Auxiliary receptor subunits: TARPs, SOL, CNIHs, SynDIG1, GSG1L

In addition to the tetrameric core of subunits that form an AMPAR, a growing number of auxiliary subunits have been identified. Auxiliary proteins are non-transient binding partners, remaining bound to the AMPARs within the postsynaptic membrane, that profoundly modulate the trafficking and electrophysiological properties of glutamatergic receptors.

The first AMPAR auxiliary subunit to be identified was stargazin (also called $\gamma 2$) (Letts et al., 1998). Stargazin is a member of the γ -auxiliary family of proteins called the transmembrane AMPAR regulatory proteins (TARPs) (Tomita et al., 2003). Based on sequence alignment and functional analyses, TARPs are divided into two groups, type I (γ -2, γ -3, γ -4, and γ -8) and type II (γ -5 and γ -7), with the related γ -1 and γ -6 proteins excluded from the family as they do not modulate AMPARs (Tomita et al., 2003; Kato et al., 2008). The functional significance of auxiliary subunits was initially recognized in “stargazer” mutant mice, which harbor mutations in stargazin that result in epilepsy, ataxia, and a lack of functional AMPARs within cerebellar granule cells (Chen et al., 2000). Subsequent studies have extended an essential role for AMPAR maturation and trafficking to include other TARP proteins (Tomita et al., 2003, 2004). Although TARPs promote surface expression of AMPARs, the synaptic retention of these receptors requires the interaction of TARPs with the key scaffolding molecule PSD-95, as well as n-PIST and S-SCAM (Schnell et al., 2002; Cuadra et al., 2004; Deng et al., 2006; Bats et al., 2007). Each of the TARPs is differentially expressed in neurons, with several tissues expressing multiple family members (Tomita et al., 2003). More recently TARPs have been shown to have dramatic effects on the agonist efficacy, activation time, deactivation rate, and desensitization rate of AMPARs (Tomita et al., 2004; Priel et al., 2005; Turetsky et al., 2005; Cho et al., 2007; Kott et al., 2007; Menuz et al., 2007; Milstein et al., 2007; Wang et al., 2008).

Proteomic analyses have since led to the discovery of several classes of novel auxiliary subunits, including the CNIH transmembrane proteins (Schwenk et al., 2009). In heterologous cells CNIH proteins markedly slow AMPAR desensitization kinetics (Schwenk et al., 2009; Shi et al., 2010; Coombs et al., 2012; Gill et al., 2012), while their genetic deletion disrupts hippocampal expression of synaptic AMPARs (Herring et al., 2013). CNIH proteins reportedly compete with TARPs for interaction with GluA2, GluA3, and GluA4 AMPAR subunits, but may concomitantly associate with the GluA1 subunit since they bind distinct sites (Schwenk et al., 2009; Herring et al., 2013). These findings suggest that CNIH and TARPs may act in concert to dictate subunit-specific trafficking, kinetics, and the strength of synaptic AMPAR-mediated transmission.

Compared with TARPs and CNIHs, much less is known regarding the function of the AMPAR-associated CKAMP44, a brain-specific type I transmembrane protein that is robustly expressed in hippocampal dentate granule cells. It can bind to each of the core AMPAR subunits, and is found in neuronal complexes that also contain stargazin (von Engelhardt et al., 2010). In striking contrast to TARPs, which accelerate recovery from the desensitized state, CKAMP44 expression slows AMPAR recovery (von Engelhardt et al., 2010).

Using high-resolution proteomic techniques, GSG1L was recently identified as a transmembrane protein bearing structural similarity to the tetraspanin TARP family proteins (Schwenk et al., 2012; Shanks et al., 2012). Functionally, GSG1L binds AMPARs and increases their surface expression (Shanks et al., 2012). Surprisingly, in contrast to their TARP relatives, but akin to CKAMP44, GSG1L slows the recovery of AMPAR from desensitization (Schwenk et al., 2012; Shanks et al., 2012).

SynDIG1 is another transmembrane protein that interacts directly with the GluA2 subunit of AMPARs. In cultured hippocampal neurons SynDIG1 supports glutamatergic synapse development, and its expression is enhanced within dendritic spines upon neuronal activity (Kalashnikova

et al., 2010). However, SynDIG1 does not modulate the trafficking of AMPARs, and its synaptogenic effects appear to be independent of its physical interaction with AMPARs, suggesting that it may not function as a traditional auxiliary subunit (Lovero et al., 2013).

In the invertebrate *Caenorhabditis elegans*, the CUB domain-containing proteins SOL-1 and SOL-2 bind the glutamate receptor homolog to modulate its desensitization kinetics (Zheng et al., 2004; Wang et al., 2012). NETO1 and NETO2 are mammalian homologs of SOL-2, and while they share regions of homology to SOL-1, they represent a distinct class of proteins (Stohr et al., 2002; Michishita et al., 2003; Wang et al., 2012). NETO proteins can affect the channel properties that are particularly characteristic of KARs, including their slow kinetics and high affinity for agonists, suggesting that NETOs are auxiliary subunits specific to these receptors (Zhang et al., 2009; Straub et al., 2011; Tang et al., 2011; Fisher and Mott, 2012, 2013).

Adhesion molecules

Embedded within synaptic membranes are several different types of cell adhesion molecules (CAMs). These molecules serve at least two functions: (1) physically linking and maintaining the pre- and postsynaptic membranes within a narrow distance, and (2) participating in cellular signaling that regulates synapse formation and glutamate receptor targeting to synapses during development and synaptic plasticity. The principal groups of synaptic CAMs are the immunoglobulin like family [IgCAMs; e.g., neurexins and neuroligins, ephrins, and Eph receptors] and the nectin-like single-pass transmembrane proteins containing extracellular Ig domains (e.g., cadherins and SynCAMs) (Dalva et al., 2007). In nascent synapses, CAMs critically regulate the contact, alignment, and maturation of synaptic membranes. The significance of CAMs persists throughout the lifetime of a synapse, as trans-synaptic interactions between pre- and postsynaptic CAMs hold the respective membranes together.

CAMs significantly influence the plasticity of synaptic connections in addition to maintaining structural fidelity. Both neurexin and neuroligin CAMs (expressed in the presynaptic and postsynaptic membranes, respectively) have been shown to interact with several synaptic regulatory molecules to modulate neuronal function. For instance, binding of neuroligin 1 to glial-secreted thrombospondin 1 in hippocampal neurons accelerates the formation rate of predominantly AMPAR-lacking synapses (also known as “silent” synapses because they do not mediate neurotransmission) (Isaac et al., 1995; Liao et al., 1995; Xu et al., 2010). Another example is the interaction of neurexin with the adaptor protein band 4.1, an actin-binding protein that helps stabilize synaptic sites by nucleating local assembly of actin/spectrin filaments (Biederer and Sudhof, 2001). Notably, neurexins and neuroligins can bind to each other, and they were the first pair of CAMs demonstrated to induce synapse formation through this interaction (Scheiffele et al., 2000; Graf et al., 2004).

In addition to being a presynaptic receptor for neuroligin, neurexin also binds members of the postsynaptic family of leucine-rich repeat transmembrane proteins (LRRTMs) (de Wit et al., 2009; Ko et al., 2009; Linhoff et al., 2009). There are four related LRRTM genes in mammals—all are enriched in brain and expressed from postnatal day one or earlier (Lauren et al., 2003). At the postsynaptic membrane, LRRTMs are part of a multiprotein complex that includes AMPA and NMDA receptor subunits, and a core scaffolding component of the PSD, PSD-95 (de Wit et al., 2009; Linhoff et al., 2009; Schwenk et al., 2012). Moreover, specific expression of LRRTM complexes along with neurexin is a potent inducer of formation of excitatory synapses (de Wit et al., 2009; Ko et al., 2009; Linhoff et al., 2009; Ko et al., 2011; Soler-Llavina et al., 2011).

Pre- and postsynaptically expressed N-cadherin mediates cell adhesion by Ca^{2+} -dependent homophilic binding to modulate the neuronal plasticity of glutamatergic synapses (Tang et al., 1998;

Bozdagi et al., 2000; Jungling et al., 2006). Recently, N-cadherin was shown to form heterophilic trans-synaptic interactions that also modulate synaptic function. Retrograde signaling through interaction of neuroligin 1 with presynaptic N-cadherin enhances presynaptic maturation (Stan et al., 2010; Aiga et al., 2011). Postsynaptic N-cadherin binds and regulates AMPAR trafficking, while also stabilizing the structure of dendritic spines through concomitant interaction with actin-cytoskeletal components (Bozdagi et al., 2000; Togashi et al., 2002; Nuriya and Huganir, 2006; Okuda et al., 2007; Saglietti et al., 2007; Brigidi et al., 2014).

The Eph receptors (EphRs) represent the largest family of receptor tyrosine kinases in the animal kingdom and also act as synaptic CAMs (Lisabeth et al., 2013). Ephrin ligands and their EphRs function in bidirectional signal transduction (i.e., modulation of both the pre- and post-synaptic cell). Alteration of EphRephrin expression and/or function affects spine morphogenesis, maintenance, and excitatory synaptic plasticity. For instance, several studies have demonstrated that EphB2 receptors associate with the NMDAR, and that ephrinB ligand application stimulates this interaction, along with recruitment of AMPARs to synaptic sites (Dalva et al., 2000; Ethell et al., 2001; Penzes et al., 2003; Grunwald et al., 2004). Moreover, EphB2 R activation regulates NMDAR-mediated signal transduction and synaptic plasticity (Grunwald et al., 2001; Henderson et al., 2001; Takasu et al., 2002). Enhancement of ephrinB-mediated signaling is also synaptogenic, increasing basal synaptic transmission and activity-induced LTP (Dalva et al., 2000; Lim et al., 2008). Using *in vivo* two-photon imaging, experience-dependent dendritic spine pruning was found to be accelerated in mice lacking ephrin-A2, and this spine loss was also found to be dependent on NMDA receptor activation (Yu et al., 2013). These findings indicate that multiple members of the ephrin-EphR family can crosstalk with glutamatergic receptors to each regulate synaptic plasticity.

These studies demonstrate that several classes of adhesion molecules can form complexes between glutamatergic receptors and scaffolding proteins. The identification of these multiprotein interactions suggests that the coupling of trans-synaptic adhesion with molecular organization of postsynaptic proteins is fundamental to the regulation of synaptic function.

Layer 2: PSD scaffolding proteins

Immediately adjacent to the laminar distribution of PSD membrane proteins is a layer of scaffolding molecules. While scaffolding molecules traditionally lack intrinsic enzymatic activity, they include one or modular domains that can form heteromeric protein complexes. Thus, scaffolding proteins not only help anchor the receptor subunits and other postsynaptic transmembrane proteins within the PSD but also link membrane-bound molecules to their downstream signaling proteins that dynamically regulate cytoarchitecture and functional plasticity. Further, these scaffold molecules interact extensively with each other to create a network that forms the physical basis of the PSD. The principal components of this PSD lamina are the MAGUK (membrane-associated guanylate kinase), GKAP (guanylate kinase-associated protein, also known as SAPAP), Shank, and Homer families of scaffold proteins.

The most extensively studied class of PSD-related scaffolding proteins are the MAGUKs. MAGUKs are structurally homologous proteins defined by the presence of three N-terminal PDZ (PSD-95/disks large/zona occludens) domains, followed by an SH3 domain, and a catalytically inactive guanylate kinase domain (Olsen and Bredt, 2003). This family includes the proteins PSD-95 (also known as SAP90), PSD-93 (also known as Chapsyn 110), SAP102, and SAP97, which are all highly enriched in the PSD (Cho et al., 1992; Kistner et al., 1993; Brenman et al., 1996; Muller et al., 1996).

EM tomography and 3D STORM (stochastic optical reconstruction microscopy) ultrastructural analyses situate PSD-95 immediately below the laminar surface of the PSD membrane (Valtschanoff and Weinberg, 2001; Petersen et al., 2003; Dani et al., 2010). At the synapse, PSD-95 orients itself into distinctive, elongated vertical filaments, which may increase the potential number of and/or facilitate the ease of interaction with other synaptic proteins (Chen et al., 2008, 2011). Soon after the discovery of PSD-95, it and other MAGUKs were found to interact with NMDARs (Kornau et al., 1995; Muller et al., 1996; Niethammer et al., 1996; Bassand et al., 1999) and so were ascribed to be NMDAR-specific anchors within the PSD. However, despite their direct binding, neither NMDAR clustering nor NMDAR neurotransmission was effected upon alteration of PSD-95 expression levels (Migaud et al., 1998; El-Husseini et al., 2000a). Focus quickly turned to the effects of PSD-95 and other MAGUKs on AMPARs. Several studies found that modulating the levels of expression of different MAGUKs in neurons caused significant changes to synaptic AMPAR targeting and function (Migaud et al., 1998; Chen et al., 2000; El-Husseini et al., 2000a; Schnell et al., 2002; Beique and Andrade, 2003; Ehrlich and Malinow, 2004; Beique et al., 2006; Elias et al., 2006).

In neurons, both PSD-95 and PSD-93 interact indirectly with AMPARs through their binding to TARPs (Chen et al., 2000; Dakoji et al., 2003), while SAP97 can bind the GluA1 subunit of AMPARs directly (Leonard et al., 1998; von Ossowski et al., 2006). Specific differences in MAGUK-mediated AMPAR and other protein interactions suggest layers of complexity in the influence of MAGUKs on synaptic plasticity. Furthermore, multiple types of post-translational modifications of MAGUKs, as well as glutamatergic receptors, greatly influence protein targeting and stability within the PSD (Chen and Roche, 2007; Anggono and Huganir, 2012; Thomas and Huganir, 2013). Palmitoylation is a reversible addition of the fatty acid palmitate to cysteine residues in target proteins (Fukata and Fukata, 2010). Both PSD-95 and PSD-93 require palmitoylation for their membrane association and specific synaptic enrichment (Topinka and Bredt, 1998; Craven et al., 1999; El-Husseini et al., 2000b; El-Husseini Ael et al., 2002). In contrast, SAP97 and SAP102, which lack palmitoylation, are widely expressed at synapses and throughout the cytosol of dendrites and axons (Muller et al., 1995, 1996). Using super-resolution microscopy the lateral distribution of scaffolding and receptor family proteins across a single PSD can be visualized *in vivo* at nanometer resolution (Betzig et al., 2006; Hess et al., 2006). These studies demonstrate that proteins across the PSD are not homogeneously distributed. Rather, the PSD consists of subsynaptic nanodomains of clustered proteins that are separated from each other by narrow gaps (Hoze et al., 2012; MacGillavry et al., 2013; Nair et al., 2013). Nanodomains of palmitoylated PSD-95 preferentially co-enrich AMPARs over NMDARs (Fukata et al., 2013; MacGillavry et al., 2013). Moreover, the expression level of PSD-95 specifically regulates AMPAR cluster size and follows the amplitude of the miniature excitatory postsynaptic current (EPSC) (Fukata et al., 2013; MacGillavry et al., 2013; Nair et al., 2013). Collectively, these studies suggest that PSD-95 can instruct the lateral architecture of the PSD as well as its function.

Further evidence supporting the role of PSD-95 as a network organizer of the PSD is found in its copious interaction with other synaptic molecules. At the synaptic surface, PSD-95 assembles membrane protein ion channels (e.g., NMDAR, AMPARs, and the auxiliary stargazin/TARP subunits) and CAMs (e.g., neuroligin and SynCAM). Deeper within the axo-dendritic laminar axis PSD-95 links additional scaffolding molecules to the PSD (Valtschanoff and Weinberg, 2001; Petersen et al., 2003; Chen et al., 2008; Dani et al., 2010). For instance, the GK domain of PSD-95 binds to most members of the GKAP family, but not to all owing to differential alternative splicing between the four GKAP members (Kim et al., 1997; Takeuchi et al., 1997). N-terminal sequences in GKAP link to the synaptic scaffolding molecule S-SCAM (Hirao et al., 1998; Wu et al., 2000). S-SCAM appears to specifically regulate trafficking of those AMPAR channels that include a

GluA2 subunit (Danielson et al., 2012). S-SCAM interacts with TARP auxiliary AMPAR subunits (Deng et al., 2006), and the microtubule motor kinesin superfamily member KIF1B (Mok et al., 2002), which may allow for microtubule-based transport of neuronal proteins to excitatory spines.

The modular domain structure of GKAPs integrates cytoskeletal association with the PSD through their C-terminal PDZ ligand-dependent binding of the Shank/ProSAP scaffolding proteins (Naisbitt et al., 1999; Tu et al., 1999). Shanks are encoded by three genes that generate multiple transcripts based on alternative splicing: Shank1 (also named Shank1a, Synamon, or SSTRIP), Shank2 (also known as ProSAP1 or CortBP1), and Shank3 (also named ProSAP2) (Boeckers et al., 1999a,b; Naisbitt et al., 1999; Yao et al., 1999). Shank family members each contain N-terminal ankyrin repeats and SH3, PDZ, proline-rich, and sterile alpha motif (SAM) domains (Boeckers et al., 1999a; Naisbitt et al., 1999). Shank proteins bind directly to the GluA1 subunit of AMPARs (Uchino et al., 2006). They are each expressed in hippocampus and cortex, but differ in abundance in other neuronal cells and peripheral tissues (Boeckers et al., 1999a,b; Naisbitt et al., 1999). Several actin-binding proteins directly interact with Shank proteins, including cortical-actin-binding protein (cortactin) (Naisbitt et al., 1999), α -fodrin (Bockers et al., 2001), actin-binding protein 1 (Abp1) (Qualmann et al., 2004), IRSp53 (an actin regulatory protein that binds Rac1 and Cdc42) (Bockmann et al., 2002; Soltau et al., 2002), and β -PIX (a guanine exchange factor for Rac1 and Cdc42) (Park et al., 2003). Overexpression of Shank promotes spine enlargement and synaptic transmission (Sala et al., 2001), whereas disrupting the interaction of Shank with actin-associated molecules stunts spine maturation (Naisbitt et al., 1999; Haeckel et al., 2008). Actin filaments form bundles in the neck of dendritic spines, and in the spine head they form an extensive lattice that interacts with the plasma membrane and the PSD through their barbed ends (Fifkova and Delay, 1982; Korobova and Svitkina, 2010). Collectively, these observations suggest that membrane-bound adhesion molecules and neurotransmitter receptors are linked to the actin cytoskeleton by a PSD-95/GKAP/Shank based network to organize the PSD and regulate synaptic function.

The Homer family of proteins (Homer1–3) is encoded by three genes with alternatively spliced variants that are highly enriched within the PSD (Brakeman et al., 1997; Kato et al., 1998; Sun et al., 1998; Xiao et al., 1998). A central coiled-coil domain expressed in Homer proteins mediates their ability to self-associate (Xiao et al., 2000). Moreover, recent X-ray crystallographic studies demonstrate that Homer and Shank interact, and recruit GKAP to form an extensive polymeric-mesh structure at synapses (Tu et al., 1999; Sala et al., 2001; Hayashi et al., 2009). As modular-domain-containing proteins, Homers serve as scaffolding proteins that link the PSD to several different receptors and channels. For instance, the N-terminal EVH1 domain of Homer binds to Shank, as well as mGluR1 and mGluR5, members of the TRPC family of ion channels, ryanodine receptor, selective L-type calcium channels, and inositol 1,4,5-trisphosphate (IP₃) receptor (Brakeman et al., 1997; Tu et al., 1998, 1999; Sala et al., 2001; Yuan et al., 2003; Yamamoto et al., 2005). IP₃ receptors are concentrated in the SER, which, as already discussed, extends into the spine and juxtaposes the cytoplasmic face of the PSD. That GKAP, Shank, and Homer are found in the “deep” cytoplasmic part of the PSD (Niethammer et al., 1996; Valtschanoff and Weinberg, 2001; Chen et al.; 2008, Dani et al., 2010) suggests that this scaffolding complex functionally couples mGluRs and IP₃Rs to mediate calcium levels in subregions of the spine (Tu et al., 1998; Worley et al., 2007). Indeed, the Homer-mediated scaffold physically associates with combinations of channels and receptors at the PSD, and is demonstrated to regulate the functional properties of these channels and their downstream signaling (Yuan et al., 2003; Sala et al., 2005; Yamamoto et al., 2005; Huang et al., 2007). Thus, through Shank, GKAP-mediated engagement with PSD-95, and with Homer coiled-coil-mediated dimerization, a structural complex is formed that is necessary for the architectural

and functional integrity of dendritic spines, and which serves as a binding platform for other signaling and adaptor proteins in the PSD.

Layer 3: PSD adaptor proteins

Among the deep laminar constituents of the PSD are the “adaptor proteins,” so-called because they function to bridge the PSD to the cytoskeleton and/or components of signal transduction. As outlined above, the cytoskeleton present throughout a dendritic spine is a dynamic network, connecting the synaptic membrane with the molecular constituents of the PSD (Fifkova and Delay, 1982; Korobova and Svitkina, 2010).

Pharmacological manipulation of the dynamics of filamentous actin can disperse glutamatergic receptors from spines, disrupt basal synaptic transmission, and block LTP (Allison et al., 1998; Kim and Lisman, 1999; Krucker et al., 2000). Thus, structural connections provided by the actin cytoskeleton have significant influence over the PSD and synaptic function. The principal scaffolding protein of the PSD, PSD-95, directly associates with numerous actin polymerization and remodeling regulatory proteins. For instance, PSD-95 binds the excitatory synapse-specific protein SynGAP, a GTPase-activating protein (GAP) for Ras, that regulates AMPAR trafficking, excitatory synaptic transmission, and dendritic spine morphology (Chen et al., 1998; Kim et al., 1998, 2003; Rumbaugh et al., 2006). The ability of SynGAP to regulate the phosphorylation state of cofilin, an actin-severing protein, may be one mechanism through which it influences spine dynamics and morphology (Carlisle et al., 2008). PSD-95 also interacts with kalirin-7, a guanine nucleotide exchange factor (GEF) for Rac1 (Penzes et al., 2001). Kalirin-7 in turn binds the trans-synaptic signaling adhesion molecule EphB2 (Penzes et al., 2003) to regulate neuronal activity-dependent changes in spine size and synaptic expression of AMPARs (Xie et al., 2007). Both IRSp53, a downstream effector of the small GTPases Rac1/Cdc42, and SPAR, an inhibitory GAP for Ras-related proteins (RAP), each promote spine growth and bind directly to PSD-95 (Pak et al., 2001; Choi et al., 2005).

Several cytoskeletal proteins have been shown to associate with specific subunits of glutamatergic receptors. The synaptic targeting of GluA1 and GluA4 subunits of AMPARs is regulated by direct interactions with the actin/spectrin-associated protein 4.1N (Shen et al., 2000; Coleman et al., 2003). The C-termini of both the NR1 and NR2B subunits of the NMDA receptor bind to α -actinin-2, a member of the spectrin–dystrophin family of actin-binding proteins that is involved in cross-linking of actin filaments (Wyszynski et al., 1997). Specific NMDAR subunits also mediate interaction with A-kinase anchor proteins (AKAPs), a family of proteins that function at the nexus of dendritic cytoarchitecture and intracellular signal transduction (Carr et al., 1992; Lin et al., 1998; Westphal et al., 1999). AKAP proteins are adaptor molecules that bind cAMP-dependent protein kinase A (PKA), protein kinase C (PKC), and the protein phosphatase calcineurin (also known as PP2B) (Sanderson and Dell'Acqua, 2011).

Through their interaction with PSD-95 and SAP97, AKAPs draw their associated enzymes to the NMDARs and AMPARs to modulate their phosphorylation/dephosphorylation status at the PSD, and ultimately regulate synaptic function (Colledge et al., 2000; Tavalin et al., 2002; Bhattacharyya et al., 2009). Another enzyme that binds the NMDARs is calcium/calmodulin-dependent protein kinase 2 (CaMKII), which is typically activated by the influx of calcium through NMDARs. CaMKII- and PKA-mediated phosphorylation of GluA1 subunits regulates AMPAR expression at synapses and is critical for several forms of synaptic plasticity and learning (Lee et al., 1998; Hayashi et al., 2000; Lee et al., 2003).

The ability to directly bind to AMPARs implicates many proteins in the functional regulation of glutamatergic synapses. Several AMPAR-interacting proteins bind particular AMPAR subunits, and so may recruit and stabilize specific subunits within the PSD to modulate the overall

properties of glutamatergic synapses. For example, NSF interacts selectively with the intracellular C-terminal domain of GluA2 (Nishimune et al., 1998; Osten et al., 1998; Song et al., 1998) to critically regulate AMPAR expression and functional plasticity at the synapse (Luscher et al., 1999). C-terminal PDZ ligands of GluA2 and GluA3 bind to specific PDZ domains of the GRIP family of proteins (also known as the ABP family) (Dong et al., 1997, 1999; Srivastava and Ziff, 1999). GRIP1 and GRIP2 are widely expressed in axons and dendrites of neurons (Wyszynski et al., 2002). While genetic ablation of both GRIP1 and GRIP2 blocks cerebellar LTD, this phenotype can be rescued upon expression of either GRIP1 or GRIP2, suggesting some degree of functional redundancy between them (Takamiya et al., 2008). Alternative splicing generates variants of GRIP1 that are palmitoylated (GRIP1b), associate with synapses, and function to accelerate AMPAR trafficking, whereas non-palmitoylated GRIP1a associates with intracellular membranes and may regulate trafficking of primarily non-synaptic pools of AMPARs (Yamazaki et al., 2001; DeSouza et al., 2002; Thomas et al., 2012). Both GRIP1 and GRIP2 are found to be expressed in non-neuronal tissues (Dong et al., 1999), indicating that these proteins function beyond the neuronal trafficking of AMPARs. Consistent with this notion is the finding that GRIP1 knock-out mice are afflicted with hemorrhagic blisters and are embryonic lethal (Takamiya et al., 2004). Furthermore, the PDZ domains of GRIP1/2 can interact with other PSD-enriched molecules and modulate their trafficking, including EphRs (Bruckner et al., 1999), GRASP1 (Ye et al., 2000), liprin-alpha (Wyszynski et al., 2002), and the palmitoyl acyltransferases DHHC5 and DHHC8 (Thomas et al., 2012), as well as metabotropic and kainate-type glutamatergic receptors (Hirbec et al., 2002, 2003).

The BAR and PDZ domain-containing protein PICK1 is another AMPAR-binding protein that can regulate the surface expression, trafficking, and synaptic function of AMPARs (Anggono and Huganir, 2012). Similar to GRIP1/2, PICK1 binds the C-terminal PDZ ligands of GluA2 and GluA3, is expressed at synaptic and non-synaptic sites in neurons, and is found in non-neuronal tissues (Dev et al., 1999; Xia et al., 1999). However, GRIP1/2 and PICK1 appear to have distinct roles in the control of AMPAR trafficking (Matsuda et al., 1999; Chung et al., 2000). GRIP1/2 play a central role in promoting the delivery and accumulation of synaptic GluA2-containing receptors (Dong et al., 1997, 1999; Thomas and Huganir, 2013), while PICK1 is important for intracellular retention of internalized receptors (Terashima et al., 2004; Citri et al., 2010) and knocking out of PICK1 eliminates cerebellar LTD (Steinberg et al., 2006). Both GRIP1/2 and PICK1 have been shown to regulate multiple forms of synaptic plasticity and learning/memory (Clem et al., 2010; Volk et al., 2010; Mejias et al., 2011). The PDZ domain of PICK1 interacts with several other proteins including PKC-alpha, mGluRs (Hirbec et al., 2002; Perroy et al., 2002), the dopamine plasma membrane transporter (Torres et al., 2001), and the netrin receptor UNC5H (Williams et al., 2003). The widespread localization of PICK1 coupled with its disparate PDZ domain-mediated interactions supports a functional role for PICK1 in the general trafficking of proteins both within and beyond the PSD.

Developmental changes in PSD function

During development, numerous proteins are concentrated at the nascent sites of synapses. This recruitment of proteins precedes the structural changes that accompany formation of a well-organized PSD. As mentioned previously, the specific localization of CAMs to create intercellular contact between pre- and postsynaptic neurons is required for synaptic organization (Siddiqui and Craig, 2011). Although the underlying mechanisms triggering the formation of synapses remains unclear, the accumulation of several CAMs (e.g., cadherin, ephrin/EphR, neurexin/neuroligin, LRRTMs) and accessory proteins (e.g. SynDIG1) is required for proper formation and/

or maturation of synapses (Dalva et al., 2000; Scheiffele et al., 2000; Graf et al., 2004; Linhoff et al., 2009; Stan et al., 2010; Aiga et al., 2011). Supporting the synaptogenic importance of these proteins are findings that gene abnormalities in CAMs result in impaired synapse and/or spine number and morphology (Dalva et al., 2007).

Once a synaptic contact has been established, the structure and composition of PSDs begins to change. Ultrastructural analyses of early postnatal brains has found that initial PSDs are small and most often found at the tips of thin dendritic protrusions (Swulius et al., 2012). As development progresses, dendritic spines grow shorter and broaden alongside their PSDs, and the expression level of many PSD proteins increases (e.g., PSD-95, CaMKII, NMDARs, AMPARs) until typically peaking along with spine maturation at 2–4 weeks after birth (Petralia et al., 2005). However, several proteins that are highly expressed in nascent synapses decline in expression with maturation. For instance, although PSD-95 is a principal scaffolding molecule in developed synapses, SAP102 predominates in neonatal neurons (Sans et al., 2000). Its expression peaks at postnatal day ten, coincident with the onset of expression of PSD-95 and PSD-93 (Sans et al., 2000). The scaffolding proteins Shank2 and Shank3 also dominate newly formed PSDs, with Shank1 primarily recruited at later stages in synapse maturation (Boeckers et al., 1999b; Sala et al., 2001; Grabrucker et al., 2011).

Another classic example of a PSD-associated protein that is reduced in development is the GluN2B subunit of NMDARs (Monyer et al., 1994; Sheng et al., 1994). Synaptic NMDARs in the early neonate comprise predominantly GluN2B in complex with SAP102, but are replaced with GluN2A–PSD-95 complexes as the synapses mature (Sans et al., 2000; Townsend et al., 2003; Yoshii et al., 2003). These temporal changes in MAGUKs play considerable role in organizing the recruitment of other molecules within the maturing synapse, which consequently have modulatory effects on AMPAR function (Elias et al., 2006). AMPARs are also developmentally regulated through alternative splicing and mRNA editing. Those AMPARs recruited to nascent synapses possess larger current flux and different functional properties than the AMPARs enriched within mature synapses (Monyer et al., 1991; Tonnes et al., 1999).

Changes in PSD constituents during synaptic plasticity

In addition to the molecular and structural changes that are incumbent to the development of PSDs, mature glutamatergic synapses are acutely modulated by neuronal activity. Increases in activity result in prolonged synaptic strengthening (i.e., LTP) (Bliss and Lomo, 1973), while decreases in activity are accompanied by persistent weakening (i.e., LTD) of synapses (Ito and Kano, 1982). This ability of synapses to change both their structure and function in response to activity is called synaptic plasticity. The concept of synaptic plasticity, proposed in the 1940s by Canadian neuroscientist Donald Hebb, is often summarized with the idiom coined by Dr. Carla Shatz “Neurons that fire together, wire together.” Hebb theorized that if a presynaptic cell persistently stimulated a postsynaptic cell, their communication would be selectively strengthened (Hebb, 1949). As a result, the functional output of plasticity applied to multiple synapses would be the specific alteration to whole networks of neurons. Based on this concept, Hebb posited that memories are stored and encoded as engrams, the biophysical changes to a neuronal network. Synaptic plasticity remains a phenomenon that is widely held to underlie the formation of memories and learning at the cellular level.

Although synaptic plasticity occurs at synapses throughout the brain, it is most extensively studied in the Schaffer collateral–CA1 glutamatergic synapses of the hippocampus. Biophysical changes in synaptic strength can be recorded using electrophysiological techniques and are

primarily attributed to the insertion and removal of synaptic AMPARs leading to the strengthening (LTP) or weakening (LTD) of synapses, respectively. Associated with this functional plasticity are structural modifications to synapses. The induction of LTP stimulates new spine growth and can increase the volume of existing spines, while LTD has the converse effect on spine structure (Fifkova and Anderson, 1981; Matsuzaki et al., 2004; Tonnesen et al., 2014). Changes in spine size are also linked with changes in the diameter of the PSD (Harris and Stevens, 1989). Importantly, these structural alterations occur in tight correlation with synaptic AMPAR content and function, suggesting they are functionally integrated phenomena.

Both hippocampal LTP and LTD are triggered by calcium entry through synaptic NMDARs (Collingridge et al., 1983; Dudek and Bear, 1992), followed by the recruitment and activation of several kinases in the PSD, including CaMKII, PKA, PKC, tyrosine kinases, and MAPKs. Opposing these kinases are phosphatases such as protein phosphatase 1 (PP1) and protein phosphatase 2B.

The most abundant kinase in the PSD is CaMKII (Chen et al., 2005). Considerable evidence suggests that CaMKII is the principal downstream target following calcium influx through NMDARs. In addition to its enzymatic activity, that is both necessary and sufficient for induction of LTP (Malinow et al., 1989; Lledo et al., 1995), CaMKII may play a structural role in plasticity by modulating spine size through interactions with the actin cytoskeleton (Okamoto et al., 2007; Pi et al., 2010). Elevated calcium in the spine triggers activity-dependent interaction of CaMKII with the GluN2B subunit of NMDAR. Significantly, disruption of this binding impairs LTP (Barria and Malinow, 2005; Zhou et al., 2007; Halt et al., 2012). These findings support the notion that anchoring CaMKII to NMDARs within the PSD positions it for optimal activation by calcium and downstream phosphorylation of PSD proteins.

A central component of plasticity that is critically regulated by phosphorylation is the AMPAR (Shepherd and Huganir, 2007). In adult rat hippocampal neurons AMPARs mainly comprise combinations of GluA1/2 or GluA2/3 heteromers, or GluA1 homomers (Wenthold et al., 1996; Lu et al., 2009). Each of these subunits can be directly phosphorylated at multiple sites by several kinases, including CaMKII, PKA, and PKC, to modulate channel conductance and/or interaction with other PSD proteins (Shepherd and Huganir, 2007). Moreover, synaptic plasticity bidirectionally regulates the phosphorylation of AMPAR, such that LTP and LTD stimulate either an increase or decrease in phosphorylation, respectively (Barria et al., 1997; Kameyama et al., 1998; Lee et al., 1998, 2000). The modulatory nature of the AMPAR phosphorylation state in plasticity is evidenced by the fact that disrupting phosphorylation of the PKA and CaMKII target sites in GluA1 causes deficits in induction of LTP and LTD, as well as deficits in spatial memory retention in mice (Lee et al., 2003). Furthermore, phosphorylation of, or mutation mimicking phosphorylation of, the PKA and/or CaMKII sites in GluA1 lowers the threshold for induction of LTP (Hu et al., 2007; Makino et al., 2011).

Concomitant with phosphorylation-state regulation of AMPARs, synaptic plasticity alters the membrane trafficking dynamics of synaptic AMPARs. Increases in synaptic strength accompanying LTP occur when AMPARs are recruited from internalized or physiologically “silent” sites into existing or new synapses (Shi et al., 1999; Hayashi et al., 2000). Conversely, induction of LTD results in the rapid endocytosis of AMPARs, and thus a diminution of synaptic responses (Carroll et al., 1999; Beattie et al., 2000; Ehlers, 2000).

AMPARs are not only dynamic in terms of trafficking in and out of the PSD, but are also highly mobile within the plane of the plasma membrane. Single-particle tracking and photoactivated localization microscopy (PALM) studies demonstrate that extrasynaptic AMPARs (those residing within the membrane but outside the PSD region) are highly mobile until they enter PSD-95-enriched synaptic microdomains, whereupon they greatly decrease their mobility (Borgdorff

and Choquet, 2002; MacGillavry et al., 2013). Several phosphorylation-dependent events and protein interactions have been suggested to modulate plasticity-dependent AMPAR trafficking within synapses. For instance, both stargazin and PSD-95 have been shown to decrease their own mobility upon entering the PSD and play an important role in the structural immobilization of receptors at synapses (Opazo et al., 2012). Furthermore, CaMKII can phosphorylate a specific splice variant of SAP97 to disrupt its interaction with AKAP79/150 to decrease AMPAR currents (Nikandrova et al., 2010). In addition, CaMKII-mediated phosphorylation of PSD-95 modulates LTP- and LTD-associated spine growth, controls the activity-dependent trafficking of PSD-95 and Shank2 out of spines, and modulates AMPAR-mediated synaptic transmission (Steiner et al., 2008).

Several theories on how LTP and LTD modulate the function and number of AMPARs within the synapse have been proposed. One possibility is that CaMKII function modifies the PSD to create slots in the membrane for receptor trafficking. Upon neuronal activation, extrasynaptic receptors would diffuse into, or out of, these pre-existing slots to modulate synaptic transmission. Following exit from the PSD slots, receptors are then available to be removed from the plasma membrane through endocytosis. Another mechanism, that needn't be exclusive from the first, is that CaMKII activation specifically phosphorylates receptors and/or auxiliary subunits to recruit and capture those complexes to slots within the PSD. Ultimately, the precise mechanisms by which LTP and LTD could promote and/or sustain the recruitment of AMPAR to specific regions within the synapse remain uncertain. Rather than a single mechanism, it is likely that several factors that control the dynamic trafficking of AMPAR converge to modulate PSD complexes as necessary for structural and functional plasticity to occur.

Conclusions

Numerous experimental approaches have provided insight into the constituents, organization, and functional regulation of the PSD. Once viewed as a fixed black mesh between neurons, the PSD has emerged as a complex and dynamic dendritic microdomain that centralizes the molecular machinery that is important for neurotransmission.

Future research endeavors in molecular neuroscience are needed to expand our functional understanding of the numerous constituents of the PSD and how they impinge on the neuronal networks that make up the brain. Interestingly, in recent human genetic studies many PSD components have been implicated in neurological and psychiatric diseases such as schizophrenia, autism, and intellectual disability. Our understanding of the basic biology of the PSD provides a strong foundation for elucidating the mechanisms underlying these conditions and for the development of therapeutic treatments for these devastating disorders.

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References

- Aiga M, Levinson JN, Bamji SX (2011) N-cadherin and neuroligins cooperate to regulate synapse formation in hippocampal cultures. *Journal of Biological Chemistry* **286**:851–858.
- Allison DW, Gelfand VI, Spector I, Craig AM (1998) Role of actin in anchoring postsynaptic receptors in cultured hippocampal neurons: differential attachment of NMDA versus AMPA receptors. *Journal of Neuroscience* **18**:2423–2436.

- Anggono V, Huganir RL (2012) Regulation of AMPA receptor trafficking and synaptic plasticity. *Current Opinion in Neurobiology* **22**:461–469.
- Barria A, Malinow R (2005) NMDA receptor subunit composition controls synaptic plasticity by regulating binding to CaMKII. *Neuron* **48**:289–301.
- Barria A, Muller D, Derkach V, Griffith LC, Soderling TR (1997) Regulatory phosphorylation of AMPA-type glutamate receptors by CaMKII during long-term potentiation. *Science* **276**:2042–2045.
- Bassand P, Bernard A, Rafiki A, Gayet D, Khrestchatsky M (1999) Differential interaction of the tSxV motifs of the NR1 and NR2A NMDA receptor subunits with PSD-95 and SAP97. *European Journal of Neuroscience* **11**:2031–2043.
- Bats C, Groc L, Choquet D (2007) The interaction between Stargazin and PSD-95 regulates AMPA receptor surface trafficking. *Neuron* **53**:719–734.
- Beattie EC, Carroll RC, Yu X, Morishita W, Yasuda H, von Zastrow M, Malenka RC (2000) Regulation of AMPA receptor endocytosis by a signaling mechanism shared with LTD. *Nature Neuroscience* **3**:1291–1300.
- Beique JC, Andrade R (2003) PSD-95 regulates synaptic transmission and plasticity in rat cerebral cortex. *Journal of Physiology* **546**:859–867.
- Beique JC, Lin DT, Kang MG, Aizawa H, Takamiya K, Huganir RL (2006) Synapse-specific regulation of AMPA receptor function by PSD-95. *Proceedings of the National Academy of Sciences of the United States of America* **103**:19535–19540.
- Betzig E, Patterson GH, Sougrat R, Lindwasser OW, Olenych S, Bonifacino JS, Davidson MW, Lippincott-Schwartz J, Hess HF (2006) Imaging intracellular fluorescent proteins at nanometer resolution. *Science* **313**:1642–1645.
- Bhattacharyya S, Biou V, Xu W, Schluter O, Malenka RC (2009) A critical role for PSD-95/AKAP interactions in endocytosis of synaptic AMPA receptors. *Nature Neuroscience* **12**:172–181.
- Biederer T, Sudhof TC (2001) CASK and protein 4.1 support F-actin nucleation on neurexins. *Journal of Biological Chemistry* **276**:47869–47876.
- Bliss TV, Lomo T (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *Journal of Physiology* **232**:331–356.
- Bockers TM, Mameza MG, Kreutz MR, Bockmann J, Weise C, Buck F, Richter D, Gundelfinger ED, Kreienkamp HJ (2001) Synaptic scaffolding proteins in rat brain. Ankyrin repeats of the multidomain Shank protein family interact with the cytoskeletal protein alpha-fodrin. *Journal of Biological Chemistry* **276**:40104–40112.
- Bockmann J, Kreutz MR, Gundelfinger ED, Bockers TM (2002) ProSAP/Shank postsynaptic density proteins interact with insulin receptor tyrosine kinase substrate IRSp53. *Journal of Neurochemistry* **83**:1013–1017.
- Boeckers TM, Kreutz MR, Winter C, Zuschratter W, Smalla KH, Sanmarti-Vila L, Wex H, Langnaese K, Bockmann J, Garner CC, Gundelfinger ED (1999a) Proline-rich synapse-associated protein-1/cortactin binding protein 1 (ProSAP1/CortBP1) is a PDZ-domain protein highly enriched in the postsynaptic density. *Journal of Neuroscience* **19**:6506–6518.
- Boeckers TM, Winter C, Smalla KH, Kreutz MR, Bockmann J, Seidenbecher C, Garner CC, Gundelfinger ED (1999b) Proline-rich synapse-associated proteins ProSAP1 and ProSAP2 interact with synaptic proteins of the SAPAP/GKAP family. *Biochemical and Biophysical Research Communications* **264**:247–252.
- Borgdorff AJ, Choquet D (2002) Regulation of AMPA receptor lateral movements. *Nature* **417**:649–653.
- Bortolotto ZA, Clarke VR, Delany CM, Parry MC, Smolders I, Vignes M, Ho KH, Miu P, Brinton BT, Fantaske R, Ogden A, Gates M, Ornstein PL, Lodge D, Bleakman D, Collingridge GL (1999) Kainate receptors are involved in synaptic plasticity. *Nature* **402**:297–301.
- Bozdagi O, Shan W, Tanaka H, Benson DL, Huntley GW (2000) Increasing numbers of synaptic puncta during late-phase LTP: N-cadherin is synthesized, recruited to synaptic sites, and required for potentiation. *Neuron* **28**:245–259.

- Brakeman PR, Lanahan AA, O'Brien R, Roche K, Barnes CA, Huganir RL, Worley PF (1997) Homer: a protein that selectively binds metabotropic glutamate receptors. *Nature* **386**:284–288.
- Brenman JE, Christopherson KS, Craven SE, McGee AW, Bredt DS (1996) Cloning and characterization of postsynaptic density 93, a nitric oxide synthase interacting protein. *Journal of Neuroscience* **16**:7407–7415.
- Brigidi GS, Sun Y, Beccano-Kelly D, Pitman K, Mobasser M, Borgland SL, Milnerwood AJ, Bamji SX (2014) Palmitoylation of delta-catenin by DHHC5 mediates activity-induced synapse plasticity. *Nature Neuroscience* **17**:522–532.
- Bruckner K, Pablo Labrador J, Scheiffele P, Herb A, Seeburg PH, Klein R (1999) EphrinB ligands recruit GRIP family PDZ adaptor proteins into raft membrane microdomains. *Neuron* **22**:511–524.
- Carlin RK, Grab DJ, Cohen RS, Siekevitz P (1980) Isolation and characterization of postsynaptic densities from various brain regions: enrichment of different types of postsynaptic densities. *Journal of Cell Biology* **86**:831–845.
- Carlisle HJ, Manzerra P, Marcora E, Kennedy MB (2008) SynGAP regulates steady-state and activity-dependent phosphorylation of cofilin. *Journal of Neuroscience* **28**:13673–13683.
- Carr DW, Stofko-Hahn RE, Fraser ID, Cone RD, Scott JD (1992) Localization of the cAMP-dependent protein kinase to the postsynaptic densities by A-kinase anchoring proteins. Characterization of AKAP 79. *Journal of Biological Chemistry* **267**:16816–16823.
- Carroll RC, Lissin DV, von Zastrow M, Nicoll RA, Malenka RC (1999) Rapid redistribution of glutamate receptors contributes to long-term depression in hippocampal cultures. *Nature Neuroscience* **2**:454–460.
- Chen BS, Roche KW (2007) Regulation of NMDA receptors by phosphorylation. *Neuropharmacology* **53**:362–368.
- Chen HJ, Rojas-Soto M, Oguni A, Kennedy MB (1998) A synaptic Ras-GTPase activating protein (p135 SynGAP) inhibited by CaM kinase II. *Neuron* **20**:895–904.
- Chen L, Chetkovich DM, Petralia RS, Sweeney NT, Kawasaki Y, Wenthold RJ, Bredt DS, Nicoll RA (2000) Stargazin regulates synaptic targeting of AMPA receptors by two distinct mechanisms. *Nature* **408**:936–943.
- Chen X, Vinade L, Leapman RD, Petersen JD, Nakagawa T, Phillips TM, Sheng M, Reese TS (2005) Mass of the postsynaptic density and enumeration of three key molecules. *Proceedings of the National Academy of Sciences of the United States of America* **102**:11551–11556.
- Chen X, Winters C, Azzam R, Li X, Galbraith JA, Leapman RD, Reese TS (2008) Organization of the core structure of the postsynaptic density. *Proceedings of the National Academy of Sciences of the United States of America* **105**:4453–4458.
- Chen X, Nelson CD, Li X, Winters CA, Azzam R, Sousa AA, Leapman RD, Gainer H, Sheng M, Reese TS (2011) PSD-95 is required to sustain the molecular organization of the postsynaptic density. *Journal of Neuroscience* **31**:6329–6338.
- Cho KO, Hunt CA, Kennedy MB (1992) The rat brain postsynaptic density fraction contains a homolog of the Drosophila discs-large tumor suppressor protein. *Neuron* **9**:929–942.
- Cho CH, St-Gelais F, Zhang W, Tomita S, Howe JR (2007) Two families of TARP isoforms that have distinct effects on the kinetic properties of AMPA receptors and synaptic currents. *Neuron* **55**:890–904.
- Choi J, Ko J, Racz B, Burette A, Lee JR, Kim S, Na M, Lee HW, Kim K, Weinberg RJ, Kim E (2005) Regulation of dendritic spine morphogenesis by insulin receptor substrate 53, a downstream effector of Rac1 and Cdc42 small GTPases. *Journal of Neuroscience* **25**:869–879.
- Choquet D, Triller A (2013) The dynamic synapse. *Neuron* **80**:691–703.
- Chung HJ, Xia J, Scannevin RH, Zhang X, Huganir RL (2000) Phosphorylation of the AMPA receptor subunit GluR2 differentially regulates its interaction with PDZ domain-containing proteins. *Journal of Neuroscience* **20**:7258–7267.
- Citri A, Bhattacharyya S, Ma C, Morishita W, Fang S, Rizo J, Malenka RC (2010) Calcium binding to PICK1 is essential for the intracellular retention of AMPA receptors underlying long-term depression. *Journal of Neuroscience* **30**:16437–16452.

- Clem RL, Anggono V, Huganir RL (2010) PICK1 regulates incorporation of calcium-permeable AMPA receptors during cortical synaptic strengthening. *Journal of Neuroscience* **30**:6360–6366.
- Coleman SK, Cai C, Mottershead DG, Haapalahti JP, Keinanen K (2003) Surface expression of GluR-D AMPA receptor is dependent on an interaction between its C-terminal domain and a 4.1 protein. *Journal of Neuroscience* **23**:798–806.
- Colledge M, Dean RA, Scott GK, Langeberg LK, Huganir RL, Scott JD (2000) Targeting of PKA to glutamate receptors through a MAGUK–AKAP complex. *Neuron* **27**:107–119.
- Collingridge GL, Kehl SJ, McLennan H (1983) Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. *Journal of Physiology* **334**:33–46.
- Collingridge GL, Olsen RW, Peters J, Spedding M (2009) A nomenclature for ligand-gated ion channels. *Neuropharmacology* **56**:2–5.
- Contractor A, Swanson G, Heinemann SF (2001) Kainate receptors are involved in short- and long-term plasticity at mossy fiber synapses in the hippocampus. *Neuron* **29**:209–216.
- Contractor A, Mulle C, Swanson GT (2011) Kainate receptors coming of age: milestones of two decades of research. *Trends in neurosciences* **34**:154–163.
- Coombs ID, Soto D, Zonouzi M, Renzi M, Shelley C, Farrant M, Cull-Candy SG (2012) Cornichons modify channel properties of recombinant and glial AMPA receptors. *Journal of Neuroscience* **32**:9796–9804.
- Craven SE, El-Husseini AE, Bredt DS (1999) Synaptic targeting of the postsynaptic density protein PSD-95 mediated by lipid and protein motifs. *Neuron* **22**:497–509.
- Cuadra AE, Kuo SH, Kawasaki Y, Bredt DS, Chetkovich DM (2004) AMPA receptor synaptic targeting regulated by stargazin interactions with the Golgi-resident PDZ protein nPIST. *Journal of Neuroscience* **24**:7491–7502.
- Dakoji S, Tomita S, Karimzadegan S, Nicoll RA, Bredt DS (2003) Interaction of transmembrane AMPA receptor regulatory proteins with multiple membrane associated guanylate kinases. *Neuropharmacology* **45**:849–856.
- Dalva MB, Takasu MA, Lin MZ, Shamah SM, Hu L, Gale NW, Greenberg ME (2000) EphB receptors interact with NMDA receptors and regulate excitatory synapse formation. *Cell* **103**:945–956.
- Dalva MB, McClelland AC, Kayser MS (2007) Cell adhesion molecules: signalling functions at the synapse. *Nature Reviews Neuroscience* **8**:206–220.
- Dani A, Huang B, Bergan J, Dulac C, Zhuang X (2010) Superresolution imaging of chemical synapses in the brain. *Neuron* **68**:843–856.
- Danielson E, Zhang N, Metallo J, Kaleka K, Shin SM, Gerges N, Lee SH (2012) S-SCAM/MAGI-2 is an essential synaptic scaffolding molecule for the GluA2-containing maintenance pool of AMPA receptors. *Journal of Neuroscience* **32**:6967–6980.
- Deng F, Price MG, Davis CF, Mori M, Burgess DL (2006) Stargazin and other transmembrane AMPA receptor regulating proteins interact with synaptic scaffolding protein MAGI-2 in brain. *Journal of Neuroscience* **26**:7875–7884.
- De Robertis E, Bennett HS (1954) Submicroscopic vesicular component in the synapse. *Federation Proceedings* **13**:35.
- DeSouza S, Fu J, States BA, Ziff EB (2002) Differential palmitoylation directs the AMPA receptor-binding protein ABP to spines or to intracellular clusters. *Journal of Neuroscience* **22**:3493–3503.
- Desmond NL, Levy WB (1986) Changes in the postsynaptic density with long-term potentiation in the dentate gyrus. *Journal of Comparative Neurology* **253**:476–482.
- Dev KK, Nishimune A, Henley JM, Nakanishi S (1999) The protein kinase C alpha binding protein PICK1 interacts with short but not long form alternative splice variants of AMPA receptor subunits. *Neuropharmacology* **38**:635–644.
- Dong H, O'Brien RJ, Fung ET, Lanahan AA, Worley PF, Huganir RL (1997) GRIP: a synaptic PDZ domain-containing protein that interacts with AMPA receptors. *Nature* **386**:279–284.

- Dong H, Zhang P, Liao D, Huganir RL (1999) Characterization, expression, and distribution of GRIP protein. *Annals of the New York Academy of Sciences* **868**:535–540.
- Dudek SM, Bear MF (1992) Homosynaptic long-term depression in area CA1 of hippocampus and effects of N-methyl-D-aspartate receptor blockade. *Proceedings of the National Academy of Sciences of the United States of America* **89**:4363–4367.
- Ehlers MD (2000) Reinsertion or degradation of AMPA receptors determined by activity-dependent endocytic sorting. *Neuron* **28**:511–525.
- Ehrlich I, Malinow R (2004) Postsynaptic density 95 controls AMPA receptor incorporation during long-term potentiation and experience-driven synaptic plasticity. *Journal of Neuroscience* **24**:916–927.
- El-Husseini AE, Schnell E, Chetkovich DM, Nicoll RA, Bredt DS (2000a) PSD-95 involvement in maturation of excitatory synapses. *Science* **290**:1364–1368.
- El-Husseini AE, Topinka JR, Lehrer-Graiwer JE, Firestein BL, Craven SE, Aoki C, Bredt DS (2000b) Ion channel clustering by membrane-associated guanylate kinases. Differential regulation by N-terminal lipid and metal binding motifs. *Journal of Biological Chemistry* **275**:23904–23910.
- El-Husseini AE, Schnell E, Dakoji S, Sweeney N, Zhou Q, Prange O, Gauthier-Campbell C, Aguilera-Moreno A, Nicoll RA, Bredt DS (2002) Synaptic strength regulated by palmitate cycling on PSD-95. *Cell* **108**:849–863.
- Elias GM, Funke L, Stein V, Grant SG, Bredt DS, Nicoll RA (2006) Synapse-specific and developmentally regulated targeting of AMPA receptors by a family of MAGUK scaffolding proteins. *Neuron* **52**:307–320.
- von Engelhardt J, Mack V, Sprengel R, Kavensonck N, Li KW, Stern-Bach Y, Smit AB, Seburg PH, Monyer H (2010) CKAMP44: a brain-specific protein attenuating short-term synaptic plasticity in the dentate gyrus. *Science* **327**:1518–1522.
- Ethell IM, Irie F, Kalo MS, Couchman JR, Pasquale EB, Yamaguchi Y (2001) EphB/syndecan-2 signaling in dendritic spine morphogenesis. *Neuron* **31**:1001–1013.
- Fifkova E, Anderson CL (1981) Stimulation-induced changes in dimensions of stalks of dendritic spines in the dentate molecular layer. *Experimental Neurology* **74**:621–627.
- Fifkova E, Delay RJ (1982) Cytoplasmic actin in neuronal processes as a possible mediator of synaptic plasticity. *Journal of Cell Biology* **95**:345–350.
- Fisher JL, Mott DD (2012) The auxiliary subunits Neto1 and Neto2 reduce voltage-dependent inhibition of recombinant kainate receptors. *Journal of Neuroscience* **32**:12928–12933.
- Fisher JL, Mott DD (2013) Modulation of homomeric and heteromeric kainate receptors by the auxiliary subunit Neto1. *Journal of Physiology* **591**:4711–4724.
- Fukata Y, Fukata M (2010) Protein palmitoylation in neuronal development and synaptic plasticity. *Nature Reviews Neuroscience* **11**:161–175.
- Fukata Y, Dimitrov A, Boncompain G, Vielemeyer O, Perez F, Fukata M (2013) Local palmitoylation cycles define activity-regulated postsynaptic subdomains. *Journal of Cell Biology* **202**:145–161.
- Geinisman Y, Morrell F, de Toledo-Morrell L (1987a) Axospinous synapses with segmented postsynaptic densities: a morphologically distinct synaptic subtype contributing to the number of profiles of “perforated” synapses visualized in random sections. *Brain Research* **423**:179–188.
- Geinisman Y, Morrell F, de Toledo-Morrell L (1987b) Synapses on dendritic shafts exhibit a perforated postsynaptic density. *Brain Research* **422**:352–356.
- Geinisman Y, de Toledo-Morrell L, Morrell F (1991) Induction of long-term potentiation is associated with an increase in the number of axospinous synapses with segmented postsynaptic densities. *Brain Research* **566**:77–88.
- Geinisman Y, Morrell F, de Toledo-Morrell L (1992) Increase in the number of axospinous synapses with segmented postsynaptic densities following hippocampal kindling. *Brain Research* **569**:341–347.
- Gill MB, Kato AS, Wang H, Bredt DS (2012) AMPA receptor modulation by cornichon-2 dictated by transmembrane AMPA receptor regulatory protein isoform. *European Journal of Neuroscience* **35**:182–194.

- Grabrucker AM, Knight MJ, Proepper C, Bockmann J, Joubert M, Rowan M, Nienhaus GU, Garner CC, Bowie JU, Kreutz MR, Gundelfinger ED, Boeckers TM (2011) Concerted action of zinc and ProSAP/Shank in synaptogenesis and synapse maturation. *EMBO Journal* **30**:569–581.
- Graf ER, Zhang X, Jin SX, Linhoff MW, Craig AM (2004) Neurexins induce differentiation of GABA and glutamate postsynaptic specializations via neuroligins. *Cell* **119**:1013–1026.
- Gray EG (1959) Axo-somatic and axo-dendritic synapses of the cerebral cortex: an electron microscope study. *Journal of Anatomy* **93**:420–433.
- Grunwald IC, Korte M, Wolfer D, Wilkinson GA, Unsicker K, Lipp HP, Bonhoeffer T, Klein R (2001) Kinase-independent requirement of EphB2 receptors in hippocampal synaptic plasticity. *Neuron* **32**:1027–1040.
- Grunwald IC, Korte M, Adelmann G, Plueck A, Kullander K, Adams RH, Frotscher M, Bonhoeffer T, Klein R (2004) Hippocampal plasticity requires postsynaptic ephrinBs. *Nature Neuroscience* **7**:33–40.
- Gulley RL, Reese TS (1981) Cytoskeletal organization at the postsynaptic complex. *Journal of Cell Biology* **91**:298–302.
- Haeckel A, Ahuja R, Gundelfinger ED, Qualmann B, Kessels MM (2008) The actin-binding protein Abp1 controls dendritic spine morphology and is important for spine head and synapse formation. *Journal of Neuroscience* **28**:10031–10044.
- Halt AR, Dallapiazza RF, Zhou Y, Stein IS, Qian H, Juntti S, Wojcik S, Brose N, Silva AJ, Hell JW (2012) CaMKII binding to GluN2B is critical during memory consolidation. *EMBO Journal* **31**:1203–1216.
- Harris KM, Stevens JK (1988) Dendritic spines of rat cerebellar Purkinje cells: serial electron microscopy with reference to their biophysical characteristics. *Journal of Neuroscience* **8**:4455–4469.
- Harris KM, Stevens JK (1989) Dendritic spines of CA 1 pyramidal cells in the rat hippocampus: serial electron microscopy with reference to their biophysical characteristics. *Journal of Neuroscience* **9**:2982–2997.
- Harris KM, Sultan P (1995) Variation in the number, location and size of synaptic vesicles provides an anatomical basis for the nonuniform probability of release at hippocampal CA1 synapses. *Neuropharmacology* **34**:1387–1395.
- Harris KM, Jensen FE, Tsao B (1992) Three-dimensional structure of dendritic spines and synapses in rat hippocampus (CA1) at postnatal day 15 and adult ages: implications for the maturation of synaptic physiology and long-term potentiation. *Journal of Neuroscience* **12**:2685–2705.
- Hayashi Y, Shi SH, Esteban JA, Piccini A, Poncer JC, Malinow R (2000) Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. *Science* **287**:2262–2267.
- Hayashi MK, Tang C, Verpelli C, Narayanan R, Stearns MH, Xu RM, Li H, Sala C, Hayashi Y (2009) The postsynaptic density proteins Homer and Shank form a polymeric network structure. *Cell* **137**:159–171.
- Hebb DO (1949) *The Organization of Behavior*. New York: Wiley.
- Henderson JT, Georgiou J, Jia Z, Robertson J, Elowe S, Roder JC, Pawson T (2001) The receptor tyrosine kinase EphB2 regulates NMDA-dependent synaptic function. *Neuron* **32**:1041–1056.
- Herring BE, Shi Y, Suh YH, Zheng CY, Blankenship SM, Roche KW, Nicoll RA (2013) Cornichon proteins determine the subunit composition of synaptic AMPA receptors. *Neuron* **77**:1083–1096.
- Hess ST, Girirajan TP, Mason MD (2006) Ultra-high resolution imaging by fluorescence photoactivation localization microscopy. *Biophysical Journal* **91**:4258–4272.
- Heuser JE, Reese TS (1977) Structure of the synapse. In: The Handbook of Physiology, the Nervous System I (Kandel, E., ed.), pp. 261–294 Bethesda, MD.: American Physiological Society.
- Hirao K, Hata Y, Ide N, Takeuchi M, Irie M, Yao I, Deguchi M, Toyoda A, Sudhof TC, Takai Y (1998) A novel multiple PDZ domain-containing molecule interacting with N-methyl-D-aspartate receptors and neuronal cell adhesion proteins. *Journal of Biological Chemistry* **273**:21105–21110.

- Hirbec H, Perestenko O, Nishimune A, Meyer G, Nakanishi S, Henley JM, Dev KK (2002) The PDZ proteins PICK1, GRIP, and syntenin bind multiple glutamate receptor subtypes. Analysis of PDZ binding motifs. *Journal of Biological Chemistry* **277**:15221–15224.
- Hirbec H, Francis JC, Lauri SE, Braithwaite SP, Coussen F, Mulle C, Dev KK, Coutinho V, Meyer G, Isaac JT, Collingridge GL, Henley JM (2003) Rapid and differential regulation of AMPA and kainate receptors at hippocampal mossy fibre synapses by PICK1 and GRIP. *Neuron* **37**:625–638.
- Hoze N, Nair D, Hosy E, Sieben C, Manley S, Herrmann A, Sibarita JB, Choquet D, Holcman D (2012) Heterogeneity of AMPA receptor trafficking and molecular interactions revealed by superresolution analysis of live cell imaging. *Proceedings of the National Academy of Sciences of the United States of America* **109**:17052–17057.
- Hu H, Real E, Takamiya K, Kang MG, Ledoux J, Huganir RL, Malinow R (2007) Emotion enhances learning via norepinephrine regulation of AMPA-receptor trafficking. *Cell* **131**:160–173.
- Huang G, Kim JY, Dehoff M, Mizuno Y, Kamm KE, Worley PF, Muallem S, Zeng W (2007) Ca²⁺ signaling in microdomains: Homer1 mediates the interaction between RyR2 and Cav1.2 to regulate excitation-contraction coupling. *Journal of Biological Chemistry* **282**:14283–14290.
- Huganir RL, Nicoll RA (2013) AMPARs and synaptic plasticity: the last 25 years. *Neuron* **80**:704–717.
- Isaac JT, Nicoll RA, Malenka RC (1995) Evidence for silent synapses: implications for the expression of LTP. *Neuron* **15**:427–434.
- Ito M, Kano M (1982) Long-lasting depression of parallel fiber-Purkinje cell transmission induced by conjunctive stimulation of parallel fibers and climbing fibers in the cerebellar cortex. *Neuroscience Letters* **33**:253–258.
- Jordan BA, Fernholz BD, Boussac M, Xu C, Grigorean G, Ziff EB, Neubert TA (2004) Identification and verification of novel rodent postsynaptic density proteins. *Molecular and Cellular Proteomics* **3**:857–871.
- Jungling K, Eulenburg V, Moore R, Kemler R, Lessmann V, Gottmann K (2006) N-cadherin transsynaptically regulates short-term plasticity at glutamatergic synapses in embryonic stem cell-derived neurons. *Journal of Neuroscience* **26**:6968–6978.
- Kalashnikova E, Lorca RA, Kaur I, Barisone GA, Li B, Ishimaru T, Trimmer JS, Mohapatra DP, Diaz E (2010) SynDIG1: an activity-regulated, AMPA-receptor-interacting transmembrane protein that regulates excitatory synapse development. *Neuron* **65**:80–93.
- Kameyama K, Lee HK, Bear MF, Huganir RL (1998) Involvement of a postsynaptic protein kinase A substrate in the expression of homosynaptic long-term depression. *Neuron* **21**:1163–1175.
- Kato A, Ozawa F, Saitoh Y, Fukazawa Y, Sugiyama H, Inokuchi K (1998) Novel members of the Vesl/Homer family of PDZ proteins that bind metabotropic glutamate receptors. *Journal of Biological Chemistry* **273**:23969–23975.
- Kato AS, Siuda ER, Nisenbaum ES, Bredt DS (2008) AMPA receptor subunit-specific regulation by a distinct family of type II TARPs. *Neuron* **59**:986–996.
- Katz Y, Menon V, Nicholson DA, Geinisman Y, Kath WL, Spruston N (2009) Synapse distribution suggests a two-stage model of dendritic integration in CA1 pyramidal neurons. *Neuron* **63**:171–177.
- Kidd FL, Coumis U, Collingridge GL, Crabtree JW, Isaac JT (2002) A presynaptic kainate receptor is involved in regulating the dynamic properties of thalamocortical synapses during development. *Neuron* **34**:635–646.
- Kim CH, Lisman JE (1999) A role of actin filament in synaptic transmission and long-term potentiation. *Journal of Neuroscience* **19**:4314–4324.
- Kim E, Naisbitt S, Hsueh YP, Rao A, Rothschild A, Craig AM, Sheng M (1997) GKAP, a novel synaptic protein that interacts with the guanylate kinase-like domain of the PSD-95/SAP90 family of channel clustering molecules. *Journal of Cell Biology* **136**:669–678.
- Kim JH, Liao D, Lau LF, Huganir RL (1998) SynGAP: a synaptic RasGAP that associates with the PSD-95/SAP90 protein family. *Neuron* **20**:683–691.

- Kim JH, Lee HK, Takamiya K, Huganir RL (2003) The role of synaptic GTPase-activating protein in neuronal development and synaptic plasticity. *Journal of Neuroscience* **23**:1119–1124.
- Kistner U, Wenzel BM, Veh RW, Cases-Langhoff C, Garner AM, Appeltauer U, Voss B, Gundelfinger ED, Garner CC (1993) SAP90, a rat presynaptic protein related to the product of the Drosophila tumor suppressor gene dlg-A. *Journal of Biological Chemistry* **268**:4580–4583.
- Ko J, Fuccillo MV, Malenka RC, Sudhof TC (2009) LRRTM2 functions as a neurexin ligand in promoting excitatory synapse formation. *Neuron* **64**:791–798.
- Ko J, Soler-Llavina GJ, Fuccillo MV, Malenka RC, Sudhof TC (2011) Neuroligins/LRRTMs prevent activity- and Ca²⁺/calmodulin-dependent synapse elimination in cultured neurons. *Journal of Cell Biology* **194**:323–334.
- Kornau HC, Schenker LT, Kennedy MB, Seuberg PH (1995) Domain interaction between NMDA receptor subunits and the postsynaptic density protein PSD-95. *Science* **269**:1737–1740.
- Korobova F, Svitkina T (2010) Molecular architecture of synaptic actin cytoskeleton in hippocampal neurons reveals a mechanism of dendritic spine morphogenesis. *Molecular Biology of the Cell* **21**:165–176.
- Kott S, Werner M, Korber C, Hollmann M (2007) Electrophysiological properties of AMPA receptors are differentially modulated depending on the associated member of the TARP family. *Journal of Neuroscience* **27**:3780–3789.
- Krucker T, Siggins GR, Halpoin S (2000) Dynamic actin filaments are required for stable long-term potentiation (LTP) in area CA1 of the hippocampus. *Proceedings of the National Academy of Sciences of the United States of America* **97**:6856–6861.
- Landis DM, Reese TS (1983) Cytoplasmic organization in cerebellar dendritic spines. *Journal of Cell Biology* **97**:1169–1178.
- Landis DM, Hall AK, Weinstein LA, Reese TS (1988) The organization of cytoplasm at the presynaptic active zone of a central nervous system synapse. *Neuron* **1**:201–209.
- Lauren J, Airaksinen MS, Saarma M, Timmusk T (2003) A novel gene family encoding leucine-rich repeat transmembrane proteins differentially expressed in the nervous system. *Genomics* **81**:411–421.
- Lauri SE, Bortolotto ZA, Bleakman D, Ornstein PL, Lodge D, Isaac JT, Collingridge GL (2001) A critical role of a facilitatory presynaptic kainate receptor in mossy fiber LTP. *Neuron* **32**:697–709.
- Lee HK, Kameyama K, Huganir RL, Bear MF (1998) NMDA induces long-term synaptic depression and dephosphorylation of the GluR1 subunit of AMPA receptors in hippocampus. *Neuron* **21**:1151–1162.
- Lee HK, Barbarosie M, Kameyama K, Bear MF, Huganir RL (2000) Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. *Nature* **405**:955–959.
- Lee HK, Takamiya K, Han JS, Man H, Kim CH, Rumbaugh G, Yu S, Ding L, He C, Petralia RS, Wenthold RJ, Gallagher M, Huganir RL (2003) Phosphorylation of the AMPA receptor GluR1 subunit is required for synaptic plasticity and retention of spatial memory. *Cell* **112**:631–643.
- Leonard AS, Davare MA, Horne MC, Garner CC, Hell JW (1998) SAP97 is associated with the alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor GluR1 subunit. *Journal of Biological Chemistry* **273**:19518–19524.
- Letts VA, Felix R, Biddlecome GH, Arikath J, Mahaffey CL, Valenzuela A, Bartlett FS, 2nd, Mori Y, Campbell KP, Frankel WN (1998) The mouse stargazer gene encodes a neuronal Ca²⁺-channel gamma subunit. *Nature Genetics* **19**:340–347.
- Li KW, Hornshaw MP, Van Der Schors RC, Watson R, Tate S, Casetta B, Jimenez CR, Gouwenberg Y, Gundelfinger ED, Smalla KH, Smit AB (2004) Proteomics analysis of rat brain postsynaptic density. Implications of the diverse protein functional groups for the integration of synaptic physiology. *Journal of Biological Chemistry* **279**:987–1002.
- Liao D, Hessler NA, Malinow R (1995) Activation of postsynaptically silent synapses during pairing-induced LTP in CA1 region of hippocampal slice. *Nature* **375**:400–404.

- Lilliu V, Pernas-Alonso R, Trelles RD, di Porzio U, Zuddas A, Perrone-Capano C (2001) Ontogeny of AMPA receptor gene expression in the developing rat midbrain and striatum. *Brain Research Molecular Brain Research* **96**:133–141.
- Lim BK, Matsuda N, Poo MM (2008) Ephrin-B reverse signaling promotes structural and functional synaptic maturation in vivo. *Nature Neuroscience* **11**:160–169.
- Lin JW, Wyszynski M, Madhavan R, Sealock R, Kim JU, Sheng M (1998) Yotiao, a novel protein of neuromuscular junction and brain that interacts with specific splice variants of NMDA receptor subunit NR1. *Journal of Neuroscience* **18**:2017–2027.
- Linhoff MW, Lauren J, Cassidy RM, Dobie FA, Takahashi H, Nygaard HB, Airaksinen MS, Strittmatter SM, Craig AM (2009) An unbiased expression screen for synaptogenic proteins identifies the LRRTM protein family as synaptic organizers. *Neuron* **61**:734–749.
- Lisabeth EM, Falivelli G, Pasquale EB (2013) Eph receptor signaling and ephrins. *Cold Spring Harbor Perspectives in Biology* **5**(9) doi: 10.1101/cshperspect.a009159.
- Lledo PM, Hjelmstad GO, Mukherji S, Soderling TR, Malenka RC, Nicoll RA (1995) Calcium/calmodulin-dependent kinase II and long-term potentiation enhance synaptic transmission by the same mechanism. *Proceedings of the National Academy of Sciences of the United States of America* **92**:11175–11179.
- Lovero KL, Blankenship SM, Shi Y, Nicoll RA (2013) SynDIG1 promotes excitatory synaptogenesis independent of AMPA receptor trafficking and biophysical regulation. *PloS ONE* **8**:e66171.
- Lu W, Shi Y, Jackson AC, Bjorgan K, During MJ, Sprengel R, Seuberg PH, Nicoll RA (2009) Subunit composition of synaptic AMPA receptors revealed by a single-cell genetic approach. *Neuron* **62**:254–268.
- Luscher C, Xia H, Beattie EC, Carroll RC, von Zastrow M, Malenka RC, Nicoll RA (1999) Role of AMPA receptor cycling in synaptic transmission and plasticity. *Neuron* **24**:649–658.
- MacGillavry HD, Song Y, Raghavachari S, Blanpied TA (2013) Nanoscale scaffolding domains within the postsynaptic density concentrate synaptic AMPA receptors. *Neuron* **78**:615–622.
- Makino Y, Johnson RC, Yu Y, Takamiya K, Huganir RL (2011) Enhanced synaptic plasticity in mice with phosphomimetic mutation of the GluA1 AMPA receptor. *Proceedings of the National Academy of Sciences of the United States of America* **108**:8450–8455.
- Malinow R, Schulman H, Tsien RW (1989) Inhibition of postsynaptic PKC or CaMKII blocks induction but not expression of LTP. *Science* **245**:862–866.
- Matsuda S, Mikawa S, Hirai H (1999) Phosphorylation of serine-880 in GluR2 by protein kinase C prevents its C terminus from binding with glutamate receptor-interacting protein. *Journal of Neurochemistry* **73**:1765–1768.
- Matsuzaki M, Ellis-Davies GC, Nemoto T, Miyashita Y, Iino M, Kasai H (2001) Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons. *Nature Neuroscience* **4**:1086–1092.
- Matsuzaki M, Honkura N, Ellis-Davies GC, Kasai H (2004) Structural basis of long-term potentiation in single dendritic spines. *Nature* **429**:761–766.
- Mejias R, Adamczyk A, Anggono V, Niranjan T, Thomas GM, Sharma K, Skinner C, Schwartz CE, Stevenson RE, Fallin MD, Kaufmann W, Pletnikov M, Valle D, Huganir RL, Wang T (2011) Gain-of-function glutamate receptor interacting protein 1 variants alter GluA2 recycling and surface distribution in patients with autism. *Proceedings of the National Academy of Sciences of the United States of America* **108**:4920–4925.
- Menon V, Musial TF, Liu A, Katz Y, Kath WL, Spruston N, Nicholson DA (2013) Balanced synaptic impact via distance-dependent synapse distribution and complementary expression of AMPARs and NMDARs in hippocampal dendrites. *Neuron* **80**:1451–1463.
- Menuz K, Stroud RM, Nicoll RA, Hays FA (2007) TARP auxiliary subunits switch AMPA receptor antagonists into partial agonists. *Science* **318**:815–817.
- Michishita M, Ikeda T, Nakashiba T, Ogawa M, Tashiro K, Honjo T, Doi K, Itohara S, Endo S (2003) A novel gene, Btcl1, encoding CUB and LDLa domains is expressed in restricted areas of mouse brain. *Biochemical and Biophysical Research Communications* **306**:680–686.

- Migaud M, Charlesworth P, Dempster M, Webster LC, Watabe AM, Makhinson M, He Y, Ramsay MF, Morris RG, Morrison JH, O'Dell TJ, Grant SG (1998) Enhanced long-term potentiation and impaired learning in mice with mutant postsynaptic density-95 protein. *Nature* **396**:433–439.
- Milstein AD, Zhou W, Karimzadegan S, Bredt DS, Nicoll JA (2007) TARP subtypes differentially and dose-dependently control synaptic AMPA receptor gating. *Neuron* **55**:905–918.
- Mok H, Shin H, Kim S, Lee JR, Yoon J, Kim E (2002) Association of the kinesin superfamily motor protein KIF1Balpha with postsynaptic density-95 (PSD-95), synapse-associated protein-97, and synaptic scaffolding molecule PSD-95/discs large/zona occludens-1 proteins. *Journal of Neuroscience* **22**:5253–5258.
- Monyer H, Seuberg PH, Wisden W (1991) Glutamate-operated channels: developmentally early and mature forms arise by alternative splicing. *Neuron* **6**:799–810.
- Monyer H, Burnashev N, Laurie DJ, Sakmann B, Seuberg PH (1994) Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. *Neuron* **12**:529–540.
- Muller BM, Kistner U, Veh RW, Cases-Langhoff C, Becker B, Gundelfinger ED, Garner CC (1995) Molecular characterization and spatial distribution of SAP97, a novel presynaptic protein homologous to SAP90 and the Drosophila discs-large tumor suppressor protein. *Journal of Neuroscience* **15**:2354–2366.
- Muller BM, Kistner U, Kindler S, Chung WJ, Kuhlendahl S, Fenster SD, Lau LF, Veh RW, Huganir RL, Gundelfinger ED, Garner CC (1996) SAP102, a novel postsynaptic protein that interacts with NMDA receptor complexes in vivo. *Neuron* **17**:255–265.
- Nair D, Hosy E, Petersen JD, Constals A, Giannone G, Choquet D, Sibarita JB (2013) Super-resolution imaging reveals that AMPA receptors inside synapses are dynamically organized in nanodomains regulated by PSD95. *Journal of Neuroscience* **33**:13204–13224.
- Naisbitt S, Kim E, Tu JC, Xiao B, Sala C, Valtschanoff J, Weinberg RJ, Worley PF, Sheng M (1999) Shank, a novel family of postsynaptic density proteins that binds to the NMDA receptor/PSD-95/GKAP complex and cortactin. *Neuron* **23**:569–582.
- Nicholson DA, Yoshida R, Berry RW, Gallagher M, Geinisman Y (2004) Reduction in size of perforated postsynaptic densities in hippocampal axospinous synapses and age-related spatial learning impairments. *Journal of Neuroscience* **24**:7648–7653.
- Niethammer M, Kim E, Sheng M (1996) Interaction between the C terminus of NMDA receptor subunits and multiple members of the PSD-95 family of membrane-associated guanylate kinases. *Journal of Neuroscience* **16**:2157–2163.
- Nikandrova YA, Jiao Y, Baucum AJ, Tavalin SJ, Colbran RJ (2010) Ca²⁺/calmodulin-dependent protein kinase II binds to and phosphorylates a specific SAP97 splice variant to disrupt association with AKAP79/150 and modulate alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-type glutamate receptor (AMPAR) activity. *Journal of Biological Chemistry* **285**:923–934.
- Nishimune A, Isaac JT, Molnar E, Noel J, Nash SR, Tagaya M, Collingridge GL, Nakanishi S, Henley JM (1998) NSF binding to GluR2 regulates synaptic transmission. *Neuron* **21**:87–97.
- Nuriya M, Huganir RL (2006) Regulation of AMPA receptor trafficking by N-cadherin. *Journal of Neurochemistry* **97**:652–661.
- Nusser Z, Lujan R, Laube G, Roberts JD, Molnar E, Somogyi P (1998) Cell type and pathway dependence of synaptic AMPA receptor number and variability in the hippocampus. *Neuron* **21**:545–559.
- Okamoto K, Narayanan R, Lee SH, Murata K, Hayashi Y (2007) The role of CaMKII as an F-actin-bundling protein crucial for maintenance of dendritic spine structure. *Proceedings of the National Academy of Sciences of the United States of America* **104**:6418–6423.
- Okuda T, Yu LM, Cingolani LA, Kemler R, Goda Y (2007) beta-Catenin regulates excitatory postsynaptic strength at hippocampal synapses. *Proceedings of the National Academy of Sciences of the United States of America* **104**:13479–13484.
- Olsen O, Bredt DS (2003) Functional analysis of the nucleotide binding domain of membrane-associated guanylate kinases. *Journal of Biological Chemistry* **278**:6873–6878.

- Opazo P, Sainlos M, Choquet D (2012) Regulation of AMPA receptor surface diffusion by PSD-95 slots. *Current Opinion in Neurobiology* 22:453–460.
- von Ossowski I, Oksanen E, von Ossowski L, Cai C, Sundberg M, Goldman A, Keinanen K (2006) Crystal structure of the second PDZ domain of SAP97 in complex with a GluR-A C-terminal peptide. *FEBS Journal* 273:5219–5229.
- Osten P, Srivastava S, Inman GJ, Vilim FS, Khatri L, Lee LM, States BA, Einheber S, Milner TA, Hanson PI, Ziff EB (1998) The AMPA receptor GluR2 C terminus can mediate a reversible, ATP-dependent interaction with NSF and alpha- and beta-SNAPs. *Neuron* 21:99–110.
- Pahl S, Tapken D, Haering SC, Hollmann M (2014) Trafficking of kainate receptors. *Membranes* 4:565–595.
- Pak DT, Yang S, Rudolph-Correia S, Kim E, Sheng M (2001) Regulation of dendritic spine morphology by SPAR, a PSD-95-associated RapGAP. *Neuron* 31:289–303.
- Palacios-Filardo J, Aller MI, Lerma J (2014) Synaptic targeting of kainate receptors. *Cerebral Cortex* doi: 10.1093/cercor/bhu244.
- Palade GE, Palay SL (1954) Electron microscopic observations of interneuronal and neuromuscular synapses. *Anatomical Record* 118:335–336.
- Palay SL (1958) The morphology of synapses in the central nervous system. *Experimental Cell Research* 14:275–293.
- Park E, Na M, Choi J, Kim S, Lee JR, Yoon J, Park D, Sheng M, Kim E (2003) The Shank family of postsynaptic density proteins interacts with and promotes synaptic accumulation of the beta PIX guanine nucleotide exchange factor for Rac1 and Cdc42. *Journal of Biological Chemistry* 278:19220–19229.
- Peng J, Kim MJ, Cheng D, Duong DM, Gygi SP, Sheng M (2004) Semiquantitative proteomic analysis of rat forebrain postsynaptic density fractions by mass spectrometry. *Journal of Biological Chemistry* 279:21003–21011.
- Penzes P, Johnson RC, Sattler R, Zhang X, Huganir RL, Kambampati V, Mains RE, Eipper BA (2001) The neuronal Rho-GEF Kalirin-7 interacts with PDZ domain-containing proteins and regulates dendritic morphogenesis. *Neuron* 29:229–242.
- Penzes P, Beeser A, Chernoff J, Schiller MR, Eipper BA, Mains RE, Huganir RL (2003) Rapid induction of dendritic spine morphogenesis by trans-synaptic ephrinB-EphB receptor activation of the Rho-GEF kalirin. *Neuron* 37:263–274.
- Perroy J, El Far O, Bertaso F, Pin JP, Betz H, Bockaert J, Fagni L (2002) PICK1 is required for the control of synaptic transmission by the metabotropic glutamate receptor 7. *EMBO Journal* 21:2990–2999.
- Peters A, Palay SL, Webster Hd (1991) *Fine Structure of the Nervous System: Neurons and Their Supporting Cells*. New York: Oxford University Press.
- Petersen JD, Chen X, Vinade L, Dosemeci A, Lisman JE, Reese TS (2003) Distribution of postsynaptic density (PSD)-95 and Ca²⁺/calmodulin-dependent protein kinase II at the PSD. *Journal of Neuroscience* 23:11270–11278.
- Petralia RS, Sans N, Wang YX, Wenthold RJ (2005) Ontogeny of postsynaptic density proteins at glutamatergic synapses. *Molecular and Cellular Neurosciences* 29:436–452.
- Pi HJ, Otmakhov N, El Gaamouch F, Lemelin D, De Koninck P, Lisman J (2010) CaMKII control of spine size and synaptic strength: role of phosphorylation states and nonenzymatic action. *Proceedings of the National Academy of Sciences of the United States of America* 107:14437–14442.
- Popov VI, Davies HA, Rogachevsky VV, Patrushev IV, Errington ML, Gabbott PL, Bliss TV, Stewart MG (2004) Remodelling of synaptic morphology but unchanged synaptic density during late phase long-term potentiation (LTP): a serial section electron micrograph study in the dentate gyrus in the anaesthetised rat. *Neuroscience* 128:251–262.
- Priel A, Kolleker A, Ayalon G, Gillor M, Osten P, Stern-Bach Y (2005) Stargazin reduces desensitization and slows deactivation of the AMPA-type glutamate receptors. *Journal of Neuroscience* 25:2682–2686.

- Qualmann B, Boeckers TM, Jeromin M, Gundelfinger ED, Kessels MM (2004) Linkage of the actin cytoskeleton to the postsynaptic density via direct interactions of Abp1 with the ProSAP/Shank family. *Journal of Neuroscience* **24**:2481–2495.
- Ramón y Cajal S (1888) Estructura de los centros nerviosos de las aves. *Revista Trimestral de Histología Normal y Patológica* **1**:1–10.
- Ramón y Cajal S (1891) Significación fisiológica de las expansiones protoplasmicas y nerviosas de la sustancia gris. *Revista de Ciencias Médicas de Barcelona* **22**:23.
- Ramón y Cajal S (1893) Neue Darstellung vom Histologischen Bau des Centralnervensystem. *Archiv für Anatomie und Entwicklungsgeschichte* **17**:319–428.
- Rumbaugh G, Adams JP, Kim JH, Huganir RL (2006) SynGAP regulates synaptic strength and mitogen-activated protein kinases in cultured neurons. *Proceedings of the National Academy of Sciences of the United States of America* **103**:4344–4351.
- Saglietti L, Dequidt C, Kamieniarz K, Rousset MC, Valnegri P, Thoumine O, Beretta F, Fagni L, Choquet D, Sala C, Sheng M, Passafaro M (2007) Extracellular interactions between GluR2 and N-cadherin in spine regulation. *Neuron* **54**:461–477.
- Sala C, Piech V, Wilson NR, Passafaro M, Liu G, Sheng M (2001) Regulation of dendritic spine morphology and synaptic function by Shank and Homer. *Neuron* **31**:115–130.
- Sala C, Roussignol G, Meldolesi J, Fagni L (2005) Key role of the postsynaptic density scaffold proteins Shank and Homer in the functional architecture of Ca^{2+} homeostasis at dendritic spines in hippocampal neurons. *Journal of Neuroscience* **25**:4587–4592.
- Sanderson JL, Dell'Acqua ML (2011) AKAP signaling complexes in regulation of excitatory synaptic plasticity. *The Neuroscientist* **17**:321–336.
- Sans NA, Montcouquiol ME, Raymond J (2000) Postnatal developmental changes in AMPA and NMDA receptors in the rat vestibular nuclei. *Brain Research Developmental Brain Research* **123**:41–52.
- Satoh K, Takeuchi M, Oda Y, Deguchi-Tawarada M, Sakamoto Y, Matsubara K, Nagasu T, Takai Y (2002) Identification of activity-regulated proteins in the postsynaptic density fraction. *Genes to Cells* **7**:187–197.
- Scheiffele P, Fan J, Choih J, Fetter R, Serafini T (2000) Neuroligin expressed in nonneuronal cells triggers presynaptic development in contacting axons. *Cell* **101**:657–669.
- Schikorski T, Stevens CF (1999) Quantitative fine-structural analysis of olfactory cortical synapses. *Proceedings of the National Academy of Sciences of the United States of America* **96**:4107–4112.
- Schmitz D, Mellor J, Nicoll RA (2001) Presynaptic kainate receptor mediation of frequency facilitation at hippocampal mossy fiber synapses. *Science* **291**:1972–1976.
- Schnell E, Sizemore M, Karimzadegan S, Chen L, Bredt DS, Nicoll RA (2002) Direct interactions between PSD-95 and stargazin control synaptic AMPA receptor number. *Proceedings of the National Academy of Sciences of the United States of America* **99**:13902–13907.
- Schwenk J, Harmel N, Zolles G, Bildl W, Kulik A, Heimrich B, Chisaka O, Jonas P, Schulte U, Fakler B, Klocker N (2009) Functional proteomics identify cornichon proteins as auxiliary subunits of AMPA receptors. *Science* **323**:1313–1319.
- Schwenk J, Harmel N, Brechet A, Zolles G, Berkefeld H, Muller CS, Bildl W, Baehrens D, Huber B, Kulik A, Klocker N, Schulte U, Fakler B (2012) High-resolution proteomics unravel architecture and molecular diversity of native AMPA receptor complexes. *Neuron* **74**:621–633.
- Shanks NF, Savas JN, Maruo T, Cais O, Hirao A, Oe S, Ghosh A, Noda Y, Greger IH, Yates JR, 3rd, Nakagawa T (2012) Differences in AMPA and kainate receptor interactomes facilitate identification of AMPA receptor auxiliary subunit GSG1L. *Cell Reports* **1**:590–598.
- Shen L, Liang F, Walensky LD, Huganir RL (2000) Regulation of AMPA receptor GluR1 subunit surface expression by a 4.1N-linked actin cytoskeletal association. *Journal of Neuroscience* **20**:7932–7940.
- Sheng M, Cummings J, Roldan LA, Jan YN, Jan LY (1994) Changing subunit composition of heteromeric NMDA receptors during development of rat cortex. *Nature* **368**:144–147.

- Sheng M, Hoogenraad CC (2007) The postsynaptic architecture of excitatory synapses: a more quantitative view. *Annual Review of Biochemistry* **76**:823–847.
- Shepherd JD, Huganir RL (2007) The cell biology of synaptic plasticity: AMPA receptor trafficking. *Annual Review of Cell and Developmental Biology* **23**:613–643.
- Shi SH, Hayashi Y, Petralia RS, Zaman SH, Wenthold RJ, Svoboda K, Malinow R (1999) Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation. *Science* **284**:1811–1816.
- Shi Y, Suh YH, Milstein AD, Isozaki K, Schmid SM, Roche KW, Nicoll RA (2010) Functional comparison of the effects of TARPs and cornichons on AMPA receptor trafficking and gating. *Proceedings of the National Academy of Sciences of the United States of America* **107**:16315–16319.
- Siddiqui TJ, Craig AM (2011) Synaptic organizing complexes. *Current Opinion in Neurobiology* **21**:132–143.
- Sihra TS, Flores G, Rodriguez-Moreno A (2014) Kainate receptors: multiple roles in neuronal plasticity. *The Neuroscientist* **20**:29–43.
- Soler-Llavina GJ, Fuccillo MV, Ko J, Sudhof TC, Malenka RC (2011) The neurexin ligands, neuroligins and leucine-rich repeat transmembrane proteins, perform convergent and divergent synaptic functions in vivo. *Proceedings of the National Academy of Sciences of the United States of America* **108**:16502–16509.
- Soltau M, Richter D, Kreienkamp HJ (2002) The insulin receptor substrate IRS-53 links postsynaptic shank1 to the small G-protein cdc42. *Molecular and Cellular Neurosciences* **21**:575–583.
- Song I, Kamboj S, Xia J, Dong H, Liao D, Huganir RL (1998) Interaction of the N-ethylmaleimide-sensitive factor with AMPA receptors. *Neuron* **21**:393–400.
- Spacek J, Harris KM (1997) Three-dimensional organization of smooth endoplasmic reticulum in hippocampal CA1 dendrites and dendritic spines of the immature and mature rat. *Journal of Neuroscience* **17**:190–203.
- Spacek J, Hartmann M (1983) Three-dimensional analysis of dendritic spines. I. Quantitative observations related to dendritic spine and synaptic morphology in cerebral and cerebellar cortices. *Anatomy and Embryology* **167**:289–310.
- Srivastava S, Ziff EB (1999) ABP: a novel AMPA receptor binding protein. *Annals of the New York Academy of Sciences* **868**:561–564.
- Stan A, Pielarski KN, Brigadski T, Wittenmayer N, Fedorchenko O, Gohla A, Lessmann V, Dresbach T, Gottmann K (2010) Essential cooperation of N-cadherin and neuroligin-1 in the transsynaptic control of vesicle accumulation. *Proceedings of the National Academy of Sciences of the United States of America* **107**:11116–11121.
- Steinberg JP, Takamiya K, Shen Y, Xia J, Rubio ME, Yu S, Jin W, Thomas GM, Linden DJ, Huganir RL (2006) Targeted in vivo mutations of the AMPA receptor subunit GluR2 and its interacting protein PICK1 eliminate cerebellar long-term depression. *Neuron* **49**:845–860.
- Steiner P, Higley MJ, Xu W, Czervionke BL, Malenka RC, Sabatini BL (2008) Destabilization of the postsynaptic density by PSD-95 serine 73 phosphorylation inhibits spine growth and synaptic plasticity. *Neuron* **60**:788–802.
- Stohr H, Berger C, Frohlich S, Weber BH (2002) A novel gene encoding a putative transmembrane protein with two extracellular CUB domains and a low-density lipoprotein class A module: isolation of alternatively spliced isoforms in retina and brain. *Gene* **286**:223–231.
- Straub C, Zhang W, Howe JR (2011) Neto2 modulation of kainate receptors with different subunit compositions. *Journal of Neuroscience* **31**:8078–8082.
- Sugiyama Y, Kawabata I, Sobue K, Okabe S (2005) Determination of absolute protein numbers in single synapses by a GFP-based calibration technique. *Nature Methods* **2**:677–684.
- Sun J, Tadokoro S, Imanaka T, Murakami SD, Nakamura M, Kashiwada K, Ko J, Nishida W, Sobue K (1998) Isolation of PSD-Zip45, a novel Homer/vesl family protein containing leucine zipper motifs, from rat brain. *FEBS Letters* **437**:304–308.
- Swulius MT, Farley MM, Bryant MA, Waxham MN (2012) Electron cryotomography of postsynaptic densities during development reveals a mechanism of assembly. *Neuroscience* **212**:19–29.

- Takamiya K, Kostourou V, Adams S, Jadeja S, Chalepakis G, Scambler PJ, Huganir RL, Adams RH (2004) A direct functional link between the multi-PDZ domain protein GRIP1 and the Fraser syndrome protein Fras1. *Nature Genetics* **36**:172–177.
- Takamiya K, Mao L, Huganir RL, Linden DJ (2008) The glutamate receptor-interacting protein family of GluR2-binding proteins is required for long-term synaptic depression expression in cerebellar Purkinje cells. *Journal of Neuroscience* **28**:5752–5755.
- Takasu MA, Dalva MB, Zigmond RE, Greenberg ME (2002) Modulation of NMDA receptor-dependent calcium influx and gene expression through EphB receptors. *Science* **295**:491–495.
- Takeuchi M, Hata Y, Hirao K, Toyoda A, Irie M, Takai Y (1997) SAPAPs. A family of PSD-95/SAP90-associated proteins localized at postsynaptic density. *Journal of Biological Chemistry* **272**:11943–11951.
- Tang L, Hung CP, Schuman EM (1998) A role for the cadherin family of cell adhesion molecules in hippocampal long-term potentiation. *Neuron* **20**:1165–1175.
- Tang M, Pelkey KA, Ng D, Ivakine E, McBain CJ, Salter MW, McInnes RR (2011) Neto1 is an auxiliary subunit of native synaptic kainate receptors. *Journal of Neuroscience* **31**:10009–10018.
- Tavalin SJ, Colledge M, Hell JW, Langeberg LK, Huganir RL, Scott JD (2002) Regulation of GluR1 by the A-kinase anchoring protein 79 (AKAP79) signaling complex shares properties with long-term depression. *Journal of Neuroscience* **22**:3044–3051.
- Terashima A, Cotton L, Dev KK, Meyer G, Zaman S, Duprat F, Henley JM, Collingridge GL, Isaac JT (2004) Regulation of synaptic strength and AMPA receptor subunit composition by PICK1. *Journal of Neuroscience* **24**:5381–5390.
- Thomas GM, Huganir RL (2013) Palmitoylation-dependent regulation of glutamate receptors and their PDZ domain-containing partners. *Biochemical Society Transactions* **41**:72–78.
- Thomas GM, Hayashi T, Chiu SL, Chen CM, Huganir RL (2012) Palmitoylation by DHHC5/8 targets GRIP1 to dendritic endosomes to regulate AMPA-R trafficking. *Neuron* **73**:482–496.
- Togashi H, Abe K, Mizoguchi A, Takaoka K, Chisaka O, Takeichi M (2002) Cadherin regulates dendritic spine morphogenesis. *Neuron* **35**:77–89.
- Tomita S, Chen L, Kawasaki Y, Petralia RS, Wenthold RJ, Nicoll RA, Bredt DS (2003) Functional studies and distribution define a family of transmembrane AMPA receptor regulatory proteins. *Journal of Cell Biology* **161**:805–816.
- Tomita S, Fukata M, Nicoll RA, Bredt DS (2004) Dynamic interaction of stargazin-like TARP with cycling AMPA receptors at synapses. *Science* **303**:1508–1511.
- Tonnes J, Stierli B, Cerletti C, Behrmann JT, Molnar E, Streit P (1999) Regional distribution and developmental changes of GluR1-flop protein revealed by monoclonal antibody in rat brain. *Journal of Neurochemistry* **73**:2195–2205.
- Tonnesen J, Katona G, Rozsa B, Nagerl UV (2014) Spine neck plasticity regulates compartmentalization of synapses. *Nature Neuroscience* **17**:678–685.
- Topinka JR, Bredt DS (1998) N-terminal palmitoylation of PSD-95 regulates association with cell membranes and interaction with K⁺ channel Kv1.4. *Neuron* **20**:125–134.
- Torres GE, Yao WD, Mohn AR, Quan H, Kim KM, Levey AI, Staudinger J, Caron MG (2001) Functional interaction between monoamine plasma membrane transporters and the synaptic PDZ domain-containing protein PICK1. *Neuron* **30**:121–134.
- Townsend M, Yoshii A, Mishina M, Constantine-Paton M (2003) Developmental loss of miniature N-methyl-D-aspartate receptor currents in NR2A knockout mice. *Proceedings of the National Academy of Sciences of the United States of America* **100**:1340–1345.
- Traynelis SF, Wollmuth LP, McBain CJ, Menniti FS, Vance KM, Ogden KK, Hansen KB, Yuan H, Myers SJ, Dingledine R (2010) Glutamate receptor ion channels: structure, regulation, and function. *Pharmacological Reviews* **62**:405–496.
- Tu JC, Xiao B, Yuan JP, Lanahan AA, Leoffert K, Li M, Linden DJ, Worley PF (1998) Homer binds a novel proline-rich motif and links group 1 metabotropic glutamate receptors with IP₃ receptors. *Neuron* **21**:717–726.

- Tu JC, Xiao B, Naisbitt S, Yuan JP, Petralia RS, Brakeman P, Doan A, Aakalu VK, Lanahan AA, Sheng M, Worley PF (1999) Coupling of mGluR/Homer and PSD-95 complexes by the Shank family of postsynaptic density proteins. *Neuron* **23**:583–592.
- Turetsky D, Garringer E, Patneau DK (2005) Stargazin modulates native AMPA receptor functional properties by two distinct mechanisms. *Journal of Neuroscience* **25**:7438–7448.
- Uchino S, Wada H, Honda S, Nakamura Y, Ondo Y, Uchiyama T, Tsutsumi M, Suzuki E, Hirasawa T, Kohsaka S (2006) Direct interaction of post-synaptic density-95/Dlg/ZO-1 domain-containing synaptic molecule Shank3 with GluR1 alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor. *Journal of Neurochemistry* **97**:1203–1214.
- Valtschanoff JG, Weinberg RJ (2001) Laminar organization of the NMDA receptor complex within the postsynaptic density. *Journal of Neuroscience* **21**:1211–1217.
- Volk L, Kim CH, Takamiya K, Yu Y, Huganir RL (2010) Developmental regulation of protein interacting with C kinase 1 (PICK1) function in hippocampal synaptic plasticity and learning. *Proceedings of the National Academy of Sciences of the United States of America* **107**:21784–21789.
- Wang R, Walker CS, Brockie PJ, Francis MM, Mellem JE, Madsen DM, Maricq AV (2008) Evolutionary conserved role for TARPs in the gating of glutamate receptors and tuning of synaptic function. *Neuron* **59**:997–1008.
- Wang R, Mellem JE, Jensen M, Brockie PJ, Walker CS, Hoerndl FJ, Hauth L, Madsen DM, Maricq AV (2012) The SOL-2/Neto auxiliary protein modulates the function of AMPA-subtype ionotropic glutamate receptors. *Neuron* **75**:838–850.
- Wenthold RJ, Petralia RS, Blahos J, II, Niedzielski AS (1996) Evidence for multiple AMPA receptor complexes in hippocampal CA1/CA2 neurons. *Journal of Neuroscience* **16**:1982–1989.
- Wenzel A, Fritschy JM, Mohler H, Benke D (1997) NMDA receptor heterogeneity during postnatal development of the rat brain: differential expression of the NR2A, NR2B, and NR2C subunit proteins. *Journal of Neurochemistry* **68**:469–478.
- Westphal RS, Tavalin SJ, Lin JW, Alto NM, Fraser ID, Langeberg LK, Sheng M, Scott JD (1999) Regulation of NMDA receptors by an associated phosphatase-kinase signaling complex. *Science* **285**:93–96.
- Williams ME, Wu SC, McKenna WL, Hinck L (2003) Surface expression of the netrin receptor UNC5H1 is regulated through a protein kinase C-interacting protein/protein kinase-dependent mechanism. *Journal of Neuroscience* **23**:11279–11288.
- de Wit J, Sylwestrak E, O'Sullivan ML, Otto S, Tiglio K, Savas JN, Yates JR, 3rd, Comoletti D, Taylor P, Ghosh A (2009) LRRTM2 interacts with Neurexin1 and regulates excitatory synapse formation. *Neuron* **64**:799–806.
- Worley PF, Zeng W, Huang G, Kim JY, Shin DM, Kim MS, Yuan JP, Kiselyov K, Muallem S (2007) Homer proteins in Ca^{2+} signaling by excitable and non-excitable cells. *Cell Calcium* **42**:363–371.
- Wu X, Hepner K, Castelino-Prabhu S, Do D, Kaye MB, Yuan XJ, Wood J, Ross C, Sawyers CL, Whang YE (2000) Evidence for regulation of the PTEN tumor suppressor by a membrane-localized multi-PDZ domain containing scaffold protein MAGI-2. *Proceedings of the National Academy of Sciences of the United States of America* **97**:4233–4238.
- Wyszynski M, Lin J, Rao A, Nigh E, Beggs AH, Craig AM, Sheng M (1997) Competitive binding of alpha-actinin and calmodulin to the NMDA receptor. *Nature* **385**:439–442.
- Wyszynski M, Kim E, Dunah AW, Passafaro M, Valtschanoff JG, Serra-Pages C, Streuli M, Weinberg RJ, Sheng M (2002) Interaction between GRIP and liprin-alpha/SYD2 is required for AMPA receptor targeting. *Neuron* **34**:39–52.
- Xia J, Zhang X, Staudinger J, Huganir RL (1999) Clustering of AMPA receptors by the synaptic PDZ domain-containing protein PICK1. *Neuron* **22**:179–187.
- Xiao B, Tu JC, Petralia RS, Yuan JP, Doan A, Breder CD, Ruggiero A, Lanahan AA, Wenthold RJ, Worley PF (1998) Homer regulates the association of group 1 metabotropic glutamate receptors with multivalent complexes of homer-related, synaptic proteins. *Neuron* **21**:707–716.

- Xiao B, Tu JC, Worley PF (2000) Homer: a link between neural activity and glutamate receptor function. *Current Opinion in Neurobiology* **10**:370–374.
- Xie Z, Srivastava DP, Photowala H, Kai L, Cahill ME, Woolfrey KM, Shum CY, Surmeier DJ, Penzes P (2007) Kalirin-7 controls activity-dependent structural and functional plasticity of dendritic spines. *Neuron* **56**:640–656.
- Xu J, Xiao N, Xia J (2010) Thrombospondin 1 accelerates synaptogenesis in hippocampal neurons through neuroligin 1. *Nature Neuroscience* **13**:22–24.
- Yamamoto K, Sakagami Y, Sugiura S, Inokuchi K, Shimohama S, Kato N (2005) Homer 1a enhances spike-induced calcium influx via L-type calcium channels in neocortex pyramidal cells. *European Journal of Neuroscience* **22**:1338–1348.
- Yamazaki M, Fukaya M, Abe M, Ikeno K, Kakizaki T, Watanabe M, Sakimura K (2001) Differential palmitoylation of two mouse glutamate receptor interacting protein 1 forms with different N-terminal sequences. *Neuroscience Letters* **304**:81–84.
- Yao I, Hata Y, Hirao K, Deguchi M, Ide N, Takeuchi M, Takai Y (1999) Synamon, a novel neuronal protein interacting with synapse-associated protein 90/postsynaptic density-95-associated protein. *Journal of Biological Chemistry* **274**:27463–27466.
- Ye B, Liao D, Zhang X, Zhang P, Dong H, Huganir RL (2000) GRASP-1: a neuronal RasGEF associated with the AMPA receptor/GRIP complex. *Neuron* **26**:603–617.
- Yoshii A, Sheng MH, Constantine-Paton M (2003) Eye opening induces a rapid dendritic localization of PSD-95 in central visual neurons. *Proceedings of the National Academy of Sciences of the United States of America* **100**:1334–1339.
- Yoshimura Y, Yamauchi Y, Shinkawa T, Taoka M, Donai H, Takahashi N, Isobe T, Yamauchi T (2004) Molecular constituents of the postsynaptic density fraction revealed by proteomic analysis using multidimensional liquid chromatography-tandem mass spectrometry. *Journal of Neurochemistry* **88**:759–768.
- Yu X, Wang G, Gilmore A, Yee AX, Li X, Xu T, Smith SJ, Chen L, Zuo Y (2013) Accelerated experience-dependent pruning of cortical synapses in ephrin-A2 knockout mice. *Neuron* **80**:64–71.
- Yuan JP, Kiselyov K, Shin DM, Chen J, Shcheynikov N, Kang SH, Dehoff MH, Schwarz MK, Seburg PH, Mualllem S, Worley PF (2003) Homer binds TRPC family channels and is required for gating of TRPC1 by IP3 receptors. *Cell* **114**:777–789.
- Yuste R (2010) *Dendritic Spines*. Cambridge, MA: MIT Press.
- Zhang W, St-Gelais F, Grabner CP, Trinidad JC, Sumioka A, Morimoto-Tomita M, Kim KS, Straub C, Burlingame AL, Howe JR, Tomita S (2009) A transmembrane accessory subunit that modulates kainate-type glutamate receptors. *Neuron* **61**:385–396.
- Zheng Y, Mellem JE, Brockie PJ, Madsen DM, Maricq AV (2004) SOL-1 is a CUB-domain protein required for GLR-1 glutamate receptor function in *C. elegans*. *Nature* **427**:451–457.
- Zhou Y, Takahashi E, Li W, Halt A, Wiltgen B, Ehninger D, Li GD, Hell JW, Kennedy MB, Silva AJ (2007) Interactions between the NR2B receptor and CaMKII modulate synaptic plasticity and spatial learning. *Journal of Neuroscience* **27**:13843–13853.

Chapter 7

Subcellular distribution of ligand- and voltage-gated ion channels

Zoltan Nusser

Summary

This chapter describes the subcellular distribution of ligand- and voltage-activated ion channels on the neuronal surface, as detected with a variety of light- and electron-microscopic immunolocalization techniques. First, a general overview is provided demonstrating a presynaptic input-dependent and postsynaptic cell type-dependent location of AMPA- and NMDA-type glutamate and GABA_A receptors in the postsynaptic densities of glutamatergic and GABAergic synapses, respectively. Next, the cell type-dependent distribution of voltage-gated channels is described: the HCN1 subunit has a uniform distribution on the somato-dendritic surface of external tufted cells of the olfactory bulb; its density increases as a function of distance from the soma in the dendrites of cortical and hippocampal pyramidal cells (PCs); and it is present exclusively in the axons of many types of GABAergic interneurons. Detailed analysis of the densities of six different Na⁺ and K⁺ channel subunits on the axo-somato-dendritic surface of hippocampal PCs revealed that each subunit has its own distribution pattern, rendering the ion channel composition of each subcellular compartment—such as the presynaptic active zone, axon terminals, axon initial segment, soma, proximal, distal dendrites, and dendritic spines—unique.

Introduction

The way in which neurons respond to continuous bombardment of synaptic input depends on their morphology, the location of synapses on their somato-dendritic surface, and their intrinsic excitability. Most neurons receive the majority of their synaptic inputs on their dendrites, and therefore the ion channel composition of the dendrites is a key determinant of their input–output transformation. Because the ion channel composition of the axons, soma, and dendrites of a neuron could be very different (see later), it is not enough to determine which ion channel subunits are expressed by a neuron—their densities in functionally defined subcellular compartments, such as presynaptic active zones, axon terminals, axon initial segment, soma, proximal, distal dendrites, and dendritic spines, must be determined. The quantitative determination of ion channel distributions is also a prerequisite for making accurate computational models of neurons.

Comparison of techniques for high-resolution localization of ion channels

Several approaches have been used to study the subcellular distribution of ligand- and voltage-gated ion channels in the central nervous system (CNS). Here, I shall provide a short overview of different techniques, highlighting their advantages and disadvantages. Electron microscopic (EM) autoradiography has been used to study the subcellular distribution of nicotinic acetylcholine receptors using high-affinity [¹²⁵I]α-bungarotoxin (Fertuck and Salpeter, 1974) and [¹²⁵I] α-scorpion toxin has been used for localizing Na⁺ channels (Boudier et al., 1992). The major limitation of this method is that only the distribution of those proteins that have high-affinity ligands/toxins can be studied. Because it is impossible to distinguish between different receptor/channel subtypes using autoradiography, recent investigations have almost exclusively focused on immunolocalization techniques with subunit/subtype-specific antibodies. Visualization of antigen–antibody complexes with fluorescent-coupled secondary antibodies (the fluorescent method) is the most widely used immunohistochemical technique. The fluorescent method has high sensitivity, colocalization of several antigens can be easily performed, and this method produces a non-diffusible marker allowing the quantitative comparison of different antigenic sites. Fluorescent reactions are analyzed with light microscopy (LM). Conventional LM has a diffraction-limited resolution of more than 0.25 μm. Recent advances in super-resolution LM imaging techniques (e.g. photoactivated localization microscopy, PALM; stochastic optical reconstruction microscopy, STORM; stimulated emission depletion microscopy, STED) overcome this limitation (Hell, 2007), making it an ideal combination for high-resolution immunolocalization. However, conventional immunofluorescent methods rely on the recognition of antigens inside thick tissue sections (usually between 10 and 70 μm), requiring tissue penetration of the immunoglobulins, which could compromise the results. Indeed, it has been repeatedly shown that conventional LM immunofluorescent methods do not allow the visualization of neurotransmitter receptors rooted in the dense protein matrix of postsynaptic densities (PSDs) or ion channels located in axon initial segments (AISs). To visualize receptors/channels in these subcellular compartments, antigen-retrieval methods (e.g., pepsin treatment, low-pH fixation, microwave irradiation etc.; Fritschy et al., 1998a; Watanabe et al., 1998; Lorincz and Nusser, 2010) must be employed. Even if they are applied, the potential uneven penetration into the depth of the tissue and into different subcellular compartments produces a degree of ambiguity when quantitative comparisons are made.

Thus, ideal techniques for high-resolution quantitative localizations should not depend on the diffusion of immunoglobulins into the sections. Such “diffusion-free” techniques are post-embedding immunolocalization and SDS-digested freeze-fracture replica immunogold labeling (SDS-FRL or FRIL; Fujimoto et al., 1996; Rash et al., 1998). In post-embedding reactions, ultrathin (from 30 to 100 nm) resin-embedded sections are reacted with the primary antibodies and the antibody–antigen complexes are visualized with either fluorescent- or gold-coupled secondary antibodies. Micheva and Smith (2007) introduced a technique called “array tomography,” which is basically a LM post-embedding immunofluorescent method on serial ultrathin sections. Although few studies have so far employed this technique to localize ion channels, the main advantage seems to be the possibility of labeling a large number of proteins within a given subcellular compartment; its limitation might be its relatively modest sensitivity. A traditionally more widely used post-embedding technique employs gold-coupled secondary antibodies and EM examination (Baude et al., 1993; Phend et al., 1995; Rubio and Wenthold, 1997; Takumi et al., 1999). The SDS-FRL technique is currently accepted as the method of choice for high-resolution quantitative localization of

Table 7.1 Comparison of methods used for immunolocalization of ion channels

Method	Resolution	Sensitivity	Multiple labeling	Quantification
LM fluorescence with confocal microscopy	>200 nm	High	Ideal (up to 4)	Possible
LM fluorescence with super-resolution microscopy	~20–40 nm	High	Not ideal (up to 2)	Possible
Array tomography	>200 nm	Moderate	Ideal (dozens)	Possible
EM peroxidase	>100 nm	High	Not possible	Not possible
EM post-embedding gold	~25 nm	Moderate	Ideal (up to 3)	Possible
EM freeze-fracture replica-labeling gold	~25 nm	Very high	Ideal (up to 3)	Possible

transmembrane proteins. It has an exceptional sensitivity (a labeling efficiency of about 100% has been reported in multiple cases) and high resolution and the large fractured plasma membrane sheets make this method amenable for quantification and surface density calculation (Tanaka et al., 2005). A summary of the different approaches to studying the subcellular distribution of ligand- and voltage-gated ion channels is provided in Table 7.1.

It is important to emphasize that irrespective of the immunolocalization technique employed, the specificity of the immunoreaction must always be tested (Rhodes and Trimmer, 2006; Fritschy, 2008; Lorincz and Nusser, 2008a).

Subcellular distribution of ionotropic glutamate receptors

Glutamate is the major excitatory neurotransmitter in the mammalian CNS. This transmitter is usually released from presynaptic axon terminals onto the cell bodies and dendrites of neurons where it exerts its influence through the activation of ion channel-forming (ionotropic) and G-protein coupled (metabotropic) receptors. The molecular, pharmacological, and biophysical properties of glutamate receptors (GluRs) have been extensively reviewed (Nakanishi, 1992; Seeburg, 1993; Hollmann and Heinemann, 1994; Pin and Duvoisin, 1995; Dingledine et al., 1999), therefore here I shall provide only a summary of some fascinating features of the subcellular distributions of AMPA and NMDA receptors.

AMPA-type glutamate receptors

The fast onset and rise time of postsynaptic AMPA receptor-mediated excitatory postsynaptic currents (EPSCs; see Chapter 8) indicate that AMPA receptors are concentrated in the PSDs of glutamatergic synapses. However, AMPA receptors are also found on extrasynaptic somatic and dendritic membranes, as probed with patch-clamp recordings (see Chapter 8). Synaptic enrichment, with a lower extrasynaptic AMPA receptor density, was confirmed by many studies using high-resolution immunolocalization techniques (Nusser et al., 1994; Phend et al., 1995; Matsubara et al., 1996; Tanaka et al., 2005; Masugi-Tokita et al., 2007; Tarusawa et al., 2009; Budisantoso et al., 2012). However, it is important to point out that the subunit composition, number, density, and subsynaptic distribution of AMPA receptors are not identical in all neurons of the CNS, but cell

type-specific and synaptic input-specific differences exist. These intriguing distribution patterns have been revealed in the past two decades by high-resolution immunolocalization experiments.

For example, Rubio and Wenthold (1997) have demonstrated that the GluA2 subunit is present in both parallel and auditory fiber synapses on dorsal cochlear nucleus fusiform cells at approximately the same density, whereas the GluA4 subunit was only detectable in auditory synapses. This result demonstrates that on a single cell, distinct glutamatergic inputs can act through AMPA receptors with different subunit compositions. A similar input-specific segregation of distinct NMDA receptor subunits has also been documented (see later). An even more widely observed phenomenon is the cell type-specific and presynaptic input-specific difference in the number and/or density of postsynaptic AMPA receptors. This was first demonstrated by Nusser et al. (1998c) in hippocampal CA3 pyramidal cells (PCs) and later by Masugi-Tokita et al. (2007) in cerebellar Purkinje cells and Tarusawa et al. (2009) in dorsal lateral geniculate nucleus relay cells. Nusser et al. (1998c) found that the number of AMPA receptors in CA3 PC commissural/associational (C/A) fiber synapses is four-fold lower than that in mossy fiber synapses of the same cell. C/A synapses had a much larger synapse-to-synapse variability in AMPA receptor number and approximately 15% of them were immunonegative, whereas mossy fiber-PC synapses were all immunopositive. A similar result was obtained in cerebellar Purkinje cells, where the climbing fiber synapses contained twice as many AMPA receptors as parallel fiber synapses (Masugi-Tokita et al., 2007). In addition, it is not only the number of AMPA receptors that can vary in a synaptic input-specific manner. Retino-geniculate and cortico-geniculate synapses have a similar number of AMPA receptors, but the two-fold larger area of the PSD in the latter results in a significantly lower AMPA receptor density in cortico-geniculate synapses (Tarusawa et al., 2009). A non-uniform intrasynaptic distribution of AMPA receptors was also reported for retino-geniculate and cortico-geniculate synapses, similar to that found in cerebellar parallel fiber (Masugi-Tokita et al., 2007) and some hippocampal synapses (Nair et al., 2013). Interestingly, detailed kinetic and diffusion modeling predicted a negligible effect of this difference in postsynaptic receptor density and subsynaptic clustering on the amplitude of postsynaptic EPSCs, indicating that the number and not the density of postsynaptic AMPA receptors is the key parameter that determines the size of the postsynaptic responses (Tarusawa et al., 2009).

To test the extent to which the postsynaptic cell type influences receptor expression, the AMPA receptor content of C/A synapses was compared between PC spines and GABAergic interneuron (IN) dendrites in the hippocampus. Synapses on IN dendrites contained about four times as many gold particles as those on PC spines (Nusser et al., 1998c). When Masugi-Tokita et al. (2007) addressed the same question by comparing the AMPA receptor content of cerebellar parallel fiber synapses on Purkinje versus stellate/basket cells, they observed a similar mean receptor number but very different receptor densities, demonstrating again that not only the number of postsynaptic AMPA receptors but also their density is regulated in a postsynaptic cell type-dependent manner.

In summary, the following view of the subcellular distribution of AMPA receptors has emerged from high-resolution immunolocalization experiments. Receptors are concentrated in glutamatergic synaptic junctions with an abrupt decrease in their density at the edge of the PSD. The molecular makeup, the number, and the density of synaptic AMPA receptors are determined by both pre- and postsynaptic factors, such that functionally distinct glutamatergic connections display characteristic patterns of receptor expression. AMPA receptors are also found on the extrasynaptic plasma membranes of the somata, dendritic shafts, and spines in some cells, albeit at a lower density, but never in membranes postsynaptic to GABAergic terminals. Intracellularly, AMPA receptors are associated with the endoplasmic reticulum, Golgi apparatus, multivesicular bodies, and the spine apparatus.

NMDA-type glutamate receptors

NMDA receptors have been in the focus of intense scientific research since the late 1970s as a consequence of their key roles in neuronal development, sensory perception, learning and memory formation, and in the pathogenesis of various CNS disorders. Since the development of the first NMDA receptor-specific ligands and the cloning of NMDA receptor subunits, physiologists, behavioral neuroscientists, and molecular biologists have joined forces to gain more insight into the role of NMDA receptors in various CNS functions and dysfunctions.

Using conventional immunohistochemical techniques (pre-embedding immunoperoxidase and fluorescent), immunolabeling for NMDA receptors was found mainly intracellularly in the somato-dendritic compartments of various cell types (Huntley et al., 1994; Petralia et al., 1994; Siegel et al., 1994). In two elegant papers in 1998, Fritschy et al. (1998a) and Watanabe et al. (1998) used two independent antigen-retrieval techniques to re-examine the subcellular distribution of NMDA receptor subunits. These studies revealed that either microwave irradiation or pepsin digestion dramatically reduced the strong intracellular labeling obtained with conventional immunoperoxidase and fluorescent methods and allowed the visualization of synaptic NMDA receptors, revealing an intensely punctate labeling of the hippocampal neuropil (Fritschy et al., 1998a; Watanabe et al., 1998). Since these experiments, it is now generally accepted that antigen retrieval methods are essential for the visualization of synaptic GABA_A receptors and GluRs when the reaction is performed in thick sections before embedding, while they are not necessary when diffusion-free techniques are used (SDS-FRL, post-embedding localization). In addition to revealing the synaptic enrichment of NMDA receptors in hippocampal synapses, Fritschy et al. (1998a) and Watanabe et al. (1998) demonstrated that the NMDA receptor composition of C/A synapses on CA3 PC spines includes the GluN1, GluN2A, and GluN2B subunits, whereas mossy fiber to CA3 PC synapses lack the GluN2B subunit, revealing that a single cell can sort distinct NMDA receptor subunits to synapses that receive distinct presynaptic inputs.

As mentioned above, the AMPA receptor content of distinct glutamatergic synapses varies depending on the pre- and postsynaptic cell type. Takumi et al. (1999) and Racca et al. (2000) examined the NMDA receptor content of Schaffer collateral to CA1 PC spine synapses. Surprisingly, the expression of AMPA and NMDA receptors at these synapses showed a very different pattern. Every Schaffer collateral synapse on CA1 PC spines contains immunoreactive NMDA receptors, but because only 75–85% of them have detectable amounts of AMPA receptors (Nusser et al., 1998c; Takumi et al., 1999; Racca et al., 2000), AMPA and NMDA receptors are colocalized only in 75–85% of these synapses. Furthermore, Takumi et al. (1999) found that the NMDA receptor content is linearly related to the diameter of the PSD, whereas the AMPA receptor content has a positive linear correlation with the area of the PSD (a second power correlation with diameter), resulting in a linear relationship between the ratio of AMPA to NMDA receptors and the diameter of the PSD. This issue was further investigated by addressing how the AMPA/NMDA ratio varies as a function of distance of the synapse from the soma. Menon et al. (2013) and Nicholson and Genisman (2009) found that the AMPA receptor content of large perforated (but not small) synapses increases, whereas the NMDA receptor content decreases, as a function of distance of the synapse from the soma, resulting in a distance-dependent increase in the synaptic AMPA/NMDA ratio.

Nyiri et al. (2003) found quantitative differences between the GluN1 subunit content of Schaffer collateral synapses in CA1 PC spines and parvalbumin (PV)-positive IN dendrites, similar to the differences in the AMPA receptor content of PC and IN synapses. The PV positive IN dendrites contained fewer gold particles with more variability, including several immunonegative synapses, whereas the spine synapses were all immunopositive. Rubio et al. (2014) demonstrated that the

number of GluN1 subunits is larger in auditory fiber synapses on cochlear nucleus fusiform cells compared to that found in parallel fiber synapses. In conclusion, these experiments revealed that, similar to AMPA receptors, the NMDA receptor content of glutamatergic synapses is determined by both pre- and postsynaptic factors. Unlike AMPA receptors, the presence of NMDA receptors has been reported in perisomatic GABAergic synapses of the hippocampus, indicating that they might play a role in regulating GABAergic neurotransmission (Szabadits et al., 2011).

Subcellular distribution of GABA_A receptors

In most brain regions, there is a large diversity in GABA-releasing neurons, which seems to be essential to fulfill the complex functional requirements of neural networks (Klausberger and Somogyi, 2008). These include the synchronization of populations of neurons, the regulation of active backpropagation of action potentials into dendrites, inhibition of dendritic Ca²⁺ electrogenesis, and shunting of excitatory synaptic inputs. Most of these diverse actions are achieved through the activation of ionotropic GABA_A receptors. GABA_A receptors are formed from pentameric assemblies of structurally distinct subunits. To date, 19 different mammalian subunit genes have been identified (α_{1-6} , β_{1-3} , γ_{1-3} , δ , ϵ , θ , π , and σ_{1-3}), allowing hundreds of thousands of possible combinations of subunits (Barnard et al., 1998; Sieghart and Sperk, 2002). Despite this, only a few dozen combinations have actually been demonstrated (McKernan and Whiting, 1996), reflecting the differential distributions of subunits among brain regions and neuronal populations (Fritschy and Mohler, 1995; Pirker et al., 2000), and also suggesting specific “rules” of subunit assembly (Jones et al., 1997; Barnard et al., 1998; Mohler et al., 1998; Kittler et al., 2002). This enormous molecular diversity has a number of important functional consequences: ligand binding, channel gating, channel conductance, mode of activation, and allosteric modulations all depend on the subunit composition of the receptors. In addition, the precise subcellular distribution of distinct GABA_A receptor subtypes varies in a presynaptic input-specific and postsynaptic cell type-specific manner. Here, I shall summarize some experimental data and provide some examples of the highly regulated cell surface expression of GABA_A receptors.

Several aspects of the subcellular distribution of GABA_A receptor subunits have been revealed by LM immunofluorescence and EM immunogold studies. The enrichment of the α_1 , α_2 , α_3 , α_6 , $\beta_{2/3}$, and γ_2 subunits has been shown in synapses opposite GABA-releasing terminals in many brain regions, including the cerebellum, globus pallidus, hippocampus, olfactory bulb, spinal cord, and neocortex (Craig et al., 1994; Fritschy and Mohler, 1995; Nusser et al., 1995, 1996; Somogyi et al., 1996; Fritschy et al., 1998a; Brunig et al., 2002; Szabadits et al., 2011; Kerti-Szigeti et al., 2014). It should be noted, however, that each of these subunits was also found in extrasynaptic dendritic and somatic plasma membranes. Even for $\alpha_1\beta_{2/3}\gamma_2$ receptors that are highly enriched in synapses, the total number of extrasynaptic receptors exceeds that found inside synaptic junctions in cerebellar granule cells (Nusser et al., 1995). This may suggest that the fraction of extrasynaptic receptors is regulated in a cell type-specific manner and may depend on the level of tonic inhibition (see later and reviews by Mody, 2001; Semyanov et al., 2004; Farrant and Nusser, 2005; Glykys and Mody, 2007). No immunoreactivity for GABA_A receptors could be detected at glutamatergic synapses in several brain regions. However, one should be cautious with the generalization, as an enrichment of the α_6 , $\beta_{2/3}$, and γ_2 subunits was found at glutamatergic mossy fiber to granule cell synapses in the cerebellum (Nusser et al., 1996, 1998a) and the α_1 and $\beta_{2/3}$ subunits in hippocampal mossy fiber synapses (Bergersen et al., 2003). The functional role of these receptors at glutamatergic synapses is as yet unknown, but their activation by synaptically released GABA has been documented (Bergersen et al., 2003; Ruiz et al., 2003). Finally, intracellular organelles such as

somatic/proximal dendritic endoplasmic reticulum and the Golgi apparatus also contain immunoreactive GABA_A receptors, consistent with the suggestion that they are synthesized in somatic/proximal dendritic regions and their transport to synapses occurs by lateral diffusion in the plasma membrane (Choquet and Triller, 2003).

It seems to be a general rule that if a neuron expresses a given GABA_A receptor in some of its synapses, the same receptor will also be present in the extrasynaptic plasma membrane at a lower density. Is the converse also true? Namely, if a neuron expresses a receptor subtype extrasynaptically, will it always be concentrated at some synapses? To examine this question, EM immunogold localization of all major GABA_A receptor subunits (α_1 , α_6 , β_2 , β_3 , γ_2 , and δ) expressed by cerebellar granule cells was carried out by Nusser et al. (1998a). The α_1 , α_6 , $\beta_{2/3}$, and γ_2 subunits were concentrated in GABAergic Golgi cell to granule cell synapses and were also present in the extrasynaptic membrane at lower concentrations. In contrast, immunogold particles for the δ subunit could not be detected in synaptic junctions, although they were abundant in the extrasynaptic dendritic and somatic membranes (see Figs 7.1 and 7.2). Similar to this, the δ subunit was also found only in extrasynaptic and perisynaptic locations in dentate gyrus granule cells and hippocampal GABAergic INs (Wei et al., 2003). The δ subunit forms receptors specifically with the α_6 and $\beta_{2/3}$ subunits in cerebellar granule cells ($\alpha_6\beta_{2/3}\delta$ and $\alpha_1\alpha_6\beta_{2/3}\delta$) and with the α_4 and β_x subunits ($\alpha_4\beta_x\delta$) in several areas of the forebrain, including the thalamus, neostriatum, and dentate gyrus, and with the α_1 and β_2 subunits in hippocampal GABAergic interneurons (Fritschy and Mohler, 1995; Pirker et al., 2000; Chandra et al., 2006; Glykys et al., 2007). For these receptor subtypes, the lack of a γ subunit may be responsible for their failure to be incorporated at the synapse. The exclusive extrasynaptic location of the δ subunit-containing receptors, together with their kinetic properties, suggest that tonic inhibition is mediated by *extrasynaptic* δ subunit-containing receptors, whereas phasic inhibition is mainly mediated by *synaptic* γ subunit-containing receptors (reviewed by Mody, 2001; Semyanov et al., 2004; Farrant and Nusser, 2005; Glykys and Mody, 2007). However, as in most cases, the emerging picture is more complex. The α_5 subunit does not seem to form channels with the δ , but with the γ_2 subunit. Despite the presence of the γ_2 subunit in $\alpha_5\beta_x\gamma_2$ receptors, they do not seem to be concentrated in synaptic junctions as judged from the diffuse α_5 labeling without detectable synaptic clustering, and from the lack of colocalization with gephyrin (Fritschy et al., 1998b; Brunig et al., 2002; Crestani et al., 2002). Thus, in hippocampal PCs, extrasynaptic $\alpha_5\beta_x\gamma_2$ receptors are likely to be responsible for tonic inhibition.

Analogous to the multiple types of glutamatergic inputs to neurons, most neurons in the CNS also receive GABAergic input from several distinct sources and express multiple GABA_A receptor subtypes. To examine whether every receptor subtype is concentrated at every GABAergic synapse on a single cell or whether selective synaptic targeting of distinct subtypes exists, the subcellular distribution of α_1 , α_2 and α_3 subunits was compared in retinal alpha ganglion cells. Koulen et al. (1996) found that these α subunits were concentrated at distinct sites, indicating an input-selective segregation of distinct GABA_A receptor subtypes on the surface of central neurons. Similar to the distribution of GluRs, not only the presynaptic input but also the postsynaptic cell type has a strong influence on the receptor expression. Klausberger et al. (2002) compared the α_1 subunit content of synapses made by PV-positive terminals on hippocampal PCs and on PV-positive INs and reported a significantly higher gold particle density in the latter synapse population.

Synapses are not static; several physiological and pathological conditions can result in dynamic and sometimes long-lasting changes in the synaptic connections between neurons. Alterations in GABAergic inhibition have been well documented in puberty, during the menstrual cycle or in pregnancy, and in a variety of pathological conditions such as epilepsy, depression, stroke, or schizophrenia (Peng et al., 2004; Maguire et al., 2005; Maguire and Mody, 2008; Clarkson et al.,

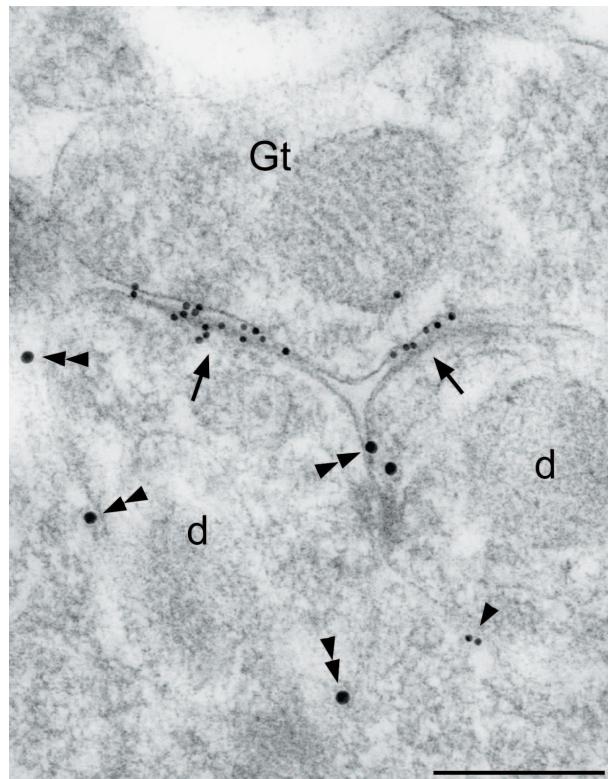


Fig. 7.1 Differential distribution of the $\beta_{2/3}$ and δ subunits of the GABA_A receptors in cerebellar granule cells. Synapses (arrows) made by a cerebellar Golgi cell terminal (Gt) with granule cell dendrites (d) show an enrichment of the $\beta_{2/3}$ subunits (small particles), but do not contain particles for the δ subunit (large particles). The presence of immunoparticles for the δ subunit (double arrowheads) at the extrasynaptic dendritic membranes demonstrates that the method is sensitive enough to visualize this subunit. Note that immunoparticles for the $\beta_{2/3}$ subunits are also associated with the extrasynaptic dendritic membranes (e.g., arrowhead). Scale bar = 0.2 μ m.

Adapted from Zoltan Nusser, Werner Sieghart, and Peter Somogyi, Segregation of different GABA_A receptors to synaptic and extrasynaptic membranes of cerebellar granule cells, *The Journal of Neuroscience*, 18(5), pp. 1693–1703, © 1998, The Society for Neuroscience.

2010; Shen et al., 2010). Lasting changes may take place (1) presynaptically by altering the probability of transmitter release, (2) postsynaptically by altering the properties and/or the quantity of receptors, or (3) the number of transmitter release sites between two neurons could also be modified. Nusser et al. (1998b) have addressed the question of whether the change in quantal size (Otis et al., 1994) following an experimental model of temporal lobe epilepsy (kindling) can be attributed to a change in the number of synaptic GABA_A receptors. Quantal analysis of evoked inhibitory postsynaptic currents (IPSCs) in hippocampal granule cells revealed a 66% increase in the quantal size after kindling, which resulted directly from a 75% increase in the number of synaptic GABA_A receptors as determined by quantitative immunogold localization (Nusser et al., 1998b). The augmented receptor number was the consequence of an enlargement (about 30%) in

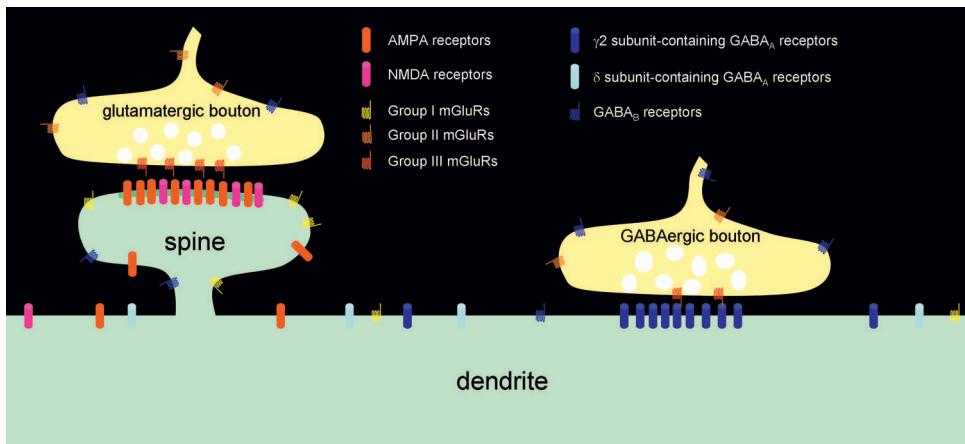


Fig. 7.2 Schematic representation of the synaptic, perisynaptic, and extrasynaptic location of ionotropic and metabotropic GABA and glutamate receptors (mGluRs).

the area of the synapses as well as an enhanced receptor density (about 35%). These results clearly demonstrate the ability of central neurons to regulate the weight of their synaptic connections by changing the number of receptors at specific synapses.

Subcellular distribution of voltage-gated ion channels

The way in which neurons respond to spatio-temporally dispersed synaptic inputs depends, to a large extent, on the properties, subcellular distribution, and densities of voltage-gated ion channels in their plasma membrane (see Chapters 9 and 12). How cellular neurophysiology has contributed to our knowledge of the functional and pharmacological properties of voltage-gated ion conductances in distinct subcellular compartments is extensively discussed in Chapter 9. Here, I shall only review recent results on the subcellular distribution of voltage-gated ion channel subunits as detected using immunolocalization methods.

Hyperpolarization-activated and cyclic nucleotide-gated (HCN) mixed cation channels

HCN channels, often termed “pacemaker” channels, are involved in a large variety of functions, including spontaneous rhythmic contraction of the heart, generation of oscillations in the brain, setting the resting membrane potential, and modulating the firing properties and the integration of synaptic inputs of central neurons (Pape, 1996; Accili et al., 2002). These channels are activated by membrane hyperpolarization (negative to -50 mV) and are modulated by intracellular cyclic nucleotides (e.g., cAMP). They are permeable to Na^+ , Ca^{2+} , and K^+ (mixed cation) and their opening results in a net inward flow of cations (I_h), causing membrane depolarization. HCN channels are built from four sub-units (HCN1–4), the homo- and heterotetrameric assemblies of which are mainly responsible for the molecular heterogeneity of these ion channels. It has been demonstrated that the subunit composition critically affects the functional properties of HCN channels, such as their activation and deactivation kinetics, and their modulation by cyclic nucleotides (Ludwig et al., 1998; Moosmang et al., 2001).

Lorincz et al. (2002) carried out quantitative EM immunogold localization to determine the distribution of HCN1 in PCs of the hippocampus and neocortex. They reported a lack of

immunoreactive HCN1 channels in the axon terminals of PCs. In contrast, the somata and proximal and distal dendrites contained significant quantities of immunogold particles, demonstrating the cellular domain dependence of HCN1 distribution. Within the dendritic tree, the density of immunogold particles was about 15-fold higher in distal compared with proximal dendritic shafts (Fig. 7.3). Proximal dendrites, in turn, have three times higher HCN1 densities than somata. These

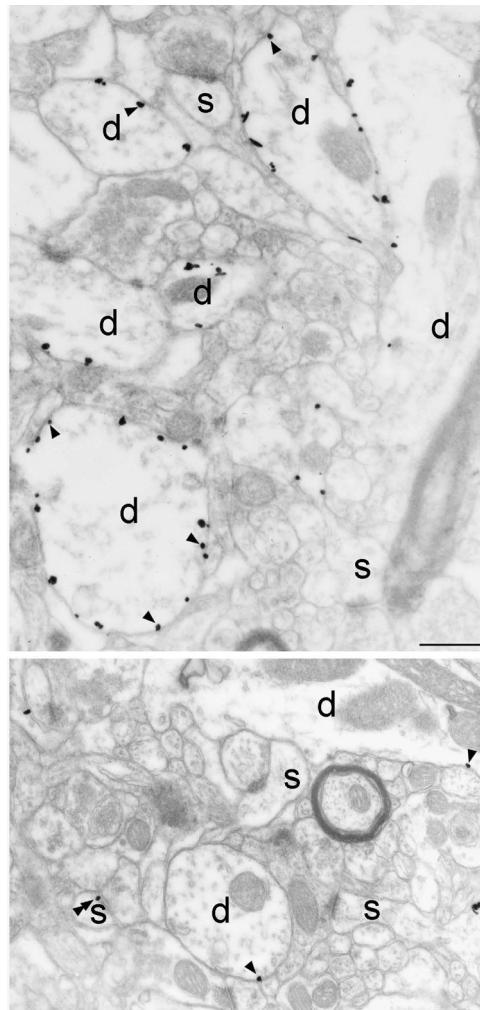


Fig. 7.3 The density of HCN1 increases as a function of distance from the soma. Distal dendrites (d) of PCs (upper panel) are labeled by a large number of immunogold particles for HCN1 (e.g., arrowheads), whereas proximal dendrites (lower panel) of approximately the same diameter contain only a small number of particles (arrowheads). Spines (s) are mainly immunonegative, but occasionally exhibit a few particles (e.g., double arrowhead). Scale bars = 0.4 μ m.

Adapted with permission from Macmillan Publishers Ltd: *Nature Neuroscience*, 5(11), Andrea Lörincz, Takuya Notomi, Gábor Tamás, Ryuichi Shigemoto and Zoltan Nusser, Polarized and compartment-dependent distribution of HCN1 in pyramidal cell dendrites, pp. 1185–1193, Copyright 2002, Nature Publishing Group.

results demonstrate that in the dendrites of hippocampal PCs, the density of HCN1 increases as a function of distance from the soma (distance dependence). In addition, distal dendritic shafts had a significantly higher HCN1 density than spines at the same average distance from the soma, revealing the subcellular compartment dependence of HCN1 density. The subcellular distribution of HCN2 in cortical and hippocampal PCs is practically identical to that of HCN1 (Notomi and Shigemoto, 2004), suggesting the heterotetrameric assembly of the HCN1 and HCN2 subunits to form functional channels. Investigations in many brain regions have revealed that the distance-dependent increase in dendritic HCN channel density is not generally valid for all neurons. For example, HCN1 is found to be present rather uniformly in the somatic and dendritic plasma membranes of external tufted and periglomerular cells of the main olfactory bulb (Holderith et al., 2003). An even more surprising finding is that HCN1 is not present at all in the somato-dendritic plasma membrane of hippocampal basket cells, but has a high density in their axon terminals and pre-terminal axons (Santoro et al., 1997; Notomi and Shigemoto, 2004). The finding of Lujan et al. (2005) adds another twist to this story; HCN1 immunolabeling in cerebellar basket cells was found throughout the axo-somato-dendritic surface, with slightly higher levels in axon terminals. The subcellular distribution of HCN1 in entorhinal cortical cells seems to be regulated during postnatal development: in young animals, the medial perforant path contains presynaptic HCN1 channels, which disappear later during development (Wilkars et al., 2012). The critical role of the HCN channel-associated subunit TRIP8b has been demonstrated in this process (Wilkars et al., 2012). These results reveal many distinct subcellular distribution patterns for HCN1 (Fig. 7.4) and demonstrate the cell-type-dependent subcellular distribution of a voltage-gated ion channel.

It is important to note that the density of I_h could vary within a single cell type, probably as a consequence of experience- or activity-dependent regulations (van Welie et al., 2004; Narayanan and Johnston, 2007; Johnston and Narayanan, 2008). Garden et al. (2008) elegantly demonstrated differences in I_h densities in stellate cells of entorhinal cortical layer 2: the density of I_h depends on the dorsoventral location of the cells and parallels the gradient in the size of their grid fields. Future quantitative high-resolution immunolocalization experiments will likely reveal variations in the densities, and probably the subcellular distribution, of HCN channels in distinct physiological states and pathological conditions.

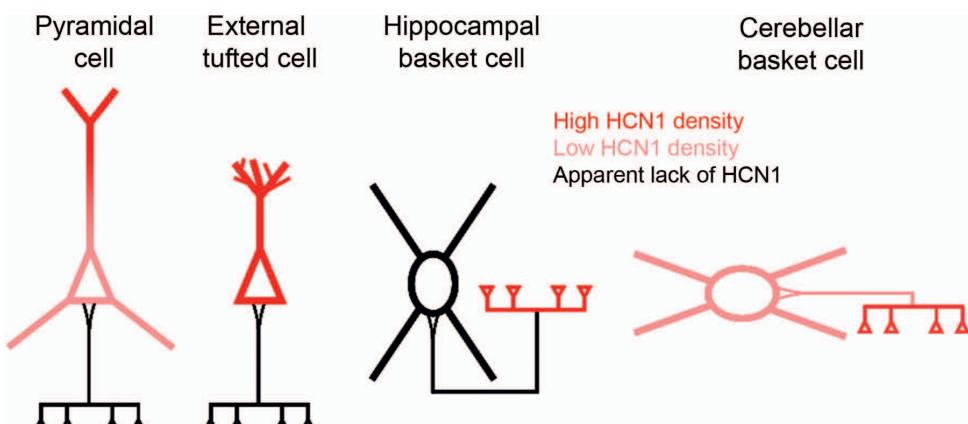


Fig. 7.4 Schematic illustration of the cell-type-dependent subcellular distributions of HCN1 in the CNS.

Voltage-gated Na⁺ channels

The electric eel electroplax voltage-gated sodium channel was the first voltage-dependent ion channel to be cloned (Noda et al., 1984). Soon after the initial cloning, a large molecular diversity was revealed for this ion channel family. Out of the 12 identified subunits ($\text{Na}_v1.1$ – $\text{Na}_v1.12$; reviewed by Goldin, 2001) five subunits show strong expression in the CNS ($\text{Na}_v1.1$, $\text{Na}_v1.2$, $\text{Na}_v1.3$, $\text{Na}_v1.5$, and $\text{Na}_v1.6$; reviewed by Trimmer and Rhodes, 2004). Some aspects of the subcellular distribution of Na⁺ channels have been revealed by autoradiography using high-affinity toxins (Angelides et al., 1988; Black et al., 1990; Boudier et al., 1992). The highest density of labeling was found in the nodes of Ranvier of myelinated axons, supporting the classical view of the axonal distribution of Na⁺ channels and their roles in active propagation of action potentials. The enrichment of Na⁺ channel subunits in the nodes of Ranvier was later confirmed by LM and EM immunolocalization studies, which also revealed their prominent concentrations in the AISs of many cell types (Jenkins and Bennett, 2001; Boiko et al., 2003; Inda et al., 2006; Kuba et al., 2006; Ogiwara et al., 2007; Van Wart et al., 2007; Kole et al., 2008; Lorincz and Nusser, 2008b, 2010; Hu et al., 2009; Kress et al., 2010), where they are part of a macromolecular complex. This AIS signaling complex and its functional role were recently reviewed in detail (Rasband, 2010; Kole and Stuart, 2012); therefore here I shall provide a brief summary focusing only on the ion channels and not the members of the auxiliary/regulatory machinery.

An intriguing feature of the distribution of Na⁺ channel subunits in the AIS is their segregation along the proximo-distal axis. The $\text{Na}_v1.1$ subunit is confined to the proximal part of the AISs of retinal ganglion cells and PV-positive GABAergic INs of the hippocampus and cerebellum. Here, the density of the $\text{Na}_v1.6$ subunit is low, and it gradually increases toward the middle and distal part of the AIS (Ogiwara et al., 2007; Van Wart et al., 2007; Lorincz and Nusser, 2008b). In cortical PCs a similar proximo-distal segregation was reported for the $\text{Na}_v1.2$ and $\text{Na}_v1.6$ subunits (Hu et al., 2009; Lorincz and Nusser, 2010), the former being located proximally close to the axon hillock, just like the $\text{Na}_v1.1$ subunit in INs. Despite the fact that these subunits show sub-AIS segregation in many cell types, Lorincz and Nusser (2008b) reported that $\text{Na}_v1.1$ is present along the entire AIS of short-axon cells of the main olfactory bulb, fully colocalizing with the $\text{Na}_v1.6$ subunit. It remains to be seen whether the segregation of these subunits is the general rule and their colocalization is the exception, or vice versa. It would also be interesting to test whether the precise sub-AIS location of these subunits within a certain cell type is fixed or if activity-dependent changes could alter their nanoscale distributions.

The studies cited above using LM immunofluorescent localizations clearly identified the $\text{Na}_v1.1$, $\text{Na}_v1.2$, and $\text{Na}_v1.6$ subunits in axonal compartments of many cell types, but failed to provide convincing evidence for their somato-dendritic locations. This was disturbing because many functional studies reported voltage-gated Na⁺ conductances in, for example, cortical and hippocampal PC somata and dendrites (see Chapter 9). A possible explanation for the discrepancy between the results of the LM fluorescent localization and *in vitro* functional studies is that the Na⁺ channels are present in the somato-dendritic compartments at low densities, below the detection threshold of the immunofluorescent method. To overcome this technical limitation, Na⁺ channel localization was carried out with SDS-FRL (Lorincz and Nusser, 2010), the most sensitive immunolocalization method available. A high density of immunogold particles labeling the $\text{Na}_v1.6$ subunit was observed in the nodes of Ranvier and AISs of hippocampal CA1 PCs, consistent with LM immunofluorescent data. With this method, gold particles were consistently found in somatic and proximal dendritic plasma membranes at densities about 40-fold lower than in the AISs (Fig. 7.5). In addition, quantitative analysis revealed a distance-dependent decrease in the density of the $\text{Na}_v1.6$

subunit along the proximo-distal axis of the apical dendrites. These SDS-FRL results provided direct evidence (1) that the $\text{Na}_v1.6$ subunit is present in axo-somato-dendritic plasma membranes, (2) for quantitative differences in the density of $\text{Na}_v1.6$ subunits between the nodes of Ranvier and AISs, (3) for the long-debated issue regarding the large difference in density of Na^+ channels in the AIS and soma, (4) that the density of $\text{Na}_v1.6$ decreases in dendrites as a function of distance from

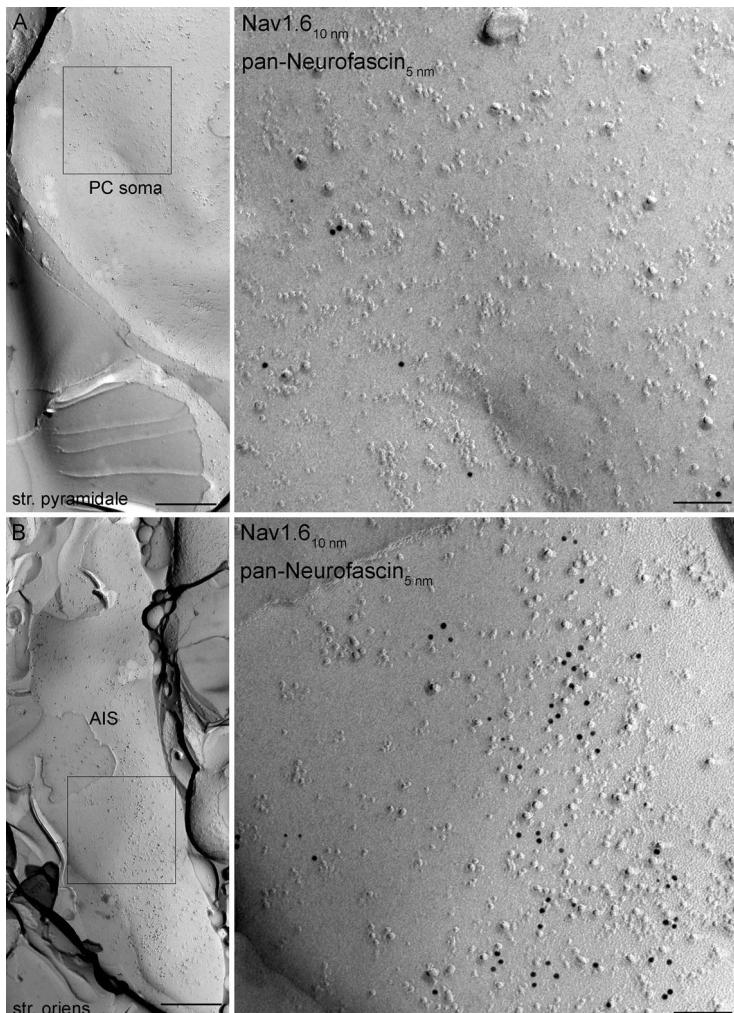


Fig. 7.5 Enrichment of the $\text{Na}_v1.6$ subunit in the AISs as revealed with SDS-FRL. (A) Low-magnification view of a somatic plasma membrane in the hippocampal CA3 area is shown on the left immunoreacted for $\text{Na}_v1.6$ and pan-Neurofascin. The boxed area is shown at higher magnification on the right. The somatic membrane contains few gold particles labeling the $\text{Na}_v1.6$ subunit. (B) A low-magnification image of an AIS immunopositive for both $\text{Na}_v1.6$ and pan-Neurofascin (left). The boxed area is shown at higher magnification on the right. The AIS has a much higher density of gold particles compared with the soma. Note that gold particles are not uniformly distributed within the AIS; there are high- and low-density areas. Scale bars: images on the left 500 nm, images on the right 100 nm.

the soma, and (5) for the lack of detectable $\text{Na}_v1.6$ subunits in dendritic spines. Similar experiments with highly sensitive localization methods will be required to reveal whether the $\text{Na}_v1.1$ and $\text{Na}_v1.2$ subunits are also present in dendritic plasma membranes at low densities, or whether they are solely axonal Na^+ channels. Currently, no information exists on the subcellular distribution of the $\text{Na}_v1.5$ subunit in central neurons.

Voltage-gated Ca^{2+} channels

Calcium channels have been classified into five different groups according to their functional and pharmacological properties (L, N, P/Q, R, and T; reviewed by Hille, 2001). Subsequently, the underlying molecular heterogeneity has been discovered by molecular cloning, revealing a total of ten α_1 subunits (Ertel et al., 2000). The α_1 subunits form ion channels together with the $\alpha_2\delta$, β , and γ subunits, creating the molecular diversity that is responsible for the well-known pharmacological and biophysical heterogeneities (Hille, 2001). The Ca_v1 family ($\text{Ca}_v1.1$, α_{1S} ; $\text{Ca}_v1.2$, α_{1C} ; $\text{Ca}_v1.3$, α_{1D} ; and $\text{Ca}_v1.4$, α_{1F}) mediate L-type Ca^{2+} currents; the Ca_v2 family mediate P/Q-type ($\text{Ca}_v2.1$, α_{1A}), N-type ($\text{Ca}_v2.2$, α_{1B}), and R-type ($\text{Ca}_v2.3$, α_{1E}) Ca^{2+} currents; and the Ca_v3 family ($\text{Ca}_v3.1$, α_{1G} ; $\text{Ca}_v3.2$, α_{1H} ; and $\text{Ca}_v3.3$; α_{1I}) mediate T-type Ca^{2+} currents (Ertel et al., 2000). *In vitro* electrophysiological and imaging experiments performed in the past two decades have led to the following general picture: the primary role of N, P/Q, and R-type Ca^{2+} channels is to mediate the release of transmitters from axon terminals, whereas L- and T-type channels are mainly responsible for the somato-dendritic influx of Ca^{2+} into most neurons (see Chapters 9 and 11). However, immunohistochemical experiments carried out in the early 1990s revealed almost identical labeling for $\text{Ca}_v1.2$, $\text{Ca}_v1.3$, $\text{Ca}_v2.1$, $\text{Ca}_v2.2$, and $\text{Ca}_v2.3$ subunits (Westenbroek et al., 1992; Hell et al., 1993; Westenbroek et al., 1995; Day et al., 1996). Cell bodies and proximal dendrites filled with strong peroxidase reaction end-products were described for all of these subunits in many cell types of the brain. As already mentioned, a similar strong intracellular staining was found for NMDA receptors in PCs, a pattern that remained identical in the corresponding knock-out mice. Thus, the prominent intracellular labeling for Ca^{2+} channels as obtained with the immunoperoxidase technique might not reflect specific antibody–antigen interactions.

Some recent experiments using high-resolution immunolocalization techniques revisited this issue. Here I shall only concentrate on experiments in which the specificity of immunolabeling was proven with either multiple antibodies or using knock-out animals. The T-type channel-forming $\text{Ca}_v3.1$ subunit was found exclusively on the somato-dendritic plasma membranes of the dorsal lateral geniculate nucleus (dLGN) and cerebellar Purkinje cells (Hildebrand et al., 2009; Parajuli et al., 2010). In dLGN relay cells, the plasma membrane-associated gold particles had apparently uniform somato-dendritic densities, whereas in Purkinje cells the density of gold particles seemed to be highest in dendritic spines. The density of the $\text{Ca}_v3.1$ subunit is cell-type-dependent: dLGN relay cells had an approximately three-fold higher density than that in local interneurons. These experiments demonstrate that voltage-gated Ca^{2+} channels also show cell type-dependent and subcellular compartment-dependent distributions.

Consistent with the functional predictions, the $\text{Ca}_v2.1$ subunit is highly enriched in presynaptic active zones (AZs) of cerebellar parallel fibers (Kulik et al., 2004; Indriati et al., 2013), hippocampal PV-positive INs (Bucurenciu et al., 2008), calyx of Held (Nakamura et al., 2014), and CA3 PCs (Holderith et al., 2012). The density of gold particles labeling the $\text{Ca}_v2.1$ subunit is constant among PC AZs and in the calyx; the larger the AZ the more Ca^{2+} channels it contains (Fig. 7.6; Holderith et al., 2012; Nakamura et al., 2014). The distribution of $\text{Ca}_v2.1$ seems to be non-uniform within hippocampal AZs, where it could form small nanoclusters.

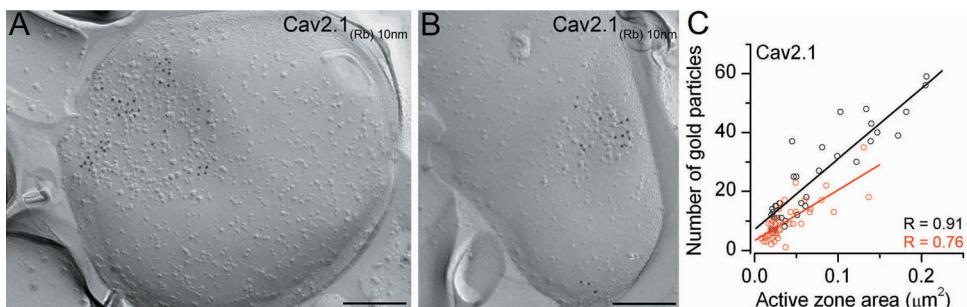


Fig. 7.6 The $\text{Ca}_v2.1$ subunit is concentrated in the presynaptic active zone of hippocampal axon terminals as revealed with SDS-FRL. (A), (B) A large axon terminal with a large AZ contains many gold particles (A) labeling the $\text{Ca}_v2.1$ subunit, whereas a much smaller terminal with a smaller AZ contains many fewer gold particles (B). Scale bars = 200 nm. (C) There is a positive linear correlation between the AZ area and the number of gold particles labeling the $\text{Ca}_v2.1$ subunit.

Adapted with permission from Macmillan Publishers Ltd: *Nature Neuroscience*, 15(7), Noemi Holderith, Andrea Lorincz, Gergely Katona, Balázs Rózsa, Akos Kulik, Masahiko Watanabe, and Zoltan Nusser, Release probability of hippocampal glutamatergic terminals scales with the size of the active zone, pp. 988–997, Copyright 2012, Nature Publishing Group.

In hippocampal PCs, this Ca^{2+} channel subunit could not be detected in somato-dendritic plasma membranes, but was readily observed in cerebellar Purkinje cells (Kulik et al., 2004; Indriati et al., 2013). In Purkinje cell somata and proximal dendrites, some of the $\text{Ca}_v2.1$ subunits were scattered and others formed high-density clusters over intra-membrane particle clusters, where they colocalized with large-conductance Ca^{2+} and voltage-activated K^+ channels (BK channels) and small-conductance Ca^{2+} and voltage-activated K^+ channels (SK₂ channels). Such a tight spatial co-clustering of Ca^{2+} and Ca^{2+} -activated K^+ channels (within about 40 nm) indicates that the activation of K^+ channels requires high concentrations of free Ca^{2+} similar to the nanodomain activation of Ca^{2+} sensors for transmitter release. The $\text{Ca}_v2.3$ subunit is also present both pre- and postsynaptically: it is mainly located in axon terminals of the interpeduncular nucleus and in dendritic shafts and spines of the striatum, amygdala, and hippocampus (Parajuli et al., 2012). The $\text{Ca}_v2.3$ subunit has similar low densities in CA1 PC basal, apical, and oblique dendrites, and its density in dendritic spines is variable (Parajuli et al., 2012). Much less is known about the sub-cellular distribution of the $\text{Ca}_v2.2$ subunit. A recent study (Lenkey et al., 2015) demonstrated its enrichment in the presynaptic AZs of hippocampal cholecystokinin (CCK)-positive GABAergic INs, where it mediates the release of GABA and acts as the effector molecule of type-1 cannabinoid receptors.

Voltage-gated K^+ channels

Potassium channels are the most diverse class of voltage-gated ion channels. Dozens of distinct subunits have been identified, creating an astonishingly large number of homo- and heteromultimeric assemblies with distinct functional and pharmacological properties (reviewed by Chandy, 1991; Hille, 2001). Some aspects of the regional and cellular distribution of several K^+ channel subunits have already been described (reviewed by Trimmer and Rhodes, 2004), therefore in this section I shall only summarize the results of experiments using EM localization of K^+ channels.

One of the first high-resolution immunolocalization studies examined the distribution of $K_v1.1$, $K_v1.2$, and $K_v3.4$ subunits in the cerebellar cortex (Laube et al., 1996). When Laube et al. (1996) compared the distribution of these subunits on cerebellar basket cell axons, the $K_v1.1$ and $K_v1.2$ subunits were found to be predominantly present in septate-like junctions within the specialized structures called Pinceau, whereas the $K_v3.4$ subunit was not concentrated in these junctions, but was rather uniformly distributed in the axonal plasma membrane of the Pinceau. None of these subunits were found on the axon collaterals of basket cells that contacted the somata and proximal dendrites of Purkinje cells, demonstrating a highly regulated subcellular distribution of K^+ channels on the surface of a neuron. However, it is difficult to generalize from this distribution, because the Pinceau is a highly specialized structure of basket cell axons surrounding Purkinje cell AISs and is exclusively found in the cerebellum.

Whether presynaptic K^+ channels are located in preterminal axons or in axon terminals, and, if they are located in axon terminals, whether they are concentrated inside or outside the AZ, are key issues for understanding their functional roles. Hu et al. (2001) has addressed this issue for BK channels and found that these channels are highly enriched in the presynaptic AZs of hippocampal Schaffer collateral synapses. *In vitro* physiological experiments revealed that these presynaptic channels are activated by local Ca^{2+} influx and contribute to the repolarization of presynaptic spikes. However, these channels are not exclusively found in presynaptic terminals; a clustered and scattered distribution was described in the somato-dendritic plasma membranes of a variety of neurons. BK channels cluster in the plasma membranes just above subsurface membrane cisternae (Kaufmann et al., 2009, 2010). The axonal distribution of the $K_v1.1$ subunit showed a different picture. This subunit is enriched in AISs of hippocampal PCs and is also found in presynaptic terminals at a six-fold lower density (Kirizs et al., 2014). However, within the presynaptic terminals, no concentration of gold particles was detected in the AZs, demonstrating that different K^+ channel subunits have distinct axon terminal distributions.

The $K_v2.1$ subunit possesses one of the most intriguing distribution patterns of all K^+ channel subunits. It has a peculiar uneven distribution on the somato-dendritic surface of almost all neurons, including α -motoneurons of the spinal cord, PCs and INs of the neocortex and hippocampus, thalamic relay cells, and neurons of the lateral posterior nucleus (Misonou et al., 2004; Muennich and Fyffe, 2004; King et al., 2014; Kirizs et al., 2014). The clustering of this subunit is regulated by phosphorylation-dependent processes. The clusters do not correspond to chemical or electrical synapses, but are apparently randomly distributed within the plasma membrane. Within AISs, this subunit forms clusters that are spatially segregated from the $K_v1.1$, $K_v1.2$, and $Na_v1.6$ subunit-enriched membrane segments (King et al., 2014; Kirizs et al., 2014). The functional consequence of such sub-AIS segregation of the $K_v2.1$ from the $K_v1.1$, $K_v1.2$, and $Na_v1.6$ subunits is unknown, but differential regulation and membrane-anchoring mechanisms can be envisaged. Within the dendritic domains of neurons, the fluorescent labeling intensity for the $K_v2.1$ subunit seems to decrease as a function of distance from the soma. Kirizs et al. (2014) have provided a quantitative surface map of the $K_v2.1$ subunit in CA1 PCs and found an almost identical overall density in AIS, somata, and proximal apical dendritic trunks, whereas all other dendritic compartments in the middle and distal stratum radiatum had no detectable levels of $K_v2.1$ labeling.

Clustered distribution of K^+ channels is not a unique feature of the $K_v2.1$ subunit, but the A-type K^+ channel-forming subunits $K_v1.4$, $K_v4.2$, and $K_v4.3$ also form clusters in the somato-dendritic membranes of many cell types. For example, clustering of the $K_v4.2$ subunit in the supraoptic nucleus (Alonso and Widmer, 1997) and the subiculum (Jinno et al., 2005) in GABAergic inhibitory synapses has been reported. In contrast, clusters of this subunit (Kollo

et al., 2008) as well as those of $K_v1.4$ (Juiz et al., 2000) and $K_v4.3$ (Kollo et al., 2006, 2008) have been detected in a variety of hippocampal, dorsal cochlear, habenular, and olfactory bulb neurons, but these clusters never overlapped with chemical or electrical synapses (Fig. 7.7). EM immunogold localization of the $K_v4.2$ and $K_v4.3$ subunits showed that gold particles were concentrated in membrane specializations between neurons. High-resolution morphological and molecular neuroanatomical experiments demonstrated that these K^+ channel-rich specializations were distinct from known chemical and electrical (gap junctions) synapses. For example, cerebellar glutamatergic climbing fibers establish $K_v4.3$ subunit-rich specializations with stellate/basket cell somata and dendrites. These specializations lack clusters of presynaptic vesicles, postsynaptic densities, and AMPA and NMDA type GluRs, essential features of known chemical glutamatergic synapses. Consistent with this, the lack of direct glutamatergic synaptic interaction between climbing fibers and stellate cells was also demonstrated by Szapiro and Barbour (2007) using *in vitro* electrophysiological approaches. The extracellular space at these K^+ channel-rich specializations was significantly narrower than that of chemical synapses (14 nm versus 21 nm), but was much wider than the space between the two membranes at gap junctions (3.5 nm). The unique clustering of these A-type K^+ channels suggests that these specializations may be the site of a novel form of communication between neurons. The widespread presence of such K^+ channel-rich specializations throughout the CNS also supports a significant role, the investigation of which will hopefully open new avenues in the field of K^+ channel research.

High-resolution immunolocalization experiments provided clear evidence for the cell type-specific distribution of voltage-gated ion channels (e.g., HCN1, $Ca_v2.1$, $Ca_v3.1$, $K_v1.1$, and $K_v1.2$), demonstrating that neurons use these channels in different ways to fulfill distinct functional requirements. I suggest that cell-type-specific differences in the subcellular distribution of an ion channel are a previously unrecognized means of increasing the functional diversity of neurons. If distinct cell types can target a single ion channel type differentially within their axo-somato-dendritic plasma membranes, the question arises as to whether a single cell that expresses a large number of molecularly distinct ion channels could distribute them differentially on its axo-somato-dendritic surface. The results of several high-resolution quantitative localization experiments from my laboratory demonstrated that six ion channels examined so far show totally different distribution patterns in CA1 PCs (Lorincz et al., 2002; Lorincz and Nusser, 2010; Kerti et al., 2012; Kirics et al., 2014). HCN1 is present in the somato-dendritic compartments and its density in the dendrites increases as a function of distance from the soma with a very prominent (supralinear) increase in the apical tufts in the stratum lacunosum-moleculare. The $K_v4.2$ subunit is also confined to the somato-dendritic compartments, but its density shows a very moderate (two-fold) increase in the apical dendrites up to the middle of the stratum radiatum, and from there the density is constant. Detectable amounts of Kir3.2 are only found in the apical dendrites of CA1 PCs, where its density increases from proximal to distal dendrites, but instead of further increasing in the stratum lacunosum-moleculare, like HCN1, its density stays constant. The $Na_v1.6$ and $K_v2.1$ subunits are present in the AIS, somata, and dendrites of PCs, but the density of $Na_v1.6$ is much higher in the AISs compared with the soma, whereas that of $K_v2.1$ is practically identical. $K_v1.1$ has a very different distribution pattern again: it is found only in the AIS and axon terminals, without detectable levels in the somato-dendritic compartments. These results reveal that each studied subunit has its own unique distribution pattern within a single cell type, resulting in distinct ion channel compositions of functionally distinct subcellular compartments (e.g., axon terminals, AZs, AIS, soma, proximal, distal apical dendrites, dendritic spines, etc.).

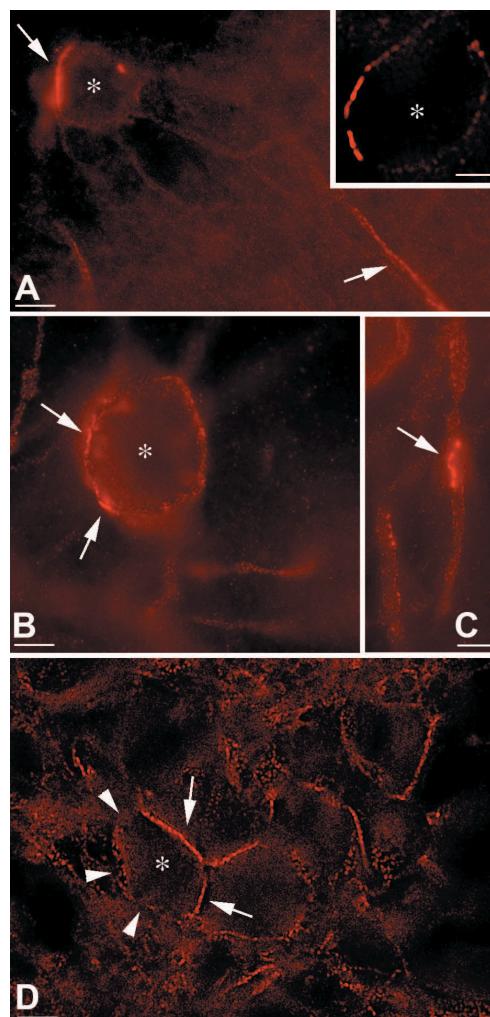


Fig. 7.7 Light-microscopic demonstration of the clustered distribution of the $K_v4.3$ subunit on the surface of central neurons. **(A)** Immunofluorescence image of the molecular layer of the cerebellar cortex. The somata (*) and dendrites of GABAergic interneurons are labeled with occasional strongly immunopositive clusters (arrows). The inset shows a high-magnification image of an interneuron soma following image deconvolution. **(B), (C)** A subpopulation of hippocampal GABAergic interneurons (*) is also immunopositive for the $K_v4.3$ subunit, also displaying inhomogeneous labeling (arrows) of the somatic (B) and dendritic (C) plasma membranes. **(D)** The medial nucleus of the habenula displays one of the most robust clusterings of $K_v4.3$ immunoreactivity. The somata of neurons (*) contain very intensely labeled clusters where they are in direct contact with some neighboring cells (arrows), whereas the immunolabeling intensity is very low at some other parts of their somatic membranes (e.g., arrowheads). Scale bars: A–D = 5 μ m, A (inset) = 2.5 μ m.

Adapted from Mihaly Kollo, Noémi B. Holderith, and Zoltan Nusser, Novel subcellular distribution pattern of A-type K^+ channels on neuronal surface, *The Journal of Neuroscience*, 26(10), pp. 2684–2691, © 2006, The Society for Neuroscience.

Conclusions

High-resolution localization studies have revealed several important principles behind the organization of GABA and glutamate receptors on the axo-somato-dendritic surface of central neurons. The segregation of distinct GABA and glutamate receptor subtypes to functionally different dendritic synapses, the exclusive extrasynaptic presence of certain GABA_A receptors, and the input-specific regulation of the number and density of postsynaptic receptors comprise just a few examples of the previously unexpected complexity in the cell surface distribution of these receptors. Although less is known about the precise subcellular distribution of voltage-gated ion channels, recent high-resolution localization studies have started to reveal their complex and highly regulated cell surface distributions with special emphasis on their cell-type-selective and ion channel subtype-selective dendritic distributions.

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References

- Accili EA, Proenza C, Baruscotti M, DiFrancesco D (2002) From funny current to HCN channels: 20 years of excitation. *News in Physiological Sciences* **17**:32–37.
- Alonso G, Widmer H (1997) Clustering of KV4.2 potassium channels in postsynaptic membrane of rat supraoptic neurons: an ultrastructural study. *Neuroscience* **77**:617–621.
- Angelides KJ, Elmer LW, Loftus D, Elson E (1988) Distribution and lateral mobility of voltage-dependent sodium channels in neurons. *Journal of Cell Biology* **106**:1911–1925.
- Barnard EA, Skolnick P, Olsen RW, Mohler H, Sieghart W, Biggio G, Braestrup C, Bateson AN, Langer SZ (1998) International union of pharmacology. XV. Subtypes of gamma-aminobutyric acid_A receptors: classification on the basis of subunit structure and receptor function. *Pharmacological Reviews* **50**:291–313.
- Baude A, Nusser Z, Roberts JD, Mulvihill E, McIlhinney RA, Somogyi P (1993) The metabotropic glutamate receptor (mGluR1a) is concentrated at perisynaptic membrane of neuronal subpopulations as detected by immunogold reaction. *Neuron* **11**:771–787.
- Bergersen L, Ruiz A, Bjaalie JG, Kullmann DM, Gundersen V (2003) GABA and GABA_A receptors at hippocampal mossy fibre synapses. *European Journal of Neuroscience* **18**:931–941.
- Black JA, Kocsis JD, Waxman SG (1990) Ion channel organization of the myelinated fiber. *Trends in Neurosciences* **13**:48–54.
- Boiko T, Van Wart A, Caldwell JH, Levinson SR, Trimmer JS, Matthews G (2003) Functional specialization of the axon initial segment by isoform-specific sodium channel targeting. *Journal of Neuroscience* **23**:2306–2313.
- Boudier JL, Le Treut T, Jover E (1992) Autoradiographic localization of voltage-dependent sodium channels on the mouse neuromuscular junction using ¹²⁵I-alpha scorpion toxin. II. Sodium distribution on postsynaptic membranes. *Journal of Neuroscience* **12**:454–466.
- Brunig I, Scotti E, Sidler C, Fritschy JM (2002) Intact sorting, targeting, and clustering of gamma-aminobutyric acid A receptor subtypes in hippocampal neurons in vitro. *Journal of Comparative Neurology* **443**:43–55.
- Bucurenciu I, Kulik A, Schwaller B, Frotscher M, Jonas P (2008) Nanodomain coupling between Ca²⁺ channels and Ca²⁺ sensors promotes fast and efficient transmitter release at a cortical GABAergic synapse. *Neuron* **57**:536–545.

- Budisantoso T, Matsui K, Kamasawa N, Fukazawa Y, Shigemoto R (2012) Mechanisms underlying signal filtering at a multisynapse contact. *Journal of Neuroscience* **32**:2357–2376.
- Chandra D, Jia F, Liang J, Peng Z, Suryanarayanan A, Werner DF, Spigelman I, Houser CR, Olsen RW, Harrison NL, Homanics GE (2006) GABA_A receptor alpha 4 subunits mediate extrasynaptic inhibition in thalamus and dentate gyrus and the action of gaboxadol. *Proceedings of the National Academy of Sciences of the United States of America* **103**:15230–15235.
- Chandy KG (1991) Simplified gene nomenclature. *Nature* **352**:26.
- Choquet D, Triller A (2003) The role of receptor diffusion in the organization of the postsynaptic membrane. *Nature Reviews Neuroscience* **4**:251–265.
- Clarkson AN, Huang BS, Macisaac SE, Mody I, Carmichael ST (2010) Reducing excessive GABA-mediated tonic inhibition promotes functional recovery after stroke. *Nature* **468**:305–309.
- Craig AM, Blackstone CD, Huganir RL, Banker G (1994) Selective clustering of glutamate and gamma-aminobutyric acid receptors opposite terminals releasing the corresponding neurotransmitters. *Proceedings of the National Academy of Sciences of the United States of America* **91**:12373–12377.
- Crestani F, Keist R, Fritschy JM, Benke D, Vogt K, Prut L, Bluthmann H, Mohler H, Rudolph U (2002) Trace fear conditioning involves hippocampal α5 GABA_A receptors. *Proceedings of the National Academy of Sciences of the United States of America* **99**:8980–8985.
- Day NC, Shaw PJ, McCormack AL, Craig PJ, Smith W, Beattie R, Williams TL, Ellis SB, Ince PG, Harpold MM, Lodge D, Volsen SG (1996) Distribution of α_{1A}, α_{1B} and α_{1E} voltage-dependent calcium channel subunits in the human hippocampus and parahippocampal gyrus. *Neuroscience* **71**:1013–1024.
- Dingledine R, Borges K, Bowie D, Traynelis SF (1999) The glutamate receptor ion channels. *Pharmacological Reviews* **51**:7–61.
- Ertel EA, Campbell KP, Harpold MM, Hofmann F, Mori Y, Perez-Reyes E, Schwartz A, Snutch TP, Tanabe T, Birnbaumer L, Tsien RW, Catterall WA (2000) Nomenclature of voltage-gated calcium channels. *Neuron* **25**:533–535.
- Farrant M, Nusser Z (2005) Variations on an inhibitory theme: phasic and tonic activation of GABA_A receptors. *Nature Reviews Neuroscience* **6**: 215–229.
- Fertuck HC, Salpeter MM (1974) Localization of acetylcholine receptor by ¹²⁵I-labeled α-bungarotoxin binding at mouse motor endplates. *Proceedings of the National Academy of Sciences of the United States of America* **71**:1376–1378.
- Fritschy JM (2008) Is my antibody-staining specific? How to deal with pitfalls of immunohistochemistry. *European Journal of Neuroscience* **28**:2365–2370.
- Fritschy J-M, Mohler H (1995) GABA_A-receptor heterogeneity in the adult rat brain: differential regional and cellular distribution of seven major subunits. *Journal of Comparative Neurology* **359**:154–194.
- Fritschy J-M, Weinmann O, Wenzel A, Benke D (1998a) Synapse-specific localization of NMDA and GABA_A receptor subunits revealed by antigen-retrieval immunohistochemistry. *Journal of Comparative Neurology* **390**:194–210.
- Fritschy JM, Johnson DK, Mohler H, Rudolph U (1998b) Independent assembly and subcellular targeting of GABA_A-receptor subtypes demonstrated in mouse hippocampal and olfactory neurons in vivo. *Neuroscience Letters* **249**:99–102.
- Fujimoto K, Umeda M, Fujimoto T (1996) Transmembrane phospholipid distribution revealed by freeze-fracture replica labeling. *Journal of Cell Science* **109**:2453–2460.
- Garden DL, Dodson PD, O'Donnell C, White MD, Nolan MF (2008) Tuning of synaptic integration in the medial entorhinal cortex to the organization of grid cell firing fields. *Neuron* **60**:875–889.
- Glykys J, Mody I (2007) Activation of GABA_A receptors: views from outside the synaptic cleft. *Neuron* **56**:763–770.
- Glykys J, Peng Z, Chandra D, Homanics GE, Houser CR, Mody I (2007) A new naturally occurring GABA(A) receptor subunit partnership with high sensitivity to ethanol. *Nature Neuroscience* **10**:40–48.

- Goldin AL (2001) Resurgence of sodium channel research. *Annual Review of Physiology* **63**:871–894.
- Hell SW (2007) Far-field optical nanoscopy. *Science* **316**:1153–1158.
- Hell JW, Westenbroek RE, Warner C, Ahljanian MK, Prystay W, Gilbert MM, Snutch TP, Catterall WA (1993) Identification and differential subcellular localization of the neuronal class C and class D L-type calcium channel $\alpha 1$ subunits. *Journal of Cell Biology* **123**:949–962.
- Hildebrand ME, Isope P, Miyazaki T, Nakaya T, Garcia E, Feltz A, Schneider T, Hescheler J, Kano M, Sakimura K, Watanabe M, Dieudonne S, Snutch TP (2009) Functional coupling between mGluR1 and Cav3.1 T-type calcium channels contributes to parallel fiber-induced fast calcium signaling within Purkinje cell dendritic spines. *Journal of Neuroscience* **29**:9668–9682.
- Hille B (2001) *Ionic Channels of Excitable Membranes*. Sunderland, MA: Sinauer Associates Inc.
- Holderith NB, Shigemoto R, Nusser Z (2003) Cell type-dependent expression of HCN1 in the main olfactory bulb. *European Journal of Neuroscience* **18**:344–354.
- Holderith N, Lorincz A, Katona G, Rozsa B, Kulik A, Watanabe M, Nusser Z (2012) Release probability of hippocampal glutamatergic terminals scales with the size of the active zone. *Nature Neuroscience* **15**:988–997.
- Hollmann M, Heinemann S (1994) Cloned glutamate receptors. *Annual Review of Neuroscience* **17**:31–108.
- Hu H, Shao LR, Chavosh S, Gu N, Trieb M, Behrens R, Laake P, Pongs O, Knaus HG, Ottersen OP, Storm JF (2001) Presynaptic Ca^{2+} -activated K^+ channels in glutamatergic hippocampal terminals and their role in spike repolarization and regulation of transmitter release. *Journal of Neuroscience* **21**:9585–9597.
- Hu W, Tian C, Li T, Yang M, Hou H, Shu Y (2009) Distinct contributions of $\text{Na}(\nu)1.6$ and $\text{Na}(\nu)1.2$ in action potential initiation and backpropagation. *Nature Neuroscience* **12**:996–1002.
- Huntley GW, Vickers JC, Janssen W, Brose N, Heinemann SF, Morrison JH (1994) Distribution and synaptic localization of immunocytochemically identified NMDA receptor subunit proteins in sensory-motor and visual cortices of monkey and human. *Journal of Neuroscience* **14**:3603–3619.
- Inda MC, DeFelipe J, Munoz A (2006) Voltage-gated ion channels in the axon initial segment of human cortical pyramidal cells and their relationship with chandelier cells. *Proceedings of the National Academy of Sciences of the United States of America* **103**:2920–2925.
- Indriati DW, Kamasawa N, Matsui K, Meredith AL, Watanabe M, Shigemoto R (2013) Quantitative localization of Cav2.1 (P/Q-type) voltage-dependent calcium channels in Purkinje cells: somatodendritic gradient and distinct somatic coclustering with calcium-activated potassium channels. *Journal of Neuroscience* **33**:3668–3678.
- Jenkins SM, Bennett V (2001) Ankyrin-G coordinates assembly of the spectrin-based membrane skeleton, voltage-gated sodium channels, and L1 CAMs at Purkinje neuron initial segments. *Journal of Cell Biology* **155**:739–746.
- Jinno S, Jeromin A, Kosaka T (2005) Postsynaptic and extrasynaptic localization of Kv4.2 channels in the mouse hippocampal region, with special reference to targeted clustering at GABAergic synapses. *Neuroscience* **134**:483–494.
- Johnston D, Narayanan R (2008) Active dendrites: colorful wings of the mysterious butterflies. *Trends in Neurosciences* **31**:309–316.
- Jones A, Korpi ER, McKernan RM, Pelz R, Nusser Z, Makela R, Mellor JR, Pollard S, Bahn S, Stephenson FA, Randall AD, Sieghart W, Somogyi P, Smith AJ, Wisden W (1997) Ligand-gated ion channel subunit partnerships: GABA_A receptor alpha6 subunit gene inactivation inhibits delta subunit expression. *Journal of Neuroscience* **17**:1350–1362.
- Juiz JM, Lujan R, Dominguez del Toro E, Fuentes V, Ballesta JJ, Criado M (2000) Subcellular compartmentalization of a potassium channel (Kv1.4): preferential distribution in dendrites and dendritic spines of neurons in the dorsal cochlear nucleus. *European Journal of Neuroscience* **12**:4345–4356.

- Kaufmann WA, Ferraguti F, Fukazawa Y, Kasugai Y, Shigemoto R, Laake P, Sexton JA, Ruth P, Wietzorek G, Knaus HG, Storm JF, Ottersen OP (2009) Large-conductance calcium-activated potassium channels in Purkinje cell plasma membranes are clustered at sites of hypolemmal microdomains. *Journal of Comparative Neurology* **515**:215–230.
- Kaufmann WA, Kasugai Y, Ferraguti F, Storm JF (2010) Two distinct pools of large-conductance calcium-activated potassium channels in the somatic plasma membrane of central principal neurons. *Neuroscience* **169**:974–986.
- Kerti K, Lorincz A, Nusser Z (2012) Unique somato-dendritic distribution pattern of Kv4.2 channels on hippocampal CA1 pyramidal cells. *European Journal of Neuroscience* **35**:66–75.
- Kerti-Szigeti K, Nusser Z, Eyre MD (2014) Synaptic GABA_A receptor clustering without the gamma2 subunit. *Journal of Neuroscience* **34**:10219–10233.
- King AN, Manning CF, Trimmer JS (2014) A unique ion channel clustering domain on the axon initial segment of mammalian neurons. *Journal of Comparative Neurology* **522**:2594–2608.
- Kirics T, Kerti-Szigeti K, Lorincz A, Nusser Z (2014) Distinct axo-somato-dendritic distributions of three potassium channels in CA1 hippocampal pyramidal cells. *European Journal of Neuroscience* **39**:1771–1783.
- Kittler JT, McAinsh K, Moss SJ (2002) Mechanisms of GABA_A receptor assembly and trafficking: implications for the modulation of inhibitory neurotransmission. *Molecular Neurobiology* **26**:251–268.
- Klausberger T, Somogyi P (2008) Neuronal diversity and temporal dynamics: the unity of hippocampal circuit operations. *Science* **321**:53–57.
- Klausberger T, Roberts JD, Somogyi P (2002) Cell type- and input-specific differences in the number and subtypes of synaptic GABA_A receptors in the hippocampus. *Journal of Neuroscience* **22**:2513–2521.
- Kole MH, Stuart G (2012) Signal processing in the axon initial segment. *Neuron* **73**:235–247.
- Kole MH, Ilschner SU, Kampa BM, Williams SR, Ruben PC, Stuart GJ (2008) Action potential generation requires a high sodium channel density in the axon initial segment. *Nature Neuroscience* **11**:178–186.
- Kollo M, Holderith NB, Nusser Z (2006) Novel subcellular distribution pattern of A-type K⁺ channels on neuronal surface. *Journal of Neuroscience* **26**:2684–2691.
- Kollo M, Holderith N, Antal M, Nusser Z (2008) Unique clustering of A-type potassium channels on different cell types of the main olfactory bulb. *European Journal of Neuroscience* **27**:1686–1699.
- Koulen P, Sassoe-Pognetto M, Grunert U, Wassle H (1996) Selective clustering of GABA_A and glycine receptors in the mammalian retina. *Journal of Neuroscience* **16**:2127–2140.
- Kress GJ, Dowling MJ, Eisenman LN, Mennerick S (2010) Axonal sodium channel distribution shapes the depolarized action potential threshold of dentate granule neurons. *Hippocampus* **20**:558–571.
- Kuba H, Ishii TM, Ohmori H (2006) Axonal site of spike initiation enhances auditory coincidence detection. *Nature* **444**:1069–1072.
- Kulik A, Nakadate K, Hagiwara A, Fukazawa Y, Lujan R, Saito H, Suzuki N, Futatsugi A, Mikoshiba K, Frotscher M, Shigemoto R (2004) Immunocytochemical localization of the α_{1A} subunit of the P/Q-type calcium channel in the rat cerebellum. *European Journal of Neuroscience* **19**:2169–2178.
- Laube G, Roper J, Pitt JC, Sewing S, Kistner U, Garner CC, Pongs O, Veh RW (1996) Ultrastructural localization of Shaker-related potassium channel subunits and synapse-associated protein 90 to septate-like junctions in rat cerebellar Pinceaux. *Brain Research Molecular Brain Research* **42**:51–61.
- Lenkey N, Kirics T, Holderith N, Mate Z, Szabo G, Vizi ES, Hajos N, Nusser Z (2015) Tonic endocannabinoid-mediated modulation of GABA release is independent of the CB1 content of axon terminals. *Nature Communications* **6**:6557 doi: 10.1038/ncomms7557.
- Lorincz A, Nusser Z (2008a) Specificity of immunoreactions: the importance of testing specificity in each method. *Journal of Neuroscience* **28**:9083–9086.
- Lorincz A, Nusser Z (2008b) Cell-type-dependent molecular composition of the axon initial segment. *Journal of Neuroscience* **28**:14329–14340.

- Lorincz A, Nusser Z (2010) Molecular identity of dendritic voltage-gated sodium channels. *Science* **238**:906–909.
- Lorincz A, Notomi T, Tamas G, Shigemoto R, Nusser Z (2002) Polarized and compartment-dependent distribution of HCN1 in pyramidal cell dendrites. *Nature Neuroscience* **5**:1185–1193.
- Ludwig A, Zong X, Jeglitsch M, Hofmann F, Biel M (1998) A family of hyperpolarization-activated mammalian cation channels. *Nature* **393**:587–591.
- Lujan R, Albasanz JL, Shigemoto R, Juiz JM (2005) Preferential localization of the hyperpolarization-activated cyclic nucleotide-gated cation channel subunit HCN1 in basket cell terminals of the rat cerebellum. *European Journal of Neuroscience* **21**:2073–2082.
- McKernan RM, Whiting PJ (1996) Which GABA_A-receptor subtypes really occur in the brain? *Trends in Neurosciences* **19**:139–143.
- Maguire J, Mody I (2008) GABA(A)R plasticity during pregnancy: relevance to postpartum depression. *Neuron* **59**:207–213.
- Maguire JL, Stell BM, Rafizadeh M, Mody I (2005) Ovarian cycle-linked changes in GABA(A) receptors mediating tonic inhibition alter seizure susceptibility and anxiety. *Nature Neuroscience* **8**:797–804.
- Masugi-Tokita M, Tarusawa E, Watanabe M, Molnar E, Fujimoto K, Shigemoto R (2007) Number and density of AMPA receptors in individual synapses in the rat cerebellum as revealed by SDS-digested freeze-fracture replica labeling. *Journal of Neuroscience* **27**:2135–2144.
- Matsubara A, Laake JH, Davanger S, Usami S, Ottersen OP (1996) Organization of AMPA receptor subunits at a glutamate synapse: a quantitative immunogold analysis of hair cell synapses in the rat organ of Corti. *Journal of Neuroscience* **16**:4457–4467.
- Menon V, Musial TF, Liu A, Katz Y, Kath WL, Spruston N, Nicholson DA (2013) Balanced synaptic impact via distance-dependent synapse distribution and complementary expression of AMPARs and NMDARs in hippocampal dendrites. *Neuron* **80**:1451–1463.
- Micheva KD, Smith SJ (2007) Array tomography: a new tool for imaging the molecular architecture and ultrastructure of neural circuits. *Neuron* **55**:25–36.
- Misonou H, Mohapatra DP, Park EW, Leung V, Zhen D, Misonou K, Anderson AE, Trimmer JS (2004) Regulation of ion channel localization and phosphorylation by neuronal activity. *Nature Neuroscience* **7**:711–718.
- Mody I (2001) Distinguishing between GABA_A receptors responsible for tonic and phasic conductances. *Neurochemical Research* **26**:907–913.
- Mohler H, Lüscher B, Fritschy JM, Benke D, Benson J, Rudolph U (1998) GABA_A-receptor assembly in vivo: lessons from subunit mutant mice. *Life Sciences* **62**:1611–1615.
- Moosmang S, Stieber J, Zong X, Biel M, Hofmann F, Ludwig A (2001) Cellular expression and functional characterization of four hyperpolarization-activated pacemaker channels in cardiac and neuronal tissues. *European Journal of Biochemistry* **268**:1646–1652.
- Muennich EA, Fyffe RE (2004) Focal aggregation of voltage-gated, Kv2.1 subunit-containing, potassium channels at synaptic sites in rat spinal motoneurones. *Journal of Physiology* **554**:673–685.
- Nair D, Hosy E, Petersen JD, Constals A, Giannone G, Choquet D, Sibarita JB (2013) Super-resolution imaging reveals that AMPA receptors inside synapses are dynamically organized in nanodomains regulated by PSD95. *Journal of Neuroscience* **33**:13204–13224.
- Nakamura Y, Harada H, Kamisawa N, Matsui K, Rothman JS, Shigemoto R, Silver RA, DiGregorio DA, Takahashi T (2014) Nanoscale distribution of presynaptic Ca channels and its impact on vesicular release during development. *Neuron* **85**:1–14.
- Nakanishi S (1992) Molecular diversity of glutamate receptors and implications for brain function. *Science* **258**:597–603.
- Narayanan R, Johnston D (2007) Long-term potentiation in rat hippocampal neurons is accompanied by spatially widespread changes in intrinsic oscillatory dynamics and excitability. *Neuron* **56**:1061–1075.

- Nicholson DA, Geinisman Y (2009) Axospinous synaptic subtype-specific differences in structure, size, ionotropic receptor expression, and connectivity in apical dendritic regions of rat hippocampal CA1 pyramidal neurons. *Journal of Comparative Neurology* **512**:399–418.
- Noda M, Shimizu S, Tanabe T, Takai T, Kayano T, Ikeda T, Takahashi H, Nakayama H, Kanaoka Y, Minamino N, et al. (1984) Primary structure of *Electrophorus electricus* sodium channel deduced from cDNA sequence. *Nature* **312**:121–127.
- Notomi T, Shigemoto R (2004) Immunohistochemical localization of Ih channel subunits, HCN1–4, in the rat brain. *Journal of Comparative Neurology* **471**:241–276.
- Nusser Z, Mulvihill E, Streit P, Somogyi P (1994) Subsynaptic segregation of metabotropic and ionotropic glutamate receptors as revealed by immunogold localization. *Neuroscience* **61**:421–427.
- Nusser Z, Roberts JDB, Baude A, Richards JG, Somogyi P (1995) Relative densities of synaptic and extrasynaptic GABA_A receptors on cerebellar granule cells as determined by a quantitative immunogold method. *Journal of Neuroscience* **15**:2948–2960.
- Nusser Z, Sieghart W, Stephenson FA, Somogyi P (1996) The α6 subunit of the GABA_A receptor is concentrated in both inhibitory and excitatory synapses on cerebellar granule cells. *Journal of Neuroscience* **16**:103–114.
- Nusser Z, Sieghart W, Somogyi P (1998a) Segregation of different GABA_A receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. *Journal of Neuroscience* **18**:1693–1703.
- Nusser Z, Hajos N, Somogyi P, Mody I (1998b) Increased number of synaptic GABA_A receptors underlies potentiation at hippocampal inhibitory synapses. *Nature* **395**:172–177.
- Nusser Z, Lujan R, Laube G, Roberts JDB, Molnar E, Somogyi P (1998c) Cell type and pathway dependence of synaptic AMPA receptor number and variability in the hippocampus. *Neuron* **21**:545–559.
- Nyiri G, Stephenson FA, Freund TF, Somogyi P (2003) Large variability in synaptic N-methyl-D-aspartate receptor density on interneurons and a comparison with pyramidal-cell spines in the rat hippocampus. *Neuroscience* **119**:347–363.
- Ogiwara I, Miyamoto H, Morita N, Atapour N, Mazaki E, Inoue I, Takeuchi T, Itohara S, Yanagawa Y, Obata K, Furuchi T, Hensch TK, Yamakawa K (2007) Na(v)1.1 localizes to axons of parvalbumin-positive inhibitory interneurons: a circuit basis for epileptic seizures in mice carrying an Scn1a gene mutation. *Journal of Neuroscience* **27**:5903–5914.
- Otis TS, De Korninck Y, Mody I (1994) Lasting potentiation of inhibition is associated with an increased number of γ-aminobutyric acid type A receptors activated during miniature inhibitory postsynaptic currents. *Proceedings of the National Academy of Sciences of the United States of America* **91**:7698–7702.
- Pape HC (1996) Queer current and pacemaker: the hyperpolarization-activated cation current in neurons. *Annual Review of Physiology* **58**:299–327.
- Parajuli LK, Fukazawa Y, Watanabe M, Shigemoto R (2010) Subcellular distribution of alpha1G subunit of T-type calcium channel in the mouse dorsal lateral geniculate nucleus. *Journal of Comparative Neurology* **518**:4362–4374.
- Parajuli LK, Nakajima C, Kulik A, Matsui K, Schneider T, Shigemoto R, Fukazawa Y (2012) Quantitative regional and ultrastructural localization of the Ca(v)2.3 subunit of R-type calcium channel in mouse brain. *Journal of Neuroscience* **32**:13555–13567.
- Peng Z, Huang CS, Stell BM, Mody I, Houser CR (2004) Altered expression of the delta subunit of the GABA_A receptor in a mouse model of temporal lobe epilepsy. *Journal of Neuroscience* **24**:8629–8639.
- Petralia RS, Wang YX, Wenthold RJ (1994) The NMDA receptor subunits NR2A and NR2B show histological and ultrastructural localization patterns similar to those of NR1. *Journal of Neuroscience* **14**:6102–6120.
- Phend KD, Rustioni A, Weinberg RJ (1995) An osmium-free method of Epon embedment that preserves both ultrastructure and antigenicity for post-embedding immunocytochemistry. *Journal of Histochemistry and Cytochemistry* **43**:283–292.

- Pin JP, Duvoisin R (1995) The metabotropic glutamate receptors: structure and functions. *Neuropharmacology* **34**:1–26.
- Pirker S, Schwarzer C, Wieselthaler A, Sieghart W, Sperk G (2000) GABA_A receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. *Neuroscience* **101**:815–850.
- Racca C, Stephenson FA, Streit P, Roberts JD, Somogyi P (2000) NMDA receptor content of synapses in stratum radiatum of the hippocampal CA1 area. *Journal of Neuroscience* **20**:2512–2522.
- Rasband MN (2010) The axon initial segment and the maintenance of neuronal polarity. *Nature Reviews Neuroscience* **11**:552–562.
- Rash JE, Yasumura T, Hudson CS, Agre P, Nielsen S (1998) Direct immunogold labeling of aquaporin-4 in square arrays of astrocyte and ependymocyte plasma membranes in rat brain and spinal cord. *Proceedings of the National Academy of Sciences of the United States of America* **95**:11981–11986.
- Rhodes KJ, Trimmer JS (2006) Antibodies as valuable neuroscience research tools versus reagents of mass distraction. *Journal of Neuroscience* **26**:8017–8020.
- Rubio ME, Wenthold RJ (1997) Glutamate receptors are selectively targeted to postsynaptic sites in neurons. *Neuron* **18**:939–950.
- Rubio ME, Fukazawa Y, Kamasawa N, Clarkson C, Molnar E, Shigemoto R (2014) Target- and input-dependent organization of AMPA and NMDA receptors in synaptic connections of the cochlear nucleus. *Journal of Comparative Neurology* **522**:4023–4042.
- Ruiz A, Fabian-Fine R, Scott R, Walker MC, Rusakov DA, Kullmann DM (2003) GABA_A receptors at hippocampal mossy fibers. *Neuron* **39**:961–973.
- Santoro B, Grant SG, Bartsch D, Kandel ER (1997) Interactive cloning with the SH3 domain of N-src identifies a new brain specific ion channel protein, with homology to Eag and cyclic nucleotide-gated channels. *Proceedings of the National Academy of Sciences of the United States of America* **94**:14815–14820.
- Seeburg PH (1993) The molecular biology of mammalian glutamate receptor channels. *Trends in Neurosciences* **16**:359–365.
- Semyanov A, Walker MC, Kullmann DM, Silver RA (2004) Tonically active GABA_A receptors: modulating gain and maintaining the tone. *Trends in Neuroscience* **27**:262–269.
- Shen H, Sabaliauskas N, Sherpa A, Fenton AA, Stelzer A, Aoki C, Smith SS (2010) A critical role for alpha4beta1delta GABAA receptors in shaping learning deficits at puberty in mice. *Science* **327**:1515–1518.
- Siegel SJ, Brose N, Janssen WG, Gasic GP, Jahn R, Heinemann SF, Morrison JH (1994) Regional, cellular, and ultrastructural distribution of N-methyl-D-aspartate receptor subunit 1 in monkey hippocampus. *Proceedings of the National Academy of Sciences of the United States of America* **91**:564–568.
- Sieghart W, Sperk G (2002) Subunit composition, distribution and function of GABA_A receptor subtypes. *Current Topics in Medicinal Chemistry* **2**:795–816.
- Somogyi P, Fritschy J-M, Benke D, Roberts JDB, Sieghart W (1996) The g2 subunit of the GABA_A receptor is concentrated in synaptic junctions containing the a1 and b2/3 subunits in hippocampus, cerebellum and globus pallidus. *Neuropharmacology* **35**:1425–1444.
- Szabadits E, Cserep C, Szonyi A, Fukazawa Y, Shigemoto R, Watanabe M, Itohara S, Freund TF, Nyiri G (2011) NMDA receptors in hippocampal GABAergic synapses and their role in nitric oxide signaling. *Journal of Neuroscience* **31**:5893–5904.
- Szapiro G, Barbour B (2007) Multiple climbing fibers signal to molecular layer interneurons exclusively via glutamate spillover. *Nature Neuroscience* **10**:735–742.
- Takumi Y, Ramirez-Leon V, Laake P, Rinvik E, Ottersen OP (1999) Different modes of expression of AMPA and NMDA receptors in hippocampal synapses. *Nature Neuroscience* **2**:618–624.
- Tanaka J, Matsuzaki M, Tarusawa E, Momiyama A, Molnar E, Kasai H, Shigemoto R (2005) Number and density of AMPA receptors in single synapses in immature cerebellum. *Journal of Neuroscience* **25**:799–807.

- Tarusawa E, Matsui K, Budisantoso T, Molnar E, Watanabe M, Matsui M, Fukazawa Y, Shigemoto R (2009) Input-specific intrasynaptic arrangements of ionotropic glutamate receptors and their impact on postsynaptic responses. *Journal of Neuroscience* **29**:12896–12908.
- Trimmer JS, Rhodes KJ (2004) Localization of voltage-gated ion channels in mammalian brain. *Annual Review of Physiology* **66**:477–519.
- Van Wart A, Trimmer JS, Matthews G (2007) Polarized distribution of ion channels within microdomains of the axon initial segment. *Journal of Comparative Neurology* **500**:339–352.
- Watanabe M, Fukaya M, Sakimura K, Manabe T, Mishina M, Inoue Y (1998) Selective scarcity of NMDA receptor channel subunits in the stratum lucidum (mossy fibre-recipient layer) of the mouse hippocampal CA3 subfield. *European Journal of Neuroscience* **10**:478–487.
- Wei W, Zhang N, Peng Z, Houser CR, Mody I (2003) Perisynaptic localization of δ subunit-containing GABA_A receptors and their activation by GABA spillover in the mouse dentate gyrus. *Journal of Neuroscience* **23**:10650–10661.
- van Welie I, van Hooft JA, Wadman WJ (2004) Homeostatic scaling of neuronal excitability by synaptic modulation of somatic hyperpolarization-activated Ih channels. *Proceedings of the National Academy of Sciences of the United States of America* **101**:5123–5128.
- Westenbroek RE, Hell JW, Warner C, Dubel SJ, Snutch TP, Catterall WA (1992) Biochemical properties and subcellular distribution of an N-type calcium channel $\alpha 1$ subunit. *Neuron* **9**:1099–1115.
- Westenbroek RE, Sakurai T, Elliott EM, Hell JW, Starr TV, Snutch TP, Catterall WA (1995) Immunochemical identification and subcellular distribution of the $\alpha 1A$ subunits of brain calcium channels. *Journal of Neuroscience* **15**:6403–6418.
- Wilkars W, Liu Z, Lewis AS, Stoub TR, Ramos EM, Brandt N, Nicholson DA, Chetkovich DM, Bender RA (2012) Regulation of axonal HCN1 trafficking in perforant path involves expression of specific TRIP8b isoforms. *PLoS ONE* **7**:e32181.

Chapter 8

Neurotransmitter-gated ion channels in dendrites

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Summary

The ability of populations of neurons in the central nervous system to rapidly and effectively share and process information is essential for brain function. The majority of communication occurs via fast chemical synapses at which neurotransmitter-gated ion channels mediate either excitatory or inhibitory effects. The biophysical properties and dendritic location of neurotransmitter receptors influence the nature of the information processing performed. In this chapter, we examine the evidence that such neurotransmitter receptors are heterogeneous, can be differentially distributed, are highly dynamic, and contribute to various forms of plasticity. We focus on receptors activated by the neurotransmitters glutamate or γ -aminobutyric acid (GABA) and discuss the factors that influence their function at synaptic and non-synaptic sites. We illustrate how the low-level biophysical properties of neurotransmitter receptors and their spatial distribution in the dendritic tree affect signal transmission, dendritic integration, and the computations performed at the single neuron and network levels.

Introduction

Neurotransmitter-gated ion channels play a key role in the processing of information within the central nervous system (CNS) because their properties govern the strength and kinetics of the synaptic conductance. Changes in the amplitude of postsynaptic currents (PSCs) are thought to mediate information storage within a network, while the time course of PSCs influences the precision of signaling, temporal integration, and input variability, which can affect the responsiveness of the neuron to changes in rate-coded signals.

The kinetic behavior, ion permeability, and conductance of the channels present at each contact, the total number of receptors exposed to neurotransmitter, the fraction of receptors that bind transmitter, and the mean number of channels open at the peak of the PSC can differ from synapse to synapse. These variables determine the duration of the conductance change, the quantal size, and the amount of charge injected into the postsynaptic cell, and therefore influence the effectiveness of the excitatory or inhibitory signaling. Neurotransmitter-gated ion channels are also present extrasynaptically and their activation can also profoundly influence neuronal signaling.

Neurotransmitter-gated ion channels, both those concentrated at synapses and those outside synapses, are found predominantly in dendrites. This has traditionally represented a challenge to experimentalists, as receptors of interest may be both physically and electrically distant

from the soma in neurons with extended dendritic trees. This inaccessibility makes direct electrical recording from the synaptic membrane difficult, while cable filtering and the activation or inactivation of voltage-gated channels in dendrites can distort the synaptic signal recorded at the soma. Nevertheless, understanding the many variables that can influence receptor activation, and hence synaptic function, is important if we wish to fully understand dendritic and neuronal function.

In this chapter we restrict our discussion to ionotropic receptors. We describe how functional heterogeneity among ionotropic glutamate and GABA receptor types, differences in their dendritic distribution, and differences in the regulation of their expression contribute to the complexity of synaptic transmission. We then discuss how the targeted distribution of receptors throughout the dendritic tree confers important properties to the functioning of neuronal dendrites. Finally, we discuss how the properties and location of heterogeneous populations of receptors throughout the dendrites of neurons are crucial for signal transmission, integration, and higher-level network function.

Fundamental properties of neurotransmitter-gated ion channels

Subunit and receptor heterogeneity

Glutamate- and GABA-activated ionotropic receptors exist as homo- or heteromeric assemblies of transmembrane subunit proteins. The different subunits are encoded by distinct genes, with additional subunit diversity resulting from alternative splicing and/or nuclear RNA editing.

N-methyl-D-aspartate (NMDA)-type glutamate receptors assemble as diheteromeric receptors from two GluN1 subunits and two GluN2 subunits (2A–2D) or as triheteromeric receptors containing two different GluN2 subunits or both GluN2 and GluN3 subunits (3A and 3B). Alpha-amino-3-hydroxy-5-methyl-4-isoazole propionic acid (AMPA)-type glutamate receptors form as homo- or heterotetramers from GluA1–GluA4 subunits, while kainate-type glutamate receptors form as homo- or heterotetramers from GluK1–GluK5 subunits, with GluK4 and GluK5 producing functional receptors only when co-assembled with GluK1, -2, or -3 (Cull-Candy et al., 2001, 2006; Traynelis et al., 2010; Smart and Paoletti, 2012; Kumar and Mayer, 2013). The ion channels are cation-selective, allowing the passage of Na^+ , K^+ , and Ca^{2+} , the exception being those containing specific edited forms of GluA2, GluK1, or GluK2 that are calcium-impermeable.

The multiplicity of ionotropic glutamate receptor subunits is mirrored by that of ionotropic GABA receptor subunits, which are grouped into eight families: $\alpha 1$ – 6 , $\beta 1$ – 3 , $\gamma 1$ – 3 , δ , ϵ , π , θ , and $\rho 1$ – 3 (Simon et al., 2004; Olsen and Sieghart, 2008). Unlike glutamate receptors, GABA_A receptors are pentameric assemblies, with the most widely expressed $\alpha\beta\gamma$ isoforms having a $2\alpha:2\beta:1\gamma$ stoichiometry (Backus et al., 1993; Chang et al., 1996; Tretter et al., 1997) and a $\gamma-\beta-\alpha-\beta-\alpha$ arrangement (anti-clockwise when viewed from the synaptic cleft) (Baumann et al., 2002; Baur et al., 2006; Smart and Paoletti, 2012). The widely expressed $\alpha\beta\delta$ isoforms have an equivalent $2\alpha:2\beta:1\delta$ stoichiometry, but the precise arrangement of subunits in native receptors is unresolved (Eaton et al., 2014; Patel et al., 2014). The ion channels of all GABA_A receptors are anion-selective; under physiological conditions the current is carried by Cl^- and HCO_3^- (reviewed in Farrant and Kaila, 2007; Kaila et al., 2014).

Early *in situ* hybridization and immunohistochemical studies showed the various glutamate and GABA receptor subunits to be distributed widely throughout the CNS. However, from the very beginning it was recognized that there could be marked regional variation in their expression (e.g.,

Wisden et al., 1992; Martin et al., 1993; Watanabe et al., 1993; Monyer et al., 1994; Fritschy and Mohler, 1995; see also Hortnagl et al., 2013). Moreover, immunolabeling (Nusser et al., 1996a,b; Fritschy et al., 1998; Kasugai et al., 2010) (see Chapter 7) as well as electrophysiological and imaging studies (Stocca and Vicini, 1998; Toth and McBain, 1998; Brickley et al., 1999; Tovar and Westbrook, 1999; Shi et al., 2001; Sobczyk et al., 2005; Zhang and Diamond, 2009; Bats et al., 2012) have demonstrated that receptors containing different subunits can be targeted to different synapses or sites on the cell surface.

How such location- or synapse-specific receptor heterogeneity arises—whether from differential trafficking of receptors within the endoplasmic reticulum prior to insertion in the surface membrane, differential trapping of receptors at synapses following lateral diffusion in the surface membrane and/or regulated local protein translation (e.g., Bannai et al., 2009; Luscher et al., 2011; Cajigas et al., 2012; Hoze et al., 2012; Cox and Racca, 2013; Holt and Schuman, 2013; Horak et al., 2014 see also Chapter 5)—is unresolved and is a topic of intense study. Nevertheless, the potential importance of such heterogeneity becomes clear when one appreciates that AMPA, NMDA, and GABA_A receptors when composed of different subunits or combinations of subunits (from within their respective gene families) can display markedly different functional characteristics (Cull-Candy et al., 2006; Farrant and Kaila, 2007; Traynelis et al., 2010; Paoletti et al., 2013; Wyllie et al., 2013). For example, while GABA_A receptors containing $\alpha 1$, $\alpha 2$, or $\alpha 3$ subunits (together with β subunit variants and the $\gamma 2$ subunit) are found at synapses, receptors containing $\alpha 4$ or $\alpha 6$ (with β and δ subunits) or $\alpha 5$ (with β and $\gamma 2$ subunits) are chiefly found outside the synapse. These extrasynaptic receptors, although distant from sites of GABA release, have a remarkably high affinity for GABA and are able to sense ambient GABA in the extracellular space, giving rise to a tonic conductance in several brain areas (reviewed in Semyanov et al., 2004; Farrant and Nusser, 2005; Brickley and Mody, 2012). They may also contribute to slower “spillover” transmission, which can be facilitated by synaptic structure (Rossi and Hamann, 1998) or by burst firing of GABA-releasing neurons (Herd et al., 2013).

Voltage dependence

With regard to signal transmission and dendritic integration, two key features of glutamate and GABA_A receptors that are strongly influenced by subunit composition are their gating kinetics and their voltage dependence. Neurons *in vivo* are never at rest; they are bombarded by synaptic input (Destexhe et al., 2003) that causes voltage fluctuations, meaning that even if these remain below the threshold for firing there is no clear “resting” membrane potential. Thus, the way in which receptor function is modified by voltage is very important.

Perhaps the best-recognized example of voltage influencing a neurotransmitter-gated ion channel is provided by NMDA receptors, which exhibit a highly nonlinear voltage dependence. At negative membrane potentials the channels are blocked by extracellular Mg²⁺ and this block is relieved upon depolarization. Thus, substantial current flow through most NMDA receptors requires coincident pre- and postsynaptic activity (glutamate release and membrane depolarization). However, because diheteromeric NMDA receptors containing GluN2C or GluN2D subunits (GluN1/GluN2C or GluN1/GluN2D) or receptors containing GluN3 subunits are blocked by extracellular Mg²⁺ less strongly than are those containing GluN2A or GluN2B subunits (GluN1/GluN2A or GluN1/GluN2B) (Kuner and Schoepfer, 1996; Qian et al., 2005; Wrighton et al., 2008; Siegler Retchless et al., 2012), these receptors can pass current in a different voltage regime. This is also the case for triheteromeric GluN3-containing receptors, which are the NMDA receptors least sensitive to Mg²⁺ (Sasaki et al., 2002; McClymont et al., 2012; Paoletti et al., 2013).

Marked voltage dependence is also seen with calcium-permeable (GluA2-lacking) AMPA receptors, which exhibit inwardly rectifying current–voltage relationships due to their voltage-dependent block by endogenous intracellular polyamines, such as spermine (Bowie and Mayer, 1995; Kamboj et al., 1995; Koh et al., 1995). Voltage can also affect the intrinsic properties of the receptors. Thus, native and recombinant calcium-impermeable AMPA receptors (Verdoorn et al., 1991; Jonas, 1993; Wyllie et al., 1993), as well as recombinant calcium-permeable AMPA receptors in the absence of intracellular spermine (e.g., Bowie and Mayer, 1995; Soto et al., 2007), can show modest outward rectification (see also Prieto and Wollmuth, 2010).

Differences in rectification that depend on subunit composition have also been described for GABA_A receptors (e.g., Verdoorn et al., 1990; Burgard et al., 1996). Notably, several studies of both native and recombinant receptors have reported outward rectification, which can be particularly pronounced at low GABA concentrations (Weiss et al., 1988; Pytel et al., 2006; Pytel and Mozrzymas, 2006; Rula et al., 2008; Pavlov et al., 2009; Ransom et al., 2010) and this is of particular importance in relation to tonically activated receptors (Pavlov et al., 2009).

Kinetic behavior

At most glutamatergic synapses the excitatory postsynaptic current (EPSC) is carried by AMPA and NMDA receptors, mediating fast and slow components, respectively (Bekkers and Stevens, 1989; Silver et al., 1992; Spruston et al., 1995). The decay of the EPSC is governed by the kinetics of receptor deactivation (Lester et al., 1990; Jonas and Spruston, 1994; Edmonds et al., 1995; Silver et al., 1996b). This conclusion was confirmed in a study of cerebellar granule cells using glutamate uncaging, where desensitization of synaptic AMPA receptors was shown to be too slow to account for the decay of the EPSC (DiGregorio et al., 2007). Nevertheless, as described below, at these synapses, as at others with multiple closely spaced release sites, slow current components can arise from spillover of glutamate from neighboring release sites (Barbour and Häusser, 1997; DiGregorio et al., 2002; Nielsen et al., 2004). Desensitization can also play a role at synapses where glutamate entrapment occurs, contributing to short-term synaptic depression (Trussell et al., 1993).

For recombinant AMPA receptors, deactivation typically takes about 0.5–1 ms (Traynelis et al., 2010). For recombinant NMDA receptors deactivation spans a 50-fold range, from about 40 ms (GluN1/GluN2A) to about 2 s (GluN1/GluN2D) (Vicini et al., 1998). For triheteromeric receptors (GluN1/GluN2A/GluN2B), which are thought to contribute EPSCs at several sites (Tovar and Westbrook, 1999; Gray et al., 2011; Rauner and Kohr, 2011; Delaney et al., 2013; Tovar et al., 2013), deactivation is intermediate between that of the two diheteromeric forms (Hansen et al., 2014; Stroebel et al., 2014). Deactivation kinetics is also influenced by the GluN1 isoform (GluN1-b is faster than GluN1-a).

For recombinant GABA_A receptors, current deactivation following brief exposure to GABA is typically bi-exponential, with weighted decay times that vary according to subunit composition. For γ -containing receptors, deactivation is strongly influenced by the nature of the α subunit. Thus, for typical “synaptic” $\alpha\beta\gamma 2L$ receptors, a near ten-fold difference in deactivation is seen for those containing $\alpha 1$ (about 6 ms) or $\alpha 2$ (about 45 ms) (Dixon et al., 2014). Direct comparison of all α subunits ($\alpha 1$ –6; in this case with $\beta 3\gamma 2L$) also reveals a wide range of decays, from around 25 ms ($\alpha 4\beta 3\gamma 2L$) to around 200 ms ($\alpha 3\beta 3\gamma 2L$) (Picton and Fisher, 2007), while a wide range of decay times are generated when expressing mixtures of α subunits (Eyre et al., 2012) (Fig. 8.1).

Although these distinct kinetic properties are thought to allow for the tailoring of synaptic responses to specific functions, relating information from recombinant receptors to the behavior of synaptic channels is not as straightforward as one might hope. First, the time course of the synaptic

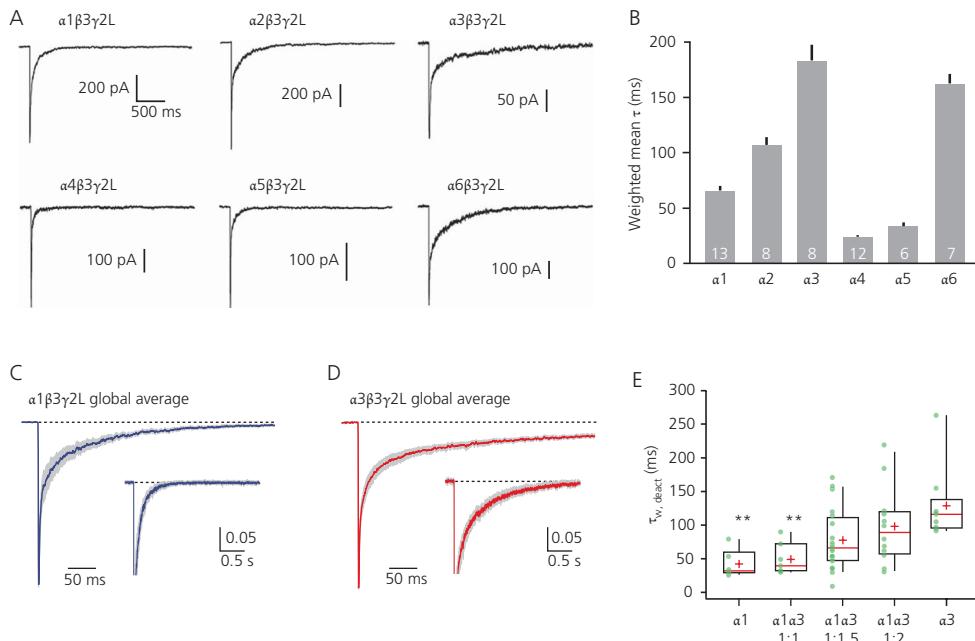


Fig. 8.1 The rate of GABA_A receptor deactivation varies with α subunit type and combination. **(A)** Currents evoked by 5-ms applications of 1 or 3 mM GABA to macropatches pulled from HEK-293T cells transiently transfected with the GABA_A receptor subunits indicated. Representative currents are shown for patches held at -70 mV. **(B)** The current decay was fitted with the sum of two exponential distributions. The weighted mean deactivation time was the sum of each time constant multiplied by its relative area. Bars represent the average \pm SEM, and the number of patches is given in each column. **(C)** Deactivation kinetics of recombinant $\alpha 1\beta 2\gamma 2L$ GABA_A receptors (1 ms, 1 mM GABA; gray shading denotes the standard error of the mean). **(D)** Same as C but for $\alpha 3\beta 2\gamma 2L$ GABA_A receptors. **(E)** Box plots showing weighted mean decay time constant ($\tau_{w,deact}$) for receptors containing $\alpha 1$ or $\alpha 3$ subunits and for receptors resulting from transfection of both $\alpha 1$ and $\alpha 3$ at various ratios. Green circles indicate individual values. Asterisks indicate a significant difference from $\alpha 3\beta 2\gamma 2L$ receptors (** $P < 0.01$, Tukey's honestly significant difference test).

Parts A and B are adapted from *Brain Research*, 1165, pp. 40–49., Amber J. Picton and Janet L. Fisher, Effect of the α subunit subtype on the macroscopic kinetic properties of recombinant GABA_A receptors, © 2007, Elsevier. With permission from Elsevier. Parts C–E are adapted from Mark D. Eyre, Massimiliano Renzi, Mark Farrant, and Zoltan Nusser, Setting the time course of inhibitory synaptic currents by mixing multiple GABA_A receptor α subunit isoforms, *The Journal of Neuroscience*, 32(17), pp. 5853–5867, Figure 3b-d, © 2012, The Society for Neuroscience.

conductance reflects not only the behavior of the receptors but also the waveform of the neurotransmitter to which they are exposed. Thus, the number and density of postsynaptic channels (Silver et al., 1996a; Nusser et al., 1997; Momiyama et al., 2003; Smith et al., 2003), the geometry of the synaptic cleft (Barbour and Häusser, 1997; Kruk et al., 1997; Cathala et al., 2005), the diffusion coefficient of the transmitter (Nielsen et al., 2004), and the spatial distribution of transmitter-binding proteins (Diamond and Jahr, 1997; Overstreet et al., 2002) can shape the waveform of transmitter in the cleft, setting the occupancy and influencing the subsequent opening of postsynaptic receptors.

The waveform of agonist exposure in experiments designed to determine the deactivation of recombinant receptors in excised membrane patches (limited to square steps, typically 1–5 ms) is distinct from the rapidly decaying waveform experienced by receptors at most “conventional” synapses (Clements et al., 1992; Rusakov and Kullmann, 1998; Mozrzymas et al., 2003; Mozrzymas, 2004; Barberis et al., 2011). A notable example of how synaptic geometry and receptor properties can influence signal transmission is seen at glomerular synapses in the cerebellum, where much of the excitatory synaptic charge is mediated by glutamate that spills over from release sites formed onto neighboring granule cells (DiGregorio et al., 2002; Nielsen et al., 2004). The prolonged glutamate transients that occur within the glomerular compartment are transduced by weakly desensitizing synaptic AMPARs (DiGregorio et al., 2007) and extrasynaptic NMDARs (Clark and Cull-Candy, 2002). These slow, spillover-mediated EPSC components, which enable mossy fiber to granule cell signaling to be sustained over an unusually wide frequency range (Saviane and Silver, 2006; Schwartz et al., 2012), have recently been shown to play a major role in encoding limb movements during locomotion (Powell et al., 2015). Diffuse activation of receptors, or “volume” transmission, is also important for mediating inhibition. Neurogliaform cells, which are found in various brain regions (Tamas et al., 2003; Capogna and Pearce, 2011), activate high-affinity GABA_A receptors by releasing GABA throughout a volume of tissue, thereby producing a sustained low concentration of GABA that is thought to underlie the unusually slow synaptic currents (Szabadics et al., 2007; Karayannis et al., 2010; Manko et al., 2012).

Second, a valid comparison between native and recombinant receptors depends on the ability to obtain an accurate recording of the electrical events occurring at the synapse. Unfortunately, for the majority of central glutamatergic synapses even such basic parameters as the amplitude and time course of the AMPA receptor-mediated conductance change are difficult to determine with any accuracy, due to passive electrical filtering and problems in achieving adequate space clamp in most neurons (Rall and Segev, 1985; Major, 1993; Spruston et al., 1993; Williams and Mitchell, 2008) (see Chapters 14 and 15). Thus, somatic recordings from neurons with extensive dendritic trees often give smaller mean EPSCs with decay times that are much larger than those seen in cells with compact electrotonic structures or with synapses close to the soma. Although it is possible to deduce the time course of the synaptic conductance from charge recovery experiments (e.g., Pearce, 1993; Häusser and Roth, 1997; Walker et al., 2002) or to estimate errors that result from dendritic cable filtering using compartmental modeling (e.g., Johnston and Brown, 1983; Jonas et al., 1993; Spruston et al., 1993; Roth and Häusser, 2001), these approaches are limited in their applicability and, in the latter case, require an accurate knowledge of neuronal morphology and synapse location. A more direct, though technically demanding, approach is to make a whole-cell recording from a dendrite close to the synaptic connection (Häusser, 1994; Benke et al., 1998; Magee, 2000; Andrasfalvy and Mody, 2006; Harnett et al., 2013; Shipman et al., 2013). While minimizing space-clamp problems, this method still does not overcome the problem of the electrode series resistance, which can also limit the quality of the voltage clamp.

Although it has been argued that excised somatic (e.g., Pugh and Raman, 2005) or dendritic patches (e.g., Spruston et al., 1995) can, in some cases, contain synaptic receptors, it seems likely that most such patches contain only extrasynaptic channels (Andrasfalvy and Magee, 2004). Receptors in such patches cannot be assumed to provide an appropriate proxy for synaptic receptors; they may differ not only in subunit composition but also in other ways that could influence their key microscopic and macroscopic properties. For example, phosphorylation of ionotropic glutamate receptors (reviewed in Salter and Kalia, 2004; Traynelis et al., 2010; Jenkins et al., 2014; Wang et al., 2014; see also Aman et al., 2014) and their association with transmembrane accessory proteins (reviewed in Jackson and Nicoll, 2011; Copits and Swanson, 2012) can variously affect their

kinetics, affinity, open probability, relative permeability, and single-channel conductance. Notably, the properties of AMPA receptors are significantly modified by their association with transmembrane proteins that function as “auxiliary subunits.” Four families of proteins have been identified: transmembrane AMPAR regulatory proteins (TARPs) (Chen et al., 2000; Tomita et al., 2005), cornichons (Schwenk et al., 2009), CKAMP44 (von Engelhardt et al., 2010), and GSG1L (Schwenk et al., 2012; Shanks et al., 2012). Of these, the best characterized are the TARPs, which exist as six isoforms, γ -2 (stargazin), γ -3, γ -4, γ -5, γ -7, and γ -8, with only partially overlapping patterns of expression in the CNS (Fukaya et al., 2005), that have been shown to differentially modulate the trafficking and functional properties of AMPARs (reviewed in Jackson and Nicoll, 2011; Straub and Tomita, 2012; Haering et al., 2014; Howe, 2015).

Dendritic targeting of receptors

From the previous section it is clear that there is great potential for functional heterogeneity among receptors and, consequently, for variation in synaptic properties. In this section we consider how the differential targeting of synapses with distinct receptor populations can contribute to the performance of complex dendritic and neuronal computations. The discussion is divided into three parts: (1) how synapses with different properties may be distributed throughout the dendritic tree, and the functional implications of this distribution; (2) the subcellular targeting of receptors within distinct dendritic branches and how this provides unique and important functions to dendritic units; and (3) the input specific targeting of receptors—where synaptic input onto the same cell, but arising from different afferent areas, may utilize different complements of receptors to carry out unique roles.

Receptor distribution along the proximal–distal axis of the dendrite

There is increasing anatomical and functional evidence that different afferent projections onto a given neuron can target different regions of the dendrites, even in non-laminar structures where gross anatomy would not predict such differences (Shepherd and Svoboda, 2005; Petreanu et al., 2009; Richardson et al., 2009; Little and Carter, 2012; MacAskill et al., 2012; Druckmann et al., 2014). Segregation of different inputs results in a computational problem for the neuron that must either be utilized or overcome. This arises from cable filtering, which, in passive dendrites, causes distal inputs to be reduced in amplitude and prolonged at the soma compared with those arising more proximally (Rall et al., 1967; Magee and Cook, 2000). In many neurons, such as cortical pyramidal neurons and striatal medium spiny neurons, this phenomenon means that synaptic inputs targeting different parts of the dendritic tree will have a distinct impact on neuronal integration (as discussed in the next section, Compartmentalization of glutamate receptor location) (Williams and Stuart, 2002; Froemke et al., 2005; Sjöström and Häusser, 2006; Nevian et al., 2007; Little and Carter, 2012; MacAskill et al., 2012; Vervaeke et al., 2012). In other cell types, such as spinal motor neurons and hippocampal CA1 pyramidal cells, the size of unitary excitatory postsynaptic potentials (EPSPs) recorded at the soma seem to be independent of synapse location (Iansek and Redman, 1973; Jack et al., 1981; Magee and Cook, 2000). This may reflect differential boosting by gradients of voltage-gated ion channels such as HCN or CaV (see Chapter 9, De Schutter and Bower, 1994; Cook and Johnston, 1999) but may also indicate a compensatory adjustment of synaptic weight throughout the dendritic tree. It has been suggested that such compensation is provided by a proximal to distal gradient of AMPA receptor subunits, particularly GluA1 subunits (Andrasfalvy et al., 2003; but see Shipman et al., 2013). This gradient results in individual synapses in distal

dendrites having roughly twice the number of AMPA receptors as proximal synapses (Magee, 2000; Andrasfalvy et al., 2003; Smith et al., 2003; Shipman et al., 2013). However, it is important to note that theoretical studies have argued that the high background synaptic conductance *in vivo* can lead to a self-cancelling of synaptic scaling (London and Segev, 2001; Williams, 2004). Moreover, it is difficult to disentangle changes in receptor number and kinetics from the effects of imperfect voltage clamp in different dendritic regions having different capacitive loads and thus imparting different degrees of low-pass filtering. In such situations it is helpful to examine charge transfer rather than current amplitude (see Spruston et al., 1993).

A dendritic gradient of synaptic weights has also been suggested for inhibitory connections. For example, in Mauthner neurons and motoneurons the number and size of inhibitory synaptic sites increases with increasing distance from the soma, in a manner similar to that seen for excitatory synapses (Triller et al., 1990; Sur et al., 1995; Alvarez et al., 1997). However, there does not seem to be an equivalent scaling of phasic inhibitory synaptic strength in hippocampal pyramidal neurons (Andrasfalvy and Mody, 2006). In contrast, however, there is anatomical and physiological evidence for a gradient of tonic inhibition in the hippocampus, with increased tonic currents in distal dendrites of pyramidal neurons (Ramos et al., 2004; Groen et al., 2014).

Compartmentalization of glutamate receptor location

As well as a global gradient of receptors along the proximal to distal axis, there is also evidence that different dendritic compartments may contain distinct complements of receptors. For example, in hippocampal area CA3, regions of dendrites in stratum oriens and stratum radiatum have synapses containing GluN1, N2A, and N2B, while in the same cells, synapses in stratum lucidum have only GluN1 and N2A (Fritschy et al., 1998; Watanabe et al., 1998). A distinct pattern of receptor placement can also be found when looking at individual dendritic branches. Oblique and basal dendrites, unlike the main apical dendrite, taper dramatically at their distal tips. The increased impedance this produces, together with the sealed end of the dendrite, means that AMPA receptor activation will result in much larger local potentials, with consequent overactivation of NMDA receptors and an increased likelihood of initiation of dendritic sodium spikes. Any increase in the number of AMPA receptors at distal synaptic locations (to normalize somatic efficacy) would exacerbate this. In hippocampal CA1 neurons this change in AMPA receptor number is associated with a decrease in NMDA receptors, coupled with a reduction in overall synaptic density with increasing distance from the branch origin (Katz et al., 2009; Menon et al., 2013). The net result is a “normalization” of synaptic efficacy *and* NMDA receptor activation throughout the dendritic tree, allowing dendritic and cellular signaling that is less dependent on location. As discussed later, this is thought to allow fundamental dendritic computations by allowing single branches to act as individual units. It is important to note, however, that other mechanisms, such as ion channel gradients, may also play a role in these processes (Magee, 2000; Williams and Stuart, 2002; Williams, 2004).

Input-specific synaptic localization

Synaptic inputs arising from different afferent areas often target distinct parts of the dendritic tree of a given neuron. Cable filtering in passive dendrites can enable such inputs to exhibit distinct integrative behaviors (Shepherd and Svoboda, 2005; Petreanu et al., 2009; Richardson et al., 2009; Little and Carter, 2012; MacAskill et al., 2012; Druckmann et al., 2014). For example, thalamic input into neocortex (Richardson et al., 2009; Little and Carter, 2012), or hippocampal input into ventral striatum (MacAskill et al., 2012) specifically targets distal portions of the dendrites. The resulting slow, filtered input may allow greater temporal summation and altered plasticity rules (Froemke et al., 2005; Evans et al., 2012).

In a number of instances, inputs arising from distinct afferent areas also have synapses with different complements of receptors. This can occur even when the synapses are intermingled on the same dendrite (Humeau et al., 2005; Richardson et al., 2009; MacAskill et al., 2012; Little and Carter, 2013; Druckmann et al., 2014) and can, for example, result in inputs from different afferents onto the same cell having markedly different unitary synaptic weights (Kumar and Huguenard, 2003; Humeau et al., 2005; Ding et al., 2008; MacAskill et al., 2012; 2014). While this could arise

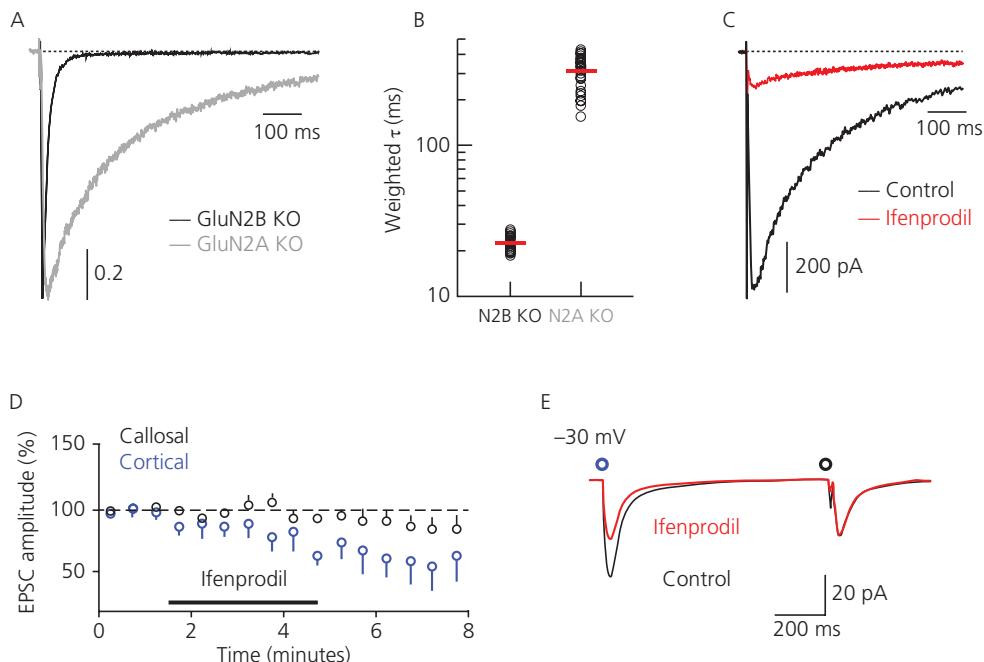


Fig. 8.2 Distinct kinetics and synapse-specific activation of EPSCs mediated by different NMDA receptors. **(A)** EPSCs in cultured hippocampal neurons from mice that lacked expression of GluN2A or GluN2B due to homologous recombination. The EPSCs are thus mediated by homogenous diheteromeric receptor populations—GluN2B-containing receptors in GluN2A knock-out (KO) neurons (gray trace) and GluN2A-containing receptors in GluN2B KO neurons (black trace). The EPSCs are superimposed and peak-scaled, highlighting the difference in their deactivation time course. **(B)** Weighted time constants of decay. **(C)** GluN2A KO EPSCs are sensitive to ifenprodil (3 μ M), as expected for GluN2B-containing receptors. **(D)** Effects of bath application of ifenprodil (3 μ M), a GluN2B subunit-specific antagonist, on pharmacologically isolated callosal and intracortical NMDA receptor-mediated EPSCs recorded in neocortical L5 pyramidal neurons in acute brain slices from rat. Pooled data points represent the ensemble average across eight experiments of ten consecutive measurements of EPSC amplitude. **(E)** Representative traces showing averaged consecutive records of EPSCs evoked in a single neuron. Note that the cortical EPSC amplitude is selectively diminished in the presence of the drug.

Parts A–C adapted from Kenneth R. Tovar, Matthew J. McGinley, and Gary L. Westbrook, Triheteromeric NMDA receptors at hippocampal synapses, *The Journal of Neuroscience*, 33(21), pp. 9150–9160, Figure 1 a, b, and d, © 2013, The Society for Neuroscience. Parts D and E adapted from Sanjay S. Kumar and John R. Huguenard, Pathway-specific differences in subunit composition of synaptic NMDA receptors on pyramidal neurons in neocortex, *The Journal of Neuroscience*, 23(31), pp. 10074–10083, Figure 3a, © 2003, The Society for Neuroscience.

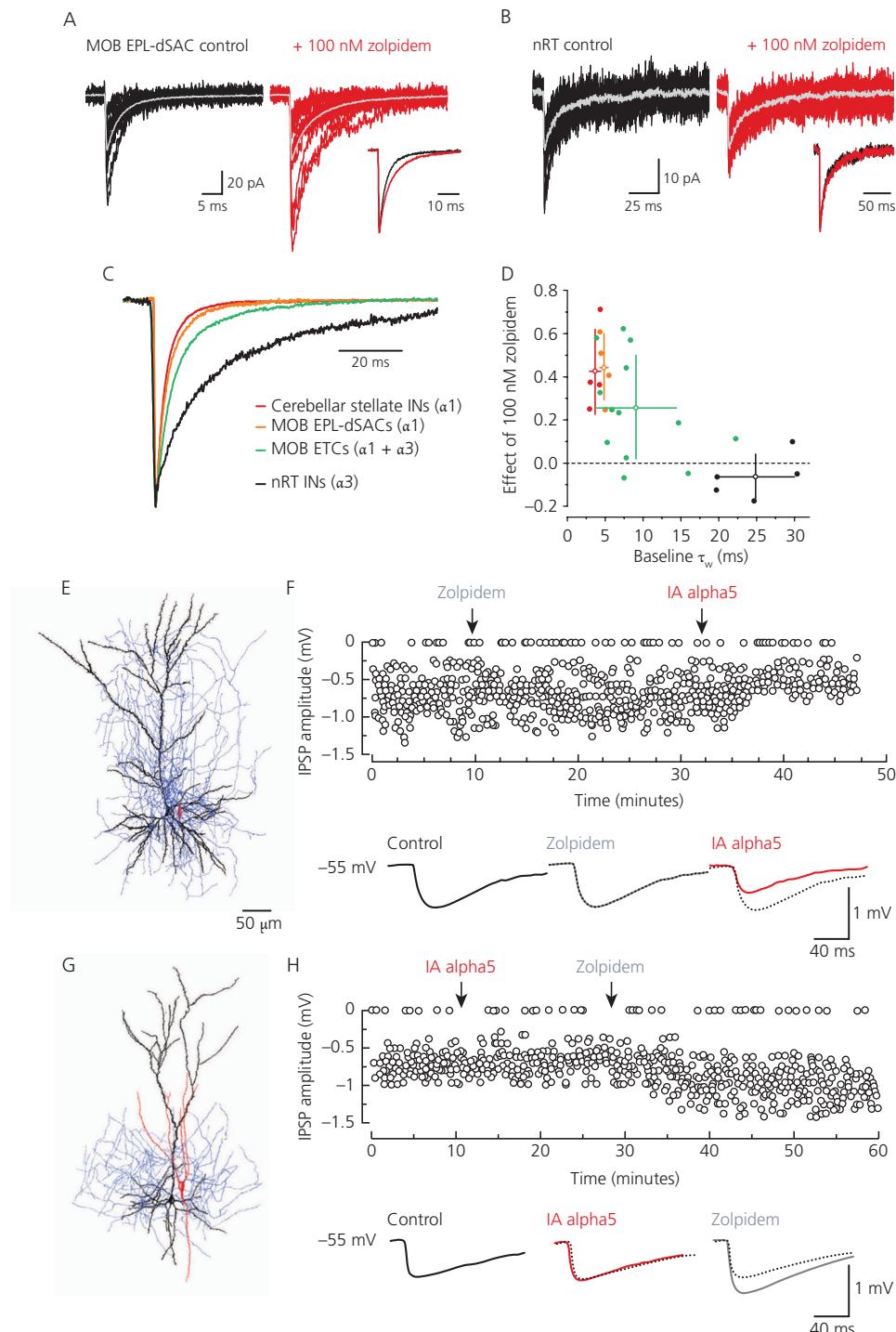


Fig. 8.3 The time course of inhibitory synaptic currents in neurons with different synaptic GABA_A receptor α subunits, and the synapse-specific expression of different α subunit isoforms. **(A)** Superimposed consecutive miniature IPSCs (mIPSCs) recorded from a main olfactory bulb external

as a result of different degrees of activity-dependent plasticity, there is evidence that it may reflect, at least in part, differences in the molecular makeup of the postsynaptic densities (for a review see Benson et al., 2001; for examples see Ding et al., 2011; Bloodgood et al., 2013; Ahrens et al., 2015).

Different inputs can activate different complements of GluN2A- and 2B-containing NMDA receptors (Kumar and Huguenard, 2003; Ding et al., 2008) (Fig. 8.2), leading to differences in both temporal summation and plasticity (Kumar and Huguenard, 2003; Evans et al., 2012; Paoletti et al., 2013). In the striatum and ventral tegmental area there is also evidence for input-specific incorporation of NMDA receptors with reduced sensitivity to Mg^{2+} (Britt et al., 2012; Yuan et al., 2013), while in the amygdala there is evidence for input-specific localization of GluA1 and GluA3 (Humeau et al., 2007). Such phenomena mean that basal AMPA and NMDA receptor signaling and the generation of events such as NMDA and sodium spikes may be concentrated to specific inputs. However, in the interpretation of these experiments it is important to consider differences in the distribution of inputs in the dendritic tree, and consequent differences in the ability to voltage clamp the responses, as well as uncertainties regarding the specificity of the pharmacological tools used. Nevertheless, there is clearly much evidence to support the notion that different subunits may be utilized to confer distinct properties to afferent inputs.

Input-specific synaptic properties are also seen at inhibitory connections. Different populations of interneurons target different areas of the dendrites in many cell types throughout the brain (Markram et al., 2004; Klausberger and Somogyi, 2008; Kepcs and Fishell, 2014). In the prototypical example, in the CA1 region of the hippocampus, parvalbumin-positive interneurons tend to

Fig. 8.3 (Continued)

plexiform layer deep short-axon cell (MOD EPL-dSAC) in an acute slice of rat brain, before (black) and after (red) the application of 100 nM zolpidem (an $\alpha 1$ -selective positive allosteric modulator); averages in gray. Inset shows peak-scaled averaged mIPSCs, demonstrating that zolpidem slowed their decay. (B) As in A but with mIPSCs recorded from a nucleus reticularis thalami (nRT) cell. The inset shows that zolpidem did not slow the mIPSC decay. (C) Peak-scaled, peak-aligned mIPSC population averages showing the different kinetics of currents in different cell types where quantitative immunolocalization revealed synaptic α subunits that were $\alpha 1$ alone, $\alpha 3$ alone or $\alpha 1$ and $\alpha 3$. (D) Plot of the effect of zolpidem as a function of the initial weighted mean time constant (τ_w) of mIPSCs. Zolpidem strongly affects the decay of mIPSCs in cells with fast kinetics [cerebellar stellate interneurons (INs), MOB EPL-dSACs] but has no effect on cells with slow kinetics (nRT cells). The effect of zolpidem on the decay of mIPSCs in MOB external tufted cells (ETCs) depends on their initial decay time constant. (E) Reconstruction of a dendrite-preferring, bitufted cell and its target pyramidal cell in an acute slice from the rat somatosensory cortex. (F) Plot of peak IPSP amplitudes in control conditions and during bath application of zolpidem and subsequent addition of IA alpha5, an $\alpha 5$ -selective inverse agonist. Average IPSPs in each condition are shown below. IPSPs elicited by dendrite-preferring bitufted cells were insensitive to zolpidem but reduced by IA alpha5. (G), (H) Same as E and F but for a multipolar adapting cell where IPSPs were insensitive to IA alpha5 but increased to zolpidem.

Parts A–D adapted from Mark D. Eyre, Massimiliano Renzi, Mark Farrant, and Zoltan Nusser, Setting the time course of inhibitory synaptic currents by mixing multiple GABA_A receptor α subunit isoforms, *The Journal of Neuroscience*, 32(17), pp. 5853–5867, Figure 11b–d, © 2012, The Society for Neuroscience. Parts E–H adapted from Afia B. Ali and Alex M. Thomson, Synaptic $\alpha 5$ subunit-containing GABA_A receptors mediate IPSPs elicited by dendrite-preferring cells in rat neocortex, *Cerebral Cortex*, 18(16), pp. 1260–1271, Figure 4, © 2008, Oxford University Press.

target peri-somatic regions of pyramidal cells, while somatostatin-positive interneurons target dendritic regions (Kawaguchi and Kubota, 1997). As discussed in the following sections, this differential targeting can have dramatic effects on the role the interneurons play in neuronal computation (e.g., Gidon and Segev, 2012; Jadi et al., 2012; Lovett-Barron et al., 2012; Muller et al., 2012; Lovett-Barron et al., 2014).

As well as targeting distinct subcellular domains, similar to excitatory input, different populations of interneurons targeting the same postsynaptic cell may also activate receptors with different subunit compositions. At different synapses—on different neurons or on individual neurons—the subunit composition of GABA_A receptors can vary (Nusser et al., 1996b; Klausberger et al., 2002; Gross et al., 2011; Eyre et al., 2012). For $\alpha\beta\gamma$ -containing receptors, the α subunit content is particularly influential. Thus, clear differences in agonist affinity, modulator pharmacology, and deactivation kinetics are seen among recombinant receptors containing distinct α subunits (Verdoorn et al., 1990; Gingrich et al., 1995; Lavoie et al., 1997; Bianchi et al., 2002; Picton and Fisher, 2007; Bright et al., 2011; Dixon et al., 2014) and this is reflected in the different properties of inhibitory postsynaptic currents (IPSCs) in neurons that express distinct α subunits or different mixtures of α subunits at their synapses (Browne et al., 2001; Mozrzymas et al., 2007; Eyre et al., 2012; Labrakakis et al., 2014) (Fig. 8.3A–D). In the case of CA1 pyramidal cells discussed earlier, synapses formed by parvalbumin-positive terminals have a large proportion of $\alpha 1$ subunits, and less $\alpha 2$, while this is reversed at parvalbumin-negative synaptic connections (Nusser et al., 1996b; Fritschy et al., 1998; Nyíri et al., 2001). This is complemented by paired recordings showing fast spiking (usually parvalbumin-positive) interneurons utilizing a high proportion of $\alpha 1$ subunit-containing receptors in their connections onto pyramidal neurons, while bitufted, regular spiking neurons do not, and instead utilize $\alpha 5$ -containing receptors (Ali and Thomson, 2008; Thomson and Jovanovic, 2010) (Fig. 8.3E–H).

Plasticity of ionotropic receptor localization

Thus far we have focused on the spatial heterogeneity of receptors and synapses. In this regard, it is important to bear in mind that the targeting of receptors is dynamic. The plasticity of targeting, at cellular, dendritic, and synaptic levels, has profound consequences for synaptic function. As discussed in Chapters 6 and 7, both glutamate and GABA receptors are subject to constitutive turnover, with rapid entry into and exit from synaptic and extrasynaptic compartments, mediated by exocytotic and endocytotic mechanisms as well as regulated lateral diffusion (Pérez-Otaño and Ehlers, 2005; Triller and Choquet, 2005; Kessels and Malinow, 2009; Smith and Kittler, 2010; Anggono and Huganir, 2012; Harris and Weinberg, 2012). The trafficking of receptors is key to various forms of activity-dependent plasticity. The most widely studied of these is “Hebbian” or associative plasticity, in which the strength of a specific excitatory synaptic connection is enhanced following correlated pre- and postsynaptic firing (long-term potentiation, LTP) or reduced in response to uncorrelated firing (long-term depression, LTD) through the insertion or removal of AMPA (Malinow and Malenka, 2002; Bredt and Nicoll, 2003; Malenka, 2003; Huganir and Nicoll, 2013) or NMDA receptors (Paoletti et al., 2013). Interestingly, these changes can also be associated with changes in calcium permeability of both AMPA and NMDA receptors, alterations in single-channel conductance, and differences in subunit composition (Benke et al., 1998; Derkach et al., 1999; Oh and Derkach, 2005; Skeberdis et al., 2006; Sobczyk and Svoboda, 2007; Harney et al., 2008; Chalifoux and Carter, 2010; Peng et al., 2010). In some cases, increased activity can also result in the rapid production of entirely new synapses with distinct complements of AMPA and NMDA receptor subunits (Kwon and Sabatini, 2011; Kozorovitskiy et al., 2012). These and similar

mechanisms have been shown to underlie various forms of experience-dependent plasticity *in vivo* (Holtmaat et al., 2006; Hofer et al., 2009; Pascoli et al., 2012; Muñoz-Cuevas et al., 2013; MacAskill et al., 2014).

The specific receptor types present at a synapse can dramatically alter the mechanisms of plasticity induction. For example, in “classical” LTP, activation of NMDA receptors is required for increases in trafficking of AMPA receptors to the synapse (Bredt and Nicoll, 2003; Malenka, 2003). However, the relative prevalence of NR2A- or NR2B-containing NMDA receptors can profoundly influence the nature of the plasticity (Pérez-Otaño and Ehlers, 2005; Evans et al., 2012; Paoletti et al., 2013). Conversely, the presence of calcium-permeable AMPA receptors lacking GluA2 can induce specific forms of plasticity without NMDA receptor activation (for review see Kullmann and Lamsa, 2007). Although plasticity at inhibitory synapses is not as well characterized as that at excitatory synapses, possibly reflecting its seemingly diverse forms (Kullmann et al., 2012; Lourenco et al., 2014; Petrini et al., 2014), it is important to note that activation of GABA_A receptors can profoundly modulate excitatory plasticity (Bar-Ilan et al., 2012; Kullmann et al., 2012).

Importantly, activity-dependent plasticity confers potential instability, as to allow unregulated LTP and LTD could result in saturation or silencing of synaptic signaling. Neurons have developed many ways to compensate for this problem. For example, overactive neurons in a network can change their intrinsic firing properties by altering the number of potassium channels in the membrane, thus maintaining activity at an ideal “set point” (Marder and Prinz, 2002; Davis, 2006). There are a number of ways in which targeting of neurotransmitter receptors also contributes to this regulation. For example, global synaptic scaling may occur, in which increased neuronal activity results in decreases in synaptic strength, while decreased activity globally increases synaptic strength, again allowing an ideal range of neuronal activity to be maintained (Turrigiano, 2008; Pozo and Goda, 2010). Finally, compensatory changes in the balance between excitation and inhibition have been shown to be a common adaptation, where increases or decreases in excitatory drive through AMPA or NMDA receptors are compensated by concurrent changes in GABA_A receptor-mediated inhibitory connectivity (Maffei et al., 2004; Gonzalez-Islas and Wenner, 2006). Elegant experiments have also shown that similar mechanisms exist *in vivo* (Desai et al., 2002; Maffei et al., 2006; Mrsic-Flogel et al., 2007; Keck et al., 2013). However, it is important to note that, in a given neuronal circuit, different connections can undergo distinct alterations via widely diverging mechanisms, and so the role of homeostatic plasticity appears to be dependent on the circuit context (Maffei et al., 2004, 2006; Pozo and Goda, 2010).

Plasticity mechanisms also play an important role in creating and maintaining the spatial distribution of receptors throughout the dendrites (see Chapter 18). While induction of classical Hebbian LTP or LTD is spatially restricted, there exists marked cooperativity throughout local dendritic regions. For example, insertion of AMPA receptors in response to coordinated activity at a single synaptic site lowers the threshold for LTP at neighboring spines (Harvey and Svoboda, 2007; Harvey et al., 2008; Govindarajan et al., 2011; Murakoshi et al., 2011). Notably, *in vivo* studies also show that experience seems to preferentially alter synapses that are in close proximity to one another, resulting in the clustering along dendrites of synapses with similar receptor properties (Chen et al., 2011; Makino and Malinow, 2011; Druckmann et al., 2014; Schoonover et al., 2014; although see Jia et al., 2010).

Homeostatic plasticity may also be expressed locally in dendrites to allow spatially precise control of synaptic properties (Ju et al., 2004; Sutton et al., 2006, 2007; Branco et al., 2008; Hou et al., 2008). Indeed, there is theoretical and experimental evidence to suggest that the induction of LTP at a synapse, in cooperation with either tonic or phasic inhibitory signaling, can weaken neighboring synapses that are inactive (Rabinowitch and Segev, 2006, 2008; Hayama et al., 2013).

Additionally, the differential distribution and regulation of ion channels can change the intrinsic excitability of local dendritic regions (see Chapter 9) (Williams, 2004; Harnett et al., 2013; 2015), and both phenomena result in a balancing of local synaptic weight in the dendrite. Finally, synaptic input from different sources can be independently regulated, allowing afferent input from different brain regions to be differentially modulated (Bartley et al., 2008; Kim and Tsien, 2008). Indeed, metaplasticity—where the induction of classical LTP or LTD at an individual synapse alters the synaptic properties in a way that determines future plastic events—can allow control over plasticity at the level of individual synapses (Abraham and Bear, 1996; Pérez-Otaño and Ehlers, 2005; Lee et al., 2010).

We have focused thus far on how the properties of individual receptors and synapses influence signaling. In the next sections we focus on: (1) how these different complements of receptors and synapses are functionally utilized; (2) how synapses interact with each other, and with the intrinsic and active properties of the dendritic tree; and (3) what role this plays in dendritic function and computation.

The role of neurotransmitter receptors in signal transmission and dendritic function

Over the last 15 years the view that AMPA receptors mediate fast excitatory synaptic transmission in the mammalian brain, with little, if any, contribution from NMDA receptors (Bliss and Collingridge, 1993), has gradually been transformed as evidence has accumulated showing that both play a role in neuronal signaling. The traditional view arose from the very reasonable assumption that only synaptic AMPA receptors, with their largely Ohmic conductance, could do the “heavy lifting” required to depolarize the membrane potential from the hyperpolarized resting levels (-75 mV measured in brain slices) to spike threshold (-45 mV). Moreover, the fact that the depolarization required to activate NMDA receptors was close to spike threshold and their activation kinetics are slow, implied that these receptors did not contribute significantly to the excitatory charge during “normal” fast synaptic transmission. Indeed, developing synapses that lacked AMPA receptors were referred to as “silent” (Liao et al., 1995; Isaac et al., 1997). Although it was realized early on that NMDA receptors play a key role in long-term synaptic plasticity (and thus learning and memory) during substantial postsynaptic depolarization (Bliss and Collingridge, 1993), at least two factors led to the initial under-appreciation of their role in synaptic integration. Firstly, due to the presence of network activity, neurons and dendrites often operate at more depolarized levels *in vivo* than they do when examined in *in vitro* preparations. Secondly, as already described above, the voltage dependence of some NMDA receptor subtypes is considerably weaker than those expressed in the largely immature preparations used in early studies.

Interplay between synaptic glutamate receptors and dendritic properties

In small, electrically compact cells such as cerebellar granule cells, whose somato-dendritic compartment is effectively isopotential (Silver et al., 1992; Cathala et al., 2003), supra-threshold synaptic integration is dominated by the powerful conductances underlying the action potential and the after-hyperpolarization potential. By contrast, in morphologically complex neurons many synapses are formed on dendrites that are electrically remote from the spike initiation zone, allowing more complex patterns of electrical behavior to arise. Integration of synaptic inputs is dependent on a range of dendritic properties, including the morphology, passive properties, and the

repertoire of ion channels they express (see Chapter 12). Since the properties of dendrites have been discussed extensively in other chapters and in several reviews (London and Häusser, 2005; Spruston, 2008; Silver, 2010), in subsequent sections we will illustrate how neurotransmitter-gated ion channels can contribute to dendritic function, with a few specific examples.

Synaptic AMPA receptors and active dendrites

Early theoretical work predicted that fast excitatory synaptic conductances, a rapid local membrane time constant, and the presence of voltage-gated Na^+ channels could enable the fine dendrites of CA1 hippocampal pyramidal cells to act as coincidence detectors (Softky, 1994). Subsequent experimental studies using direct whole-cell dendritic recording confirmed that fine dendrites of cortical pyramidal cells have high impedance but a rapid membrane time constant, resulting in local dendritic EPSPs that are substantially larger and faster than at the soma (Williams and Stuart, 2002; Nevian et al., 2007). Experiments using glutamate uncaging to mimic synaptic input confirmed that Na^+ channels on basolateral CA1 dendrites mediate rapidly rising dendritic spikes (Golding and Spruston, 1998; Gasparini et al., 2004; Losonczy and Magee, 2006). Moreover, the activation of synaptic inputs onto a dendritic branch must be highly coincident to trigger a Na^+ spike (Losonczy and Magee, 2006). These studies show how rapid AMPA receptor-mediated synaptic conductances can combine with passive and active dendritic properties to form a local dendritic coincidence detector.

Role of NMDA receptors in signal transmission

In cerebellar granule cells, the NMDA receptor-mediated component of the synaptic conductance undergoes developmental maturation with a switch from GluN2A/B- to GluN2C-containing receptors (Farrant et al., 1994; Monyer et al., 1994). This subunit change weakens the Mg^{2+} block and the voltage-dependent properties of the NMDA receptors (Cathala et al., 2000; Yamada et al., 2001), enabling them to pass depolarizing current even at relatively hyperpolarized potentials. Early studies indicated that these receptors contributed to single EPSPs and high-frequency mossy fiber bursts (D'Angelo et al., 1995). More recent quantification of the relative contributions of AMPA and NMDA receptor-mediated components revealed that more than 50% of the charge is mediated by the latter during sustained signaling at physiologically relevant rates (Schwartz et al., 2012). Moreover, modeling NMDA receptors with a weak Mg^{2+} block demonstrates that they conduct substantially more charge than immature GluN2A/B-containing receptors (Schwartz et al., 2012). The slower decays of NMDA compared with AMPA receptor-mediated conductances enable more effective temporal integration, extending the bandwidth of sustained rate-coded signaling to lower frequencies (Schwartz et al., 2012). GluN2C-containing NMDA receptors with a weak Mg^{2+} block are also found in the input layer of the neocortex (Binshtok et al., 2006). As at cerebellar mossy fiber–granule cell synapses, the synaptic NMDA receptor-mediated conductances in L4 spiny stellate cells convey a large fraction of the synaptic charge, even for single synaptic events (Feldmeyer et al., 1999). Such studies show that NMDA receptors often contribute a substantial component of the excitatory synaptic charge, and that their contribution is particularly large at synapses where the GluN2C (or GluN2D) subunits are expressed.

Properties of NMDA receptor-mediated dendritic signaling

Synaptic NMDA receptors can contribute to both linear and nonlinear dendritic behavior. The voltage-dependent properties of the receptors can counteract the effects of the depolarization-induced reduction in driving force that is particularly apparent in thin dendrites (Cook and

Johnston, 1999; Gasparini et al., 2004), and can help linearize subthreshold integration (Cash and Yuste, 1999). Interestingly, it is the slow time course of conductance rather than the fast local membrane time constant that sets the temporal integration window in fine dendrites that receive synapses with a substantial NMDA receptor-mediated component (Branco and Häusser, 2011). This allows synaptic inputs to be integrated over 10 ms or more (Polsky et al., 2004, 2009; Branco and Häusser, 2011). Synaptic NMDA receptors can also directly drive regenerative dendritic depolarization producing “NMDA spikes” (Schiller et al., 2000). Unlike voltage-gated Na^+ and Ca^{2+} channels, NMDA receptor channels can only be opened when glutamate (and glycine) is bound and the membrane is depolarized. This restricts NMDA spikes to those branches that receive synaptic input and prevents their active propagation (Larkum et al., 2009), although their relatively long duration ensures their passive spread is quite effective (Fig. 8.4). The idea that NMDA spikes are local and can perform branch-specific thresholding is attractive as it could substantially enhance the computational power of a neuron, enabling each dendritic branch to act as a nonlinear thresholding unit (Schiller and Schiller, 2001; Polsky et al., 2004) and the whole dendritic tree and soma to operate in a manner that is analogous to a two-layer neural network (Poirazi et al., 2003).

Experiments using two-photon glutamate uncaging or dendritic recording in cortical slices suggest that the threshold for activating NMDA spikes is high, requiring a substantial fraction of the synapses present on an apical dendritic branch (Nevian et al., 2007; Larkum et al., 2009; Branco and Häusser, 2011), even though these fine dendrites have the highest impedance. Moreover, in L5 pyramidal cells, activation of multiple branches is required to trigger a Ca^{2+} spike, which couples the electrically remote tuft to the soma (Larkum et al., 1999). Modeling suggests that NMDA spikes are sensitive to inhibitory synaptic input (Rhodes, 2006), and inhibition is strong *in vivo* (Borg-Graham et al., 1998; Haider et al., 2013). Together, these observations suggest that NMDA spikes are difficult to activate and occur only following spatio-temporally clustered synaptic input.

In the barrel cortex, local changes in synaptic AMPA receptors on the dendrites of L2/3 pyramidal cells have been found to occur during sensory experience (Makino and Malinow, 2011), suggesting that plasticity mechanisms could specifically enhance the strength of clusters of synapses. However, such clustered strengthening would seem unlikely in visual and auditory cortices, where inputs with distinct receptive fields are interspersed on the same dendritic branch (Jia et al., 2010; Chen et al., 2011). Nevertheless, recent *in vivo* studies have established that sensory-evoked NMDA spikes can occur in the dendrites of spiny stellate cells (Lavzin et al., 2012) and L2/3 pyramidal cells (Smith et al., 2013; Palmer et al., 2014) (Fig. 8.4). It is possible that a relatively weak level of clustering, as quantified anatomically in L4 neurons (Schoonover et al., 2014), is sufficient to trigger NMDA spikes. However, it is also possible that the ongoing network activity present *in vivo* enhances the likelihood of NMDA spikes, explaining how they can occur in sensory systems where clustering of synaptic input appears weak.

Cortical networks in intact animals are intrinsically active, so neurons are continually bombarded by a background level of synaptic input (Destexhe et al., 2003). Theoretical studies using biologically detailed models of pyramidal cells have shown that background synaptic input at rates observed *in vivo* could make the occurrence of NMDA spikes more likely *in vivo* than *in vitro*, where networks are largely quiescent (Larkum et al., 1999; Farinella et al., 2014; Palmer et al., 2014). This occurs because background synaptic input depolarizes the dendrite (Major et al., 2008) and introduces glutamate-bound NMDA receptors across the dendritic tree, lowering the threshold for NMDA spikes, increasing their duration, and allowing them to actively spread to neighboring branches (Farinella et al., 2014). Balanced background inhibition partially counteracts these effects, suggesting that the level of inhibition and disinhibition of the tuft (Gentet et al., 2012) could control the nature of synaptic integration (Lovett-Barron et al., 2012) and prevent runaway excitation. These results suggest that under quiescent network conditions NMDA spikes have a

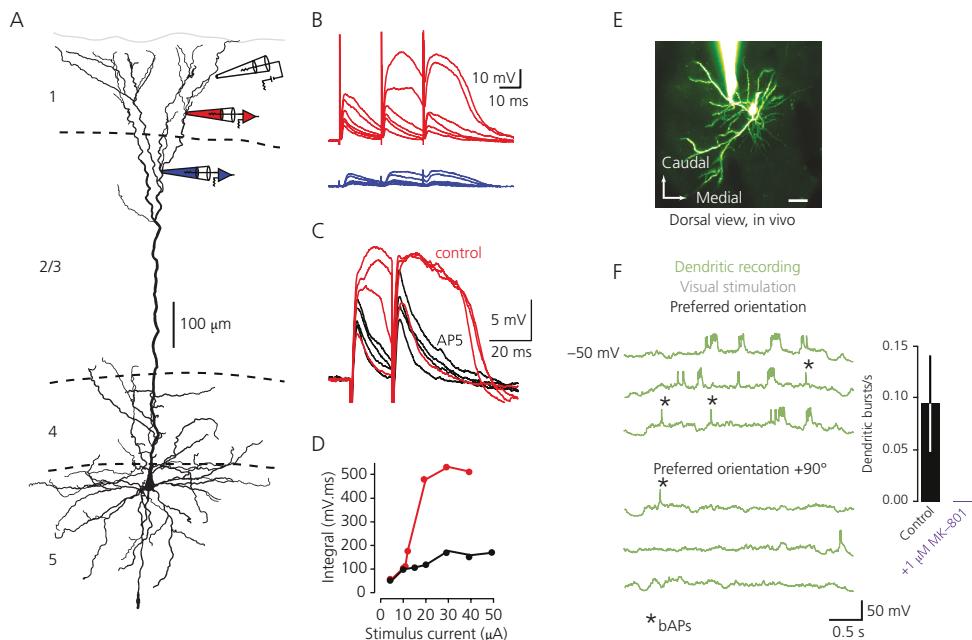


Fig. 8.4 NMDA spikes in cortical pyramidal cells *in vitro* and *in vivo*. **(A)** Reconstruction of a biocytin-filled L5 pyramidal neuron showing dendritic recording pipettes near the third bifurcation (875 µm from the soma, red) and near the second bifurcation (715 µm from the soma, blue). An extracellular stimulation electrode (black) is shown about 100 µm distal to the distal recording electrode. **(B)** Responses to a gradual increase of the extracellular stimulus (from 4 to 9 µA) recorded in both electrodes. **(C)** Dendritic recording from another pyramidal neuron after the second bifurcation (807 µm from the soma, red), similar to the recording shown in (A). An extracellular synaptic stimulation electrode was positioned about 100 µm from the recording electrode. Gradually increasing the extracellular stimulation strength (from 10 to 40 µA) led to broad regenerative spikes that were blocked by the addition of 50 µM d-2-amino-5-phosphonopentanoic acid (AP5). **(D)** Integral of the voltage responses to a paired stimulation before (red) and after application of AP5 for recordings shown in (C). The integral was measured over the entire response. **(E)** A two-photon microscopy image of a L2/3 pyramidal neuron in the mouse visual cortex *in vivo*, obtained by filling with Alexa Fluor 594 and dendritic patch-clamp recording at 100 µm from the soma (maximum intensity projection) (scale bar = 20 µm). **(F)** Dendritic activity evoked by square-wave grating visual stimuli exhibited reliable orientation-tuned burst-spiking events. The bar chart shows that when 1 µM MK-801 was included in the recording pipette, the visually evoked responses contained fewer dendritic bursts, confirming that these do not occur when NMDA receptors are blocked. Note, in recordings with MK-801 orientation tuning was not determined and the responses were averaged over all of the stimulus presentations for both conditions.

Parts A–D adapted from Matthew E. Larkum, Thomas Nevian, Maya Sandler, Alon Polsky, and Jackie Schiller, Synaptic integration in tuft dendrites of layer 5 pyramidal neurons: a new unifying principle, *Science*, 325(5941) pp. 756–760, © 2009, The American Association for the Advancement of Science. Reprinted with permission from AAAS. Parts E and F adapted with permission from Macmillan Publishers Ltd: *Nature*, 503(7474), Spencer L. Smith, Ikuko T. Smith, Tiago Branco & Michael Häusser, Dendritic spikes enhance stimulus selectivity in cortical neurons *in vivo*, pp. 115–120, © 2013, Nature Publishing Group.

high threshold and are localized to particular branches, consistent with experiments in acute slices (Nevian et al., 2007; Larkum et al., 2009; Branco and Häusser, 2011). In contrast, when cortical networks operate around their normal level of activity *in vivo*, the NMDA spike threshold is lower, allowing activation by distributed synaptic input. These characteristics are consistent with *in vivo* measurements showing that NMDA spikes are typically associated with multibranch activation (Lavzin et al., 2012; Palmer et al., 2014) and, as we will discuss later, have important consequences for dendritic computation.

The role of GABA_A receptors in signal transmission

Classically, GABA_A receptors are viewed as inhibitory, as their activation typically leads to a reduction in the probability of the firing of action potentials. This is, of course, a grotesque oversimplification. Their effects are far more varied and subtle than this blanket description implies. In the CNS, principal cells receive hundreds or thousands of inhibitory synapses that are non-uniformly distributed across their surface, according to cell-type-specific patterns of innervation by different interneurons (Klausberger and Somogyi, 2008) that contribute to feedforward or feedback circuits (Buzsaki, 1984; Stokes and Isaacson, 2010; Fino and Yuste, 2011; Isaacson and Scanziani, 2011). Although a given dendritic region may receive input from multiple interneuron types, these synapses often exhibit distinct temporal dynamics that depend on the identities of the presynaptic cells. Moreover, the activity of these interneurons can change differentially during alterations in network states (Somogyi et al., 2014).

One way in which GABA_A receptors generate inhibition is through membrane hyperpolarization. This reflects the presence of a Cl⁻ extruding mechanism, primarily the neuron-specific K⁺-Cl⁻ co-transporter 2 (KCC2), which establishes an equilibrium potential for Cl⁻ that is more negative than the resting membrane potential. The hyperpolarizing inhibitory postsynaptic potential (IPSP) generated at the synapse is attenuated as it spreads along the dendritic membrane. Importantly, variation in the expression and localization of Cl⁻ transporters (both between neurons and spatially within individual cells), activity-driven collapse of the Cl⁻ gradient, and the obligatory outward flow of HCO₃⁻ (Farrant and Kaila, 2007; Kaila et al., 2014) means that robust hyperpolarizing actions of GABA are not necessarily always generated (e.g., Martina et al., 2001; Chavas and Marty, 2003; Gullidge and Stuart, 2003; but see Glickfeld et al., 2009; Song et al., 2011). Moreover, site- and voltage-dependent interaction with voltage-gated ion channels can greatly influence the inhibitory efficacy of GABA_A receptor-mediated postsynaptic potentials (Williams and Stuart, 2003).

Irrespective of the membrane potential or the transmembrane gradients of the permeant Cl⁻ and HCO₃⁻ ions, GABA_A receptor activation results in an increase in membrane conductance that produces a “shunting” inhibition. This decreases the membrane time and space constants of the cell and thus limits the temporal and spatial summation of depolarizing responses driven by EPSCs or other currents. Although any increase in synaptic conductance is highly local, due to the cable properties of the dendrites its effects can be felt at remote sites (Koch et al., 1983; Williams, 2004; Gidon and Segev, 2012). Indeed, when multiple inhibitory synapses contact the dendritic tree the inhibitory effect can spread, to the extent that, somewhat counter-intuitively, the maximal effect of inhibition may be seen at locations that lack inhibitory synapses (Gidon and Segev, 2012).

From dendritic neurotransmitter receptors to computation

Early theoretical work showed that networks of simple point neurons could, in principle, perform all computable operations (McCulloch and Pitts, 1943). The fact that the neurons within these networks only performed linear summation followed by nonlinear thresholding operations led to the

idea (that still lingers in some areas of theoretical neuroscience) that all but the simplest computations arise from network connectivity rather than from the neurons themselves. As experimental and theoretical methods for studying dendrites have improved, evidence has accumulated that individual neurons can perform a wide range of computations, many of which arise from the interaction between neurotransmitter receptors and other dendritic properties (London and Häusser, 2005; Silver, 2010).

Sublinear and linear dendritic integration

Point neurons typically perform near-linear (additive) summation of synaptic input. Synaptic inputs that are spatially distributed onto passive dendritic trees also tend to be integrated linearly because the space constant for brief synaptic conductances is short, ensuring they act on the soma as independent current sources. However, sublinear integration of synaptic input does occur when a substantial number of excitatory synaptic inputs converge onto a dendritic branch, due to the reduction in driving force during large local EPSPs. Such sublinear dendritic integration of AMPA receptor-mediated synaptic input has been observed in cerebellar interneurons (Abrahamsson et al., 2012; Vervaeke et al., 2012). Sublinear dendritic behavior could be used to compute functions that cannot be separated using linear operations alone (Caze et al., 2013) and to generate an optimal response to input spike trains with second-order correlations between presynaptic inputs (Ujfaluissy and Lengyel, 2011). However, in excitatory neurons, sublinear dendritic properties are often counteracted by voltage-activated dendritic conductances, including NMDA receptor-mediated conductances, which play an important role in this process (Cash and Yuste, 1999). Such linear dendritic integration is useful for conserving linear representations and performing linear–nonlinear thresholding operations.

GABA_A receptor-mediated inhibition is particularly important in setting the number of synaptic inputs required to trigger an action potential. Although increasing the amount of inhibitory conductance scales down subthreshold EPSPs in a multiplicative manner, it usually introduces a subtractive offset in the rate-coded input–output (I–O) relationship. This occurs because the inhibitory conductance behaves like a fixed current during continuous firing, as a result of the voltage being “clamped” to around the threshold voltage by the powerful conductances activated during spiking (Holt and Koch, 1997; Mitchell and Silver, 2003). As inhibitory interneurons receive both feedforward and feedback excitation their firing rate can track the local network activity. This network activity-dependent behavior ensures that the I–O relationships of neurons in the local network do not saturate during strong network drive, a feature that is important for preventing information loss (Papadopoulou et al., 2011; Billings et al., 2014).

Gain modulation and nonlinear dendritic integration

Multiplication of synaptic inputs is essential for a wide range of computations. This operation is reflected as a change in the slope of the neuronal I–O relationship. An increase in the slope corresponds to a multiplicative operation, while a decrease in slope corresponds to a division (Silver, 2010). These arithmetic operations are often referred to collectively as gain modulation. Modulation of neuronal gain alters the sensitivity of a neuron to changes in its driving input without altering its selectivity or receptive field properties. For temporally coincident signaling, neuronal gain can alter the time window for temporal integration, specifically altering the sharpness of the cut-off, thereby governing the temporal properties of signaling (Shu et al., 2003). A number of distinct mechanisms have been shown to be involved in gain modulation for rate-coded signaling, including synaptic noise (Chance et al., 2002; Mitchell and Silver, 2003), short-term synaptic

plasticity (Abbott et al., 1997; Rothman et al., 2009), dendritic nonlinearities (Schiller et al., 2000; Larkum et al., 2004; Losonczy and Magee, 2006) and shunting inhibition (Murphy and Miller, 2003; Rothman et al., 2009). Dendritic neurotransmitter-gated ion channels play a role in all of these mechanisms.

Effect of synaptic noise on neuronal gain

Excitatory and inhibitory synaptic conductances arising from ongoing network activity can be present even during quiet wakefulness, when sensory input is minimal. This “background” synaptic input reduces the input resistance of the neuron and depolarizes the membrane potential from the hyperpolarized level typically measured under quiescent conditions in acute slices (London and Segev, 2001; Destexhe et al., 2003). Moreover, phasic synaptic conductances introduce pronounced fluctuations in membrane potential, increasing membrane noise (Destexhe et al., 2003). Fluctuations in the membrane potential arising from background synaptic input enables synaptic inputs to cross the threshold and trigger an action potential even when the time-averaged mean membrane potential is well below the threshold (Destexhe et al., 2003). Increasing the level of noise by increasing background synaptic input reduces the neuronal gain by lowering the threshold and introducing a more graded transition from sub-threshold to suprathreshold behavior (Anderson et al., 2000). Dynamic clamp experiments established that when excitatory and inhibitory background synaptic input is perfectly balanced, the subtractive offset introduced by the conductance counteracts the additive effects of increasing noise, having a purely multiplicative effect on the I-O relationship (Chance et al., 2002). Although the recurrent structure and extensive local connectivity of inhibition found in cortical networks suggest that they are well placed to deliver a balanced input, the precise relationship of AMPA/NMDA and GABA_A receptor-mediated conductances and how this balance changes with time is poorly understood. This is because the interpretation of attempts to quantify excitatory and inhibitory synaptic conductances *in vivo* is complicated by the difficulty of controlling dendritic voltage from somatic recordings (Williams and Mitchell, 2008; Chadderton et al., 2014).

Inhibition-mediated gain modulation and short-term plasticity

Simply altering the level of inhibitory conductance can lead to changes in neuronal gain under certain conditions (Silver, 2010). When EPSCs are large and fast, GABA_A receptor-mediated conductance can alter the gain of a rate-coded I-O relationship, because the variance of random synaptic input trains increases linearly with the mean rate (Mitchell and Silver, 2003). When combined with an inhibitory conductance this input-frequency-dependent increase in noise produces a multiplicative scaling of the I-O relationship, as well as a residual additive component (Mitchell and Silver, 2003). Of note, rectification of GABA_A receptors (as already discussed) may affect the way in which the GABA_A receptor-mediated conductance can perform additive or divisive operations (Pavlov et al., 2009). GABA_A receptor-mediated gain modulation can also arise when nonlinearity is present in the excitatory synaptic input (Murphy and Miller, 2003; Rothman et al., 2009). At mossy fiber-granule cell synapses short-term synaptic depression (STD) of the AMPA receptor-mediated component introduces a saturating nonlinear relationship between synaptic conductance and the input rate (Rothman et al., 2009) (Fig. 8.5A,B). When the tonic inhibitory conductance, mediated by α6-containing GABA_A receptors in granule cells, was mimicked using dynamic clamping the frequency-dependent nonlinear behavior of the excitatory synaptic input converted an inhibition-mediated subtractive shift in the I-O relationship into a divisive scaling

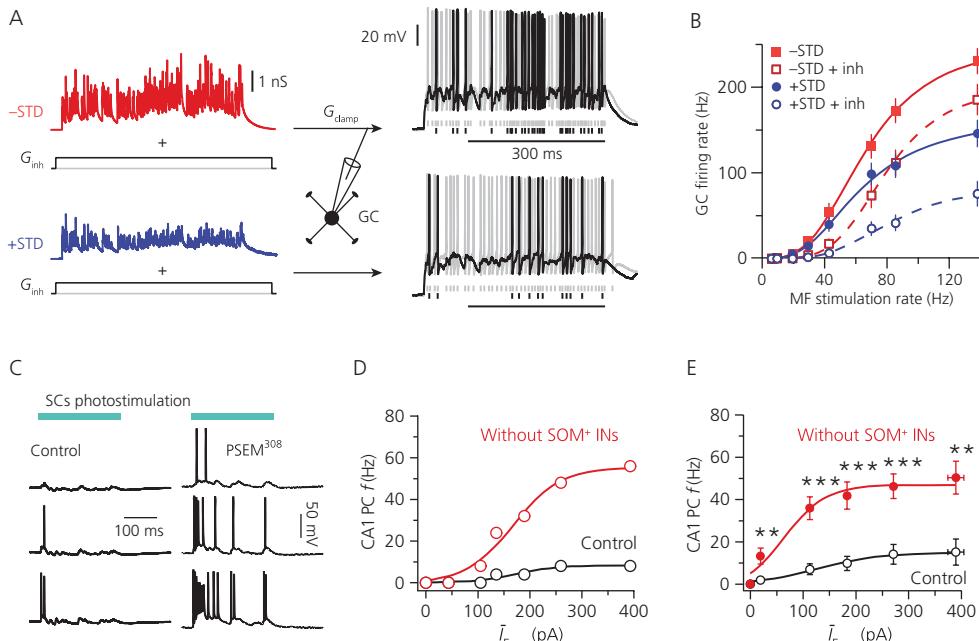


Fig. 8.5 Inhibition-mediated neuronal gain modulation. **(A)** The sum of four independent synaptic trains (each $f = 86$ Hz) with and without short-term depression (STD) injected into a cerebellar granule cell (GC) by means of dynamic clamp (G_{clamp}) with and without tonic inhibition (black and gray, respectively; $G_{\text{inh}} = 500$ pS). Right vertical ticks indicate spike times. Horizontal bars indicate output rate measurement window. $V_{\text{rest}} = -79$ mV. **(B)** Average input–output relationships ($n = 9$) with and without STD (blue and red, respectively) and tonic inhibition (inh, open and filled symbols, respectively; error bars are \pm standard error of the mean). Lines are fits to a Hill function. **(C)** Current-clamp recordings from a CA1 pyramidal cell (CA1PC) during photostimulation of channelrhodopsin-2-expressing CA3 Schaffer collaterals (SCs), before and after pharmacogenetic silencing of somatostatin-positive (SOM+) interneurons with PSEM308. **(D)** Input–output function for a single representative CA1PC. **(E)** Input–output relationship for a population of CA1PCs before and after silencing SOM+ interneurons; note the change in gain (error bars indicate \pm SEM; ** $P < 0.01$, *** $P < 0.001$, ANOVA with Tukey's test).

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of the output rate (Rothman et al., 2009). At input frequencies up to around 100 Hz a substantial part of the mossy fiber–granule cell STD is due to desensitization of AMPA receptors (Saviane and Silver, 2006; DiGregorio et al., 2007). Thus, a nonlinearity introduced by a conformational change in a synaptic AMPA receptor alters the mathematical operations performed by tonic inhibition at the neuronal level.

Dendritic targeting interneurons and neuronal gain modulation

Distinct interneuron classes innervate proximal and distal regions of pyramidal cells and these can perform different operations on sensory responses. Parvalbumin-expressing interneurons, which form synapses on the soma and proximal dendrites of pyramidal cells, influence spike timing and oscillations (Cobb et al., 1995; Pouille and Scanziani, 2001). In primary visual cortex, optogenetic activation of parvalbumin-expressing interneurons, modulates the gain of visual responses without altering their orientation selectivity (Atallah et al., 2012; Wilson et al., 2012). However, stronger activations of these interneurons can introduce subtractive components that affect orientation selectivity (Lee et al., 2012). Dendrite-targeting somatostatin (SOM)-expressing interneurons can also have subtractive (Wilson et al., 2012) and multiplicative effects on visual responses (Lee et al., 2012). The precise arithmetic operation performed by distinct groups of interneurons on sensory signals depends on a number of factors. These include the relationship between the sensory variable and the synaptic current (Murphy and Miller, 2003), synaptic plasticity (Rothman et al., 2009) and whether the interneurons within a network are co-activated by a particular stimulus. Each of these factors can introduce nonlinearities that transform additive neuronal I-O operations into multiplicative operations (Silver 2010). Indeed, at the level of a neuronal I-O relationship, increasing or decreasing the activity of parvalbumin-expressing interneurons produces a largely additive offset, while modulating dendritic SOM interneurons tends to multiplicatively scale the I-O relationship (Lee et al., 2012; Lovett-Barron et al., 2012) (Fig. 8.5C–E). The relatively strong effects of SOM-expressing interneurons arise from multiple powerful synapses on the distal tuft (Silbergberg and Markram, 2007), which theoretical work suggests are particularly effective at generating centripetal inhibition (Gidon and Segev, 2012). These studies show that inhibition delivered to different regions of the dendritic tree can be configured in different ways to perform distinct operations on sensory stimuli. However, at present there does not seem to be a simple rule that relates inhibitory synapse location to a particular type of arithmetic operation.

NMDA spikes and dendritic amplification

Excitatory synaptic inputs that are spatio-temporally clustered on a dendritic tree produce a greater dendritic depolarization than equivalent inputs that are more uniformly distributed. Thus, unsurprisingly, in acute slices, voltage-dependent NMDA receptor-mediated conductance is preferentially activated by stimulation of clustered inputs or paired-pulse glutamate uncaging protocols (Polsky et al., 2004; Branco et al., 2010). Given the relatively short space constant in fine dendrites (Nevian et al., 2007), it is likely that NMDA receptors can enhance the dendritic gain locally, potentially enabling the neuron to respond selectively to spatio-temporally clustered inputs. Indeed, when the gain of a dendritic branch is sufficiently high, NMDA receptors can sustain nonlinear regenerative dendritic events, introducing a thresholding nonlinearity, which could enhance the ability of the neuron to discriminate patterns of activity (Poirazi et al., 2003).

Dendritic depolarization and an enhanced NMDA receptor conductance over the dendritic tree are both likely to occur during active network states, due to elevated excitatory synaptic input. This will tend to increase the global dendritic gain. Modeling of L5 pyramidal cells suggests that such gain changes could substantially increase the spatial and temporal integration window for synaptic inputs onto the distal tuft (Farinella et al., 2014). Thus, when network activity and distributed NMDA receptor-mediated conductance is low, synaptic NMDA receptors mediate local amplification. By contrast, when network (or subnetwork) activity and the distributed NMDA receptor-mediated conductance is elevated, synaptic NMDA receptors mediate global dendritic amplification. Such widespread increases in dendritic gain could enable spatially distributed

sensory-evoked synaptic inputs (Jia et al., 2010; Chen et al., 2011; Palmer et al., 2014) to evoke NMDA spikes and to be integrated effectively (Farinella et al., 2014). Balanced levels of inhibition, matching those recorded from SOM-positive and neurogliaform interneurons *in vivo*, are predicted to partially counteract these gain changes, enabling inhibition to tune the spatio-temporal integration window of the pyramidal cell tuft and prevent unconstrained positive feedback. Thus, dendritic NMDA receptors and dendritic targeting interneurons are likely to play key roles in setting the properties of dendritic integration *in vivo*, to perform both local and global dendritic gain modulation and to enable pyramidal cells to change their computational properties as a function of the network state.

A number of studies have now established that NMDA spikes are often distributed across more than one dendritic branch *in vivo* (Lavzin et al., 2012; Grienberger et al., 2014; Palmer et al., 2014). These findings are consistent with the idea that gain modulation by synaptic NMDA receptors is more widespread *in vivo* than predicted from experiments in acute brain slices. However, it is possible that spatially distributed Na^+ and Ca^{2+} channels also contribute to the spread of NMDA spikes across multiple branches (Xu et al., 2012) and that transient and sustained K^+ channels dampen them (Harnett et al 2013) (see Chapters 12 and 13). Whatever the precise spatial extent of NMDA spikes, *in vivo* recordings clearly establish that they are involved in sensory processing. Whisker deflection evokes regenerative events in the dendrites of spiny stellate cells in L4 of mouse barrel cortex that are sensitive to hyperpolarization and NMDA receptor antagonists (Lavzin et al., 2012). Multibranch NMDA spikes have also been recorded in L2/3 pyramidal cells in somatosensory cortex during hindlimb stimulation (Palmer et al., 2014). Elegant use of local uncaging of an intracellular receptor antagonist directly confirmed the involvement of NMDA receptors (Palmer et al., 2014). Dendritic patch-clamp recordings from L2/3 cells in the visual cortex have also shown that dendritic NMDA spikes are evoked by visual stimuli (Smith et al., 2013) (Fig. 8.4E,F). Pairing of L1 input and hindlimb stimulation activates the dendritic tuft of L2/3 cells, suggesting that NMDA spikes are also involved in combining L1 inputs and bottom-up sensory inputs in pyramidal cells (Palmer et al., 2014). This result is consistent with established ideas on L5 pyramidal cell function (Larkum et al., 2009), and the findings from barrel cortex in awake animals (although Ca^{2+} spikes may play a key role in these latter cells) (Xu et al., 2012). These studies show that NMDA spikes are widespread in the cortex and that the dendritic amplification they confer plays a role in sensory processing and in the combining of cortical and thalamic input.

Dendritic neurotransmitter receptors and network function

The properties of excitatory and inhibitory synaptic receptors not only influence the computations that individual neurons can perform but also play important roles in network function. In this section we select a few examples to illustrate how the properties of specific neurotransmitter-gated ion channels can affect high-level function.

Additive operations and network function

The additive shifts in the I-O relationship that perisomatic inhibition can perform are important for a number of network properties (Silver, 2010). Thresholding is perhaps the most fundamental of operations: by setting the number of inputs required to trigger a spike this property affects both information flow and the manner in which information is represented. Low thresholds tend to facilitate information transmission while high thresholds aid sparse representations. Sparse codes, which are used in many areas of the brain, are advantageous as the network activity patterns exhibit

little overlap and are energy efficient (Olshausen and Field, 2004). Inhibition plays a key role in setting the trade-off between information transmission and sparse coding, allowing sparse encoding without loss of information within cerebellar networks (Billings et al., 2014). A close match between inhibitory and excitatory synaptic conductances is likely to be particularly important for signal propagation through cortical networks, where large numbers of synaptic inputs are required to reach the threshold. Additive operations arising from shifts in the threshold are particularly powerful when combined with probabilistic Poisson-like population codes, allowing a range of network computations including encoding uncertainty in sensory variables and multisensory integration (Ma et al., 2006; Seilheimer et al., 2014).

Dendritic gain and network function

Recent *in vivo* studies have revealed that NMDA receptor-mediated conductances, which alter dendritic gain in an activity-dependent manner, play important roles in feature detection in a range of different systems. In the rodent barrel cortex, NMDA spikes in spiny stellate cells contribute to the selectivity of whisker tuning (Lavzin et al., 2012), while in L2/3 pyramidal cells in the primary visual cortex they sharpen orientation selectivity (Smith et al., 2013). These results, together with the finding that cortical neurons with specific receptive field properties are preferentially connected, forming subnetworks (Ko et al., 2013), suggest that both the synaptic connectivity and nonlinear dendritic amplification are important for feature detection. While most studies focus on the dendritic computations mediated by nonlinear NMDA receptor-mediated conductances, a recent theoretical study suggests that NMDA spikes could act as optimal filters for reconstructing the presynaptic population activity from synaptic inputs in cortical networks with synchronized up and down states (Ujfaluissy and Lengyel, 2011).

Simulations predict that the neurotransmitter and voltage dependence of dendritic NMDA receptors could provide a mechanism that enables L5 pyramidal cells to change their integrative properties as a function of the network state (Farinella et al., 2014). This could potentially enable thalamo-cortical and cortico-cortical inputs to be integrated in pyramidal cells over longer time scales than previously envisaged, and for subgroups of L5 pyramidal cells located within activated patches of cortex to communicate selectively. The concept of “functional connectivity” is attractive because it would enable the same network to perform many different functions, thereby providing much more powerful network processing than a fixed “hard wired” network (Haider and McCormick, 2009). Thus, by enabling network state-dependent synaptic integration, NMDA receptors illustrate how the fundamental properties of neurotransmitter-gated ion channels could play a role in high-level emergent properties such as attention (Haider and McCormick, 2009; Farinella et al., 2014).

Multiplicative neuronal operations, which can be performed by inhibition in the presence of a change in noise, by short-term plasticity, or by coactivation of interneurons with the stimulus, are essential for a wide range of network behaviors (Fig. 8.5). At its most basic level, modulation of neuronal gain allows the limited dynamic range of the neuron to be matched to the synaptic input. This prevents saturation and ensures efficient information flow through the network (Fairhall et al., 2001; Billings et al., 2014). For spike-timing-based codes, neuronal gain can determine the temporal properties of signal propagation through a network (Diesmann et al., 1999). Thus, inhibition-based neuronal gain modulation may also enable a network to route information in different ways depending on the state of the network (Haider and McCormick, 2009). Neuronal gain modulation mediated by inhibition is involved in divisive normalization, an operation that is ubiquitous in sensory processing (Carandini and Heeger, 2012). This enables neuronal selectivity to be maintained under a wide range

of conditions, with contrast invariance of orientation tuning being the most extensively studied example of this. Indeed, multiplicative neuronal operations are essential for a wide range of network computations (Salinas and Thier, 2000; Silver, 2010), including the coordinate transforms that are required for complex multimodal behavior such as visually guided reaching tasks (Brotchie et al., 1995; Salinas and Abbott, 1995).

Conclusions

The studies reviewed here demonstrate a rapid evolution in our understanding of the role of dendritic neurotransmitter-gated ion channels in neuronal signaling and information processing. Although we have been able to cover only a fraction of the published work, it is clear that the interplay between the biophysical properties of the receptors themselves, the plasticity of their expression and location, together with the passive and active properties of dendrites, enables individual neurons to perform complex spatio-temporal computations. Recent *in vivo* studies demonstrate that these computations play important roles in information processing within a range of sensory systems. Clearly, an appreciation of the fundamental properties and heterogeneity of neurotransmitter-gated ion channels is important for a mechanistic understanding of high-level brain function. In the future it will be exciting to explore how the receptor properties we have described in this chapter contribute to the adaptive functional connectivity of networks and to the computations they carry out, as both of these functions are thought to play key roles in sensory processing and the generation of behavior.

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References

- Abbott LF, Varela JA, Sen K, Nelson SB (1997) Synaptic depression and cortical gain control. *Science* **275**:220–224.
- Abraham WC, Bear MF (1996) Metaplasticity: the plasticity of synaptic plasticity. *Trends in Neurosciences* **19**:126–130.
- Abrahamsson T, Cathala L, Matsui K, Shigemoto R, Digregorio DA (2012) Thin dendrites of cerebellar interneurons confer sublinear synaptic integration and a gradient of short-term plasticity. *Neuron* **73**:1159–1172.
- Ahrens S, Jaramillo S, Yu K, Ghosh S, Hwang G-R, Paik R, Lai C, He M, Huang ZJ, Li B (2015) ErbB4 regulation of a thalamic reticular nucleus circuit for sensory selection. *Nature Neuroscience* **18**:104–111.
- Ali AB, Thomson AM (2008) Synaptic α 5 subunit-containing GABA_A receptors mediate IPSPs elicited by dendrite-preferring cells in rat neocortex. *Cerebral Cortex* **18**:1260–1271.
- Alvarez FJ, Dewey DE, Harrington DA, Fyffe RE (1997) Cell-type specific organization of glycine receptor clusters in the mammalian spinal cord. *Journal of Comparative Neurology* **379**:150–170.
- Aman TK, Maki BA, Ruffino TJ, Kasperek EM, Popescu GK (2014) Separate intramolecular targets for protein kinase A control N-methyl-D-aspartate receptor gating and Ca²⁺ permeability. *Journal of Biological Chemistry* **289**:18805–18817.
- Anderson JS, Lampl I, Gillespie DC, Ferster D (2000) The contribution of noise to contrast invariance of orientation tuning in cat visual cortex. *Science* **290**:1968–1972.
- Andrasfalvy BK, Magee JC (2004) Changes in AMPA receptor currents following LTP induction on rat CA1 pyramidal neurones. *Journal of Physiology* **559**:543–554.

- Andrasfalvy BK, Mody I (2006) Differences between the scaling of miniature IPSCs and EPSCs recorded in the dendrites of CA1 mouse pyramidal neurons. *Journal of Physiology* **576**:191–196.
- Andrasfalvy BK, Smith MA, Borchardt T, Sprengel R, Magee JC (2003) Impaired regulation of synaptic strength in hippocampal neurons from GluR1-deficient mice. *Journal of Physiology* **552**:35–45.
- Anggono V, Huganir RL (2012) Regulation of AMPA receptor trafficking and synaptic plasticity. *Current Opinion in Neurobiology* **22**:461–469.
- Atallah BV, Bruns W, Carandini M, Scanziani M (2012) Parvalbumin-expressing interneurons linearly transform cortical responses to visual stimuli. *Neuron* **73**:159–170.
- Backus KH, Arigoni M, Drescher U, Scheurer L, Malherbe P, Mohler H, Benson JA (1993) Stoichiometry of a recombinant GABA_A receptor deduced from mutation-induced rectification. *NeuroReport* **5**:285–288.
- Bannai H, Levi S, Schweizer C, Inoue T, Launey T, Racine V, Sibarita JB, Mikoshiba K, Triller A (2009) Activity-dependent tuning of inhibitory neurotransmission based on GABA_AR diffusion dynamics. *Neuron* **62**:670–682.
- Bar-Ilan L, Gidon A, Segev I (2012) The role of dendritic inhibition in shaping the plasticity of excitatory synapses. *Frontiers in Neural Circuits* **6**:118.
- Barberis A, Petrini EM, Mozrzymas JW (2011) Impact of synaptic neurotransmitter concentration time course on the kinetics and pharmacological modulation of inhibitory synaptic currents. *Frontiers in Cellular Neuroscience* **5**:6.
- Barbour B, Häusser M (1997) Intersynaptic diffusion of neurotransmitter. *Trends in Neurosciences* **20**:377–384.
- Bartley AF, Huang ZJ, Huber KM, Gibson JR (2008) Differential activity-dependent, homeostatic plasticity of two neocortical inhibitory circuits. *Journal of Neurophysiology* **100**:1983–1994.
- Bats C, Soto D, Studniarczyk D, Farrant M, Cull-Candy SG (2012) Channel properties reveal differential expression of TARPed and TARPress AMPARs in stargazer neurons. *Nature Neuroscience* **15**:853–861.
- Baumann SW, Baur R, Sigel E (2002) Forced subunit assembly in $\alpha 1\beta 2\gamma 2$ GABA_A receptors. Insight into the absolute arrangement. *Journal of Biological Chemistry* **277**:46020–46025.
- Baur R, Minier F, Sigel E (2006) A GABA_A receptor of defined subunit composition and positioning: concatenation of five subunits. *FEBS Letters* **580**:1616–1620.
- Bekkers JM, Stevens CF (1989) NMDA and non-NMDA receptors are co-localized at individual excitatory synapses in cultured rat hippocampus. *Nature* **341**:230–233.
- Benke TA, Luthi A, Isaac JT, Collingridge GL (1998) Modulation of AMPA receptor unitary conductance by synaptic activity. *Nature* **393**:793–797.
- Benson DL, Colman DR, Huntley GW (2001) Molecules, maps and synapse specificity. *Nature Reviews Neuroscience* **2**:899–909.
- Bianchi MT, Haas KF, Macdonald RL (2002) $\alpha 1$ and $\alpha 6$ subunits specify distinct desensitization, deactivation and neurosteroid modulation of GABA_A receptors containing the delta subunit. *Neuropharmacology* **43**:492–502.
- Billings G, Piasini E, Lorincz A, Nusser Z, Silver RA (2014) Network structure within the cerebellar input layer enables lossless sparse encoding. *Neuron* **83**:960–974.
- Binshtok AM, Fleidervish IA, Sprengel R, Gutnick MJ (2006) NMDA receptors in layer 4 spiny stellate cells of the mouse barrel cortex contain the NR2C subunit. *Journal of Neuroscience* **26**:708–715.
- Bliss TV, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* **361**:31–39.
- Bloodgood BL, Sharma N, Browne HA, Trepman AZ, Greenberg ME (2013) The activity-dependent transcription factor NPAS4 regulates domain-specific inhibition. *Nature* **503**:121–125.
- Borg-Graham LJ, Monier C, Fregnac Y (1998) Visual input evokes transient and strong shunting inhibition in visual cortical neurons. *Nature* **393**:369–373.

- Bowie D, Mayer ML (1995) Inward rectification of both AMPA and kainate subtype glutamate receptors generated by polyamine-mediated ion channel block. *Neuron* **15**:453–462.
- Branco T, Häusser M (2011) Synaptic integration gradients in single cortical pyramidal cell dendrites. *Neuron* **69**:885–892.
- Branco T, Staras K, Darcy KJ, Goda Y (2008) Local dendritic activity sets release probability at hippocampal synapses. *Neuron* **59**:475–485.
- Branco T, Clark BA, Häusser M (2010) Dendritic discrimination of temporal input sequences in cortical neurons. *Science* **329**:1671–1675.
- Bredt DS, Nicoll RA (2003) AMPA receptor trafficking at excitatory synapses. *Neuron* **40**:361–379.
- Brickley SG, Cull-Candy SG, Farrant M (1999) Single-channel properties of synaptic and extrasynaptic GABA_A receptors suggest differential targeting of receptor subtypes. *Journal of Neuroscience* **19**:2960–2973.
- Brickley SG, Mody I (2012) Extrasynaptic GABA_A receptors: their function in the CNS and implications for disease. *Neuron* **73**:23–34.
- Bright DP, Renzi M, Bartram J, McGee TP, MacKenzie G, Hosie AM, Farrant M, Brickley SG (2011) Profound desensitization by ambient GABA limits activation of δ-containing GABA_A receptors during spillover. *Journal of Neuroscience* **31**:753–763.
- Britt JP, Benaliouad F, McDevitt RA, Stuber GD, Wise RA, Bonci A (2012) Synaptic and behavioral profile of multiple glutamatergic inputs to the nucleus accumbens. *Neuron* **76**:790–803.
- Brotchie PR, Andersen RA, Snyder LH, Goodman SJ (1995) Head position signals used by parietal neurons to encode locations of visual stimuli. *Nature* **375**:232–235.
- Browne SH, Kang J, Akk G, Chiang LW, Schulman H, Huguenard JR, Prince DA (2001) Kinetic and pharmacological properties of GABA_A receptors in single thalamic neurons and GABA_A subunit expression. *Journal of Neurophysiology* **86**:2312–2322.
- Burgard EC, Tietz EI, Neelands TR, Macdonald RL (1996) Properties of recombinant γ-aminobutyric acid_A receptor isoforms containing the α5 subunit subtype. *Molecular Pharmacology* **50**:119–127.
- Buzsaki G (1984) Feed-forward inhibition in the hippocampal formation. *Progress in Neurobiology* **22**:131–153.
- Cajigas IJ, Tushev G, Will TJ, tom Dieck S, Fuerst N, Schuman EM (2012) The local transcriptome in the synaptic neuropil revealed by deep sequencing and high-resolution imaging. *Neuron* **74**:453–466.
- Capogna M, Pearce RA (2011) GABA_{A,slow}: causes and consequences. *Trends in Neurosciences* **34**:101–112.
- Carandini M, Heeger DJ (2012) Normalization as a canonical neural computation. *Nature Reviews Neuroscience* **13**:51–62.
- Cash S, Yuste R (1999) Linear summation of excitatory inputs by CA1 pyramidal neurons. *Neuron* **22**:383–394.
- Cathala L, Brickley S, Cull-Candy S, Farrant M (2003) Maturation of EPSCs and intrinsic membrane properties enhances precision at a cerebellar synapse. *Journal of Neuroscience* **23**:6074–6085.
- Cathala L, Holderith NB, Nusser Z, DiGregorio DA, Cull-Candy SG (2005) Changes in synaptic structure underlie the developmental speeding of AMPA receptor-mediated EPSCs. *Nature Neuroscience* **8**:1310–1318.
- Cathala L, Misra C, Cull-Candy S (2000) Developmental profile of the changing properties of NMDA receptors at cerebellar mossy fiber-granule cell synapses. *Journal of Neuroscience* **20**:5899–5905.
- Caze RD, Humphries M, Gutkin B (2013) Passive dendrites enable single neurons to compute linearly non-separable functions. *PLoS Computational Biology* **9**:e1002867.
- Chadderton P, Schaefer AT, Williams SR, Margrie TW (2014) Sensory-evoked synaptic integration in cerebellar and cerebral cortical neurons. *Nature Reviews Neuroscience* **15**:71–83.

- Chalifoux JR, Carter AG (2010) GABA_B receptors modulate NMDA receptor calcium signals in dendritic spines. *Neuron* **66**:101–113.
- Chance FS, Abbott LF, Reyes AD (2002) Gain modulation from background synaptic input. *Neuron* **35**:773–782.
- Chang Y, Wang R, Barot S, Weiss DS (1996) Stoichiometry of a recombinant GABA_A receptor. *Journal of Neuroscience* **16**:5415–5424.
- Chavas J, Marty A (2003) Coexistence of excitatory and inhibitory GABA synapses in the cerebellar interneuron network. *Journal of Neuroscience* **23**:2019–2031.
- Chen L, Chetkovich DM, Petralia RS, Sweeney NT, Kawasaki Y, Wenthold RJ, Bredt DS, Nicoll RA (2000) Stargazin regulates synaptic targeting of AMPA receptors by two distinct mechanisms. *Nature* **408**:936–943.
- Chen X, Leischner U, Rochefort NL, Nelken I, Konnerth A (2011) Functional mapping of single spines in cortical neurons in vivo. *Nature* **475**:501–505.
- Clark BA, Cull-Candy SG (2002) Activity-dependent recruitment of extrasynaptic NMDA receptor activation at an AMPA receptor-only synapse. *Journal of Neuroscience* **22**:4428–4436.
- Clements JD, Lester RA, Tong G, Jahr CE, Westbrook GL (1992) The time course of glutamate in the synaptic cleft. *Science* **258**:1498–1501.
- Cobb SR, Buhl EH, Halasy K, Paulsen O, Somogyi P (1995) Synchronization of neuronal activity in hippocampus by individual GABAergic interneurons. *Nature* **378**:75–78.
- Cook EP, Johnston D (1999) Voltage-dependent properties of dendrites that eliminate location-dependent variability of synaptic input. *Journal of Neurophysiology* **81**:535–543.
- Copits BA, Swanson GT (2012) Dancing partners at the synapse: auxiliary subunits that shape kainate receptor function. *Nature Reviews Neuroscience* **13**:675–686.
- Cox DJ, Racca C (2013) Differential dendritic targeting of AMPA receptor subunit mRNAs in adult rat hippocampal principal neurons and interneurons. *Journal of Comparative Neurology* **521**:1954–2007.
- Cull-Candy S, Brickley S, Farrant M (2001) NMDA receptor subunits: diversity, development and disease. *Current Opinion in Neurobiology* **11**:327–335.
- Cull-Candy S, Kelly L, Farrant M (2006) Regulation of Ca²⁺-permeable AMPA receptors: synaptic plasticity and beyond. *Current Opinion in Neurobiology* **16**:288–297.
- D'Angelo E, De Filippi G, Rossi P, Taglietti V (1995) Synaptic excitation of individual rat cerebellar granule cells *in situ*: evidence for the role of NMDA receptors. *Journal of Physiology* **484**:397–413.
- Davis GW (2006) Homeostatic control of neural activity: from phenomenology to molecular design. *Annual Review of Neuroscience* **29**:307–323.
- Delaney AJ, Sedlak PL, Autuori E, Power JM, Sah P (2013) Synaptic NMDA receptors in basolateral amygdala principal neurons are triheteromeric proteins: physiological role of GluN2B subunits. *Journal of Neurophysiology* **109**:1391–1402.
- Derkach V, Barria A, Soderling TR (1999) Ca²⁺/calmodulin-kinase II enhances channel conductance of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate type glutamate receptors. *Proceedings of the National Academy of Sciences of the United States of America* **96**:3269–3274.
- Desai NS, Cudmore RH, Nelson SB, Turrigiano GG (2002) Critical periods for experience-dependent synaptic scaling in visual cortex. *Nature Neuroscience* **5**:783–789.
- De Schutter E, Bower JM (1994) Simulated responses of cerebellar Purkinje cells are independent of the dendritic location of granule cell synaptic inputs. *Proceedings of the National Academy of Sciences of the United States of America* **91**:4736–4740.
- Destexhe A, Rudolph M, Pare D (2003) The high-conductance state of neocortical neurons in vivo. *Nature Reviews Neuroscience* **4**:739–751.
- Diamond JS, Jahr CE (1997) Transporters buffer synaptically released glutamate on a submillisecond time scale. *Journal of Neuroscience* **17**:4672–4687.

- Diesmann M, Gewaltig MO, Aertsen A (1999) Stable propagation of synchronous spiking in cortical neural networks. *Nature* **402**:529–533.
- DiGregorio DA, Nusser Z, Silver RA (2002) Spillover of glutamate onto synaptic AMPA receptors enhances fast transmission at a cerebellar synapse. *Neuron* **35**:521–533.
- DiGregorio DA, Rothman JS, Nielsen TA, Silver RA (2007) Desensitization properties of AMPA receptors at the cerebellar mossy fiber granule cell synapse. *Journal of Neuroscience* **27**:8344–8357.
- Ding J, Peterson JD, Surmeier DJ (2008) Corticostriatal and thalamostriatal synapses have distinctive properties. *Journal of Neuroscience* **28**:6483–6492.
- Ding JB, Oh W-J, Sabatini BL, Gu C (2011) Semaphorin 3E-Plexin-D1 signaling controls pathway-specific synapse formation in the striatum. *Nature Neuroscience* **15**:215–223.
- Dixon C, Sah P, Lynch JW, Keramidas A (2014) GABA_A receptor α and γ subunits shape synaptic currents via different mechanisms. *Journal of Biological Chemistry* **289**:5399–5411.
- Druckmann S, Feng L, Lee B, Yook C, Zhao T, Magee JC, Kim J (2014) Structured synaptic connectivity between hippocampal regions. *Neuron* **81**:629–640.
- Eaton MM, Bracamontes J, Shu HJ, Li P, Mennerick S, Steinbach JH, Akk G (2014) γ-Aminobutyric acid type A α4, β2, and δ subunits assemble to produce more than one functionally distinct receptor type. *Molecular Pharmacology* **86**:647–656.
- Edmonds B, Gibb AJ, Colquhoun D (1995) Mechanisms of activation of glutamate receptors and the time course of excitatory synaptic currents. *Annual Review of Physiology* **57**:495–519.
- von Engelhardt J, Mack V, Sprengel R, Kavenstock N, Li KW, Stern-Bach Y, Smit AB, Seburg PH, Monyer H (2010) CKAMP44: a brain-specific protein attenuating short-term synaptic plasticity in the dentate gyrus. *Science* **327**:1518–1522.
- Evans RC, Morera-Herreras T, Cui Y, Du K, Sheehan T, Kotalski JH, Venance L, Blackwell KT (2012) The effects of NMDA subunit composition on calcium influx and spike timing-dependent plasticity in striatal medium spiny neurons. *PLoS Computational Biology* **8**:e1002493.
- Eyre MD, Renzi M, Farrant M, Nusser Z (2012) Setting the time course of inhibitory synaptic currents by mixing multiple GABA_A receptor α subunit isoforms. *Journal of Neuroscience* **32**:5853–5867.
- Fairhall AL, Lewen GD, Bialek W, de Ruyter Van Steveninck RR (2001) Efficiency and ambiguity in an adaptive neural code. *Nature* **412**:787–792.
- Farinella M, Ruedt DT, Gleeson P, Lanore F, Silver RA (2014) Glutamate-bound NMDARs arising from in vivo-like network activity extend spatio-temporal integration in a L5 cortical pyramidal cell model. *PLoS Computational Biology* **10**:e1003590.
- Farrant M, Kaila K (2007) The cellular, molecular and ionic basis of GABA_A receptor signalling. *Progress in Brain Research* **160**:59–87.
- Farrant M, Nusser Z (2005) Variations on an inhibitory theme: phasic and tonic activation of GABA_A receptors. *Nature Reviews Neuroscience* **6**:215–229.
- Farrant M, Feldmeyer D, Takahashi T, Cull-Candy SG (1994) NMDA-receptor channel diversity in the developing cerebellum. *Nature* **368**:335–339.
- Feldmeyer D, Egger V, Lubke J, Sakmann B (1999) Reliable synaptic connections between pairs of excitatory layer 4 neurones within a single “barrel” of developing rat somatosensory cortex. *Journal of Physiology* **521**:169–190.
- Fino E, Yuste R (2011) Dense inhibitory connectivity in neocortex. *Neuron* **69**:1188–1203.
- Fritschy JM, Mohler H (1995) GABA_A-receptor heterogeneity in the adult rat brain: differential regional and cellular distribution of seven major subunits. *Journal of Comparative Neurology* **359**:154–194.
- Fritschy JM, Weinmann O, Wenzel A, Benke D (1998) Synapse-specific localization of NMDA and GABA_A receptor subunits revealed by antigen-retrieval immunohistochemistry. *Journal of Comparative Neurology* **390**:194–210.
- Froemke RC, Poo M-M, Dan Y (2005) Spike-timing-dependent synaptic plasticity depends on dendritic location. *Nature* **434**:221–225.

- Fukaya M, Yamazaki M, Sakimura K, Watanabe M (2005) Spatial diversity in gene expression for VDCC γ subunit family in developing and adult mouse brains. *Neuroscience Research* **53**:376–383.
- Gasparini S, Migliore M, Magee JC (2004) On the initiation and propagation of dendritic spikes in CA1 pyramidal neurons. *Journal of Neuroscience* **24**:11046–11056.
- Gentet LJ, Kremer Y, Taniguchi H, Huang ZJ, Staiger JF, Petersen CC (2012) Unique functional properties of somatostatin-expressing GABAergic neurons in mouse barrel cortex. *Nature Neuroscience* **15**:607–612.
- Gidon A, Segev I (2012) Principles governing the operation of synaptic inhibition in dendrites. *Neuron* **75**:330–341.
- Gingrich KJ, Roberts WA, Kass RS (1995) Dependence of the GABA_A receptor gating kinetics on the α -subunit isoform: implications for structure-function relations and synaptic transmission. *Journal of Physiology* **489**:529–543.
- Glickfeld LL, Roberts JD, Somogyi P, Scanziani M (2009) Interneurons hyperpolarize pyramidal cells along their entire somatodendritic axis. *Nature Neuroscience* **12**:21–23.
- Golding NL, Spruston N (1998) Dendritic sodium spikes are variable triggers of axonal action potentials in hippocampal CA1 pyramidal neurons. *Neuron* **21**:1189–1200.
- Gonzalez-Islas C, Wenner P (2006) Spontaneous network activity in the embryonic spinal cord regulates AMPAergic and GABAergic synaptic strength. *Neuron* **49**:563–575.
- Govindarajan A, Israely I, Huang S-Y, Tonegawa S (2011) The dendritic branch is the preferred integrative unit for protein synthesis-dependent LTP. *Neuron* **69**:132–146.
- Gray JA, Shi Y, Usui H, During MJ, Sakimura K, Nicoll RA (2011) Distinct modes of AMPA receptor suppression at developing synapses by GluN2A and GluN2B: single-cell NMDA receptor subunit deletion in vivo. *Neuron* **71**:1085–1101.
- Grienberger C, Chen X, Konnerth A (2014) NMDA receptor-dependent multidendrite Ca²⁺ spikes required for hippocampal burst firing in vivo. *Neuron* **81**:1274–1281.
- Groen MR, Paulsen O, Perez-Garcí E, Nevian T, Wortel J, Dekker MP, Mansvelder HD, van Ooyen A, Meredith RM (2014) Development of dendritic tonic GABAergic inhibition regulates excitability and plasticity in CA1 pyramidal neurons. *Journal of Neurophysiology* **112**:287–299.
- Gross A, Sims RE, Swinny JD, Sieghart W, Bolam JP, Stanford IM (2011) Differential localization of GABA_A receptor subunits in relation to rat striatopallidal and pallidopallidal synapses. *European Journal of Neuroscience* **33**:868–878.
- Gulledge AT, Stuart GJ (2003) Excitatory actions of GABA in the cortex. *Neuron* **37**:299–309.
- Haering SC, Tapken D, Pahl S, Hollmann M (2014) Auxiliary subunits: shepherding AMPA receptors to the plasma membrane. *Membranes* **4**:469–490.
- Haider B, McCormick DA (2009) Rapid neocortical dynamics: cellular and network mechanisms. *Neuron* **62**:171–189.
- Haider B, Häusser M, Carandini M (2013) Inhibition dominates sensory responses in the awake cortex. *Nature* **493**:97–100.
- Hansen KB, Ogden KK, Yuan H, Traynelis SF (2014) Distinct functional and pharmacological properties of Triheteromeric GluN1/GluN2A/GluN2B NMDA receptors. *Neuron* **81**:1084–1096.
- Harnett MT, Xu NL, Magee JC, Williams SR (2013) Potassium channels control the interaction between active dendritic integration compartments in layer 5 cortical pyramidal neurons. *Neuron* **79**:516–529.
- Harnett MT, Magee JC, Williams SR (2015) Distribution and function of HCN channels in the apical dendritic tuft of neocortical pyramidal neurons. *Journal of Neuroscience* **35**:1024–1037.
- Harney SC, Jane DE, Anwyll R (2008) Extrasynaptic NR2D-containing NMDARs are recruited to the synapse during LTP of NMDAR-EPSCs. *Journal of Neuroscience* **28**:11685–11694.
- Harris KM, Weinberg RJ (2012) Ultrastructure of synapses in the mammalian brain. *Cold Spring Harbor Perspectives in Biology* **4**(5): a005587.

- Harvey CD, Svoboda K (2007) Locally dynamic synaptic learning rules in pyramidal neuron dendrites. *Nature* **450**:1195–1200.
- Harvey CD, Yasuda R, Zhong H, Svoboda K (2008) The spread of Ras activity triggered by activation of a single dendritic spine. *Science* **321**:136–140.
- Häusser M (1994) Kinetics of excitatory synaptic currents in Purkinje cells studied using dendritic patch-clamp recording. *Society for Neuroscience Abstracts* **20**:891.
- Häusser M, Roth A (1997) Estimating the time course of the excitatory synaptic conductance in neocortical pyramidal cells using a novel voltage jump method. *Journal of Neuroscience* **17**:7606–7625.
- Hayama T, Noguchi J, Watanabe S, Takahashi N, Hayashi-Takagi A, Ellis-Davies GCR, Matsuzaki M, Kasai H (2013) GABA promotes the competitive selection of dendritic spines by controlling local Ca^{2+} signaling. *Nature Neuroscience* **16**:1409–1416.
- Herd MB, Brown AR, Lambert JJ, Belelli D (2013) Extrasynaptic GABA_A receptors couple presynaptic activity to postsynaptic inhibition in the somatosensory thalamus. *Journal of Neuroscience* **33**:14850–14868.
- Hofer SB, Mrsic-Flogel TD, Bonhoeffer T, Hübener M (2009) Experience leaves a lasting structural trace in cortical circuits. *Nature* **457**:313–317.
- Holt CE, Schuman EM (2013) The central dogma decentralized: new perspectives on RNA function and local translation in neurons. *Neuron* **80**:648–657.
- Holt GR, Koch C (1997) Shunting inhibition does not have a divisive effect on firing rates. *Neural Computation* **9**:1001–1013.
- Holtmaat A, Wilbrecht L, Knott GW, Welker E, Svoboda K (2006) Experience-dependent and cell-type-specific spine growth in the neocortex. *Nature* **441**:979–983.
- Horak M, Petralia RS, Kaniakova M, Sans N (2014) ER to synapse trafficking of NMDA receptors. *Frontiers in Cellular Neuroscience* **8**:394.
- Hortnagl H, Tasan RO, Wieselthaler A, Kirchmair E, Sieghart W, Sperk G (2013) Patterns of mRNA and protein expression for 12 GABA_A receptor subunits in the mouse brain. *Neuroscience* **236**:345–372.
- Hou Q, Zhang D, Jarzylo L, Huganir RL, Man H-Y (2008) Homeostatic regulation of AMPA receptor expression at single hippocampal synapses. *Proceedings of the National Academy of Sciences of the United States of America* **105**:775–780.
- Howe JR (2015) Modulation of non-NMDA receptor gating by auxiliary subunits. *Journal of Physiology* **593**:61–72.
- Hoze N, Nair D, Hosy E, Sieben C, Manley S, Herrmann A, Sibarita JB, Choquet D, Holcman D (2012) Heterogeneity of AMPA receptor trafficking and molecular interactions revealed by superresolution analysis of live cell imaging. *Proceedings of the National Academy of Sciences of the United States of America* **109**:17052–17057.
- Huganir RL, Nicoll RA (2013) AMPARs and synaptic plasticity: the last 25 years. *Neuron* **80**:704–717.
- Humeau Y, Herry C, Kemp N, Shaban H, Fourcaudot E, Bissière S, Lüthi A (2005) Dendritic spine heterogeneity determines afferent-specific Hebbian plasticity in the amygdala. *Neuron* **45**:119–131.
- Humeau Y, Reisel D, Johnson AW, Borchardt T, Jensen V, Gebhardt C, Bosch V, Gass P, Bannerman DM, Good MA, Hvalby Ø, Sprengel R, Lüthi A (2007) A pathway-specific function for different AMPA receptor subunits in amygdala long-term potentiation and fear conditioning. *Journal of Neuroscience* **27**:10947–10956.
- Iansek R, Redman SJ (1973) The amplitude, time course and charge of unitary excitatory post-synaptic potentials evoked in spinal motoneurone dendrites. *Journal of Physiology* **234**:665–688.
- Isaac JT, Crair MC, Nicoll RA, Malenka RC (1997) Silent synapses during development of thalamocortical inputs. *Neuron* **18**:269–280.
- Isaacson JS, Scanziani M (2011) How inhibition shapes cortical activity. *Neuron* **72**:231–243.
- Jack JJ, Redman SJ, Wong K (1981) The components of synaptic potentials evoked in cat spinal motoneurones by impulses in single group Ia afferents. *Journal of Physiology* **321**:65–96.

- Jackson AC, Nicoll RA (2011) The expanding social network of ionotropic glutamate receptors: TARPs and other transmembrane auxiliary subunits. *Neuron* **70**:178–199.
- Jadi M, Polksky A, Schiller J, Mel BW (2012) Location-dependent effects of inhibition on local spiking in pyramidal neuron dendrites. *PLoS Computational Biology* **8**:e1002550.
- Jenkins MA, Wells G, Bachman J, Snyder JP, Jenkins A, Huganir RL, Oswald RE, Traynelis SF (2014) Regulation of GluA1 alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor function by protein kinase C at serine-818 and threonine-840. *Molecular Pharmacology* **85**:618–629.
- Jia H, Rochefort NL, Chen X, Konnerth A (2010) Dendritic organization of sensory input to cortical neurons in vivo. *Nature* **464**:1307–1312.
- Johnston D, Brown TH (1983) Interpretation of voltage-clamp measurements in hippocampal neurons. *Journal of Neurophysiology* **50**:464–486.
- Jonas P (1993) Glutamate receptors in the central nervous system. *Annals of the New York Academy of Sciences* **707**:126–135.
- Jonas P, Spruston N (1994) Mechanisms shaping glutamate-mediated excitatory postsynaptic currents in the CNS. *Current Opinion in Neurobiology* **4**:366–372.
- Jonas P, Major G, Sakmann B (1993) Quantal components of unitary EPSCs at the mossy fibre synapse on CA3 pyramidal cells of rat hippocampus. *Journal of Physiology* **472**:615–663.
- Ju W, Morishita W, Tsui J, Gaietta G, Deerinck TJ, Adams SR, Garner CC, Tsien RY, Ellisman MH, Malenka RC (2004) Activity-dependent regulation of dendritic synthesis and trafficking of AMPA receptors. *Nature Neuroscience* **7**:244–253.
- Kaila K, Price TJ, Payne JA, Puskarjov M, Voipio J (2014) Cation-chloride cotransporters in neuronal development, plasticity and disease. *Nature Reviews Neuroscience* **15**:637–654.
- Kamboj SK, Swanson GT, Cull-Candy SG (1995) Intracellular spermine confers rectification on rat calcium-permeable AMPA and kainate receptors. *Journal of Physiology* **486**:297–303.
- Karayannidis T, Elfant D, Huerta-Ocampo I, Teki S, Scott RS, Rusakov DA, Jones MV, Capogna M (2010) Slow GABA transient and receptor desensitization shape synaptic responses evoked by hippocampal neurogliaform cells. *Journal of Neuroscience* **30**:9898–9909.
- Kasugai Y, Swinny JD, Roberts JD, Dalezios Y, Fukazawa Y, Sieghart W, Shigemoto R, Somogyi P (2010) Quantitative localisation of synaptic and extrasynaptic GABA_A receptor subunits on hippocampal pyramidal cells by freeze-fracture replica immunolabelling. *European Journal of Neuroscience* **32**:1868–1888.
- Katz Y, Menon V, Nicholson DA, Geinisman Y, Kath WL, Spruston N (2009) Synapse distribution suggests a two-stage model of dendritic integration in CA1 pyramidal neurons. *Neuron* **63**:171–177.
- Kawaguchi Y, Kubota Y (1997) GABAergic cell subtypes and their synaptic connections in rat frontal cortex. *Cerebral Cortex* **7**:476–486.
- Keck T, Keller GB, Jacobsen RI, Eysel UT, Bonhoeffer T, Hübener M (2013) Synaptic scaling and homeostatic plasticity in the mouse visual cortex in vivo. *Neuron* **80**:327–334.
- Kepcs A, Fishell G (2014) Interneuron cell types are fit to function. *Nature* **505**:318–326.
- Kessels HW, Malinow R (2009) Synaptic AMPA receptor plasticity and behavior. *Neuron* **61**:340–350.
- Kim J, Tsien RW (2008) Synapse-specific adaptations to inactivity in hippocampal circuits achieve homeostatic gain control while dampening network reverberation. *Neuron* **58**:925–937.
- Klausberger T, Somogyi P (2008) Neuronal diversity and temporal dynamics: the unity of hippocampal circuit operations. *Science* **321**:53–57.
- Klausberger T, Roberts JD, Somogyi P (2002) Cell type- and input-specific differences in the number and subtypes of synaptic GABA(A) receptors in the hippocampus. *Journal of Neuroscience* **22**:2513–2521.
- Ko H, Cossell L, Baragli C, Antolik J, Clopath C, Hofer SB, Mrsic-Flogel TD (2013) The emergence of functional microcircuits in visual cortex. *Nature* **496**:96–100.
- Koch C, Poggio T, Torre V (1983) Nonlinear interactions in a dendritic tree: localization, timing, and role in information processing. *Proceedings of the National Academy of Sciences of the United States of America* **80**:2799–2802.

- Koh DS, Burnashev N, Jonas P (1995) Block of native Ca^{2+} -permeable AMPA receptors in rat brain by intracellular polyamines generates double rectification. *Journal of Physiology* **486**:305–312.
- Kozorovitskiy Y, Saunders A, Johnson CA, Lowell BB, Sabatini BL (2012) Recurrent network activity drives striatal synaptogenesis. *Nature* **485**:646–650.
- Kruk PJ, Korn H, Faber DS (1997) The effects of geometrical parameters on synaptic transmission: a Monte Carlo simulation study. *Biophysical Journal* **73**:2874–2890.
- Kullmann DM, Lamsa KP (2007) Long-term synaptic plasticity in hippocampal interneurons. *Nature Reviews Neuroscience* **8**:687–699.
- Kullmann DM, Moreau AW, Bakiri Y, Nicholson E (2012) Plasticity of inhibition. *Neuron* **75**:951–962.
- Kumar J, Mayer ML (2013) Functional insights from glutamate receptor ion channel structures. *Annual Review of Physiology* **75**:313–337.
- Kumar SS, Huguenard JR (2003) Pathway-specific differences in subunit composition of synaptic NMDA receptors on pyramidal neurons in neocortex. *Journal of Neuroscience* **23**:10074–10083.
- Kuner T, Schoepfer R (1996) Multiple structural elements determine subunit specificity of Mg^{2+} block in NMDA receptor channels. *Journal of Neuroscience* **16**:3549–3558.
- Kwon H-B, Sabatini BL (2011) Glutamate induces de novo growth of functional spines in developing cortex. *Nature* **474**:100–104.
- Labrakakis C, Rudolph U, De Koninck Y (2014) The heterogeneity in GABA_A receptor-mediated IPSC kinetics reflects heterogeneity of subunit composition among inhibitory and excitatory interneurons in spinal lamina II. *Frontiers in Cellular Neuroscience* **8**:424.
- Larkum ME, Nevian T, Sandler M, Polsky A, Schiller J (2009) Synaptic integration in tuft dendrites of layer 5 pyramidal neurons: a new unifying principle. *Science* **325**:756–760.
- Larkum ME, Senn W, Luscher HR (2004) Top-down dendritic input increases the gain of layer 5 pyramidal neurons. *Cerebral Cortex* **14**:1059–1070.
- Larkum ME, Zhu JJ, Sakmann B (1999) A new cellular mechanism for coupling inputs arriving at different cortical layers. *Nature* **398**:338–341.
- Lavoie AM, Tingey JJ, Harrison NL, Pritchett DB, Twyman RE (1997) Activation and deactivation rates of recombinant GABA_A receptor channels are dependent on α -subunit isoform. *Biophysical Journal* **73**:2518–2526.
- Lavzin M, Rapoport S, Polsky A, Garion L, Schiller J (2012) Nonlinear dendritic processing determines angular tuning of barrel cortex neurons in vivo. *Nature* **490**:397–401.
- Lee M-C, Yasuda R, Ehlers MD (2010) Metaplasticity at single glutamatergic synapses. *Neuron* **66**:859–870.
- Lee S-H, Kwan AC, Zhang S, Phoumthipphavong V, Flannery JG, Masmanidis SC, Taniguchi H, Huang ZJ, Zhang F, Boyden ES, Deisseroth K, Dan Y (2012) Activation of specific interneurons improves V1 feature selectivity and visual perception. *Nature* **488**:379–383.
- Lester RA, Clements JD, Westbrook GL, Jahr CE (1990) Channel kinetics determine the time course of NMDA receptor-mediated synaptic currents. *Nature* **346**:565–567.
- Liao D, Hessler NA, Malinow R (1995) Activation of postsynaptically silent synapses during pairing-induced LTP in CA1 region of hippocampal slice. *Nature* **375**:400–404.
- Little JP, Carter AG (2012) Subcellular synaptic connectivity of layer 2 pyramidal neurons in the medial prefrontal cortex. *Journal of Neuroscience* **32**:12808–12819.
- Little JP, Carter AG (2013) Synaptic mechanisms underlying strong reciprocal connectivity between the medial prefrontal cortex and basolateral amygdala. *Journal of Neuroscience* **33**:15333–15342.
- London M, Häusser M (2005) Dendritic computation. *Annual Review of Neuroscience* **28**:503–532.
- London M, Segev I (2001) Synaptic scaling in vitro and in vivo. *Nature Neuroscience* **4**:853–855.
- Losonczy A, Magee JC (2006) Integrative properties of radial oblique dendrites in hippocampal CA1 pyramidal neurons. *Neuron* **50**:291–307.

- Lourenco J, Pacioni S, Rebola N, van Woerden GM, Marinelli S, DiGregorio D, Bacci A (2014) Non-associative potentiation of perisomatic inhibition alters the temporal coding of neocortical layer 5 pyramidal neurons. *PLoS Biology* 12:e1001903.
- Lovett-Barron M, Turi GF, Kaifosh P, Lee PH, Bolze F, Sun XH, Nicoud JF, Zemelman BV, Sternson SM, Losonczy A (2012) Regulation of neuronal input transformations by tunable dendritic inhibition. *Nature Neuroscience* 15:423–430, S421–S423.
- Lovett-Barron M, Kaifosh P, Kheirbek MA, Danielson N, Zaremba JD, Reardon TR, Turi GF, Hen R, Zemelman BV, Losonczy A (2014) Dendritic inhibition in the hippocampus supports fear learning. *Science* 343:857–863.
- Luscher B, Fuchs T, Kilpatrick CL (2011) GABA_A receptor trafficking-mediated plasticity of inhibitory synapses. *Neuron* 70:385–409.
- Ma WJ, Beck JM, Latham PE, Pouget A (2006) Bayesian inference with probabilistic population codes. *Nature Neuroscience* 9:1432–1438.
- MacAskill AF, Little JP, Cassel JM, Carter AG (2012) Subcellular connectivity underlies pathway-specific signaling in the nucleus accumbens. *Nature Neuroscience* 15:1624–1626.
- MacAskill AF, Cassel JM, Carter AG (2014) Cocaine exposure reorganizes cell type- and input-specific connectivity in the nucleus accumbens. *Nature Neuroscience* 17:1198–1207.
- McClymont DW, Harris J, Mellor IR (2012) Open-channel blockade is less effective on GluN3B than GluN3A subunit-containing NMDA receptors. *European Journal of Pharmacology* 686:22–31.
- McCulloch WS, Pitts W (1943) A logical calculus of the ideas immanent in nervous activity. *Bulletin of Mathematical Biophysics*, 5:115–133.
- Maffei A, Nelson SB, Turrigiano GG (2004) Selective reconfiguration of layer 4 visual cortical circuitry by visual deprivation. *Nature Neuroscience* 7:1353–1359.
- Maffei A, Nataraj K, Nelson SB, Turrigiano GG (2006) Potentiation of cortical inhibition by visual deprivation. *Nature* 443:81–84.
- Magee JC (2000) Dendritic integration of excitatory synaptic input. *Nature Reviews Neuroscience* 1:181–190.
- Magee JC, Cook EP (2000) Somatic EPSP amplitude is independent of synapse location in hippocampal pyramidal neurons. *Nature Neuroscience* 3:895–903.
- Major G (1993) Solutions for transients in arbitrarily branching cables: III. Voltage clamp problems. *Biophysical Journal* 65:469–491.
- Major G, Polsky A, Denk W, Schiller J, Tank DW (2008) Spatiotemporally graded NMDA spike/plateau potentials in basal dendrites of neocortical pyramidal neurons. *Journal of Neurophysiology* 99:2584–2601.
- Makino H, Malinow R (2011) Compartmentalized versus global synaptic plasticity on dendrites controlled by experience. *Neuron* 72:1001–1011.
- Malenka RC (2003) Synaptic plasticity and AMPA receptor trafficking. *Annals of the New York Academy of Sciences* 1003:1–11.
- Malinow R, Malenka RC (2002) AMPA receptor trafficking and synaptic plasticity. *Annual Review of Neuroscience* 25:103–126.
- Manko M, Bienvenu TC, Dalezios Y, Capogna M (2012) Neurogliaform cells of amygdala: a source of slow phasic inhibition in the basolateral complex. *Journal of Physiology* 590:5611–5627.
- Marder E, Prinz AA (2002) Modeling stability in neuron and network function: the role of activity in homeostasis. *BioEssays* 24:1145–1154.
- Markram H, Toledo-Rodriguez M, Wang Y, Gupta A, Silberberg G, Wu C (2004) Interneurons of the neocortical inhibitory system. *Nature Reviews Neuroscience* 5:793–807.
- Martin LJ, Blackstone CD, Levey AI, Huganir RL, Price DL (1993) AMPA glutamate receptor subunits are differentially distributed in rat brain. *Neuroscience* 53:327–358.
- Martina M, Royer S, Pare D (2001) Cell-type-specific GABA responses and chloride homeostasis in the cortex and amygdala. *Journal of Neurophysiology* 86:2887–2895.

- Menon V, Musial TF, Liu A, Katz Y, Kath WL, Spruston N, Nicholson DA (2013) Balanced synaptic impact via distance-dependent synapse distribution and complementary expression of AMPARs and NMDARs in hippocampal dendrites. *Neuron* **80**:1451–1463.
- Mitchell SJ, Silver RA (2003) Shunting inhibition modulates neuronal gain during synaptic excitation. *Neuron* **38**:433–445.
- Momiyama A, Silver RA, Häusser M, Notomi T, Wu Y, Shigemoto R, Cull-Candy SG (2003) The density of AMPA receptors activated by a transmitter quantum at the climbing fibre–Purkinje cell synapse in immature rats. *Journal of Physiology* **549**:75–92.
- Monyer H, Burnashev N, Laurie DJ, Sakmann B, Seeburg PH (1994) Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. *Neuron* **12**:529–540.
- Mozrzymas JW (2004) Dynamism of GABA_A receptor activation shapes the “personality” of inhibitory synapses. *Neuropharmacology* **47**:945–960.
- Mozrzymas JW, Zarnowska ED, Pytel M, Mercik K (2003) Modulation of GABA_A receptors by hydrogen ions reveals synaptic GABA transient and a crucial role of the desensitization process. *Journal of Neuroscience* **23**:7981–7992.
- Mozrzymas JW, Barberis A, Vicini S (2007) GABAergic currents in RT and VB thalamic nuclei follow kinetic pattern of alpha3- and alpha1-subunit-containing GABA_A receptors. *European Journal of Neuroscience* **26**:657–665.
- Mrsic-Flogel TD, Hofer SB, Ohki K, Reid RC, Bonhoeffer T, Hübener M (2007) Homeostatic regulation of eye-specific responses in visual cortex during ocular dominance plasticity. *Neuron* **54**:961–972.
- Muller C, Beck H, Coulter D, Remy S (2012) Inhibitory control of linear and supralinear dendritic excitation in CA1 pyramidal neurons. *Neuron* **75**:851–864.
- Muñoz-Cuevas FJ, Athilingam J, Piscopo D, Wilbrecht L (2013) Cocaine-induced structural plasticity in frontal cortex correlates with conditioned place preference. *Nature Neuroscience* **16**:1367–1369.
- Murakoshi H, Wang H, Yasuda R (2011) Local, persistent activation of Rho GTPases during plasticity of single dendritic spines. *Nature* **472**:100–104.
- Murphy BK, Miller KD (2003) Multiplicative gain changes are induced by excitation or inhibition alone. *Journal of Neuroscience* **23**:10040–10051.
- Nevian T, Larkum ME, Polsky A, Schiller J (2007) Properties of basal dendrites of layer 5 pyramidal neurons: a direct patch-clamp recording study. *Nature Neuroscience* **10**:206–214.
- Nielsen TA, DiGregorio DA, Silver RA (2004) Modulation of glutamate mobility reveals the mechanism underlying slow-rising AMPAR EPSCs and the diffusion coefficient in the synaptic cleft. *Neuron* **42**:757–771.
- Nusser Z, Sieghart W, Benke D, Fritschy JM, Somogyi P (1996b) Differential synaptic localization of two major γ-aminobutyric acid type A receptor α subunits on hippocampal pyramidal cells. *Proceedings of the National Academy of Sciences of the United States of America* **93**:11939–11944.
- Nusser Z, Sieghart W, Stephenson FA, Somogyi P (1996a) The α6 subunit of the GABA_A receptor is concentrated in both inhibitory and excitatory synapses on cerebellar granule cells. *Journal of Neuroscience* **16**:103–114.
- Nusser Z, Cull-Candy S, Farrant M (1997) Differences in synaptic GABA_A receptor number underlie variation in GABA mini amplitude. *Neuron* **19**:697–709.
- Nyíri G, Freund TF, Somogyi P (2001) Input-dependent synaptic targeting of α2-subunit-containing GABA_A receptors in synapses of hippocampal pyramidal cells of the rat. *European Journal of Neuroscience* **13**:428–442.
- Oh MC, Derkach VA (2005) Dominant role of the GluR2 subunit in regulation of AMPA receptors by CaMKII. *Nature Neuroscience* **8**:853–854.
- Olsen RW, Sieghart W (2008) International Union of Pharmacology. LXX. Subtypes of γ-aminobutyric acid_A receptors: classification on the basis of subunit composition, pharmacology, and function. Update. *Pharmacological Reviews* **60**:243–260.

- Olshausen BA, Field DJ (2004) Sparse coding of sensory inputs. *Current Opinion in Neurobiology* **14**:481–487.
- Overstreet LS, Westbrook GL, Jones MV (2002) Measuring and modeling the spatiotemporal profile of GABA at the synapse. In: *Transmembrane Transporters* (Quick MW, ed.), pp 259–275. Hoboken, NJ: Wiley-Liss Inc.
- Palmer LM, Shai AS, Reeve JE, Anderson HL, Paulsen O, Larkum ME (2014) NMDA spikes enhance action potential generation during sensory input. *Nature Neuroscience* **17**:383–390.
- Paoletti P, Bellone C, Zhou Q (2013) NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease. *Nature Reviews Neuroscience* **14**:383–400.
- Papadopoulou M, Cassenaer S, Nowotny T, Laurent G (2011) Normalization for sparse encoding of odors by a wide-field interneuron. *Science* **332**:721–725.
- Pascoli V, Turiault M, Lüscher C (2012) Reversal of cocaine-evoked synaptic potentiation resets drug-induced adaptive behaviour. *Nature* **481**:71–75.
- Patel B, Mortensen M, Smart TG (2014) Stoichiometry of δ subunit containing GABA_A receptors. *British Journal of Pharmacology* **171**:985–994.
- Pavlov I, Savtchenko LP, Kullmann DM, Semyanov A, Walker MC (2009) Outwardly rectifying tonically active GABA_A receptors in pyramidal cells modulate neuronal offset, not gain. *Journal of Neuroscience* **29**:15341–15350.
- Pearce RA (1993) Physiological evidence for two distinct GABA_A responses in rat hippocampus. *Neuron* **10**:189–200.
- Peng Y, Zhao J, Gu Q-H, Chen R-Q, Xu Z, Yan J-Z, Wang S-H, Liu S-Y, Chen Z, Lu W (2010) Distinct trafficking and expression mechanisms underlie LTP and LTD of NMDA receptor-mediated synaptic responses. *Hippocampus* **20**:646–658.
- Pérez-Otaño I, Ehlers MD (2005) Homeostatic plasticity and NMDA receptor trafficking. *Trends in Neurosciences* **28**:229–238.
- Petreanu L, Mao T, Sternson SM, Svoboda K (2009) The subcellular organization of neocortical excitatory connections. *Nature* **457**:1142–1145.
- Petrini EM, Ravasenga T, Hausrat TJ, Iurilli G, Olcese U, Racine V, Sibarita JB, Jacob TC, Moss SJ, Benfenati F, Medini P, Kneussel M, Barberis A (2014) Synaptic recruitment of gephyrin regulates surface GABA_A receptor dynamics for the expression of inhibitory LTP. *Nature Communications* **5**:3921.
- Picton AJ, Fisher JL (2007) Effect of the α subunit subtype on the macroscopic kinetic properties of recombinant GABA_A receptors. *Brain Research* **1165**:40–49.
- Poirazi P, Brannon T, Mel BW (2003) Pyramidal neuron as two-layer neural network. *Neuron* **37**:989–999.
- Polksy A, Mel BW, Schiller J (2004) Computational subunits in thin dendrites of pyramidal cells. *Nature Neuroscience* **7**:621–627.
- Polksy A, Mel B, Schiller J (2009) Encoding and decoding bursts by NMDA spikes in basal dendrites of layer 5 pyramidal neurons. *Journal of Neuroscience* **29**:11891–11903.
- Pouille F, Scanziani M (2001) Enforcement of temporal fidelity in pyramidal cells by somatic feed-forward inhibition. *Science* **293**:1159–1163.
- Powell K, Mathy A, Duguid I, Häusser M (2015) Synaptic representation of locomotion in single cerebellar granule cells. eLife Sciences Publications Limited; **4**:e07290.
- Pozo K, Goda Y (2010) Unraveling mechanisms of homeostatic synaptic plasticity. *Neuron* **66**:337–351.
- Prieto ML, Wollmuth LP (2010) Gating modes in AMPA receptors. *Journal of Neuroscience* **30**:4449–4459.
- Pugh JR, Raman IM (2005) GABA_A receptor kinetics in the cerebellar nuclei: evidence for detection of transmitter from distant release sites. *Biophysical Journal* **88**:1740–1754.
- Pytel M, Mozrzymas JW (2006) Membrane voltage differently affects mIPSCs and current responses recorded from somatic excised patches in rat hippocampal cultures. *Neuroscience Letters* **393**:189–193.
- Pytel M, Mercik K, Mozrzymas JW (2006) Membrane voltage modulates the GABA_A receptor gating in cultured rat hippocampal neurons. *Neuropharmacology* **50**:143–153.

- Qian A, Buller AL, Johnson JW (2005) NR2 subunit-dependence of NMDA receptor channel block by external Mg²⁺. *Journal of Physiology* **562**:319–331.
- Rabinowitch I, Segev I (2006) The endurance and selectivity of spatial patterns of long-term potentiation/depression in dendrites under homeostatic synaptic plasticity. *Journal of Neuroscience* **26**:13474–13484.
- Rabinowitch I, Segev I (2008) Two opposing plasticity mechanisms pulling a single synapse. *Trends in Neurosciences* **31**:377–383.
- Rall W, Burke RE, Smith TG, Nelson PG, Frank K (1967) Dendritic location of synapses and possible mechanisms for the monosynaptic EPSP in motoneurons. *Journal of Neurophysiology* **30**:1169–1193.
- Rall W, Segev I (1985) Space-clamp problems when voltage clamping branched neurons with intracellular microelectrodes. In: *Voltage and Patch Clamping with Microelectrodes* (Smith TG, Lecar H, Redman SJ, Gage P, eds), pp. 191–215. Bethesda, MD: American Physiological Society.
- Ramos B, Lopez-Tellez JF, Vela J, Baglietto-Vargas D, del Rio JC, Ruano D, Gutierrez A, Vitorica J (2004) Expression of α5 GABA_A receptor subunit in developing rat hippocampus. *Brain Research Developmental Brain Research* **151**:87–98.
- Ransom CB, Wu Y, Richerson GB (2010) Postdepolarization potentiation of GABA_A receptors: a novel mechanism regulating tonic conductance in hippocampal neurons. *Journal of Neuroscience* **30**:7672–7684.
- Rauner C, Kohr G (2011) Triheteromeric NR1/NR2A/NR2B receptors constitute the major N-methyl-D-aspartate receptor population in adult hippocampal synapses. *Journal of Biological Chemistry* **286**:7558–7566.
- Rhodes P (2006) The properties and implications of NMDA spikes in neocortical pyramidal cells. *Journal of Neuroscience* **26**:6704–6715.
- Richardson RJ, Blundon JA, Bayazitov IT, Zakharenko SS (2009) Connectivity patterns revealed by mapping of active inputs on dendrites of thalamorecipient neurons in the auditory cortex. *Journal of Neuroscience* **29**:6406–6417.
- Rossi DJ, Hamann M (1998) Spillover-mediated transmission at inhibitory synapses promoted by high affinity α6 subunit GABA_A receptors and glomerular geometry. *Neuron* **20**:783–795.
- Roth A, Häusser M (2001) Compartmental models of rat cerebellar Purkinje cells based on simultaneous somatic and dendritic patch-clamp recordings. *Journal of Physiology* **535**:445–472.
- Rothman JS, Cathala L, Steuber V, Silver RA (2009) Synaptic depression enables neuronal gain control. *Nature* **457**:1015–1018.
- Rula EY, Lagrange AH, Jacobs MM, Hu N, Macdonald RL, Emeson RB (2008) Developmental modulation of GABA_A receptor function by RNA editing. *Journal of Neuroscience* **28**:6196–6201.
- Rusakov DA, Kullmann DM (1998) Extrasynaptic glutamate diffusion in the hippocampus: ultrastructural constraints, uptake, and receptor activation. *Journal of Neuroscience* **18**:3158–3170.
- Salinas E, Abbott LF (1995) Transfer of coded information from sensory to motor networks. *Journal of Neuroscience* **15**:6461–6474.
- Salinas E, Thier P (2000) Gain modulation: a major computational principle of the central nervous system. *Neuron* **27**:15–21.
- Salter MW, Kalia LV (2004) Src kinases: a hub for NMDA receptor regulation. *Nature Reviews Neuroscience* **5**:317–328.
- Sasaki YF, Rothe T, Premkumar LS, Das S, Cui J, Talantova MV, Wong HK, Gong X, Chan SF, Zhang D, Nakanishi N, Sucher NJ, Lipton SA (2002) Characterization and comparison of the NR3A subunit of the NMDA receptor in recombinant systems and primary cortical neurons. *Journal of Neurophysiology* **87**:2052–2063.
- Saviane C, Silver RA (2006) Fast vesicle reloading and a large pool sustain high bandwidth transmission at a central synapse. *Nature* **439**:983–987.
- Schiller J, Schiller Y (2001) NMDA receptor-mediated dendritic spikes and coincident signal amplification. *Current Opinion in Neurobiology* **11**:343–348.

- Schiller J, Major G, Koester HJ, Schiller Y (2000) NMDA spikes in basal dendrites of cortical pyramidal neurons. *Nature* **404**:285–289.
- Schoonover CE, Tapia J-C, Schilling VC, Wimmer V, Blazeski R, Zhang W, Mason CA, Bruno RM (2014) Comparative strength and dendritic organization of thalamocortical and corticocortical synapses onto excitatory layer 4 neurons. *Journal of Neuroscience* **34**:6746–6758.
- Schwartz EJ, Rothman JS, Dugue GP, Diana M, Rousseau C, Silver RA, Dieudonne S (2012) NMDA receptors with incomplete Mg²⁺ block enable low-frequency transmission through the cerebellar cortex. *Journal of Neuroscience* **32**:6878–6893.
- Schwenk J, Harmel N, Zolles G, Bildl W, Kulik A, Heimrich B, Chisaka O, Jonas P, Schulte U, Fakler B, Klocker N (2009) Functional proteomics identify cornichon proteins as auxiliary subunits of AMPA receptors. *Science* **323**:1313–1319.
- Schwenk J, Harmel N, Brechet A, Zolles G, Berkefeld H, Muller CS, Bildl W, Baehrens D, Huber B, Kulik A, Klocker N, Schulte U, Fakler B (2012) High-resolution proteomics unravel architecture and molecular diversity of native AMPA receptor complexes. *Neuron* **74**:621–633.
- Seilheimer RL, Rosenberg A, Angelaki DE (2014) Models and processes of multisensory cue combination. *Current Opinion in Neurobiology* **25**:38–46.
- Semyanov A, Walker MC, Kullmann DM, Silver RA (2004) Tonically active GABA_A receptors: modulating gain and maintaining the tone. *Trends in Neurosciences* **27**:262–269.
- Shanks NF, Savas JN, Maruo T, Cais O, Hirao A, Oe S, Ghosh A, Noda Y, Greger IH, Yates JR, 3rd, Nakagawa T (2012) Differences in AMPA and kainate receptor interactomes facilitate identification of AMPA receptor auxiliary subunit GSG1L. *Cell Reports* **1**:590–598.
- Shepherd GM, Svoboda K (2005) Laminar and columnar organization of ascending excitatory projections to layer 2/3 pyramidal neurons in rat barrel cortex. *Journal of Neuroscience* **25**:5670–5679.
- Shi S, Hayashi Y, Esteban JA, Malinow R (2001) Subunit-specific rules governing AMPA receptor trafficking to synapses in hippocampal pyramidal neurons. *Cell* **105**:331–343.
- Shipman SL, Herring BE, Suh YH, Roche KW, Nicoll RA (2013) Distance-dependent scaling of AMPARs is cell-autonomous and GluA2 dependent. *Journal of Neuroscience* **33**:13312–13319.
- Shu Y, Hasenstaub A, McCormick DA (2003) Turning on and off recurrent balanced cortical activity. *Nature* **423**:288–293.
- Siegle Retchless B, Gao W, Johnson JW (2012) A single GluN2 subunit residue controls NMDA receptor channel properties via intersubunit interaction. *Nature Neuroscience* **15**:406–413, S401–S402.
- Silberberg G, Markram H (2007) Disynaptic inhibition between neocortical pyramidal cells mediated by Martinotti cells. *Neuron* **53**:735–746.
- Silver RA (2010) Neuronal arithmetic. *Nature Reviews Neuroscience* **11**:474–489.
- Silver RA, Traynelis SF, Cull-Candy SG (1992) Rapid-time-course miniature and evoked excitatory currents at cerebellar synapses in situ. *Nature* **355**:163–166.
- Silver RA, Cull-Candy SG, Takahashi T (1996a) Non-NMDA glutamate receptor occupancy and open probability at a rat cerebellar synapse with single and multiple release sites. *Journal of Physiology* **494**:231–250.
- Silver RA, Colquhoun D, Cull-Candy SG, Edmonds B (1996b) Deactivation and desensitization of non-NMDA receptors in patches and the time course of EPSCs in rat cerebellar granule cells. *Journal of Physiology* **493**:167–173.
- Simon J, Wakimoto H, Fujita N, Lalande M, Barnard EA (2004) Analysis of the set of GABA_A receptor genes in the human genome. *Journal of Biological Chemistry* **279**:41422–41435.
- Sjöström PJ, Häusser M (2006) A cooperative switch determines the sign of synaptic plasticity in distal dendrites of neocortical pyramidal neurons. *Neuron* **51**:227–238.
- Skeberdis VA, Chevaleyre V, Lau CG, Goldberg JH, Pettit DL, Suadicani SO, Lin Y, Bennett MVL, Yuste R, Castillo PE, Zukin RS (2006) Protein kinase A regulates calcium permeability of NMDA receptors. *Nature Neuroscience* **9**:501–510.

- Smart TG, Paoletti P (2012) Synaptic neurotransmitter-gated receptors. *Cold Spring Harbor Perspectives in Biology* 4(3): a009662.
- Smith KR, Kittler JT (2010) The cell biology of synaptic inhibition in health and disease. *Current Opinion in Neurobiology* 20:550–556.
- Smith MA, Ellis-Davies GCR, Magee JC (2003) Mechanism of the distance-dependent scaling of Schaffer collateral synapses in rat CA1 pyramidal neurons. *Journal of Physiology* 548:245–258.
- Smith SL, Smith IT, Branco T, Häusser M (2013) Dendritic spikes enhance stimulus selectivity in cortical neurons in vivo. *Nature* 503:115–120.
- Sobczyk A, Svoboda K (2007) Activity-dependent plasticity of the NMDA-receptor fractional Ca^{2+} current. *Neuron* 53:17–24.
- Sobczyk A, Scheuss V, Svoboda K (2005) NMDA receptor subunit-dependent $[\text{Ca}^{2+}]$ signaling in individual hippocampal dendritic spines. *Journal of Neuroscience* 25:6037–6046.
- Softky W (1994) Sub-millisecond coincidence detection in active dendritic trees. *Neuroscience* 58:13–41.
- Somogyi P, Katona I, Klausberger T, Lasztoczi B, Viney TJ (2014) Temporal redistribution of inhibition over neuronal subcellular domains underlies state-dependent rhythmic change of excitability in the hippocampus. *Philosophical Transactions of the Royal Society B: Biological Sciences* 369:20120518.
- Song I, Savtchenko L, Semyanov A (2011) Tonic excitation or inhibition is set by GABA_A conductance in hippocampal interneurons. *Nature Communications* 2:376.
- Soto D, Coombs ID, Kelly L, Farrant M, Cull-Candy SG (2007) Stargazin attenuates intracellular polyamine block of calcium-permeable AMPA receptors. *Nature Neuroscience* 10:1260–1267.
- Spruston N (2008) Pyramidal neurons: dendritic structure and synaptic integration. *Nature Reviews Neuroscience* 9:206–221.
- Spruston N, Jaffe DB, Williams SH, Johnston D (1993) Voltage- and space-clamp errors associated with the measurement of electrotonically remote synaptic events. *Journal of Neurophysiology* 70:781–802.
- Spruston N, Jonas P, Sakmann B (1995) Dendritic glutamate receptor channels in rat hippocampal CA3 and CA1 pyramidal neurons. *Journal of Physiology* 482:325–352.
- Stocca G, Vicini S (1998) Increased contribution of NR2A subunit to synaptic NMDA receptors in developing rat cortical neurons. *Journal of Physiology* 507:13–24.
- Stokes CC, Isaacson JS (2010) From dendrite to soma: dynamic routing of inhibition by complementary interneuron microcircuits in olfactory cortex. *Neuron* 67:452–465.
- Straub C, Tomita S (2012) The regulation of glutamate receptor trafficking and function by TARPs and other transmembrane auxiliary subunits. *Current Opinion in Neurobiology* 22:488–495.
- Stroebel D, Carvalho S, Grand T, Zhu S, Paoletti P (2014) Controlling NMDA receptor subunit composition using ectopic retention signals. *Journal of Neuroscience* 34:16630–16636.
- Sur C, Triller A, Korn H (1995) Morphology of the release site of inhibitory synapses on the soma and dendrite of an identified neuron. *Journal of Comparative Neurology* 351:247–260.
- Sutton MA, Ito HT, Cressy P, Kempf C, Woo JC, Schuman EM (2006) Miniature neurotransmission stabilizes synaptic function via tonic suppression of local dendritic protein synthesis. *Cell* 125:785–799.
- Sutton MA, Taylor AM, Ito HT, Pham A, Schuman EM (2007) Postsynaptic decoding of neural activity: eEF2 as a biochemical sensor coupling miniature synaptic transmission to local protein synthesis. *Neuron* 55:648–661.
- Szabadics J, Tamas G, Soltesz I (2007) Different transmitter transients underlie presynaptic cell type specificity of $\text{GABA}_{A,\text{slow}}$ and $\text{GABA}_{A,\text{fast}}$. *Proceedings of the National Academy of Sciences of the United States of America* 104:14831–14836.
- Tamas G, Lorincz A, Simon A, Szabadics J (2003) Identified sources and targets of slow inhibition in the neocortex. *Science* 299:1902–1905.
- Thomson AM, Jovanovic JN (2010) Mechanisms underlying synapse-specific clustering of GABA_A receptors. *European Journal of Neuroscience* 31:2193–2203.

- Tomita S, Adesnik H, Sekiguchi M, Zhang W, Wada K, Howe JR, Nicoll RA, Bredt DS (2005) Stargazin modulates AMPA receptor gating and trafficking by distinct domains. *Nature* **435**:1052–1058.
- Toth K, McBain CJ (1998) Afferent-specific innervation of two distinct AMPA receptor subtypes on single hippocampal interneurons. *Nature Neuroscience* **1**:572–578.
- Tovar KR, Westbrook GL (1999) The incorporation of NMDA receptors with a distinct subunit composition at nascent hippocampal synapses in vitro. *Journal of Neuroscience* **19**:4180–4188.
- Tovar KR, McGinley MJ, Westbrook GL (2013) Triheteromeric NMDA receptors at hippocampal synapses. *Journal of Neuroscience* **33**:9150–9160.
- Traynelis SF, Wollmuth LP, McBain CJ, Menniti FS, Vance KM, Ogden KK, Hansen KB, Yuan H, Myers SJ, Dingledine R (2010) Glutamate receptor ion channels: structure, regulation, and function. *Pharmacological Reviews* **62**:405–496.
- Tretter V, Ehya N, Fuchs K, Sieghart W (1997) Stoichiometry and assembly of a recombinant GABA_A receptor subtype. *Journal of Neuroscience* **17**:2728–2737.
- Triller A, Choquet D (2005) Surface trafficking of receptors between synaptic and extrasynaptic membranes: and yet they do move! *Trends in Neurosciences* **28**:133–139.
- Triller A, Seitanidou T, Franksson O, Korn H (1990) Size and shape of glycine receptor clusters in a central neuron exhibit a somato-dendritic gradient. *New Biologist* **2**:637–641.
- Trussell LO, Zhang S, Raman IM (1993) Desensitization of AMPA receptors upon multiquantal neurotransmitter release. *Neuron* **10**:1185–1196.
- Turriagiano GG (2008) The self-tuning neuron: synaptic scaling of excitatory synapses. *Cell* **135**:422–435.
- Ujfalussy BB, Lengyel M (2011) Active dendrites: adaptation to spike-based communication. In: *Advances in Neural Information Processing Systems 24* (Shawe-Taylor J, Zemel R, Bartlett P, Pereira F, Weinberger K, eds), pp. 1188–1196. La Jolla, CA: Neural Information Processing Systems Foundation Inc.
- Verdoorn TA, Draguhn A, Ymer S, Seeburg PH, Sakmann B (1990) Functional properties of recombinant rat GABA_A receptors depend upon subunit composition. *Neuron* **4**:919–928.
- Verdoorn TA, Burnashev N, Monyer H, Seeburg PH, Sakmann B (1991) Structural determinants of ion flow through recombinant glutamate receptor channels. *Science* **252**:1715–1718.
- Vervaeke K, Lorincz A, Nusser Z, Silver RA (2012) Gap junctions compensate for sublinear dendritic integration in an inhibitory network. *Science* **335**:1624–1628.
- Vicini S, Wang JF, Li JH, Zhu WJ, Wang YH, Luo JH, Wolfe BB, Grayson DR (1998) Functional and pharmacological differences between recombinant N-methyl-D-aspartate receptors. *Journal of Neurophysiology* **79**:555–566.
- Walker HC, Lawrence JJ, McBain CJ (2002) Activation of kinetically distinct synaptic conductances on inhibitory interneurons by electrotonically overlapping afferents. *Neuron* **35**:161–171.
- Wang JQ, Guo ML, Jin DZ, Xue B, Fibach EE, Mao LM (2014) Roles of subunit phosphorylation in regulating glutamate receptor function. *European Journal of Pharmacology* **728**:183–187.
- Watanabe M, Inoue Y, Sakimura K, Mishina M (1993) Distinct distributions of five N-methyl-D-aspartate receptor channel subunit mRNAs in the forebrain. *Journal of Comparative Neurology* **338**:377–390.
- Watanabe M, Fukaya M, Sakimura K, Manabe T, Mishina M, Inoue Y (1998) Selective scarcity of NMDA receptor channel subunits in the stratum lucidum (mossy fibre-recipient layer) of the mouse hippocampal CA3 subfield. *European Journal of Neuroscience* **10**:478–487.
- Weiss DS, Barnes EM, Jr., Hablitz JJ (1988) Whole-cell and single-channel recordings of GABA-gated currents in cultured chick cerebral neurons. *Journal of Neurophysiology* **59**:495–513.
- Williams SR (2004) Spatial compartmentalization and functional impact of conductance in pyramidal neurons. *Nature Neuroscience* **7**:961–967.
- Williams SR, Mitchell SJ (2008) Direct measurement of somatic voltage clamp errors in central neurons. *Nature Neuroscience* **11**:790–798.
- Williams SR, Stuart GJ (2002) Dependence of EPSP efficacy on synapse location in neocortical pyramidal neurons. *Science* **295**:1907–1910.

- Williams SR, Stuart GJ (2003) Voltage- and site-dependent control of the somatic impact of dendritic IPSPs. *Journal of Neuroscience* **23**:7358–7367.
- Wilson NR, Runyan CA, Wang FL, Sur M (2012) Division and subtraction by distinct cortical inhibitory networks *in vivo*. *Nature* **488**:343–348.
- Wisden W, Laurie DJ, Monyer H, Seuberg PH (1992) The distribution of 13 GABA_A receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon. *Journal of Neuroscience* **12**:1040–1062.
- Wrighton DC, Baker EJ, Chen PE, Wyllie DJ (2008) Mg²⁺ and memantine block of rat recombinant NMDA receptors containing chimeric NR2A/2D subunits expressed in *Xenopus laevis* oocytes. *Journal of Physiology* **586**:211–225.
- Wyllie DJ, Traynelis SF, Cull-Candy SG (1993) Evidence for more than one type of non-NMDA receptor in outside-out patches from cerebellar granule cells of the rat. *Journal of Physiology* **463**:193–226.
- Wyllie DJ, Livesey MR, Hardingham GE (2013) Influence of GluN2 subunit identity on NMDA receptor function. *Neuropharmacology* **74**:4–17.
- Xu NL, Harnett MT, Williams SR, Huber D, O'Connor DH, Svoboda K, Magee JC (2012) Nonlinear dendritic integration of sensory and motor input during an active sensing task. *Nature* **492**:247–251.
- Yamada K, Fukaya M, Shimizu H, Sakimura K, Watanabe M (2001) NMDA receptor subunits GluR ϵ 1, GluR ϵ 3 and GluR ζ 1 are enriched at the mossy fibre-granule cell synapse in the adult mouse cerebellum. *European Journal of Neuroscience* **13**:2025–2036.
- Yuan T, Mameli M, Connor EC, Dey PN, Verpelli C, Sala C, Pérez-Otaño I, Lüscher C, Bellone C (2013) Expression of cocaine-evoked synaptic plasticity by GluN3A-containing NMDA receptors. *Neuron* **80**:1025–1038.
- Zhang J, Diamond JS (2009) Subunit- and pathway-specific localization of NMDA receptors and scaffolding proteins at ganglion cell synapses in rat retina. *Journal of Neuroscience* **29**:4274–4286.

Chapter 9

Dendritic voltage-gated ion channels

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Summary

This chapter will focus on the types and distributions of the main voltage-gated ion channels currently known to exist within the dendrites of CA1 pyramidal neurons, neocortical L5 pyramidal neurons, cerebellar Purkinje cells, and olfactory bulb mitral cells. The focus of this chapter is intentionally limited to these four distinctly different types of central neurons because a substantial amount of high-quality information is available concerning their dendritic voltage-gated channels and because they express a wide range of different types of dendritic electrogenesis. The chapter begins with a short survey of the known dendritic voltage-gated ion channel types and their modulation. The physiologically relevant biophysical properties and some pharmacology of these ion channels are tabulated. These sections are followed by a look at the distributions of these channels across the entire axis of the four cell types. The final section introduces some current ideas on how dendritic voltage-gated channels might impact on the physiological functioning of these neurons.

Introduction

In most central neurons, incoming synaptic inputs are widely distributed across the full extent of complicated dendritic arborizations. The role of dendrites in the integration of such widespread synaptic activity has been the topic of much experimental and theoretical study (reviewed in Johnston et al., 1996; Yuste and Denk, 1995; Magee et al., 1998; Häusser et al., 2000). The fundamental importance of dendritic integration in the functioning of neurons has ensured a continued interest in this issue. Recently, our understanding of the integrative properties of neuronal dendrites has been greatly expanded by the detailed characterizations of many dendritic voltage-gated ion channel populations (Migliore and Shepherd, 2002). This new information has provided an improved appreciation of the factors involved in the coordination and transformation of incoming synaptic input within dendrites.

Voltage-gated and calcium-gated ion channels in dendrites

Four types of voltage-gated channels that have been found to regulate dendritic excitability: voltage-gated sodium (Na_v) channels, calcium (Ca_v) channels, and potassium (K_v) channels, as well as hyperpolarization-activated cation (HCN) channels. Calcium-gated (K_{Ca}) potassium channels also affect the excitability of dendrites.

Na_v channels

There are four different Na_v channel α-subunits expressed at high levels in the central nervous system (CNS), namely Na_v1.1, -1.2, -1.3, and -1.6 (reviewed in Trimmer and Rhodes 2004). While the activation properties of the different Na⁺ channels are quite similar, a good deal of variation can be found in their inactivation properties (see Table 9.1). The relatively uniform activation properties of Na⁺ channels produce a fast inward current that dramatically increases the excitability of dendrites, allowing for the generation and propagation of action potentials as well as synaptic potential shaping. Dendritic Na⁺ channels have many of the same biophysical and pharmacological characteristics as those reported for other neuronal tetrodotoxin (TTX)-sensitive channels (reviewed in Marban et al., 1998; Fozard and Hanck, 1996). Most of the channels (>80%) are available to be activated at the resting membrane potential (RMP) and significant channel activation begins with depolarizations of around 20 mV. These channels have rapid activation and inactivation kinetics and an underlying single-channel conductance of approximately 15 pS (Huguenard et al., 1989; Stuart and Häusser, 1994; Stuart and Sakmann, 1994; Magee and Johnston, 1995; Bischofberger and Jonas, 1997; Gasparini and Magee, 2002). See Table 9.1.

Although most channel properties appear to be fairly uniform along the somato-dendritic axis, there are two key exceptions in CA1 pyramidal neurons. In these cells, Na⁺ channels from both the somatic and dendritic membrane possess a separate inactivation state that requires seconds instead of milliseconds for full recovery (Colbert et al., 1997; Jung et al., 1997). Interestingly, the fraction of channels that enter this slow inactivation state is much greater in dendrites compared with the soma, but the time for recovery from this inactivation at resting membrane potentials is similar in both compartments (Colbert et al., 1997; Mickus et al., 1999). As will be discussed in more detail later, the differences between the channel populations in the soma and dendrites appear to be the result of differing levels of phosphorylation (Colbert and Johnston, 1998) rather than different Na_v channel subunits (Hu et al., 2009; Lorincz and Nusser, 2010). Whatever the mechanism, this difference in Na_v channel inactivation underlies the frequency dependence of action potential backpropagation observed in pyramidal dendrites (Spruston et al., 1995; Callaway and Ross, 1995). In addition to the location-dependent slow inactivation, there is also a hyperpolarizing shift in the steady-state activation properties of dendritic Na⁺ channels in these cells, such that the voltage range of activation of more distal Na_v channels is shifted to about 8 mV more hyperpolarized than proximal channels (Gasparini and Magee, 2002). Again, this shift also appears to be due to a reduced level of Na_v channel phosphorylation in the more distal channels.

In all central neurons examined so far dendritic as well as somatic Na_v channels are blocked by low concentrations of TTX (in the high nM range; Stuart and Häusser, 1994; Stuart and Sakmann, 1994; Magee and Johnston, 1995; Bischofberger and Jonas, 1997). Thus, in most respects dendritic Na_v channels appear to be fairly standard neuronal TTX-sensitive Na⁺ channels. In addition to fast-inactivating Na_v channels, there is quite good evidence of a less standard, persistent Na⁺ current in both neocortical pyramidal neurons and Purkinje cells (Llinás and Sugimori, 1980; Strastrom et al., 1985; Schwindt and Crill, 1995; reviewed in Crill, 1996), primarily localized to the axo-somatic region of neocortical pyramidal neurons (Stuart and Sakmann, 1994). There are fewer data confirming the presence (or absence) of a persistent Na⁺ current in CA1 pyramidal neurons or mitral cells.

Na_v channels can be modulated by kinases and G-proteins, and such modulation generally produces quite significant changes in their voltage-dependent properties (Cantrell and Catterall, 2002). The most common form, usually measured in cell bodies or expression systems, is a

down-modulation by neuromodulators like dopamine and 5-hydroxytryptamine (5-HT) that results in a protein kinase A (PKA)- or PKC-dependent alteration in inactivation properties which, in the end, reduces the amount of available somatic Na^+ current (Cantrell et al., 1996; Carr et al., 2003). The matter is less clear for dendritic Na^+ channels. There are reports in L5 neurons of the prefrontal cortex that 5-HT inhibits somatic Na^+ currents as well as dendritic backpropagating action potentials, while DA has been found to have no effect on spike backpropagation in the same cells (Carr et al., 2002; Gullede and Stuart, 2003). Also, PKC activation in CA1 pyramidal neurons has been reported to speed recovery from the prolonged inactivation state, reducing the drop-off of Na^+ current during 20-Hz trains from 65% to 25%, and virtually eliminating the frequency dependence of action potential backpropagation (Colbert and Johnston, 1998). Further adding to the complexity, non-specific inhibition of dendritic kinases was found to enhance Na_v channel activation by producing a hyperpolarizing shift in the voltage range of activation in CA1 dendrites (Gasparini and Magee, 2002). More recently, a CaMKII-dependent shift in both the steady-state activation and inactivation curves has been reported in somatic Na^+ channels of CA1 pyramidal neurons (Xu et al., 2005).

Ca_v channels

The relatively slower, more prolonged inward current provided by Ca_v channels further adds to the excitability of dendritic membranes, allowing more prolonged single action potentials and bursts of multiple action potentials to occur (Dunlap et al., 1995; Trimmer and Rhodes, 2004; reviewed in Huguenard, 1996). These channels also provide a pathway for Ca^{2+} influx in addition to that mediated by agonist-gated ion channels. Ca^{2+} imaging studies and a variety of voltage-clamp recordings have found a mixture of several of the known types of Ca^{2+} channels (i.e., $\text{Ca}_v1.2$ and 1.3, L-types; $\text{Ca}_v2.1$, P/Q-type; $\text{Ca}_v2.2$, N-type; $\text{Ca}_v2.3$, R-type; and $\text{Ca}_v3.1$, 3.2, and 3.3, T-types) to be non-uniformly distributed throughout the dendrites of all neurons thus far tested. See Table 9.1 for a comparison of channel properties.

T-type ($\text{Ca}_v3.x$)

A Ca^{2+} current and associated Ca^{2+} influx that begins activating at relatively hyperpolarized potentials has been found in several neuronal dendrites (Magee and Johnston, 1995; Mouginot et al., 1997; Bindokas et al., 1993; Markram and Sakmann, 1994; Magee et al., 1998; Eilers et al., 1995; Kavalali et al., 1997; Williams and Stuart, 2000a). The corresponding low-voltage-activated (LVA) single-channel activity has a small conductance (~9 pS) and a higher Ca:Ba permeability ratio (>1) than other Ca^{2+} channel types (Magee and Johnston, 1995; Kavalali et al., 1997; Mouginot et al., 1997). In 2 mM Ca^{2+} , the LVA channels begin activating near rest (>-70 mV) with relatively slow activation (~5 ms at 0 mV) and rapid inactivation kinetics (~30 ms at -60 mV) (Kavalali et al., 1997; Mouginot et al., 1997; Magee, unpublished observations). Only a small fraction of the population is available at rest (<20% at -70 mV, 2 mM CaCl), however, due to a hyperpolarized inactivation range (Kavalali et al., 1997; Mouginot et al., 1997; Magee, unpublished observations). Compared with other Ca^{2+} channel types, the LVA channels show uniquely slow deactivation kinetics ($\tau_{\text{deact}} = \sim 4$ ms at -60 mV) (Magee and Johnston, 1995; Kavalali et al., 1997). Displaying a somewhat distinctive pharmacology, they have a high sensitivity to NiCl ($\text{IC}_{50} \sim 50$ μM) as well as a nearly complete insensitivity to ω -conotoxin MVIIIC and dihydropyridines (Magee and Johnston, 1995; Avery and Johnston, 1996; Kavalali et al., 1997). Together these properties strongly suggest that these are T-type Ca^{2+} channels.

Table 9.1 Channel properties

Channel	Voltage dependence			Kinetics ^a		Pharmacology	Modulation
	Activation ^b	Inactivation ^c	Activation	Deactivation	Inactivation		
Na	>-45 mV	>80%	<1 ms	<1 ms	~1 ms	TTX	PKC
Ca _T	>-70 mV	<25%	5 ms	5 ms	25 ms	Ni	?
Ca _L	>-60 mV	~100%	~1 ms	<1 ms	Minimal	Dihydrop.	PKA ^d
Ca _N	>-20 mV	>70% ^d	~1 ms	<1 ms	50 ms	GVIA	G-protein
Ca _P	>-40 mV	>90% ^d	~1 ms	<1 ms	100s ms	Aga IVA	G-protein
Ca _R	>-40 mV	>50% ^d	~1 ms	<1 ms	50 ms	Ni	?
K _{transient}	>-50 mV	>70%	~1 ms	<1 ms	~7 ms	4-AP	Kinases
K _D	>-50 mV	>70%	~1 ms	<1 ms	~20 ms	αDTX	Kinases ^d
K _S	>-30 mV	~100%	~3 ms	<1 ms	Minimal	TEA	Kinases ^d
H	<-50 mV	~100%	~50 ms ^e	5 ms ^e	None	ZD7288	CNTs

TTX, tetrodotoxin; Aga IVA, ω -agatoxin IVA; Dihydrop., dihydropyridines; GVIA, ω -conotoxin GVIA; 4-AP, 4-amino pyridine; αDTX, alpha-dendrotoxin; TEA, tetraethylammonium; PKA, PKC, protein kinases A and C.

^aDetermined at 0, -60, and -20 mV, respectively at ~22°C.

^bPotentials where channels start activating.

^cPercentage of population available at -70 mV.

^dDetermined in non-dendritic preparation.

^eAt -80 and -45 mV at 35°C for HCN1-predominant channels (pyramids, Purkinje). Channels composed of HCN2 or -4 will show about ten times slower activation (thalamocortical). See text for references.

L-types ($\text{Ca}_v1.2$ and 1.3)

The other more easily identified dendritic channel type is a large-conductance, high-voltage activated (HVA) channel (~25 pS, in 110 mM Ba) that mediates a sustained Ca^{2+} influx in a variety of dendrites (Bindokas et al., 1993; Bischofberger and Schild, 1995; Christie et al., 1995; Markram et al., 1995; Magee and Johnston, 1995, Magee et al., 1996; Kavalali et al., 1997; Williams and Stuart, 2000). This is a relatively rapid activating (~1 ms at 0 mV) and deactivating current that, due to a lack of voltage-dependent inactivation, is quite prolonged. The single-channel open time distributions are generally best fitted by a double exponential function, suggesting that there are two kinetically separable open states, one fast and the other slow (~0.5 and ~3.0 ms) (Magee and Johnston, 1995; see also Marrion and Tavalin, 1998). These channels are thought to be L-type Ca^{2+} channels because they exhibit a high sensitivity to dihydropyridines, have a lower Ni^{2+} sensitivity, and are insensitive to ω -conotoxin MVIIIC (Magee and Johnston, 1995; Kavalali et al., 1997). The two isoforms of CNS L-type Ca_v channels ($\text{Ca}_v1.2$ and -1.3) present markedly different activation ranges and some distinct pharmacology. The $\text{Ca}_v1.3$ channels begin activating (in ~2 mM Ca^{2+}) at substantially more hyperpolarized potentials (>-60 mV versus >-30 mV) and show a greatly reduced affinity for dihydropyridines (EC_{50} ~2 μM versus ~100 nM) than $\text{Ca}_v1.2$ channels (Xu and Lipscombe, 2001). A Ca_v current matching the properties of the α_{1d} is found to regulate resting $[\text{Ca}]_i$ in the dendrites of CA1 pyramidal neurons and also appears to be found in perigranular cells of the olfactory bulb (Avery and Johnston, 1996; Magee et al., 1996; Schnee and Ricci 2003; Murphy and Isaacson 2004).

P/Q-, N-, and R-types ($\text{Ca}_v2.1$, $\text{Ca}_v2.2$, $\text{Ca}_v2.3$)

A dendritic Ca_v current that is neither a transient current activated at hyperpolarized potentials nor a prolonged dihydropyridine-sensitive current is likely to be some mixture of P/Q-, N-, and R-type Ca_v channels (Usowicz et al., 1992; Bindokas et al., 1993; Bischofberger and Schild, 1995; Christie et al., 1995; Magee and Johnston, 1995; Markram et al., 1995; Kavalali et al., 1997; Isaacson and Strowbridge, 1998). These HVA currents are mediated by rapidly activating (time to peak ~1 ms at 0 mV) channels with unitary conductances around 15 pS (110 mM BaCl) (Usowicz et al., 1992; Magee and Johnston, 1995; Kavalali et al., 1997). They show a range of inactivation rates, with $\text{Ca}_v2.3$ being the most rapid and $\text{Ca}_v2.1$ showing very slow inactivation kinetics (Usowicz et al., 1992; Magee and Johnston, 1995; Kavalali et al., 1997; see also Randall and Tsien, 1997). The voltage ranges of activation for this group of channels are somewhat homogenous ($\text{Ca}_v2.1$ and $\text{Ca}_v2.3$ start activating at >-40 mV) with $\text{Ca}_v2.2$ channels perhaps having a relatively more depolarized activation range (these start activating at >-20 mV) (Randall and Tsien, 1997; Liu and Campbell, 1998). These channels can be isolated on the basis of their pharmacology. $\text{Ca}_v2.1$ channels show a unique sensitivity to ω -agatoxin IVA, while $\text{Ca}_v2.2$ are highly sensitive to ω -conotoxin GVIA, and both are sensitive to ω -conotoxin MVIIIC (Usowicz et al., 1992; Magee and Johnston, 1995; Kavalali et al., 1997). The $\text{Ca}_v2.3$ channels do not appear to be sensitive to any of these toxins but do show a high sensitivity to Ni^{2+} as well as the toxin SNX-482 (Magee and Johnston, 1995; Kavalali et al., 1997; Sochivko et al., 2002).

Modulation of Ca_v channels

Ca_v channels are well-established targets of a variety of modulators (G-proteins, kinases, phosphatases, lipid modulators) (Welsby et al., 2003; for review see Catterall, 2000). Several neuromodulators have been shown to inhibit dendritic Ca_v channels in isolated dendrosomes of hippocampal neurons and action potential-induced Ca^{2+} influx into stratum pyramidale (Chen and Lambert, 1997; Kavalali et al., 1997). In these studies, metabotropic glutamate, GABA_B, somatostatin,

serotonin, and adenosine receptor activation reduced the HVA Ca^{2+} current and associated Ca^{2+} influx by 12–50% of control. These are all G-protein-mediated modulators and they appear to have their largest impact on dendritic $\text{Ca}_v2.2$ channels. In cultured mitral cells norepinephrine has been shown to reduce depolarization-induced dendritic Ca^{2+} influx via α_2 -adrenoceptor activation while having less of an effect on somatic Ca^{2+} influx (Bischofberger and Schild, 1995). This is presumably through the well-described α_2 inhibition of $\text{Ca}_v2.2$ channels. Another recent study has shown a pronounced adrenoceptor (α_2 -AR) enhancement of Ca_v1 (L-type) channel-mediated Ca^{2+} influx into the proximal dendrites of CA1 pyramidal neurons. This PKA-dependent modulation was particularly strong within dendritic spines, implicating the presence of an interesting complex of Ca_v1 channels, β -AR, and other modulatory components within synaptic compartments (Hoogland and Saggau, 2004).

K_v channels

K_v channels are the main regulators of both dendritic and soma/axonal excitability (see Table 9.1 for a comparison of their properties). The wide variety of electogenesis observed in central neurons is in large part the result of the wide variety of K_v channels available (K_v1 , K_v2 , K_v3 , and K_v4 families) (Storm, 1990; Jan and Jan, 1997; Mathie et al., 1998; Trimmer and Rhodes, 2004). Most, but not necessarily all, of the main types of K_v channels have been found in neuronal dendrites.

Transient current K_v subtypes ($\text{K}_v1.1$, 1.2 ; $\text{K}_v3.3$; $\text{K}_v4.2$, 4.3)

Dendritic voltage-clamp recordings from CA1 and neocortical L5 pyramidal neurons as well as Purkinje cells have found large outward currents that have rapid kinetics of activation ($\tau_{\text{act}} = 1$ –3 ms), deactivation ($\tau_{\text{deact}} < 3$ ms), and inactivation ($\tau_{\text{inact}} = 6$ –20 ms) (Hoffman et al., 1997; Bekkers, 2000; Korngreen and Sakmann, 2000; Martina et al., 2003; Chen and Johnston, 2004; Harnett et al., 2013; see also Martina et al., 1998). Steady-state activation and inactivation curves indicate that these transient currents begin activating at around –40 to –50 mV and that under control conditions a large fraction of the population is available at rest (~60% in CA1 pyramidal cells and >90% in the Purkinje cells) (Hoffman et al., 1997; Martina et al., 2003; Chen and Johnston, 2004). The steady-state inactivation properties of the transient current in L5 pyramidal dendrites of juvenile rats are shifted in a hyperpolarized direction such that there are only about 20% available at rest in the most proximal dendritic regions (<250 μm) while fewer than 5% are available in the middle regions (250–400 μm) (Bekkers, 2000). However, such a difference was not observed in recordings from adult animals, where steady-state properties were similar through the apical dendrites (Harnett et al., 2013). The somatic regions of pyramidal neurons also appear to contain a significant density of a current that shows similarly rapid activation but with slower inactivation ($\tau_{\text{inact}} \sim 100$ ms) (Golding et al., 1999; Bekkers and Delaney, 2001; Chen and Johnston, 2004). This more slowly inactivating component of the transient current is also sensitive to dendrotoxin (and to low micromolar concentrations of 4-amino pyridine, 4-AP) and thus can tentatively be characterized as a D-type current carried by K_v1 subunits (Hoffman et al., 1997; Bekkers and Delaney, 2001; see also Martina et al., 1998). In CA1 and L5 dendrites, the largest fraction of the transient component possesses a pharmacological profile [more sensitive to 4-AP than tetraethylammonium (TEA) but with a higher sensitivity to Ba^{2+}] that supports its identification as an A-type K^+ current composed of K_v4 subunits (Hoffman et al., 1997; Gasparini et al., 2007; Harnett et al., 2013; see also Martina et al., 1998). The transient outward current in Purkinje cell dendrites showed a pharmacological profile that was different from the CA1 cells in that these currents were sensitive to low concentrations of both TEA and 4-AP and is thus consistent with α -subunits from the K_v3 family (Martina et al., 2003).

Sustained current K_v subtypes ($K_v2.1$, 2.2 ; $K_v3.4$; K_v7)

Another general class of outward current commonly found in neuronal dendrites is a sustained current that can activate somewhat more slowly ($\tau_{act} > 2$ ms) and that shows very little if any voltage-dependent inactivation (τ_{inact} is hundreds of milliseconds to seconds) (Bischofberger and Jonas, 1997; Hoffman et al., 1997; Martina et al., 1998; Bekkers, 2000; Martina et al., 2003; Chen and Johnston, 2004; Harnett et al., 2013). Steady-state activation and inactivation curves from CA1 and L5 pyramidal neurons indicate that depolarizations of >40 mV are required for activation of the sustained component (Hoffman et al., 1997; Harnett et al., 2013). The outward current in mitral cell primary dendrites begins activating at about -50 mV (Bischofberger and Jonas, 1997). In CA1 dendrites, the sustained component has voltage-dependent and pharmacological properties (more sensitive to TEA than 4-AP) that are more similar to a delayed-rectifier-type K^+ current perhaps carried by K_v2 subunits (Hoffman et al., 1997; Harnett et al., 2013; see also Martina et al., 1998). These dendrites also appear to contain a relatively non-inactivating channel activity that is sensitive to muscarinic receptor activation, thus providing strong evidence that M-currents are also present in CA1 dendrites (Chen and Johnston, 2004). Finally, Purkinje cell dendrites also possess a slowly inactivating component (hundreds of milliseconds) that appears to be sensitive to TEA (Martina et al., 2003; McKay and Turner, 2004).

K_{Ca} channels

The Ca-dependent K^+ (K_{Ca}) channel types are involved in action potential repolarization, the generation of afterhyperpolarization potential (AHP) currents of different durations, and spike frequency adaptation. In comparison with the K_v channel types, less is known about K_{Ca} channels in the dendrites of central neurons (but see Andreasen and Lambert, 1995a; Sah and Bekkers, 1996; Schwindt and Crill, 1997; Benhassine and Berger, 2005). There are at least three different channel types of interest and they have been well characterized in neuronal somata (Storm, 1987; reviewed in Sah, 1996; Marrion and Tavalin, 1998). The first is a large-conductance channel (100–400 pS; large conductance Ca^{2+} and voltage-activated K^+ channel, or BK channel) that has a substantial voltage dependence along with its relatively low Ca^{2+} sensitivity (0.6–10 μM). From published data it appears that most dendritic arborizations contain some density of BK channels (Sah and Bekkers, 1996; Poolos and Johnston, 1999; Womack and Khodakhah, 2004; Benhassine and Berger, 2005). A second, smaller conductance channel (5–20 pS; calcium-dependent small-conductance K channel, or SK channel) has relatively little voltage dependence and is more sensitive to Ca^{2+} (100–600 nM). While there are no direct channel recordings from dendrites there is substantial indirect evidence that SK channels are present in many neuronal dendrites (Womack and Khodakhah, 2003, Cai et al., 2004). A third channel type (I_{AHP}) apparently has an even smaller single-channel conductance (3–7 pS) and is activated by even lower concentrations of Ca^{2+} (the exact sensitivity is undetermined). The time courses of the currents are variable, with BK currents appearing as the most transient, followed by SK, and finally I_{AHP} which can be quite prolonged. BK channels are sensitive to charybdotoxin (ChTx) and low concentrations of TEA, while SK channels are primarily blocked by apamin. I_{AHP} is not blocked by apamin but is inhibited by high concentrations of TEA and a host of neuromodulators like norepinephrine.

Modulation of K_v channels

K_v channel subunits contain numerous potential phosphorylation sites. $K_v4.2$, which is the most likely molecular identity of the transient K_v current component in CA1 pyramidal neurons, has sites for PKA, PKC, CaMK and MAPK phosphorylation (Baldwin et al., 1991; Yuan et al., 2002; Schrader et al., 2002; Varga et al., 2004). Neuromodulator (β -adrenoceptor, DA, and muscarinic)

activation of PKA and PKC and the subsequent convergent activation of MAPK shift the voltage range of activation of the dendritic transient K⁺ current component about 15 mV depolarized and increase the amplitude of dendritic action potentials (Hoffman and Johnston, 1998, 1999). Both the transient and sustained K⁺ currents in pyramidal neurons also appear to be heavily down-modulated by lipid messengers like arachidonic acid and prostaglandin E2 (Ramakers and Storm, 2002; Chen and Bazan, 2005; see also Oliver et al., 2004). Interestingly, the voltage range of activation for transient channels located in the distal dendrites of CA1 pyramidal neurons is shifted 10–15 mV hyperpolarized compared with that from the soma/proximal (up to 100 μm) dendrites, perhaps due to location dependences in the modulatory state of the channels (Hoffman et al., 1997). PKA and PKC activation do not significantly affect the sustained component. As with Na⁺ channel modulation, kinase activation increases dendritic excitability in CA1 pyramidal neurons. As expanded in more detail later, the presence of such highly modulated channels in dendritic arbors provides neurons with a highly transformable excitability.

Hyperpolarization-activated cation channels and inwardly rectifying K⁺ channels

Hyperpolarization-activated (I_h) and inwardly rectifying K⁺ channels (IRK) participate in setting the resting membrane potential as well as other basic membrane properties such as input resistance and membrane polarization rates (Holt and Eatock, 1995; Pape, 1996; Robinson and Siegelbaum, 2003). Although they are very different channel types, they are considered together here because they have similar functional consequences, with both channels tending to stabilize the membrane potential in response to deviations from the resting potential. Both HCN and IRK channels have also been shown to have a large impact on the integration of synaptic activity (Magee 1998, 1999; Stuart and Spruston, 1998; Swindt and Crill, 1997; Williams and Stuart, 2000a; Williams et al., 2002; Nolan et al., 2003, 2004; Harnett et al., 2015). In the soma and apical dendrites of pyramidal and thalamocortical neurons membrane hyperpolarizations evoke inward currents that are slowly activating and deactivating and virtually non-inactivating (Magee, 1998; Williams and Stuart, 2000a,b). Activation curves demonstrate that a significant fraction (~25%) of this hyperpolarization-activated current (I_h) is active near rest, and in CA1 neurons the voltage range of activation is relatively more hyperpolarized in the distal dendrites (similar to the shift observed for dendritic A-type K⁺ channels). As with other I_h types, dendritic I_h channels demonstrated a mixed Na⁺/K⁺ conductance that is sensitive to low concentrations of external CsCl and ZD7288 (Gasparini and DiFrancesco, 1997; Magee, 1998). It appears that I_h channels with similar properties are found in the dendrites of both neocortical pyramidal neurons and cerebellar Purkinje cells (Schwindt and Crill, 1997; Stuart and Spruston, 1998; Häusser and Clark, 1997; Kole et al., 2006; Harnett et al., 2015). Indeed, a non-stationary fluctuation analysis (NSFA) suggests that single-channel conductance is quite small (680 fS) and that peak open probability approaches 1 (0.92) for I_h channels in the dendrites of neocortical pyramidal neurons (Kole et al., 2006).

Recent voltage-clamp recordings from CA1 and L5 dendrites have supplemented a good deal of indirect evidence that suggests both standard and G-protein-activated inward-rectifying K⁺ channels (IRK and GIRK, respectively) are present in neuronal dendrites (Takigawa and Alzheimer, 1999, 2003; Chen and Johnston, 2005; Varga et al., 2009; Makara and Magee, 2013). IRK and GIRK channels are K⁺ selective and display rapid activation kinetics and little if any inactivation, and of course most isoforms show extremely low levels of outward current compared with inward (Holt and Eatock, 1995; Chen and Johnston, 2005; reviewed in Bichet et al., 2003). These channels are in general sensitive to low concentrations of BaCl (<100 μM) and many dendritic electrical properties are likewise sensitive to Ba (Gasparini et al., 2007).

The I_h activation range shows subunit-specific sensitivity to intracellular cAMP and cGMP, and has been shown in a variety of non-dendritic preparations to be modulated by many neurotransmitters and modulators (Pape, 1996). Increases in cAMP or cGMP (such as during β -adrenoceptor activation or application of nitric oxide) shift the activation curve in a depolarized direction increasing the amount of I_h channel activation present at resting membrane potential V_m . Decreases in cyclic nucleotides have the opposite effect. An increase in resting I_h activation with increased intracellular cAMP concentrations has been observed in CA1 dendrites (Magee, unpublished observations), but was not observed in L5 dendrites (Kole et al., 2006).

Subcellular channel distributions

Because voltage-gated ion channels are differentially distributed across the plasma membranes of various neurons, the integrative properties and firing behavior of central neurons vary greatly between different cell types. As examples of this diversity, we shall examine in detail a few representative neurons which display distinctly different types of electrical signaling and for which there is substantial evidence concerning the dendritic distribution of voltage-gated ion channels. The discussion is presented in a qualitative manner because methodological differences among the various studies make it difficult to directly compare the specific channel densities ($pS/\mu m^2$).

Hippocampal CA1 pyramidal neurons

Cell-attached patch-clamp recordings from CA1 pyramidal neurons have indicated that Na^+ channel density is maintained at a fairly constant level from the axon initial segment to about 500 μm (total dendritic length is about 550 μm) into the dendritic field (Magee and Johnston, 1995; Colbert and Johnston, 1996; Bittner et al., 2012) (Fig. 9.1A). Immunohistochemical data indicate that the main Na^+ channel subunit present in the apical dendrites is $Na_v1.6$ (Hu et al., 2009; Lorincz and Nusser, 2010). These data fit well with the recording of TTX-sensitive dendritic action potentials and correlated Ca^{2+} influx (Richardson et al., 1987; Herraras, 1990; Jaffe et al., 1992; Turner et al., 1991; Colling and Wheal, 1994; Spruston et al., 1995; Mackenzie and Murphy, 1998). In spite of this relatively uniform Na^+ channel density and a more hyperpolarized activation range, the dendritic propagation of action potentials is less robust and the dendritic threshold for action potential initiation is higher than in the axonal regions of the neurons (Magee and Johnston, 1997; Golden and Spruston, 1998; Mackenzie and Murphy, 1998; Gasparini and Magee, 2002, 2004). The presence of a high density of axonal Na_v channels together with an elevated dendritic K_v channel density appears to be largely responsible for this situation.

The other main type of inward current-carrying channels, Ca^{2+} channels, also appear to be maintained at a fairly constant density (although substantially lower than Na^+ channels) from soma to 350 μm into the apical dendrites (Magee and Johnston, 1995) (Fig. 9.1B). While this is true for the total Ca^{2+} current, voltage-clamp recordings and fluorescence imaging studies suggest that the particular subtypes of Ca^{2+} channels present change with distance from the soma (Christie et al., 1995; Magee and Johnston, 1995). The Ca^{2+} current of the more proximal regions of the cell appears to be carried predominately by L-type and N-type Ca_v channels, while the Ni^{2+} -sensitive channel types (T and R) appear to play a more dominant role in the more distal regions of the cell (Christie et al., 1995; Magee and Johnston, 1995; Kavalali et al., 1997; Takahashi and Magee, 2009). A substantial density of N and P/Q Ca_v channel types has been reported for isolated dendritic segments (Kavalali et al., 1997).

While inward current density appears to be relatively constant across the dendritic axis, the total outward current density is not. The recorded density of the transient K^+ current component

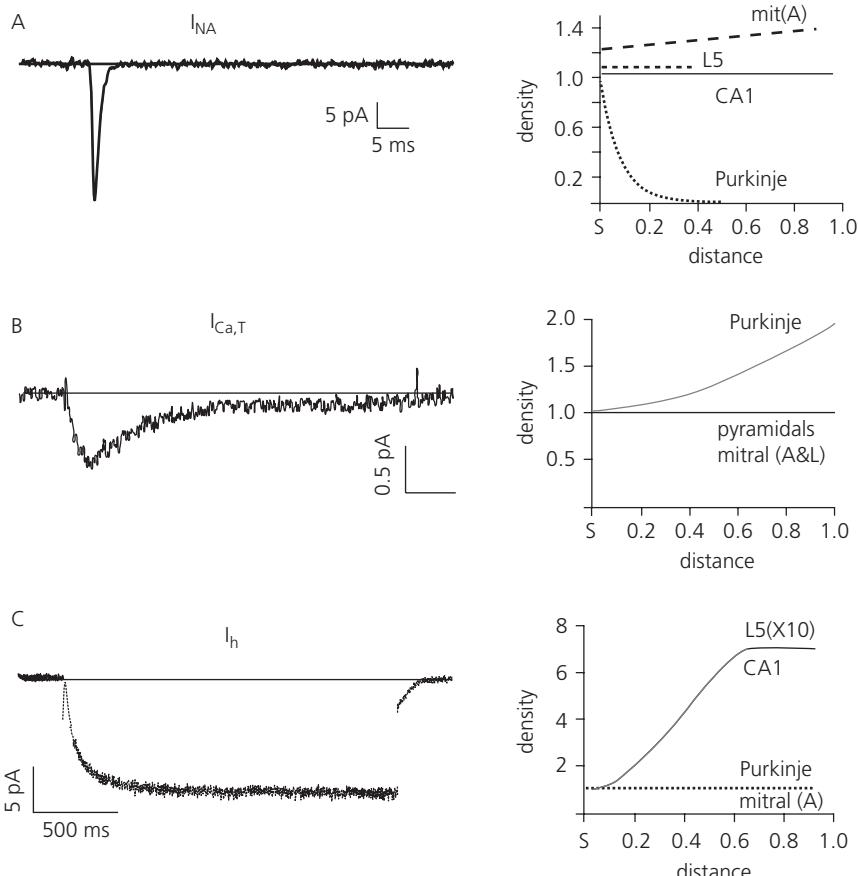


Fig. 9.1 Inward currents in dendrites. **(A)** Representative Na^+ current (left; average of ten traces evoked by step depolarizations from -90 to -10 mV) and schematized distribution of channel density along the dendrites of four cell types (right). **(B)** Representative T-type Ca^{2+} current (left; average of 30 traces evoked by step depolarizations from -90 to -60 mV in 2.5 mM CaCl_2) and distribution of total composite Ca^{2+} current comprising all channel subtypes (right). **(C)** Representative hyperpolarization-activated current recorded in cell-attached patch mode from CA1 pyramidal dendrites (left; average of five traces evoked by step hyperpolarizations from -50 to -110 mV in 110 mM KCl) and distribution (right). No data are available for I_h in mitral cells. For the distribution plots, the hypothesized channel density is plotted relative to the somatic density as a function of relative distance from the soma. The maximum distance (1.0) is equal to the maximum length of the dendritic arbor for each cell type. Mitral_(A) and Mitral_(L) are apical and lateral dendrites, respectively.

increases linearly with distance from the soma to a density that is more than five-fold higher at about $350\text{ }\mu\text{m}$ into the apical dendrites (Hoffman et al., 1997) (Fig. 9.2A). The current density of the sustained component, however, remains relatively constant (Hoffman et al., 1997) (Fig. 9.2B). Thus, total outward current density increases dramatically with distance away from the soma. Many studies, employing a combination of techniques, have suggested that K_v4 subunits, and $K_v4.2$

in particular, underlie the increased density of dendritic transient outward current (Chen et al., 2006; Jung et al., 2008; Sun et al., 2011) although immunohistochemical studies found only a modest two-fold increase in the density of $K_v4.2$ in CA1 pyramidal neurons (Kerti et al., 2011).

Ca-dependent K^+ currents have not so far been directly recorded from CA1 pyramidal dendrites so it is impossible to be quantitative about their densities. However, we can infer from more

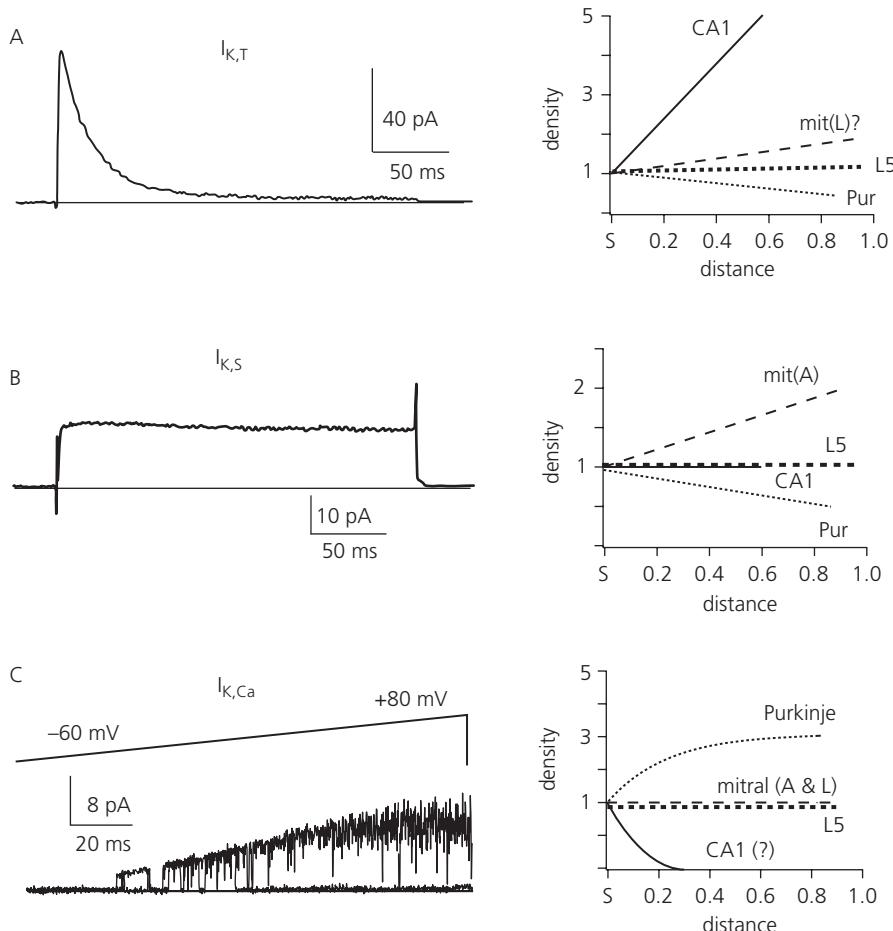


Fig. 9.2 Outward currents in dendrites. (A) Representative transient K^+ current (left; average of 30 traces evoked by step depolarizations from -90 to $+50$ mV) and schematized distribution of channel density along the dendrites of four cell types (right; available data for neocortical pyramidal neurons is limited to the proximal third of the arbor). (B) Representative sustained K^+ current [left; average of 30 traces evoked by step depolarizations from -90 to $+50$ mV from the same patch as in A₁ using standard subtraction procedures described in Hoffmann et al. (1997)] and distribution (right; available data for neocortical pyramidal neurons are again limited to the proximal third of the arbor). (C) Representative putative Ca^{2+} -dependent K^+ current recorded in cell-attached patch mode from CA1 pyramidal dendrites (left; two superimposed traces evoked by ramp depolarizations from -60 to $+80$ mV, recorded from the proximal dendrite of a 2-week-old rat) and distribution (right). Hypothesized channel density is plotted as in Fig. 9.1. Mitral_(A) and Mitral_(L) are apical and lateral dendrites, respectively.

indirect data that the density of these channels is very low in more distal regions of the dendrites (Fig. 9.2C). Voltage recordings of more distal dendritic action potentials rarely show any substantial AHPs, even though a significant Ca^{2+} influx can be recorded (Wong and Stewart, 1992; Andersen and Lambert, 1995b; Spruston et al., 1995; Magee and Johnston, 1997; Tsubokawa and Ross, 1997; Poolos and Johnston, 1999; but see Andersen and Lambert, 1995a). Furthermore, any AHP that is present in the dendrites is more sensitive to I_h channel blockers than apamin, charybdotoxin, or high concentrations of BAPTA (Tsubokawa and Ross, 1997; Magee, 1998; Poolos and Johnston, 1999). Again, these data suggest that the channels underlying Ca-dependent AHPs are present at low densities in the dendrites compared with the somatic compartment where large AHPs are generated. Finally, BK and SK channels have been shown to colocalize with N- and L-type Ca_v channels, respectively, in CA1 pyramidal neurons and in other neuronal preparations (Sah, 1996; Marrion and Tavalin, 1998). The relatively lower N- and L-type Ca_v channel densities present in the more distal dendrites may explain why BK- and SK-type K_{Ca} channel activities are hard to find in CA1 pyramidal dendrites.

Like the transient K_v component, I_h density increases dramatically with distance from the soma in the apical dendrites. In CA1 pyramidal neurons I_h density has been observed to increase nearly seven-fold from soma to 350 μm distal, and this maximum density is maintained into the apical tuft regions (up to 500 μm from the soma) (Magee, 1998; Bittner et al., 2012) (Fig. 9.1C). This observation is supported by multiple reports of an elevated membrane sag during dendritic hyperpolarizations (a classic hallmark of I_h) (Andersen and Lambert, 1995b; Tsubokawa and Ross, 1996; Magee, 1998; Tsubokawa et al., 1999; Bittner et al., 2012; Kupferman et al., 2014). These regional differences can be removed by I_h blockade. Similar data are available for inward-rectifying K^+ currents where the amount of constitutive GIRK channel activity was found to be significantly larger in the apical dendrites than in the soma and low concentrations of BaCl_2 (50 μM) have been shown to have a larger impact on the R_N and the membrane kinetics of dendrites compared with that of the soma (Losconzy and Magee, 2004; Chen and Johnston, 2005). Thus it appears that the density of inward-rectifying K channels could also increase with distance away from the soma.

Neocortical L5 pyramidal neurons

TTX-sensitive Na^+ currents have been recorded at a fairly constant density in neocortical pyramidal neurons from the soma to about 500 μm (the total dendritic length is approximately 1 mm, depending on the cortical region) (Stuart and Sakmann, 1994) (Fig. 9.1A). Neocortical neurons also show frequency-dependent backpropagation (Stuart et al., 1997a; Larkrum et al., 2001), and this phenomenon has been shown to be dependent on the prolonged Na^+ channel inactivation previously discussed (Colbert et al., 1997; Jung et al., 1997a). Thus the distribution of dendritic Na^+ channels in neocortical pyramidal neurons appears to be similar to that reported for CA1 pyramidal neurons. Evidence for the presence of Ca_v channels (T-, N-, P/Q-, as well as L-type) has also been found across the entire dendritic arbor of neocortical neurons, including the distal tuft and spines (Markram et al., 1995; Schiller et al., 1997, 1998; Perez-Garci et al., 2013) (Fig. 9.1B). While there have been no direct quantitative measurements of relative current density, there is enough Ca^{2+} current present in even the most distal regions of neocortical dendrites to generate dendritic calcium spikes (Schiller et al., 1997; Perez-Garci et al., 2013). Importantly, at least one component (Ca_v1 or L-type) of this complex current is powerfully inhibited by GABA_B -regulated G_i -subunit interaction (Perez-Garci et al., 2006; Perez-Garci et al., 2013).

Cell-attached and outside-out patch recordings have shown that both transient and sustained K^+ current components can be recorded from the apical dendrites, with the sustained component

maintaining a fairly constant density (Bekkers, 2000; Harnett et al., 2013) (Fig 9.2A,B). Recent evidence suggest that the sustained component density decreases rapidly with distance from the soma (Schaefer et al 2007). The transient component, has been shown to either increase with a slope of 2.3 pA/100 μm (somatic amplitude was 4.7 pA) (Bekkers, 2000) or maintain a relatively constant density from the proximal trunk regions the apical tuft (Korngreen and Sakmann, 2000; Harnett et al., 2013; Fig. 9.2A).

Direct dendritic recordings of K_{Ca} channels indicate that BK channels are homogeneously distributed across the somato-dendritic axis of neocortical pyramidal neurons from juvenile rats (about 4 weeks old) (Benhassine and Berger, 2005). This is in spite of the fact that dendritic action potentials rarely show AHPs that are anywhere near the amplitude recorded from the soma (Stuart and Sakmann, 1994; Schiller et al., 1997; Stuart et al., 1997a).

As with CA1 pyramidal neurons, there is solid evidence that I_h channels are present in the dendritic arbors of neocortical pyramidal neurons (Stuart and Spruston, 1998; Schwindt and Crill, 1997; Zhu, 2000; Kole et al., 2006; Harnett et al., 2015). In fact, an elevated dendritic I_h density was required for a realistic neuronal model to match whole-cell data recorded from the soma and dendrites (Stuart and Spruston, 1998) (Fig. 9.1C). A fluctuation analysis was used to estimate the microscopic properties of I_h channels in L5 pyramidal neurons, and this analysis revealed an approximately 60-fold increase in I_h channel density from proximal to distal dendritic regions, which is very similar to that observed for immunogold-labeled HCN1 particles in these same cells (Lorincz et al., 2002; Kole et al., 2006). It has recently been shown that this elevated I_h channel density is maintained into the apical tuft regions (Harnett et al., 2015). Finally a Ba^{2+} -sensitive voltage-dependent inward rectification has been observed in isolated neocortical dendrites (Takigawa and Alzheimer, 1999). This rectification is thought to be the result of dendritic inward-rectifying K^+ channels.

Cerebellar Purkinje cells

Purkinje cells have a substantially different distribution of voltage-gated ion channels from pyramidal neurons. The most prominent difference is that the Na_v channel density decreases rapidly with distance from the soma (Stuart and Häusser, 1994; Callaway and Ross, 1997; Lev Ram et al., 1992) (Fig. 9.1A). This, along with the unique dendritic morphology of Purkinje cells, determines that Na -dependent action potentials initiated in the soma-axonal region do not actively propagate into the dendrites (Llinás and Sugimori, 1979, 1980; Stuart and Häusser, 1994; Callaway et al., 1995; Roth and Häusser, 2001). This does not, however, mean that Purkinje dendrites are incapable of active electogenesis. In fact, dendritic recordings and imaging studies have shown Ca -dependent action potentials and associated dendritic Ca^{2+} influx that are insensitive to TTX (Llinás and Sugimori, 1979, 1980; Lev Ram et al., 1992; Mitgaard et al., 1993; Callaway et al., 1995). These studies demonstrated that although Purkinje dendrites are relatively devoid of Na_v channels, they contain a substantial density of Ca_v channels (Fig. 9.1B). Later studies revealed that the Ca_v channels located in the arbor are mainly P/Q- and T-type Ca_v channels (Usowicz et al., 1992; Bindokas et al., 1993; Mouginot et al., 1997; Watanabe et al., 1998; Womack and Khodakhah, 2004). The T-type channels are capable of generating a highly localized Ca^{2+} influx in response to subthreshold synaptic activity, while both channel types are involved in the large Ca^{2+} influx associated with the dendritic calcium spikes (although P-type channels play the predominant role). It has been hypothesized that the dendritic Ca_v channel density may be elevated when compared with that of the soma, especially in localized areas where there may be “hotspots” of particularly high Ca_v channel density (Llinás and Sugimori, 1980; Jaeger et al., 1997).

The dendritic calcium spiking is terminated by a prominent AHP that is presumably generated by a high density of K_{Ca} channels (BK and SK) located within the proximal regions of the dendrite

(Llinas and Sugimori, 1980; Jaeger et al., 1997; Womack and Khodakhah, 2003) (Fig. 9.2C). Although it appears to decrease substantially with distance, a high K^+ current density of been recorded as far as 220 μm into the dendritic arbor of Purkinje cells (the total dendritic length is about 250 μm in the 2–3-week-old rats used in the experiments). As mentioned above, this current is composed of two distinct components (fast and slow inactivating), and the available evidence suggest that the sustained K_v channel current is primarily located in the somatic and proximal dendritic regions where it is involved in fast spike repolarization. The more transient component seems to decrease less rapidly than the sustained one (Midtgård et al., 1993; Midtgård, 1994; Jaeger et al., 1997; Watanabe et al., 1998; Martina et al., 2003; McKay and Turner, 2004).

From voltage recordings of approximately similar hyperpolarization-induced membrane potential “sag,” there appears to be a uniform density of I_h in the somatic and dendritic compartments (Häusser and Clark, 1997; Williams et al., 2002) (Fig. 9.1C). Little information is available concerning the distribution of inwardly rectifying K^+ currents in the dendrites on Purkinje cells.

In summary, because of a low density of dendritic Na_v channels and an extremely unfavorable branching pattern, Purkinje cell dendrites do not actively backpropagate Na -dependent action potentials. Instead, slowly inactivating Ca -channel types in the dendrites provide a prolonged inward current that generates Ca^{2+} spikes that are terminated once the intracellular Ca^{2+} concentration becomes sufficiently elevated to activate Ca -dependent K^+ channels. The threshold voltages required for the generation of these dendritic Ca spikes appear to be regulated by a transient outward current located in the dendrites themselves. This type of dendritic electrogenesis contrasts sharply with that of the pyramidal cell types discussed earlier.

Olfactory bulb mitral cells

These cells represent yet another class of dendritic electroresponsivity that is also a result of a different complement and distribution of voltage-gated ion channels. They have two populations of dendrites (apical and lateral branches) that both appear to have full complements of voltage-gated ion channels, although there may be some subtle differences. Like the pyramidal neurons discussed earlier, mitral cell apical dendrites have a constant Na_v channel density across the somato-dendritic axis (Bischofberger and Jonas, 1997) (Fig. 9.1A). Unlike the pyramidal neurons, the K_v channel density (which appears to be comparatively quite high) is primarily composed of slower activating currents (similar to the sustained K_v component already discussed) that increase slightly (about two-fold) across the apical dendrite (Bischofberger and Jonas, 1997) (Fig. 9.2A,B). Because of the slow activation kinetics of these currents, the amplitude of action potentials in the apical dendrite is relatively insensitive to K_v channel blockade, while the duration is prolonged. The complement and density of Na_v and K_v channels of both the somatic and apical dendritic compartments of mitral cells is similar to that of CA1 pyramidal somata. Although K_{Ca} currents have not been directly recorded, the presence of similarly sized fast AHPs in both the soma and dendritic recordings suggest that the density of these channels is also uniform throughout mitral cells (Chen et al., 1997; Bischofberger and Jonas 1997; Isaacson and Strowbridge, 1998; Christie and Westbrook, 2003) (Fig. 9.2C). The presence of N-type Ca_v channels in mitral cell dendrites (see later) supports the idea that these channels (BK type) are colocalized.

There appears to be an inhomogeneous distribution of Ca_v channel subtypes in these neurons. Imaging data from cultured mitral cells suggested that the density of L-type Ca_v channels is restricted to the proximal regions of the cell, while N-type channels are distributed throughout the arborization (Bischofberger and Schild, 1995; Isaacson and Strowbridge, 1998). Additional studies have shown that P/Q-type channels are also found along with N-type Ca_v channels in the dendrites

and that these channels play an important role in dendro-dendritic inhibition in the olfactory bulb (Isaacson and Strowbridge, 1998). There is no evidence of T-type Ca_v channels in mitral cell dendrites. Finally, action potential-evoked Ca^{2+} signals have been reported to decrease with distance from the soma in the primary and lateral dendrites until the distal tuft region, where influx appears to increase back to somatic levels (Isaacson and Strowbridge, 1998). This preliminary data suggest that Ca_v channel density may be lower in the non-tuft regions of the dendritic arbor of mitral cells (Fig. 9.1B).

Action potentials also backpropagate down the more lengthy (1–2 mm) lateral dendrites of mitral cells (Christie and Westbrook, 2003; Debarbieux et al., 2003; Djurisic et al., 2004). Ca^{2+} imaging and direct recordings from the more proximal regions have shown a TTX-sensitive propagation that is complete throughout the entire branch, although there may be a small amount of attenuation (Christie and Westbrook, 2003; Debarbieux et al., 2003). These signals also appear to be more sensitive to 4-AP, suggesting that there may be a higher or at least more effective density of A-type K_v channels in lateral compared with apical dendrites of mitral cells (Christie and Westbrook, 2003). The presence of backpropagating action potential-associated Ca^{2+} signals throughout the entire lateral branch indicates that voltage-gated Ca_v channels are also present in the lateral dendrites of these cells.

Location-dependent channel modulation

While there is no reason to suspect that specific channel types are differentially modulated depending on their location within the cell, the non-uniformity of channel densities suggests that similar location-dependent differences in receptor types and associated second messenger systems may exist. In fact, Na_v channels, the transient K_v component, N-type Ca_v channels, and I_h channels all appear to be differentially modulated across the somato-dendritic axis (Bischofberger and Schild, 1995; Hoffman et al., 1997; Colbert and Johnston, 1998; Magee, 1998; Gasparini and Magee, 2000). There appears to be only a small direct impact of PKC- and PKA-dependent phosphorylation on the properties of dendritic Na_v and A-type K_v channels (Colbert and Johnston, 1998; Hoffman and Johnston, 1998), and for dendritic I_h there appears to be less cAMP-dependent channel modulation through what is thought to be a direct modulation by cAMP (Magee, 1998). One cannot, however, rule out less PKA-dependent phosphorylation, as the exact mechanism of cAMP modulation of I_h has not been convincingly established (Pape, 1996). Therefore, all three of these channel types could be in a relatively less phosphorylated state. This may be indicative of elevated dendritic phosphatase activity or lower concentrations of other second messengers (i.e., cAMP). Although such a situation may be somewhat of an over-simplification, given the diversity and complexity of modulatory mechanisms, large regional differences in basal channel modulatory state do exist in CA1 pyramidal neurons.

Dendritic plasticity

Voltage-gated ion channels in dendrites play an important role in both the processing and storage of information. Through a complicated interplay with neuronal morphology and synaptic properties these channels determine the pattern of action potential output by filtering, amplifying, and in other ways modifying the integration of electrical signals delivered by synapses (Golding and Spruston, 1998; Magee, 1999, 2000; Larkrum et al., 2001; Williams et al., 2002; Ariav et al., 2003; Williams and Stuart, 2003; Gasparini and Magee, 2004; Polksy et al., 2004; Losonczy and Magee, 2006; Takahashi and Magee, 2009; Branco et al., 2010; Vaidya and Johnston, 2013). Because of this,

the active membrane properties of dendrites could play as large a role as any other single factor, including synaptic weight, in determining the final impact of a given synaptic input.

Furthermore, the active properties of dendrites are intricately involved in both the induction and expression of various forms of synaptic and non-synaptic (membrane excitability) plasticity. For the induction of synaptic plasticity, dendritic voltage-gated ion channels allow action potentials generated in the output region of the neuron to propagate back into the arbor providing a feedback signal to the region of the cell receiving the input (Magee and Johnston, 1997; Golding et al., 2002). Also, local initiation of dendritic spikes has been shown to be capable of inducing both long-term potentiation (LTP) and long-term depression (LTD) in pyramidal neurons (Golding et al., 2002; Holthoff et al., 2004; Gordon, et al., 2006; Brandalise and Gerber, 2014; Gambino et al., 2014). Thus, by shaping the integration of incoming synaptic input and by providing both associative and non-associative Ca^{2+} influx mechanisms, the active properties of dendrites are heavily involved in determining the impact of a given synaptic input.

In addition to this role in the induction of synaptic plasticity, certain behavioral paradigms and/or LTP/LTD induction protocols can induce long-lasting changes in the properties of dendritic channel. Examples of this form of plasticity have been found in both pyramidal and Purkinje neurons where the activation of kinase signaling pathways (PKA, PKC, MAPK) by synaptic input patterns leads to the expression of both long-term synaptic plasticity and modulation of specific dendritic ion channels (Aizenman and Linden, 2000; Wang et al., 2003; Zang and Linden, 2003; Frick et al., 2004; Fan et al., 2005; Brager and Johnston, 2007; Narayanan and Johnston, 2007; Narayanan and Johnston, 2008; Otsu et al., 2014) (Fig. 9.3). The induction of long-term changes in active dendritic properties is not limited to synaptic input protocols but can also follow increased postsynaptic action potential firing and particular forms of channel modulation (Yasuda et al., 2003).

Highly correlated input patterns onto apical oblique and basal dendrites of CA1 pyramidal cells evoke local fast sodium spikes whose propagation strength varies among the different radial oblique branches of the perisomatic region (Golding and Spruston, 1998; Ariav et al., 2003; Losonczy and Magee, 2006). The strength with which the local sodium spike propagates to the soma was also found to be a plastic feature of dendrites that can be strengthened by associative pairing of dendritic spikes with backpropagating action potentials or muscarinic acetylcholine receptor activation (branch strength potentiation) (Losonczy et al., 2008). These results raise the intriguing possibility that dendritic plasticity might participate in memory formation in the hippocampus by providing a mechanism to store the specific, complex features of the environment encoded in highly correlated input patterns. Furthermore, *in vivo* manipulations have shown that an increase in foraging and exploratory activity induces this form of plasticity in dendritic branch excitability in CA1 pyramidal neurons (Makara et al., 2009). In summary, *in vivo* experience produces a use-dependent plasticity of excitability in selective compartments of the dendritic arborization (Holt and Schuman, 2013), which may allow neurons to store higher-order statistical features encoded within the synaptic input they receive.

This ability of activity patterns to significantly modify active dendritic properties allows neurons to dynamically change the way they process and store information, transforming a neuron's functional role within its particular network. Because the channels themselves (voltage ranges and kinetics of activation and inactivation, as well as channel subunit composition and density) are easily and widely modulated, dendritic integration of incoming synaptic input can vary from one condition to another very quickly and over long periods of time. This highly effective mechanism permits information to be stored in the pattern of dendritic membrane excitability, presumably increasing the ability of neurons to perform the appropriate computation for a given condition.

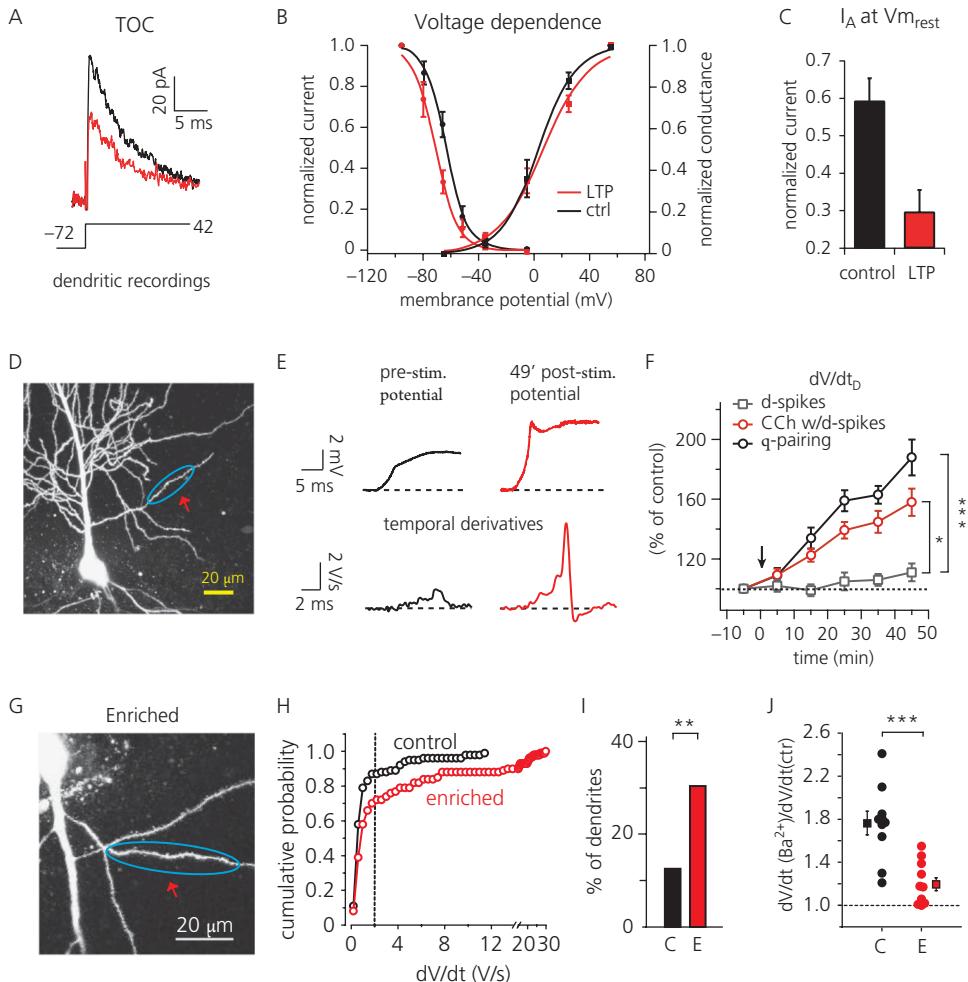


Fig. 9.3 Branch-specific dendritic plasticity. (A–C) LTP protocols reduce the amplitude of the dendritic transient outward current (TOC) evoked from resting membrane potentials (A) due to a hyperpolarizing shift in the inactivation curve following LTP induction (B, C). (D–F) A localized synaptic stimulation protocol (stimulated branch indicated with circle and arrow; D) leads to a branch-specific enhancement of dendritic sodium spike propagation (E) due to a downregulation of TOC as evidenced by a long-term increase in somatically recorded sodium spike components (F; evoked potentials and dV/dt). (G–J) Housing rats in an enriched environment (labeled E) leads to branch-specific dendritic plasticity (H) that is reflected in an increased presence of strongly excitable dendritic branches compared with control housing (labeled C) (I; elevated branch sodium spike dV/dt evidenced by a positive shift in the cumulative distribution and increased percentage of branches showing >2 V/s branch sodium spikes). (J) A reduced impact of TOC pharmacological blockade (150 mM Ba²⁺), suggests that the enriched housing reduces TOC amplitude.

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References

- Aizenman CD, Linden DJ (2000) Rapid synaptically driven increases in the intrinsic excitability of cerebellar deep nuclear neurons. *Nature Neuroscience* **3**:109–111.
- Andreasen M, Lambert JDC (1995a) The excitability of CA1 pyramidal cell dendrites is modulated by a local Ca^{2+} -dependent K^+ conductance. *Brain Research* **698**:193–203.
- Andreasen M, Lambert JDC (1995b) Regenerative properties of pyramidal cell dendrites in area CA1 of the rat hippocampus. *Journal of Physiology* **483**:421–441.
- Ariav G, Polksky A, Schiller J (2003) Submillisecond precision of the input-output transformation function mediated by fast sodium dendritic spikes in basal dendrites of CA1 pyramidal neurons. *Journal of Neuroscience* **23**:7750–7758.
- Avery RB, Johnston D (1996) Multiple channel types contribute to the low-voltage-activated calcium current in hippocampal CA3 pyramidal neurons. *Journal of Neuroscience* **16**:5567–5582.
- Baldwin TJ, Tsaur ML, Lopez GA, Jan LY (1991) Characterization of a mammalian cDNA for an inactivating voltage-sensitive K^+ channel. *Neuron*, **7**:471–483.
- Bekkers JM (2000) Distribution and activation of voltage-gated potassium channels in cell-attached and outside-out patches from large layer 5 cortical pyramidal neurons of the rat. *Journal of Physiology* **525**:611–620.
- Bekkers JM, Delaney AJ (2001) Modulation of excitability by alpha-dendrotoxin-sensitive potassium channels in neocortical pyramidal neurons. *Journal of Neuroscience* **21**:6553–6560.
- Benhassine N, Berger T (2005) Homogeneous distribution of large-conductance calcium-dependent potassium channels on soma and apical dendrite of rat neocortical layer 5 pyramidal neurons. *European Journal of Neuroscience* **21**:914–926.
- Bichet D, Haass FA, Jan LY (2003) Merging functional studies with structures of inward rectifier K^+ channels. *Nature Reviews Neuroscience* **4**:957–967.
- Bindokas VP, Brorson JR, Miller RJ (1993) Characteristics of voltage sensitive calcium channels in dendrites of cultured rat cerebellar neurons. *Neuropharmacology* **32**:1213–1220.
- Bischofberger J, Jonas P (1997) Action potential propagation into the presynaptic dendrites of rat mitral cells. *Journal of Physiology* **504**:359–365.
- Bischofberger J, Schild D (1995) Different spatial patterns of $[\text{Ca}^{2+}]$ increase caused by N- and L-type Ca^{2+} channel activation in frog olfactory bulb neurones. *Journal of Physiology* **487**:305–387.
- Bittner K, Andrasfalvy B, Magee JC (2012) Ion channel gradients in the apical tuft region of CA1 pyramidal neurons. *PLoS ONE* **7**: e46652.
- Brager DH, Johnston D (2007) Plasticity of intrinsic excitability during long-term depression is mediated through mGluR-dependent changes in $I(h)$ in hippocampal CA1 pyramidal neurons. *Journal of Neuroscience* **27**:13926–13937.
- Branco T, Clark BA, Häusser M (2010) Dendritic discrimination of temporal input sequences in cortical neurons. *Science* **329**:1671–1675.
- Brandalise F, Gerber U (2014) Mossy fiber-evoked subthreshold responses induce timing-dependent plasticity at hippocampal CA3 recurrent synapses. *Proceedings of the National Academy of Sciences of the United States of America* **111**:4303–4308.
- Cai X, Liang CW, Muralidharan S, Kao JPY, Tang C-M, Thompson SM (2004) Unique roles of SK and Kv4.2 potassium channels in dendritic integration. *Neuron* **44**:351–364.
- Callaway JC, Ross WN (1995) Frequency-dependent propagation of sodium action potentials in dendrites of hippocampal CA1 pyramidal neurons. *Journal of Neurophysiology* **74**:1395–1403.
- Callaway JC, Ross WN (1997) Spatial distribution of synaptically activated sodium concentration changes in cerebellar Purkinje neurons. *Journal of Neurophysiology* **77**:145–152.
- Callaway JC, Lasser-Ross N, Ross WN (1995) IPSPs strongly inhibit climbing fiber-activated $[\text{Ca}^{2+}]_i$ increases in the dendrites of cerebellar Purkinje neurons. *Journal of Neuroscience* **15**:2777–2787.

- Cantrell AR, Catterall WA (2002) Neuromodulation of Na^+ channels: an unexpected form of cellular plasticity. *Nature Reviews Neuroscience* 2:397–407.
- Cantrell AR, Ma JY, Scheuer T, Catterall WA (1996) Muscarinic modulation of sodium current by activation of protein kinase C in rat hippocampal neurons. *Neuron* 16:1019–1026.
- Carr DB, Cooper DC, Ulrich SL, Spruston N, Surmeier DJ (2002) Serotonin receptor activation inhibits sodium current and dendritic excitability in prefrontal cortex via a protein kinase C-dependent mechanism. *Journal of Neuroscience* 22:6846–6855.
- Carr DB, Day M, Cantrell AR, Held J, Scheuer T, Catterall WA, Surmeier DJ (2003) Transmitter modulation of slow, activity-dependent alterations in sodium channel availability endows neurons with a novel form of cellular plasticity. *Neuron* 39:793–806.
- Catterall WA (2000) Structure and regulation of voltage-gated Ca^{2+} channels. *Annual Review of Cell and Developmental Biology* 16:521–555.
- Chen C, Bazan NG (2005) Endogenous PGE2 regulates membrane excitability and synaptic transmission in hippocampal CA1 pyramidal neurons. *Journal of Neurophysiology* 93:929–941.
- Chen H, Lambert NA (1997) Inhibition of dendritic calcium influx by activation of G-protein-coupled receptors in the hippocampus. *Journal of Neurophysiology* 78:3484–3488.
- Chen X, Johnston D (2004) Properties of single voltage-dependent K^+ channels in dendrites of CA1 pyramidal neurones of rat hippocampus. *Journal of Physiology* 559:187–203.
- Chen X, Johnston D (2005) Constitutively active GIRK channels in dendrites of hippocampal CA1 pyramidal neurons. *Journal of Neuroscience* 25:3787–3792.
- Chen WR, Midtgård J, Shepherd GM (1997) Forward and backward propagation of dendritic impulses and their synaptic control in mitral cells. *Science* 278:463–467.
- Chen X, Yuan LL, Zhao C, Birnbaum SG, Frick A, Jung WE, Schwarz TL, Sweatt JD, Johnston D (2006) Deletion of Kv4.2 gene eliminates dendritic A-type K^+ current and enhances induction of long-term potentiation in hippocampal CA1 pyramidal neurons. *Journal of Neuroscience* 26:12143–12151.
- Christie JM, Westbrook GL (2003) Regulation of backpropagating action potentials in mitral cell lateral dendrites by A-type potassium currents. *Journal of Neurophysiology* 89:2466–2472.
- Christie BR, Eliot LS, Ito K, Miyakawa H, Johnston D (1995) Different Ca^{2+} channels in soma and dendrites of hippocampal pyramidal neurons mediate spike-induced Ca^{2+} influx. *Journal of Neurophysiology* 73:2553–2557.
- Colbert CM, Johnston D (1996) Axonal action-potential initiation and Na^+ channel densities in the soma and axon initial segment of subiculum pyramidal neurons. *Journal of Neuroscience* 16:6676–6686.
- Colbert CM, Johnston D (1998) Protein kinase C activation decreases activity-dependent attenuation of dendritic Na^+ current in hippocampal CA1 pyramidal neurons. *Journal of Neurophysiology* 79:491–495.
- Colbert CM, Magee JC, Hoffman DA, Johnston D (1997) Slow recovery from inactivation of Na^+ channels underlies the activity-dependent attenuation of dendritic action potentials in hippocampal CA1 pyramidal neurons. *Journal of Neuroscience* 17:6512–6521.
- Colling SB, Wheal HV (1994) Fast sodium action potentials are generated in the distal apical dendrites of rat hippocampal CA1 pyramidal cells. *Neuroscience Letters* 172:73–76.
- Crill WE (1996) Persistent sodium current in mammalian central neurons. *Annual Review of Physiology* 58:349–362.
- Debarbieux F, Audinat E, Charpak S (2003) Action potential propagation in dendrites of rat mitral cells in vivo. *Journal of Neuroscience* 23:5553–5560.
- Djurisic M, Antic S, Chen WR, Zecevic D (2004) Voltage imaging from dendrites of mitral cells: EPSP attenuation and spike trigger zones. *Journal of Neuroscience* 24:6703–6714.
- Dunlap K, Luebke JI, Turner TJ (1995) Exocytotic Ca^{2+} channels in mammalian central neurons. *Trends in Neurosciences* 18:89–98.
- Eilers J, Augustine GJ, Konnerth A (1995) Subthreshold synaptic Ca^{2+} signaling in fine dendrites and spines of cerebellar Purkinje neurons. *Nature* 373:155–158.

- Fan Y, Fricker D, Brager DH, Chen X, Lu HC, Chitwood RA, Johnston D (2005) Activity-dependent decrease of excitability in rat hippocampal neurons through increases in I(h). *Nature Neuroscience* 8:1542–1551.
- Fozzard HA, Hanck DA (1996) Structure and function of voltage-dependent sodium channels: comparison of brain II and cardiac isoforms. *Physiological Reviews* 76:887–926.
- Frick A, Magee JC, Johnston D (2004) Long-term potentiation is accompanied by an enhanced local excitability of pyramidal neuron dendrites. *Nature Neuroscience* 7:126–132.
- Gambino F, Pagès S, Kehayas V, Baptista D, Tatti R, Carleton A, Holtmaat A (2014) Sensory-evoked LTP driven by dendritic plateau potentials in vivo. *Nature* 515:116–119.
- Gasparini S, DiFrancesco D (1997) Action of the hyperpolarization-activated current (Ih) blocker ZD 7288 in hippocampal CA1 neurons. *European Journal of Physiology* 435:99–106.
- Gasparini S, Magee JC (2002) Phosphorylation-dependent differences in the activation properties of distal and proximal dendritic Na⁺ channels in rat CA1 hippocampal neurons. *Journal of Physiology* 541:665–672.
- Gasparini S, Magee JC (2004) On the initiation and propagation of dendritic spikes in CA1 pyramidal neurons. *Journal of Neuroscience* 24:1–11.
- Gasparini S, Losonczy A, Chen X, Johnston D, Magee JC (2007) Associative pairing enhances AP backpropagation into radial oblique dendrites of CA1 pyramidal neurons. *Journal of Physiology* 580:787–800.
- Golding NL, Spruston N (1998) Dendritic sodium spikes are variable triggers of axonal action potentials in hippocampal CA1 pyramidal neurons. *Neuron* 21:1189–1200.
- Golding NL, Jung HY, Mickus T, Spruston N (1999) Dendritic calcium spike initiation and repolarization are controlled by distinct potassium channel subtypes in CA1 pyramidal neurons. *Journal of Neuroscience* 19:8789–8798.
- Golding NL, Staff NP, Spruston N (2002) Dendritic spikes as a mechanism for cooperative long-term potentiation. *Nature* 418:326–331.
- Gordon U, Polsky A, Schiller J. (2006) Plasticity compartments in basal dendrites of neocortical pyramidal neurons. *Journal of Neuroscience* 26:12717–12726.
- Gulledge AT, Stuart GJ (2003) Action potential initiation and propagation in layer 5 pyramidal neurons of the rat prefrontal cortex: absence of dopamine modulation. *Journal of Neuroscience* 23:11363–11372.
- Harnett MT, Xu N-L, Magee JC, Williams SR (2013) Potassium channels control the interaction between active dendritic integration compartments in L5 cortical pyramidal neurons. *Neuron* 79:516–529.
- Harnett MT, Magee JC, Williams SR (2015) Distribution and function of HCN channels in the apical dendritic tuft of neocortical pyramidal neurons. *Journal of Neuroscience* 35:1024–1037.
- Häusser M, Clark BA (1997) Tonic synaptic inhibition modulates neuronal output pattern and spatiotemporal synaptic integration. *Neuron* 19:665–678.
- Häusser M, Spruston N, Stuart GJ (2000) Diversity and dynamics of dendritic signaling. *Science* 290:739–744.
- Herreras O (1990) Propagating dendritic action potential mediates synaptic transmission in CA1 pyramidal cells in situ. *Journal of Neurophysiology* 64:1429–1441.
- Hoffman DA, Johnston D (1998) Downregulation of transient K⁺ channels in dendrites of hippocampal CA1 pyramidal neurons by activation of PKA and PKC. *Journal of Neuroscience* 18:3521–3528.
- Hoffman DA, Johnston D (1999) Neuromodulation of dendritic action potentials. *Journal of Neurophysiology* 81:408–411.
- Hoffman DA, Magee JC, Colbert C, Johnston D (1997) K⁺ channel regulation of signal propagation in dendrites of hippocampal pyramidal neurons. *Nature* 387:869–875.
- Holt JR, Eatock RA (1995) Inwardly rectifying currents of saccular hair cells from the leopard frog. *Journal of Neurophysiology* 73:1484–1502.

- Holt CE, Schuman EM (2013) The central dogma decentralized: new perspectives on RNA function and local translation in neurons. *Neuron* **80**:648–657.
- Holthoff K, Kovalchuk Y, Yuste R, Konnerth A (2004) Single-shock LTD by local dendritic spikes in pyramidal neurons of mouse visual cortex. *Journal of Physiology* **560**:27–36.
- Hoogland TM, Saggau P (2004) Facilitation of L-type Ca^{2+} channels in dendritic spines by activation of beta2 adrenergic receptors. *Journal of Neuroscience* **24**:8416–8427.
- Hu W, Tian C, Li T, Yang M, Hou H, Shu Y (2009) Distinct contributions of $\text{Na}(\nu)1.6$ and $\text{Na}(\nu)1.2$ in action potential initiation and backpropagation. *Nature Neuroscience* **12**:996–1002.
- Huguenard JR (1996) Low-threshold calcium currents in central nervous system neurons. *Annual Review of Physiology* **58**:329–348.
- Huguenard JR, Hamill OP, Prince DA (1989) Sodium channels in dendrites of rat cortical pyramidal neurons. *Proceedings of the National Academy of Sciences of the United States of America* **86**:2473–2477.
- Isaacson JS, Strowbridge BW (1998) Olfactory reciprocal synapses: dendritic signaling in the CNS. *Neuron* **20**:749–761.
- Jaeger D, De Schutter E, Bower JM (1997) The role of synaptic and voltage-gated currents in the control of Purkinje cell spiking: a modeling study. *Journal of Neuroscience* **17**:91–106.
- Jaffe DB, Johnston D, Lasser-Ross N, Lisman JE, Miyakawa H, Ross WN (1992) The spread of Na^+ spikes determines the pattern of dendritic Ca^{2+} entry into hippocampal neurons. *Nature* **357**:244–246.
- Jan LY, Jan YN (1997) Voltage-gated and inwardly rectifying potassium channels. *Journal of Physiology* **505**:267–282.
- Johnston D, Magee JC, Colbert C, Christie BR (1996) Active properties of neuronal dendrites. *Annual Review of Neuroscience* **19**:165–186.
- Jung H-Y, Mickus T, Spruston N (1997) Prolonged Na^+ channel inactivation contributes to dendritic action potential attenuation in hippocampal pyramidal neurons. *Journal of Neuroscience* **17**:6639–6646.
- Jung SC, Kim J, Hoffman DA (2008) Rapid, bidirectional remodeling of synaptic NMDA receptor subunit composition by A-type K^+ channel activity in hippocampal CA1 pyramidal neurons. *Neuron* **60**:657–671.
- Kavalali ET, Zhuo M, Bito H, Tsien RW (1997) Dendritic Ca^{2+} channels characterized by recordings from isolated hippocampal dendritic segments. *Neuron* **18**:651–663.
- Kerti K, Lorincz A, Nusser Z (2011) Unique somato-dendritic distribution pattern of Kv4.2 channels on hippocampal CA1 pyramidal cells. *European Journal of Neuroscience* **35**:66–75.
- Kole MHP, Hallerman S, Stuart GJ (2006) Single Ih channels in pyramidal neuron dendrites: Properties, distribution and impact on action potential output. *Journal of Neuroscience* **26**:1677–1687.
- Korngreen A, Sakmann B (2000) Voltage-gated K^+ channels in layer 5 neocortical pyramidal neurones from young rats: subtypes and gradients. *Journal of Physiology* **525**:621–639.
- Kupferman JV, Basu J, Russo MJ, Guevarra J, Cheung SK, Siegelbaum SA (2014) Reelin signaling specifies the molecular identity of the pyramidal neuron distal dendritic compartment. *Cell* **158**:1335–1347.
- Larkum ME, Zhu JJ, Sakmann B (2001) Dendritic mechanisms underlying the coupling of the dendritic with the axonal action potential initiation zone of adult rat layer 5 pyramidal neurons. *Journal of Physiology* **533**:447–466.
- Lev-Ram V, Miyakawa H, Lasser-Ross N, Ross WN (1992) Calcium transients in cerebellar Purkinje neurons evoked by intracellular stimulation. *Journal of Neurophysiology* **68**:1167–1177.
- Llinás R, Sugimori M (1979) Calcium conductances in Purkinje cell dendrites: their role in development and integration. *Progress in Brain Research* **51**:323–334.
- Llinás R, Sugimori M (1980) Electrophysiological properties of *in vitro* Purkinje cell dendrites in mammalian cerebellar slices. *Journal of Physiology* **305**:197–213.
- Lorincz A, Notomi T, Tamas G, Shigemoto R, Nusser Z (2002) Polarized and compartment-dependent distribution of HCN-1 in pyramidal cell dendrites. *Nature Neuroscience* **5**:1185–1189.

- Lorincz A, Nusser Z (2010) Molecular identity of dendritic voltage-gated sodium channels. *Science* **328**:906–909.
- Losonczy A, Magee JC (2006) The integrative properties of radial oblique dendrites in hippocampal CA1 pyramidal neurons. *Neuron* **50**:291–307.
- Losonczy A, Makara J, Magee, JC (2008) Compartmentalized dendritic plasticity and neuronal input feature storage. *Nature* **452**:436–442.
- McKay BE, Turner RW (2004) Kv3 K⁺ channels enable burst output in rat cerebellar Purkinje cells. *European Journal of Neuroscience* **20**:729–739.
- Mackenzie PJ, Murphy TH (1998) High safety factor for action potential conduction along axons but not dendrites of cultured hippocampal and cortical neurons. *Journal of Neurophysiology* **80**:2089–2101.
- Magee JC (1998) Dendritic hyperpolarization-activated currents modify the integrative properties of hippocampal CA1 pyramidal neurons. *Journal of Neuroscience* **18**:1–12.
- Magee JC (1999) Temporal integration of widespread synaptic input is normalized by a nonuniform I_h density in hippocampal CA1 neurons. *Nature Neuroscience* **6**:508–514.
- Magee JC (2000) Dendritic integration of excitatory synaptic input. *Nature Reviews Neuroscience* **1**:181–190.
- Magee JC, Johnston D (1995) Characterization of single voltage-gated Na⁺ and Ca²⁺ channels in apical dendrites of rat CA1 pyramidal neurons. *Journal of Physiology* **487**:67–90.
- Magee JC, Johnston D (1997) A synaptically controlled, associative signal for Hebbian plasticity in hippocampal neurons. *Science* **275**:209–213.
- Magee JC, Avery RB, Christie BR, Johnston D (1996) Dihydropyridine-sensitive, voltage-gated Ca²⁺ channels contribute to the resting intracellular Ca²⁺ concentration of hippocampal CA1 pyramidal neurons. *Journal of Neurophysiology* **76**:3460–3470.
- Magee JC, Hoffman D, Colbert C, Johnston D (1998) Electrical and calcium signaling in dendrites of hippocampal pyramidal neurons. *Annual Review of Physiology* **60**:327–346.
- Makara JK, Magee JC (2013) Branch-specific dendritic integration in hippocampal CA3 pyramidal neurons. *Neuron* **80**:1438–1450.
- Makara JK, Losonczy A, Wen Q, Magee JC (2009) Effect of enriched environment on branch strength plasticity in rat hippocampal CA1 pyramidal neurons. *Nature Neuroscience* **12**:1485–1487.
- Marban E, Yamagishi T, Tomaselli GF (1998) Structure and function of voltage-gated sodium channels. *Journal of Physiology* **508**:647–657.
- Markram H, Sakmann B (1994) Calcium transients in dendrites of neocortical neurons evoked by single subthreshold excitatory postsynaptic potentials via low-voltage-activated calcium channels. *Proceedings of the National Academy of Sciences of the United States of America* **91**:5207–5211.
- Markram H, Helm PJ, Sakmann B (1995) Dendritic calcium transients evoked by single back-propagating action potentials in rat neocortical pyramidal neurons. *Journal of Physiology* **485**:1–20.
- Marrion NV, Tavalin SJ (1998) Selective activation of Ca²⁺-activated K⁺ channels by co-localized Ca²⁺ channels in hippocampal neurons. *Nature* **395**:900–905.
- Martina M, Schultz JH, Ehmke H, Monyer H, Jonas P (1998) Functional and molecular differences between voltage-gated K⁺ channels of fast-spiking interneurons and pyramidal neurons of rat hippocampus. *Journal of Neuroscience* **18**:8111–8125.
- Martina M, Yao GL, Bean BP (2003) Properties and functional role of voltage-dependent potassium channels in dendrites of rat cerebellar Purkinje neurons. *Journal of Neuroscience* **23**:5698–5707.
- Mathie A, Wooltorton JR, Watkins CS (1998) Voltage-activated potassium channels in mammalian neurons and their block by novel pharmacological agents. *General Pharmacology* **30**:13–24.
- Mickus T, Jung H, Spruston N (1999) Properties of slow, cumulative sodium channel inactivation in rat hippocampal CA1 pyramidal cells. *Biophysical Journal* **76**:846–860.
- Midtgård J (1994) Processing of information from different sources: spatial synaptic integration in the dendrites of vertebrate CNS. *Trends in Neurosciences* **17**:166–173.

- Midtgård J, Lasser-Ross N, Ross WN** (1993) Spatial distribution of Ca^{2+} influx in turtle Purkinje cell dendrites in vitro: role of a transient outward current. *Journal of Neurophysiology* **70**:2455–2469.
- Migliore M, Shepherd GM** (2002) Emerging rules for the distributions of active dendritic conductances. *Nature Reviews Neuroscience* **3**:362–370.
- Mouginot D, Bossu J, Gähwiler BH** (1997) Low-threshold Ca^{2+} currents in dendritic recordings from Purkinje cells in rat cerebellar slice cultures. *Journal of Neuroscience* **17**:160–170.
- Murphy GJ, Isaacson JS** (2004) Role of dendritic calcium spikes in intragranular inhibition. *Society for Neuroscience Abstracts* **67**:15.
- Narayanan R, Johnston D** (2007) Long-term potentiation in rat hippocampal neurons is accompanied by spatially widespread changes in intrinsic oscillatory dynamics and excitability. *Neuron* **56**:1061–1075.
- Narayanan R, Johnston D** (2008) The h channel mediates location dependence and plasticity of intrinsic phase response in rat hippocampal neurons. *Journal of Neuroscience* **28**:5846–5860.
- Nolan MF, Malleret G, Lee KH, Gibbs E, Dudman JT, Santoro B, Yin D, Thompson RF, Siegelbaum SA, Kandel ER, Morozov A** (2003) The hyperpolarization-activated HCN1 channel is important for motor learning and neuronal integration by cerebellar Purkinje cells. *Cell* **115**:551–564.
- Nolan MF, Malleret G, Dudman JT, Buhl, D.L., Santoro B, Gibbs E, Vronskaya, S., Buzsaki, G., Siegelbaum SA, Kandel ER, Morozov A** (2004) A behavioral role for dendritic integration: HCN1 channels constrain spatial memory and plasticity at inputs to distal dendrites of CA1 pyramidal neurons. *Cell* **119**:719–732.
- Oliver D, Lien CC, Soom M, Baukrowitz T, Jonas P, Fakler B** (2004) Functional conversion between A-type and delayed rectifier K^+ channels by membrane lipids. *Science* **304**:265–270.
- Otsu Y, Marcaggi P, Feltz A, Isope P, Kollo M, Nusser Z, Mathieu B, Kano M, Tsujita M, Sakimura K, Dieudonné S** (2014) Activity-dependent gating of calcium spikes by A-type K^+ channels controls climbing fiber signaling in Purkinje cell dendrites. *Neuron* **84**:137–151.
- Pape H-C** (1996) Queer current and pacemaker: the hyperpolarization-activated cation current in neurons. *Annual Review of Physiology* **58**:299–327.
- Pérez-Garcí E, Gassmann M, Bettler B, Larkum ME** (2006) The GABAB1b isoform mediates long-lasting inhibition of dendritic Ca^{2+} spikes in layer 5 somatosensory pyramidal neurons. *Neuron* **50**:603–616.
- Pérez-Garcí E, Larkum ME, Nevian T** (2013) Inhibition of dendritic Ca^{2+} spikes by GABAB receptors in cortical pyramidal neurons is mediated by a direct $\text{G}\iota/\text{o}-\beta$ -subunit interaction with Cav1 channels. *Journal of Physiology* **591**:1599–1612.
- Polksy A, Mel BW, Schiller J** (2004) Computational subunits in thin dendrites of pyramidal cells. *Nature Neuroscience* **7**:621–627.
- Poolos NP, Johnston D** (1999) Calcium-activated potassium conductances contribute to action potential repolarization at the soma but not the dendrites of hippocampal CA1 pyramidal neurons. *Journal of Neuroscience* **19**:5205–5212.
- Ramakers GM, Storm JF** (2002) A postsynaptic transient $\text{K}(+)$ current modulated by arachidonic acid regulates synaptic integration and threshold for LTP induction in hippocampal pyramidal cells. *Proceedings of the National Academy of Sciences of the United States of America* **99**:10144–10149.
- Randall AD, Tsien RW** (1997) Contrasting biophysical and pharmacological properties of T-type and R-type calcium channels. *Neuropharmacology* **36**:879–893.
- Richardson TL, Turner RW, Miller JJ** (1987) Action potential discharge in hippocampal CA1 pyramidal neurons: current source-density analysis. *Journal of Neurophysiology* **58**:981–986.
- Robinson R, Siegelbaum S** (2003) Hyperpolarization-activated cation currents: from molecules to physiological function. *Annual Review of Physiology* **65**:453–480.
- Roth A, Häusser M** (2001) Compartmental models of rat cerebellar Purkinje cells based on simultaneous somatic and dendritic patch-clamp recordings. *Journal of Physiology* **535**:445–447.
- Sah P** (1996) Ca^{2+} -activated K^+ currents in neurons: types, physiological roles and modulation. *Trends in Neurosciences* **19**:150–154.

- Sah P, Bekkers JM (1996) Apical dendritic location of slow afterhyperpolarization current in hippocampal pyramidal neurons: implications for the integration of long-term potentiation. *Journal of Neuroscience* **16**:4537–4542.
- Schiller J, Schiller Y, Stuart G, Sakmann B (1997) Calcium action potentials restricted to distal apical dendrites of rat neocortical pyramidal neurons. *Journal of Physiology* **505**:605–616.
- Schiller J, Schiller Y, Clapham DE (1998) NMDA receptors amplify calcium influx into dendritic spines during associative pre- and postsynaptic activation. *Nature Neuroscience* **1**:114–118.
- Schnee ME, Ricci AJ (2003) Biophysical and pharmacological characterization of voltage-gated calcium currents in turtle auditory hair cells. *Journal of Physiology* **549**:697–717.
- Schrader LA, Anderson AE, Varga AW, Levy M, Sweatt JD (2002) The other half of Hebb: K⁺ channels and the regulation of neuronal excitability in the hippocampus. *Molecular Neurobiology* **25**:51–66.
- Schwindt PC, Crill WE (1995) Amplification of synaptic current by persistent sodium conductance in apical dendrite of neocortical neurons. *Journal of Neurophysiology* **74**:2220–2224.
- Schwindt PC, Crill WE (1997) Modification of current transmitted from apical dendrite to soma by blockade of voltage- and Ca²⁺-dependent conductances in rat neocortical pyramidal neurons. *Journal of Neurophysiology* **78**:187–198.
- Sochivko D, Pereverzev A, Smyth N, Gissel C, Schneider T, Beck H (2002) The Ca(V)2.3 Ca(2+) channel subunit contributes to R-type Ca(2+) currents in murine hippocampal and neocortical neurones. *Journal of Physiology* **542**:699–710.
- Spruston N, Schiller Y, Stuart G, Sakmann B (1995) Activity-dependent action potential invasion and calcium influx into hippocampal CA1 dendrites. *Science* **268**:297–300.
- Storm JF (1987) Action potential repolarization and a fast after-hyperpolarization in rat hippocampal pyramidal cells. *Journal of Physiology* **385**:733–759.
- Storm JF (1990) Potassium currents in hippocampal pyramidal cells. *Progress in Brain Research* **83**:161–187.
- Strafstrom CE, Schwindt PC, Chubb MC, Crill WE (1985) Properties of persistent sodium conductance and calcium conductance of layer V neurons from cat sensorimotor cortex in vitro. *Journal of Neurophysiology* **53**:152–170.
- Stuart GJ, Häusser M (1994) Initiation and spread of sodium action potentials in cerebellar Purkinje cells. *Neuron* **13**:703–712.
- Stuart GJ, Sakmann B (1994) Active propagation of somatic action potentials into neocortical pyramidal cell dendrites. *Nature* **367**:69–72.
- Stuart G, Spruston N (1998) Determinants of voltage attenuation in neocortical pyramidal neuron dendrites. *Journal of Neuroscience* **18**:3501–3510.
- Stuart G, Schiller J, Sakmann B (1997a) Action potential initiation and propagation in rat neocortical pyramidal neurons. *Journal of Physiology* **505**:617–632.
- Stuart G, Spruston N, Sakmann B, Häusser M (1997b) Action potential initiation and backpropagation in neurons of the mammalian CNS. *Trends in Neurosciences* **20**:125–131.
- Sun W, Maffie JK, Lin L, Petralia RS, Rudy B, Hoffman DA (2011) DPP6 establishes the A-type K⁺ current gradient critical for the regulation of dendritic excitability in CA1 hippocampal neurons. *Neuron* **71**:1102–1115.
- Takahashi H, Magee JC (2009) Pathway interactions and synaptic plasticity in the distal tuft regions of CA1 pyramidal neurons. *Neuron* **62**:102–111.
- Takigawa T, Alzheimer C (1999) G protein-activated inwardly rectifying K⁺ (GIRK) currents in dendrites of rat neocortical pyramidal cells. *Journal of Physiology* **517**:385–390.
- Takigawa T, Alzheimer C (2003) Interplay between activation of GIRK current and deactivation of Ih modifies temporal integration of excitatory input in CA1 pyramidal cells. *Journal of Neurophysiology* **89**:2238–2244.

- Trimmer JS, Rhodes KJ (2004) Localization of voltage-gated ion channels in mammalian brain. *Annual Review of Physiology* **66**:477–519.
- Tsubokawa H, Ross WN (1996) IPSPs modulate spike backpropagation and associated $[Ca^{2+}]_i$ changes in the dendrites of hippocampal CA1 pyramidal neurons. *Journal of Neurophysiology* **76**:2896–2906.
- Tsubokawa H, Ross WN (1997) Muscarinic modulation of spike backpropagation in the apical dendrites of hippocampal CA1 pyramidal neurons. *Journal of Neuroscience* **17**:5782–5791.
- Tsubokawa H, Miura M, Kano M (1999) Elevation of intracellular Na^+ induced by hyperpolarization at the dendrites of pyramidal neurones of mouse hippocampus. *Journal of Physiology* **517**:135–142.
- Turner RW, Meyers DER, Richardson TL, Barker JL (1991) The site for initiation of action potential discharge over the somatodendritic axis of rat hippocampal CA1 pyramidal neurons. *Journal of Neuroscience* **11**:2270–2280.
- Usowicz MM, Sugimori M, Cherksey B, Llinás R (1992) P-type calcium channels in the somata and dendrites of adult cerebellar Purkinje cells. *Neuron* **9**:1185–1199.
- Vaidya SP, Johnston D (2013) Temporal synchrony and gamma-to-theta power conversion in the dendrites of CA1 pyramidal neurons. *Nature Neuroscience* **16**:1812–1820.
- Varga AW, Yuan LL, Anderson AE, Schrader LA, Wu GY, Gatchel JR, Johnston D, Sweatt JD (2004) Calcium-calmodulin-dependent kinase II modulates Kv4.2 channel expression and upregulates neuronal A-type potassium currents. *Journal of Neuroscience* **24**:3643–3654.
- Varga V, Losonczy A, Zemelman G, Borhegyi Z, Nyiri G, Magee JC, Freund T (2009) Fast synaptic subcortical control of cortical circuits. *Science* **326**:449–453.
- Wang Z, Xu N-L, Wu C, Duan S, Poo M-M (2003) Bidirectional changes in spatial dendritic integration accompanying long-term synaptic modifications. *Neuron* **37**:463–472.
- Watanabe S, Takagi H, Miyasho T, Kirino Y, Kudo Y, Miyakawa H (1998) Differential roles of two types of voltage-gated Ca^{2+} channels in the dendrites of Purkinje neurons. *Brain Research* **79**:43–55.
- Welsby PJ, Wang H, Wolfe JT, Colbran RJ, Johnson ML, Barrett PQ (2003) A mechanism for the direct regulation of T-type calcium channels by Ca^{2+} /calmodulin-dependent kinase II. *Journal of Neuroscience* **23**:10116–10121.
- Williams SR, Stuart GJ (2000a) Action potential backpropagation and somato-dendritic distribution of ion channels in thalamocortical neurons. *Journal of Neuroscience* **20**:1307–1317, 2000.
- Williams SR, Stuart GJ (2000b) Site independence of EPSP time course is mediated by dendritic I(h) in neocortical pyramidal neurons. *Journal of Neurophysiology* **83**:3177–3182.
- Williams SR, Stuart GJ (2003) Voltage- and site-dependent control of the somatic impact of dendritic IPSPs. *Journal of Neuroscience* **23**:7358–7367.
- Williams SR, Christensen SR, Stuart GJ, Häusser M (2002) Membrane potential bistability is controlled by the hyperpolarization-activated current I(H) in rat cerebellar Purkinje neurons in vitro. *Journal of Physiology* **539**:469–483.
- Womack MD, Khodakhah K (2003) Somatic and dendritic small-conductance calcium-activated potassium channels regulate the output of cerebellar Purkinje neurons. *Journal of Neuroscience* **23**:2600–2607.
- Womack MD, Khodakhah K (2004) Dendritic control of spontaneous bursting in cerebellar Purkinje cells. *Journal of Neuroscience* **24**:3511–3521.
- Wong RKS, Stewart M (1992) Different firing patterns generated in dendrites and somata of CA1 pyramidal neurons in guinea-pig hippocampus. *Journal of Physiology* **457**:675–687.
- Xu W, Lipscombe D (2001) Neuronal Ca(V)1.3alpha(1) L-type channels activate at relatively hyperpolarized membrane potentials and are incompletely inhibited by dihydropyridines. *Journal of Neuroscience* **21**:5944–5951.
- Xu J, Kang N, Jiang L, Nedergaard M, Kang J (2005) Activity-dependent long-term potentiation of intrinsic excitability in hippocampal CA1 pyramidal neurons. *Journal of Neuroscience* **25**:1750–1760.

- Yasuda R, Sabatini B, Svoboda K (2003) Plasticity of calcium channels in dendritic spines. *Nature Neuroscience* **6**:948–955.
- Yuan LL, Adams JP, Swank M, Sweatt JD, Johnston D (2002) Protein kinase modulation of dendritic K⁺ channels in hippocampus involves a mitogen-activated protein kinase pathway. *Journal of Neuroscience* **22**:4860–4868.
- Yuste R, Denk W (1995) Dendritic spines as basic functional units of neuronal integration. *Nature* **375**:682–684.
- Yuste R, Tank DW (1996) Dendritic integration in mammalian neurons, a century after Cajal. *Neuron* **16**:701–716.
- Zhang W, Linden DJ (2003) The other side of the engram: experience-driven changes in neuronal intrinsic excitability. *Nature Reviews Neuroscience* **4**:885–900.
- Zhu JJ (2000) Maturation of layer 5 neocortical pyramidal neurons: amplifying salient layer 1 and layer 4 inputs by Ca²⁺ action potentials in adult rat tuft dendrites. *Journal of Physiology* **526**:571–587.

Chapter 10

Biochemical compartmentalization in dendrites

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Summary

Dendrites have both an electrical and a biochemical character, which are closely linked. In this chapter we discuss dendritic structures as compartments for chemical signals such as concentration changes of ions or other second messengers, which control enzyme activity and signaling cascades. In particular, we focus on the question to what extent these signals can be confined to only part of the dendritic tree. Such ‘compartmentalization’ is considered the basis of local modifications of dendritic properties, in particular to achieve input-specific changes in synaptic strength. Following an introduction we first discuss general factors that affect compartmentalization of chemical signals, including diffusion, intracellular binding, and removal mechanisms. We then provide examples of measurements of dendritic ion and second messenger signaling, with the main focus on calcium signaling, for which the most detailed information is available from imaging studies. Subsequently, in an attempt to define functional units, we present an overview of the different spatial scales of dendritic compartmentalization, spanning a range of three orders of magnitude. Finally, we discuss examples of how chemical signals are used for dendritic information processing.

Introduction

The central nervous system (CNS) has evolved powerful activity-dependent plasticity mechanisms that enable higher brain functions such as learning and memory. Prominent cellular mechanisms of neural plasticity are long-term potentiation (LTP) and its counterpart long-term depression (LTD), which are long-lasting changes in the strength of synaptic communication between neurons that can be induced by distinct patterns of electrical stimulation of afferent fibers or sensory stimulation (Holtmaat and Svoboda, 2009). As these synaptic weight changes can be expressed at individual synapses from among the thousands of synapses on a single neuron there is a practically unlimited number of ways for neural circuits to reconfigure their functional connectivity matrix—this is thought to underlie the huge information processing and storage capacity of the brain. The precise anatomical features of neurons, in particular dendrites and dendritic spines, are critical in this regard because they confer upon neurons the ability to confine or segregate biochemical signals and thereby implement thousands of more or less independently adjustable functional microcompartments.

Electrical and biochemical activity are tightly coupled in neurons (Fig. 10.1A). A key link is provided by calcium ions (Ca^{2+}), which flow into neurons whenever they become synaptically excited or generate action potentials (APs). Entering the cytosol via ionotropic receptors and voltage-gated channels, Ca^{2+} strongly influences a wide variety of intracellular signaling cascades that

can regulate essentially all aspects of neuronal function, including neuronal excitability. Likewise, synaptic activity also stimulates G-protein-coupled (metabotropic) receptors, which leads to the formation of second messengers such as inositol 1,4,5-trisphosphate (IP_3) or cyclic AMP (cAMP) (Fig. 10.1B). These second messengers in turn can powerfully modify neuronal excitability on both short and long time scales (see Chapters 8 and 9).

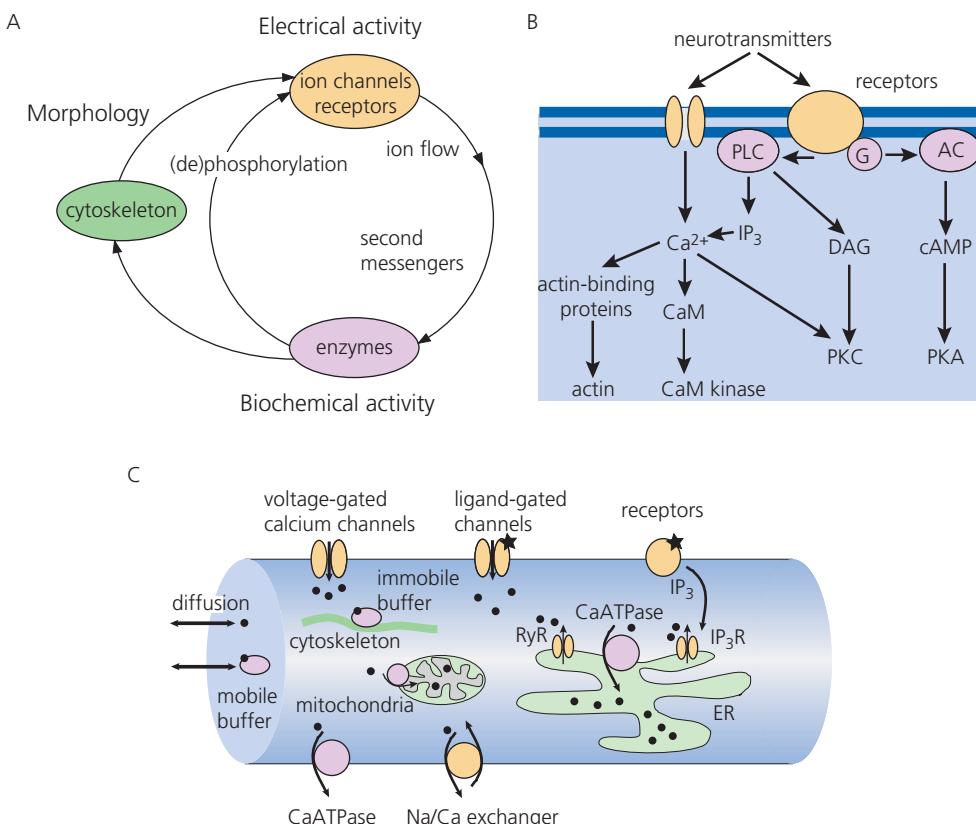


Fig. 10.1 Overview of second messenger signaling pathways. (A) Coupling of electrical signaling, chemical signaling, and morphology. Ion fluxes and receptor activation cause changes in the concentration of ions and second messengers. Activation of cytosolic enzymes in turn leads to modifications of membrane proteins or cytoskeletal proteins. (B) Main pathways of protein kinase activation via G-protein-coupled receptors. Production of cAMP by adenylate cyclase (AC) is controlled by G-proteins and activates cAMP-dependent protein kinase (PKA). Activation of phospholipase C (PLC) leads to generation of diacylglycerol (DAG), which activates protein kinase C (PKC), and of inositol 1,4,5-trisphosphate (IP_3), which causes release of calcium from internal stores via the IP_3 receptor. Among various other effects Ca^{2+} binds to calmodulin (CaM) and activates CaM kinases. Ca^{2+} is also involved in the regulation of actin-binding proteins. (C) Components of neuronal Ca^{2+} homeostasis. Calcium ions are indicated by black dots. Pathways for Ca^{2+} entry are voltage- and ligand-gated ion channels and release from endoplasmic reticulum (ER) via IP_3 receptors or ryanodine receptors (RyR). Cytosolic calcium diffuses and binds to mobile or fixed buffers. Clearance mechanisms include extrusion across the plasma membrane via a Ca -ATPase and a Na^+/Ca^{2+} exchanger, uptake into the ER via a Ca -ATPase, and uptake into mitochondria via an uniporter.

Thus, neurons are endowed with transduction mechanisms that allow them to effectively transform fast and transient electrical signals into changes in biochemical activity, for instance through the activation of kinases and phosphatases that are present at the sites of Ca^{2+} entry or second messenger production. The activation of these biochemical pathways can lead to morphological changes in dendritic spines via remodeling of the actin-based cytoskeletal network (Matus, 2000; Kasai et al., 2003; Oertner and Matus, 2005; Carlisle and Kennedy, 2005; Bosch and Hayashi, 2012; see Chapter 19), as well as to changes in the composition and properties of synaptic membrane proteins (Makino and Malinow, 2009; Choquet and Triller, 2013). Calcium ions have a central role as second messengers because they can enter the cytosol through various pathways, including voltage-dependent calcium channels and release from intracellular stores, and they interact with numerous proteins (Fig. 10.1C).

Importantly, these biochemical effects, including the feedback on neuronal excitability, can occur over an extremely wide time scale, ranging from milliseconds to the lifespan of the animal. They engage a wide variety of molecular effectors and cell-biological processes, including nearly instant and short-lived actions on Ca^{2+} -dependent conductances such as voltage-gated K^+ and Ca^{2+} channels, but also more delayed and long-term effects via local protein synthesis (see Chapter 5) and gene expression (Deisseroth et al., 2003).

Neurons are highly polarized structures having nerve terminals, axon, soma, and dendrites as specialized cellular compartments. It is obvious that chemical signals such as changes in Ca^{2+} concentration can be spatially confined to these different functional compartments (Wang and Augustine, 1999). More recently, it has become clear that subdivisions into functional compartments also exist within dendritic trees. Such compartmentalization enables a neuron to spatially segregate signaling cascades and to restrict their effects to particular dendritic regions, for example a dendritic spine or an individual branch (Branco and Häusser, 2010). This is especially relevant for the induction of various forms of synaptic plasticity that depend on second messenger signals. For example, several prominent forms of LTP and LTD depend on postsynaptic Ca^{2+} and can display input specificity according to the Hebbian rule that only synapses that experience near-coincident pre- and postsynaptic activity become modified (Häusser et al., 2000; Sjöström and Nelson, 2002; Johnston et al., 2003). The spatial profile of Ca^{2+} in these cases will largely determine how far synaptic plasticity spreads to inactive neighboring synapses. In view of the complexity of the results obtained with different protocols for inducing synaptic plasticity, it seems likely that the precise spatio-temporal patterns of the relevant signaling molecules need to be known in order to understand the overall effect.

The following questions naturally arise. What is the spatial extent of localized chemical signals that are initiated in small neuronal compartments such as dendrites and dendritic spines? How far do they spread and how long do they persist? What are the cellular mechanisms involved? Is compartmentalization dynamically regulated and how is it related to the induction and maintenance of synaptic plasticity and eventually to neural computation and animal behavior?

During the last decade there have been great advances in the experimental tools needed to address these questions and they continue to develop at a rapid pace. A variety of optical techniques have been developed that make it possible both to measure and to manipulate the electrical and biochemical activity inside small neuronal compartments, supplementing, and increasingly supplanting, traditional electrophysiological and pharmacological approaches to study neuronal signaling pathways. The large palette of synthetic small molecule fluorescent indicators (Tsien, 1989) has been complemented by a new class of genetically encoded biosensors based on fluorescent proteins (Zhang et al., 2002; Miyawaki, 2003; Looger and Griesbeck, 2012). For example, a variety of highly sensitive Ca^{2+} indicators have been developed (reviewed in Grienberger and Konnerth, 2012) that are based either on a single-fluorescent protein design (Chen et al., 2013) or on

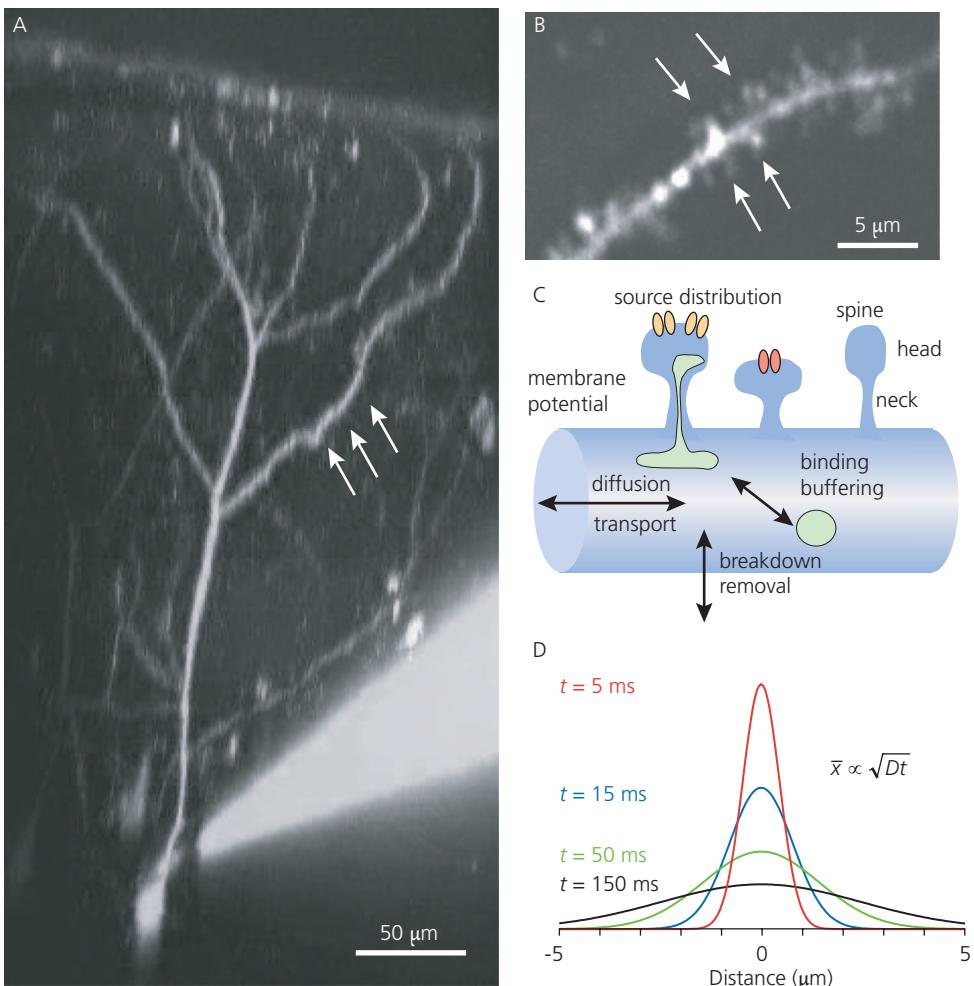


Fig. 10.2 Factors contributing to compartmentalization. **(A)** Example of a side-projection of a fluorescently labeled pyramidal neuron in the neocortex of an anesthetized rat obtained with a two-photon microscope. The neuron was filled via a whole-cell patch pipette. Local activation of a dendritic branch is schematically indicated. **(B)** High-magnification two-photon image of a spiny dendritic branch of a pyramidal neuron *in vivo*. Activation of a subset of dendritic spines is schematically indicated. **(C)** Schematic illustrating the main determinants of compartmentalization following a spatially confined increase in ion or second messenger concentration, for example after local synaptic activation of a dendritic branch as indicated in parts A and B. **(D)** The square-root law of diffusion illustrated for the example of buffered calcium diffusion. The theoretical Gaussian distributions of the diffusional spread following a brief calcium injection at the origin are shown at four time points after the injection. The apparent diffusion constant was assumed to be $0.02 \mu\text{m}^2/\text{ms}$. Note that for a ten times longer time period the curves broaden by only a factor of about three ($\sqrt{10}$).

tandem-fluorescent protein constructs that utilize Förster resonance energy transfer (FRET) to transform the conformational change induced by Ca^{2+} binding into a fluorescence signal. Probes based on FRET changes have also been designed for reporting other second messenger concentrations or enzymatic activity. In a parallel development, microscopy techniques have also undergone tremendous improvement: multi-photon microscopy now permits imaging of dendrites and synapses in the intact brain over many days and even months (Fig. 10.2A, B; Helmchen and Denk, 2005; Kerr and Denk, 2008). Moreover, the advent of super-resolution microscopy makes it possible to image synaptic structures and their molecular dynamics beyond the classic diffraction barrier of light microscopy (Kittel et al., 2006; reviewed in Hell, 2007; Dani et al., 2010; Maglione and Sigrist, 2013; Tønnesen and Nägerl, 2013), which is absolutely critical because of the sub-micron spatial dimensions of CNS synapses (see Fig. 10.5).

Of the methods to manipulate neural activity, photolysis of caged compounds provides a means to deliver chemical substances in a very rapid and spatially controlled way inside three-dimensional brain tissue. Caged compounds have been designed for a variety of molecules, including nucleotides, neurotransmitters, IP_3 , Ca^{2+} , and nitric oxide (Nerbonne, 1996), and two-photon uncaging of glutamate has been particularly useful for studying the physiology of dendritic spines (Matsuzaki et al., 2004; Kwon and Sabatini, 2011; Tønnesen et al., 2014). A new generation of chromatically specific compounds makes it possible to probe neuronal function by uncaging glutamate and gamma-aminobutyric acid (GABA) at different wavelengths (Amatrudo et al., 2015). Finally, the development of optogenetic approaches using a variety of engineered opsins for spatio-temporal control of neuronal activity has enormously expanded the possibilities for probing neural circuit dynamics (Bernstein and Boyden, 2011; Deisseroth, 2011; Fenno et al., 2011), making it possible to both control and monitor the electrical activity of neurons remotely with light using voltage actuators and sensors (Oertner, 2011; Hochbaum et al., 2014). By combining these refined optical techniques with traditional electrophysiological and biochemical approaches, local events inside dendritic microcompartments can now be studied in much greater detail and under more realistic experimental conditions *in vitro* and *in vivo*. Furthermore, the investigation of chemical compartmentalization in the intact brain may allow us to resolve its behavioral relevance. Given the great diversity of dendritic morphology and the differences in expression pattern and subcellular distribution of proteins, the extent and functional importance of compartmentalization vary widely between different neuronal cell types. We cannot account for this variety here. Rather than listing all different cell types we prefer to focus on the general principles of second messenger signaling in dendrites and then discuss characteristic examples in certain cell types.

Determinants of compartmentalization

A wide variety of factors contribute to compartmentalization in dendrites, including the spatial distribution of synaptic input, dendritic morphology, intracellular diffusion, and the production and clearance of second messengers (Fig. 10.2C). The aim of this section is to provide a general introduction that is applicable to any diffusible dendritic messenger. More specific information about various second messenger substances is given in the next section.

Spatio-temporal activation pattern

The initial location of a particular chemical signal depends on the spatio-temporal activation pattern of the receptors, membrane conductances, or enzymes that mediate this signal. The dendritic

distribution of these proteins (see Chapters 7–9) is therefore of crucial importance, together with the pattern of afferent innervation. Furthermore, work over the last 10 years has shown that synaptic receptors are highly mobile on the surface of neuronal membranes, and that their mobility is regulated by neuronal activity (Choquet and Triller, 2013).

In some cases dendritic trees are clearly innervated in a topographic manner (Borst and Egelhaaf, 1994). In the mammalian neocortex, the precise patterning of inputs is known for connections from inhibitory interneurons onto the dendrites of pyramidal cells, which target characteristic locations (Douglas and Martin, 1998; Klausberger, 2009). In the cerebellum, Purkinje cells receive anatomically segregated excitatory input from parallel fibers and climbing fibers (Palay and Chan-Palay, 1974). In other cell types, for example neurons in the lateral nucleus of the amygdala (Humeau et al., 2005), inputs from various afferent projections are dispersed and mixed throughout the dendritic tree. However, for many cell types the dendritic innervation patterns have not been fully determined. In addition, even if the anatomical organization of afferent input is known, the functional activation patterns that occur under *in vivo* conditions have only begun to be mapped (reviewed in Grienberger et al., 2015).

What is clear, however, is that stimulation of specific afferent pathways can result in local activation of postsynaptic dendrites (schematically indicated in Fig. 10.2). This activation may appear as the local production of second messengers by G-protein-coupled receptors, which is mostly confined to the sites of presynaptic transmitter release. It may also involve postsynaptic ionic currents, the spatial pattern of which is influenced by the presence of voltage-dependent ion channels in dendrites (see Chapter 9). For example, excitatory postsynaptic potentials (EPSPs) may locally reach the threshold for activation of voltage-dependent channels, including *N*-methyl-D-aspartate (NMDA)-type glutamate receptor channels, causing spatially restricted “local spikes” and concomitant localized calcium influx (Häusser et al., 2000; Yuste et al., 2000; Schiller and Schiller, 2001; Major et al., 2013). Backpropagating APs, on the other hand, lead to a more widespread dendritic calcium influx depending on how far APs invade the dendrites (Häusser et al., 2000; Sjöström and Nelson, 2002; Waters et al., 2005). The electrical properties of dendritic membranes are thus of vital importance in determining the spread of these calcium signals (see Chapters 12 and 13).

Clearly, not only is the distribution of sources important but also the spatial organization of effector proteins. It is by now well established that signaling proteins can be locally synthesized and degraded in dendrites, held in place by anchoring proteins, and translocated following synaptic stimulation (Steward and Schuman, 2003; Horton and Ehlers, 2004). In addition, the speed and duration of second messenger production could be of relevance for the activation of effector proteins. Second messenger production by enzymes is usually relatively slow, on a time scale of hundreds of milliseconds or longer (Kasai and Petersen, 1994). In contrast, influx of Ca^{2+} during an AP can be as brief as a millisecond. It may be important to distinguish between such brief pulse-like injections of Ca^{2+} and slower changes in Ca^{2+} concentration, for example during prolonged membrane depolarizations. The reason is that intracellular target proteins with different Ca^{2+} -binding kinetics are activated differentially depending on whether calcium ions enter the cytosol rapidly or slowly (Markram et al., 1998; Sabatini et al., 2002). Targets with fast binding rates are favored by brief injections of Ca^{2+} because they outcompete slower proteins on a short time scale (see also the subsection Binding affinities and kinetics).

Morphology

The anatomical structure of dendrites is a major factor controlling the diffusional spread of second messenger molecules. The length and diameter of dendritic branches, their branching pattern,

and the density of spines influence the ability of a neuron to spatially confine chemical signals. Importantly, excitatory neurons generally bear dendritic spines whereas inhibitory cells tend to have smooth dendrites (Douglas and Martin, 1998). This morphological distinction may reflect different requirements for biochemical compartmentalization in these cell types. On the other hand, synaptically induced calcium transients in smooth dendrites of neocortical and cerebellar interneurons have also been shown to be confined to micrometer compartments (Goldberg and Yuste, 2005; Soler-Llavina and Sabatini, 2006). This finding suggests that compartmentalization on the synaptic scale could be a general concept, although achieved by various mechanisms.

Dendritic spines typically host a single synapse at their bulbous head, which is connected to the parent dendrite via a thin and elongated stalk, called the spine neck, with a diameter of between 50 and 500 nm and a length of 0.1–3 μm (Fig. 10.2C). Depending on these wide-ranging dimensions, many spine necks impose a major diffusion barrier even to highly diffusible molecules (Tønnesen et al., 2014). Similarly, dendritic branches can also feature ultrathin constrictions (down to a width of 200 nm). Hence spines and dendritic segments are small volumes that can be diffusionally isolated, enabling neurons to amplify chemical signals in the sense that the same transmembrane ion flux—or the same amount of enzymatic second messenger production—causes larger rises in concentration if confined to a smaller volume. In other words, the surface-to-volume ratio is a crucial determinant of the absolute concentrations reached (Anwar et al., 2014). For dendrites, which are approximately cylindrical, the surface-to-volume ratio depends roughly inversely on their radius. It should be noted that the accessible cytosolic volume is reduced due to the presence of intracellular organelles; the relative degree of volume exclusion in spines and dendritic shafts caused by intracellular organelles remains unknown. Structural changes in the dendrites and spines (see Chapter 19) could have a pronounced effect on the local amplitude of chemical signals if they alter the surface-to-volume ratio or affect the diffusional isolation of the microcompartment. These changes can be synergistic or cancel each other out, depending on their extent and direction. Finally, the branching pattern of a dendritic tree defines not only the electrotonic but also the “chemical distance” between any two points on the dendritic tree. In analogy to the propagation of electrical potentials, the spread of chemical signals in the dendritic tree will depend not only on the density of branching but also on the relative diameters of parent and daughter dendrites at branch points, with increases in diameter causing further dilution.

The degree of chemical isolation between two sites also critically depends on the nature of the messenger substance, in particular its diffusive properties. Two synapses along a dendritic branch may be far apart with respect to a low-diffusible messenger but experience similar concentration levels in the case of a highly diffusible substance. For extracellularly diffusing neurotransmitter molecules the geometric parameters of the extracellular space determine how far these molecules “spill over” and bind to extrasynaptic dendritic receptors (Syková 2004). In the extreme case of a freely diffusible messenger, compartmentalization is bypassed altogether. Nitric oxide (NO), for instance, is a gaseous and membrane-permeant messenger implicated in the induction of synaptic plasticity (Schuman and Madison, 1991; O’Dell et al., 1991; Hardingham et al., 2013). From its diffusion coefficient (Table 10.1), its rate of production, and its approximate lifetime of a few seconds one can estimate that NO spreads about 100–200 μm before it degrades (Ledo et al., 2004). Therefore, it may also affect synapses that have not participated in the induction of synaptic plasticity located either on neighboring dendritic branches of the same neuron or on branches of neighboring cells. None of these special cases of “volume transmission,” including local modulation of synaptic transmission mediated by retrograde messengers (Ohno-Shosaku and Kano, 2014), will be further discussed here.

Diffusion

We now turn our attention to the mechanisms that affect the intracellular spread of chemical signals in dendrites once they have been generated locally. We limit our description to a few fundamental ideas. A more comprehensive treatment of diffusion and buffering can be found in Koch (1998). We do not consider active transport mechanisms in dendrites, which operate on a slower time scale (Kapitein and Hoogenraad, 2011). For simplicity we consider the one-dimensional case. Since dendrites are typically long, thin cylindrical structures, such a description is often a useful approximation.

Mobile substances in the cytosol spread by diffusion. Diffusion is a probabilistic process due to the thermal agitation of molecules. If we are interested in the temporal change of the concentration $[C(x,t)]$ of a diffusible substance C at a position x along a dendrite, we have to take the difference of influx and efflux from both sides, which both depend on the local concentration gradient. The resulting balance equation is the *diffusion equation*:

$$\frac{\partial[C(x,t)]}{\partial t} = D \frac{\partial^2[C(x,t)]}{\partial x^2} \quad (10.1)$$

where D is the diffusion coefficient. For large molecules (molecular weight $M > 1,000$) D mainly depends on the radius of the molecule, and therefore on the cube root of M . Ions have relatively small diffusion coefficients, partially due to the formation of hydration shells in aqueous solution. Typical aqueous diffusion coefficients for ions, second messengers, and proteins range from 0.1 to 100 $\mu\text{m}^2/\text{ms}$ (Table 10.1). Cytoplasmic diffusion coefficients are generally several-fold smaller because of the higher viscosity and physical restrictions in the cytosol (Woolf and Greer, 1994; Biess et al., 2011). In addition, binding to immobile proteins further slows down diffusion of a substance (see the discussion on buffering later). For example, the apparent diffusion coefficient of calcium in the cytosol is only about 0.02 $\mu\text{m}^2/\text{ms}$ due to intracellular binding (Allbritton et al., 1992). In the case of a local injection of a substance into an infinite cylinder, the solution of the diffusion equation is a Gaussian distribution of the substance along the dendrite that broadens with time (Fig. 10.2D). An important characteristic of this spreading is that the mean displacement \bar{x} from the point of injection is proportional to the square root of time:

$$\bar{x} \propto \sqrt{Dt}. \quad (10.2)$$

Hence, a substance with a diffusion coefficient of 0.1 $\mu\text{m}^2/\text{ms}$ spreads about 0.3 μm in 1 ms, 1 μm in 10 ms, 3 μm in 100 ms, and 10 μm in 1 s. This illustrates that—given that intracellular messengers have lifetimes of less than a second to a few seconds—chemical signals can be quite effectively compartmentalized in long dendrites solely due to this square-root law of the diffusional processes. The dilution of the messenger concentration during spreading further limits the spatial range of substrate activation.

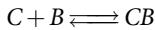
Buffering

Second messengers and ions act by binding to target molecules, causing conformational and functional changes. For H^+ and for Ca^{2+} the concentration of binding sites can be so large that most ions are bound and only a small fraction remain free. This means that pH and the intracellular free calcium concentration ($[\text{Ca}^{2+}]_i$) are effectively buffered. Many Ca^{2+} -binding proteins have reversible binding of calcium as their only known function and are commonly referred to as “calcium buffers.”

Table 10.1 Aqueous (D_{aqu}) and cytosolic (D_{cyto}) diffusion coefficients for several ions, second messengers, and proteins. References also refer to citations therein

Substance	$D_{\text{aqu}} (\mu\text{m}^2/\text{ms})$	$D_{\text{cyto}} (\mu\text{m}^2/\text{ms})$	References
NO	2.6	2.2 (in tissue)	Santos et al. (2011)
H ⁺	8	0.1–0.2 (buffered)	Irving et al. (1990)
Na ⁺	1.9	1.15	Goodman et al. (2005)
K ⁺	1.96	1.3–1.73	Hodgkin and Keynes (1953), Koch (1998)
Cl ⁻	2	2	Koch (1998), Kuner and Augustine (2000)
Ca ²⁺	0.6	0.223 (free) 0.01–0.05 (buffered)	Koch (1998) Allbritton et al. (1992), Murthy et al. (2000)
IP ₃	0.72	0.28	Allbritton et al. (1992), Woolf and Greer (1994)
cAMP	0.72	0.33–0.78	Bacskai et al. (1993), Woolf and Greer (1994)
Calmodulin	0.13	0.045	Woolf and Greer (1994)
CaMKII	0.034	0.006	Woolf and Greer (1994)
PKA	0.1	0.032	Woolf and Greer (1994)

We now ask how intracellular buffering affects compartmentalization in dendrites. The binding of a substance C to a buffer B can be described by a second-order binding scheme:



Binding occurs with an association rate k_{on} and the complex CB dissociates with a rate k_{off} . The affinity of B for binding C is given by the dissociation constant $K_B = k_{\text{off}}/k_{\text{on}}$. If we include binding to a single buffer species in our description of the spatio-temporal dynamics of $[C]$ (Equation 10.1) we obtain the *reaction-diffusion equations*:

$$\begin{aligned} \frac{\partial [C]}{\partial t} &= D_C \frac{\partial^2 [C]}{\partial x^2} - k_{\text{on}} [C] \cdot [B] + k_{\text{off}} [CB] \\ \frac{\partial [B]}{\partial t} &= D_m \frac{\partial^2 [B]}{\partial x^2} + k_{\text{on}} [C] \cdot [B] - k_{\text{off}} [CB] \\ [B]_T &= [B] + [CB] \end{aligned} \quad (10.3)$$

Square brackets denote concentrations, which depend on x and t . We assume that the diffusion coefficient of both the free and the bound buffer is D_m and that the total buffer concentration is $[B]_T$ which in general is spatially inhomogeneous but in the following is considered constant. Equation 10.3 is a relatively complex coupled system of partial differential equations, which is difficult to solve analytically. This reflects the enormous increase in complexity of signaling when intracellular binding sites are present at a high density. Useful approximations of Equation 3 can be derived on different spatial scales and with appropriate assumptions.

A frequent assumption is that the substance and its substrate are in chemical equilibrium at all points in time and in space. Although this assumption is not justified for very brief time periods or on small spatial scales ($<1 \mu\text{m}$; see the subsection Calcium domains) it holds reasonably well on a larger scale. In chemical equilibrium the concentration of the bound form of the buffer is given by:

$$[CB] = \frac{[C] \cdot [B]_T}{[C] + K_B} \quad (10.4)$$

The effectiveness of a buffer to maintain the concentration at a given level (its binding capacity) is given by the ratio of the change in buffer-bound concentration to the change in free concentration of the substance. This ratio is called the “buffering capacity” or “binding ratio” κ_B and is calculated as the first derivative of Equation 10.4 with respect to $[C]$ (Neher 1995):

$$\kappa_B = \frac{\partial [CB]}{\partial [C]} = \frac{[B]_T K_B}{([C] + K_B)^2} \quad (10.5)$$

At a given concentration of C , κ_B depends on the total concentration and the dissociation constant of the buffer. For low concentrations ($[C] \ll K_B$) the binding ratio simplifies to $[B]_T / K_B$. Later in the chapter we will discuss experimental estimates of dendritic Ca^{2+} -binding ratios.

How can we use the concept of binding ratios to describe diffusion in dendrites in the presence of buffering? By adding the two equations in Equation 10.3 and considering that:

$$\partial [CB] / \partial t = \kappa_B \partial [C] / \partial t$$

the following differential equation for the spatiotemporal dynamics of $[C]$ is obtained:

$$(1 + \kappa_B) \frac{\partial [C]}{\partial t} = D_C \frac{\partial^2 [C]}{\partial x^2} + D_m \frac{\partial^2 [CB]}{\partial x^2} \quad (10.6)$$

In the case of immobile buffers ($D_m = 0$) this readily reduces to a simple diffusion equation:

$$\frac{\partial [C]}{\partial t} = D_{app} \frac{\partial^2 [C]}{\partial x^2} \quad (10.7)$$

with an apparent diffusion coefficient

$$D_{app} = \frac{D_C}{1 + \kappa_B} \quad (10.8)$$

Thus, on a spatial scale where chemical equilibrium is reached, the diffusion of substances is slowed down in the presence of binding to immobile intracellular proteins (Matthews et al., 2013). Intuitively this is clear, because fixed buffers hold the substances in place and thus hinder their diffusional spread. When both mobile and fixed buffers are considered, Equation 10.6 can be simplified to a diffusion equation with an apparent diffusion coefficient given by (Irving et al., 1990; Gabso et al., 1997):

$$D_{app} = \frac{D_C + \kappa_m D_m}{(1 + \kappa_f + \kappa_m)} \quad (10.9)$$

where D_m denotes the diffusion coefficient of the mobile buffer and κ_m and κ_f are the binding ratios of the mobile and fixed buffer, respectively. In the case of a mobile buffer with $D_m > D_C / (1 + \kappa_f)$ the apparent diffusion of a substance is faster than when only a fixed buffer is present. The explanation is that a highly mobile buffer captures the substance near the source and facilitates the diffusional spread by transporting it in “piggyback” fashion. In summary, intracellular buffers can either slow down or facilitate the diffusional spread of a substance depending on their concentration,

affinity, and mobility. This leaves room for speculation that the spatial extent of dendritic calcium compartments may not be stationary but dynamically regulated depending on the expression level of Ca^{2+} -binding proteins (Simons et al., 2009).

Binding affinities and kinetics

As we have seen, the diffusion of a second messenger substance is affected by the presence of intracellular binding sites. The actual activation pattern of intracellular targets, however, also depends on the properties of the targets themselves. For example, the activation of a low-affinity binding protein may be more restricted in space than that of a high-affinity protein because it needs higher messenger concentrations for substantial binding. In addition, the activation pattern of target molecules can become far more complex if one considers kinetic effects. For example, the rate constants of Ca^{2+} -binding proteins vary over several orders of magnitude ($k_{\text{on}} \approx 10^6\text{--}10^9/(M\ s)$, $k_{\text{off}} \approx 1/\text{s}$ to $10^3/\text{s}$; Falke et al., 1994). Markram et al. (1998) explored the competition for incoming calcium ions between multiple proteins with different binding kinetics prior to full equilibration. Using numerical simulations they found that proteins with fast kinetics initially outcompete slower proteins, in particular during brief, pulse-like calcium injections such as those evoked by APs. In a subsequent slower phase calcium ions are passed over to slower targets, although these may be bypassed altogether if powerful calcium removal mechanisms are present. Another situation where such non-equilibrium effects are significant is Ca^{2+} -binding in the immediate vicinity of open calcium channels (Naraghi and Neher, 1997; see the subsection Calcium domains). In other words, different proteins may “see” quite different signals depending on their localizations, their affinities, and their relative kinetic properties. Sequential binding of calcium has been investigated in detail in muscle (Falke et al., 1994), but its possible role in dendritic signaling has so far not been studied. For further exploration of non-equilibrium effects, it will be essential to determine not only the localization and affinity but also the kinetic properties of Ca^{2+} -binding proteins in dendrites (Faas and Mody, 2012).

Further complexity may arise from multiple binding sites on one protein. The Ca^{2+} -binding protein calmodulin, for example, has four Ca^{2+} -binding sites with different affinities, which bind calcium in a cooperative way (Faas et al., 2011). The Ca^{2+} -calmodulin complex is a versatile activator of a variety of enzymes (Gosh and Greenberg, 1995). Due to cooperative binding to multiple sites, the activation of calmodulin depends steeply on the calcium concentration, confining it to regions of high calcium concentration (Gamble and Koch, 1987; Koch, 1998).

Removal mechanisms

Following a period of activity, neurons have to reestablish the resting concentrations of ions and second messengers in order to maintain the ability to produce electrical and chemical signals. This is achieved through ion pump mechanisms or enzymatic breakdown. The simplest mathematical description of removal from the cytosol is a non-saturable mechanism, which depends linearly on messenger concentration with a single rate constant γ (in units of per second). Such a removal mechanism can be considered as the low-concentration limit of a saturable pump or enzyme, for example one following Michaelis–Menten kinetics. Although a simplification, this approximation often provides a reasonable fit to experimental data, for example to calcium transients measured in dendrites or nerve terminals (Helmchen and Tank, 2011). The removal rate depends on the surface to volume ratio of the cellular compartment, being faster in thinner dendrites. Therefore, the structural subdivision of dendrites into many small volumes not only amplifies concentration changes but also facilitates rapid signaling.

How do removal mechanisms affect the diffusional spread of messengers? A dendritic segment in this case resembles a leaky pipe because the messenger substance is either degraded or extruded while diffusing. If we include a linear removal mechanism in Equation 7—with both a mobile and a fixed buffer present—we obtain the *linearized reaction-diffusion equation*:

$$(1 + \kappa_f + \kappa_m) \frac{\partial [C]}{\partial t} = (D_C + \kappa_m D_m) \frac{\partial^2 [C]}{\partial x^2} - \gamma [C] \quad (10.10)$$

This equation is equivalent to the cable equation (see Chapters 12 and 13). Therefore, a useful analogy between chemical and electrical signaling can be drawn (Kasai and Petersen, 1994; Zador and Koch, 1994): the diffusion coefficient relates inversely to the intracellular resistivity, the removal rate corresponds to the inverse of the membrane resistance, and buffers act similarly to capacitance (for more details see Koch, 1998). In the idealized case of an infinite cylinder the chemical equivalents of space and time constants can be defined based on cable theory. The “chemical length constant” or diffusion length is given by:

$$\lambda_{ch} = \sqrt{D_{app} \tau_{ch}} = \sqrt{\frac{D_C + \kappa_m D_m}{\gamma}} \quad (10.11)$$

where the time constant is defined as:

$$\tau_{ch} = \frac{1 + \kappa_m + \kappa_f}{\gamma} \quad (10.12)$$

The diffusion length is small for a large removal rate γ . Inserting reasonable numbers into Equation 10.11 yields a wide range of diffusion lengths for different messengers. For buffered Ca^{2+} the diffusion length is a few micrometers or less. In contrast, IP_3 as well as cAMP or cGMP have diffusion lengths of tens of micrometers or more (Allbritton et al., 1992; Kasai and Petersen, 1994). Therefore, Ca^{2+} can act as a short-range messenger, while IP_3 and cAMP have to be considered rather as long-range messengers (Kasai and Petersen, 1994). Notably, even the longest diffusion lengths are at least an order of magnitude shorter than typical electrical space constants, indicating that, in general, dendritic chemical signals are much more confined than electrical signals.

These considerations assume a localized increase in the concentration of a messenger molecule. If the concentration rises in concert over large parts of the dendritic tree, for example in the case of calcium transients evoked by backpropagating APs, the diffusional term in Equation 10.10 can be neglected entirely, and the description reduces to a simple single-compartment model (Regehr and Tank, 1994; Helmchen and Tank, 2011). The calcium transients in this model have an exponential decay with a time constant given by Equation 10.12.

The approximations of the reaction–diffusion equations and of removal mechanisms given in this section may provide a reasonable description of second messenger dynamics for individual dendritic segments and in the low-concentration regime. The underlying assumptions, however, do not hold for real dendritic trees. Deviations from the above equations arise from the complex geometry of dendrites and spines as well as from nonlinearities such as saturation of buffers or pumps. For more realistic and detailed models of second messenger dynamics in dendrites the full differential equations—possibly including saturable removal mechanisms—have to be solved based on realistic morphological reconstructions and using numerical methods (Blackwell, 2013). This can be done either for single dendritic branches or spines (Gamble and Koch, 1987; Woolf and Greer, 1994) or for entire dendritic trees using compartmental models of reconstructed cells (De Schutter and Smolen, 1998).

Examples from dendritic ion and second messenger signaling

In this section we discuss examples of measurements of the spatio-temporal dynamics of the concentrations of ions or second messengers in dendrites. The emphasis will be on what is known—from imaging or uncaging experiments—about diffusion, buffering, and removal mechanisms in dendrites.

Changes in ion concentration

Changes in ion concentration evoked by neuronal activity may have a variety of effects. They can cause shifts in the reversal potentials by changing the concentration gradients across the plasma membrane, and they may directly affect membrane conductances through binding. Changes in ion concentration in small dendrites and dendritic spines can be large and rapid, so that intradendritic diffusion of ions cannot be neglected when calculating membrane potential dynamics. The electrical cable model in this case must be generalized by including ion diffusion. For details on such an electrodiffusion model consult Sejnowski and Qian (1992).

pH

Due to the pH sensitivity of most enzymes and ion channels, the intracellular pH needs to be tightly regulated in cells (Chesler, 2003). Not surprisingly, high values of pH buffering have been reported for muscles and neurons, corresponding to a binding ratio of more than 10,000 (Irving et al., 1990; Tombaugh, 1998). Diffusion of protons in the cytoplasm is therefore relatively slow (see Table 10.1). Neuronal activity is typically associated with a fall in intracellular pH (Chesler, 2003), and it is likely that such pH changes occur locally in dendrites (Chesler and Kaila, 1992; Tombaugh, 1998). A possible function of intracellular acidification could be a negative feedback on the electroresponsiveness and dendritic Ca^{2+} influx mediated by the pH sensitivity of voltage-gated calcium channels (Tombaugh, 1998). While early studies have used pH-sensitive electrodes or small-molecule indicators, more recent studies have employed newly available genetically encoded pH sensors (Tantama et al., 2012).

Sodium

The spatial distribution of changes in Na^+ concentration in dendrites has been monitored using the Na^+ -sensitive dye SBFI (sodium-binding benzofuran isophthalate). Lasser-Ross and Ross (1992) observed large somatic Na^+ transients in cerebellar Purkinje neurons, but they were not able to detect dendritic changes in $[\text{Na}^+]_i$ evoked by fast sodium spikes or during calcium APs. This is consistent with a low density of Na^+ channels in these dendrites. Dendritic influx of Na^+ was, however, observed upon stimulation of climbing fibers and parallel fibers, localized to dendritic branches in the latter case (Callaway and Ross, 1997). This Na^+ influx was attributed to the activation of ligand-gated channels. Diffusion away from entry sites was rapid, which is in contrast to synaptic-ally evoked, locally confined increases in $[\text{Ca}^{2+}]_i$ (Eilers et al., 1995), reflecting the differences in the apparent cytosolic diffusion coefficients of Na^+ and Ca^{2+} . Dendritic Na^+ imaging has been also used to map the maturation of innervation fields of climbing fibers on single Purkinje cells during development (Scelfo et al., 2003).

In hippocampal pyramidal neurons, widespread increases in $[\text{Na}^+]_i$ have been reported during backpropagation of APs (Rose, 2003). Furthermore, two-photon imaging of SBFI revealed that suprathreshold synaptic stimulation causes $[\text{Na}^+]_i$ to increase in the stimulated dendritic branch, with particularly high values in active spines (Rose and Konnerth, 2001b). These signals depended on the coincidence of backpropagating APs and synaptic stimulation, apparently being mediated

by NMDA receptor activation. In most of these studies Na^+ transients displayed a relatively slow decay of several seconds. Because little is known about intracellular Na^+ buffering, it is unclear in how far SBFI buffering alters Na^+ transients. The physiological role of increased $[\text{Na}^+]_i$ also remains elusive. Besides affecting $[\text{Na}^+]_i$ -dependent mechanisms such as the $\text{Na}^+/\text{Ca}^{2+}$ exchanger, local increases in $[\text{Na}^+]_i$ have been implicated in the induction of cerebellar LTD (Linden et al., 1993) and in the regulation of NMDA receptor currents (Yu and Salter, 1998).

Chloride

The regulation of cell volume, intracellular pH, GABAergic reversal potential, and stabilization of the resting membrane potential are among the many physiological functions of chloride ions (Cl^-). Transient influx of Cl^- into neurons is mainly mediated by GABA_A and glycine receptors. Chloride clearance mechanisms include chloride–bicarbonate exchangers, co-transporters, and an ATP-driven chloride pump. An uneven distribution of these removal mechanisms between the soma and dendrites of cultured hippocampal neurons has been suggested to generate a gradient of intracellular chloride concentration ($[\text{Cl}^-]_i$) at rest (Hara et al., 1992). Such a standing gradient, as well as transient local increases in $[\text{Cl}^-]_i$ due to receptor activation, could shift the reversal potential for Cl^- in dendritic regions, thus determining whether activation of GABAergic inputs cause hyperpolarizing or depolarizing synaptic potentials (Sejnowski and Qian, 1992; Staley et al., 1995; Kaila et al., 1997). Long-lasting increases in $[\text{Cl}^-]_i$ have also been implicated in seizure generation (Ben-Ari, 2006). The open questions regarding intracellular Cl^- signaling and its effects can now be addressed by *in vitro* and *in vivo* application of fluorescent chloride indicators, including many new variants of genetically encoded chloride indicators (Kuner and Augustine, 2000; Arosio and Ratto, 2014).

Calcium

The most detailed information is available for the mechanisms involved in dendritic calcium handling, although our knowledge about the molecular identity and spatial distribution of proteins involved in Ca^{2+} buffering and removal is still incomplete. The pathways of Ca^{2+} entry and their dendritic distribution are discussed in Chapters 7 and 9. Here we focus on the following questions. What happens to calcium ions after they enter the cytosol? What is known about the diffusion of calcium ions and of Ca^{2+} -binding proteins in dendrites? How strongly is $[\text{Ca}^{2+}]_i$ buffered in dendrites? How rapidly, and by what mechanisms, is cytosolic Ca^{2+} removed?

Calcium diffusion

Relatively low apparent diffusion coefficients of Ca^{2+} were found in cytosolic extracts from *Xenopus* oocytes (Allbritton et al., 1992) and in dendrites of cultured hippocampal neurons (Murthy et al., 2000; Table 10.1). Presumably this is caused by rather immobile endogenous buffers. The mobility of endogenous Ca^{2+} -binding proteins is a critical factor in shaping the spatio-temporal spread of Ca^{2+} (see Equation 10.9). Using brief and local intracellular injections of Ca^{2+} into axons of *Aplysia* neurons, Gabso et al. (1997) determined an upper limit of $0.016 \mu\text{m}^2/\text{ms}$ for the diffusion coefficient of endogenous Ca^{2+} buffers, suggesting that they are either immobilized or of high molecular weight. In dendrites of neocortical and hippocampal pyramidal neurons Ca^{2+} buffers were found to be rather stationary because no significant washout of buffers was observed through whole-cell pipettes (Helmchen et al., 1996; Lee et al., 2000). Relatively immobile pools of Ca^{2+} buffers have also been recently reported for dentate granule cells in the hippocampus (Matthews et al., 2013) and cerebellar granule cell dendrites (poorly mobile calretinin; Arendt et al., 2013).

In contrast, fluorescently labeled parvalbumin is highly mobile in dendrites of cerebellar Purkinje neurons (Schmidt et al., 2003a). As a caveat, due to its high diffusibility this Ca^{2+} buffer possibly washes out significantly during whole-cell recordings. Parvalbumin is special in several aspects as it is a Ca^{2+} buffer with slow kinetics that is specifically expressed in subpopulations of GABAergic neurons. Due to its slow binding kinetics, parvalbumin initially acts as a sink, accelerating the decay of $[\text{Ca}^{2+}]_i$ following brief influx but delaying the build-up of $[\text{Ca}^{2+}]_i$ during repetitive stimulation on a longer time scale (Lee et al., 2000; Schmidt et al., 2003a). Parvalbumin in the latter case may facilitate the spread of Ca^{2+} , extending the size of functional compartments (Schmidt et al., 2003a). In general, the repertoire of endogenous Ca^{2+} -binding proteins is critical for determining the lifetime and spread of local calcium transients. Neurons therefore possibly regulate the size of functional calcium compartments by changing their expression profile of Ca^{2+} -binding proteins (Schmidt et al., 2003a).

Calcium buffering

The endogenous Ca^{2+} -binding capacity (Equation 10.5) can be determined by systematically varying the amount of an exogenous buffer, for example a Ca^{2+} indicator, and studying the effect on the intracellular changes in $[\text{Ca}^{2+}]_i$ evoked by a constant calcium influx (Neher, 1995). Depending on the “baseline buffering” of the endogenous buffers, lower or higher concentrations of added buffer will cause a significant reduction and prolongation of the transients (see Equation 10.12). For dendrites of pyramidal neurons, endogenous Ca^{2+} -binding ratios in the range of 50–180 have been reported. On the level of dendritic spines an even lower value of about 20 was found (Sabatini et al., 2002). Low levels of endogenous Ca^{2+} buffers enable large and fast Ca^{2+} transients evoked by single APs ($> 1 \mu\text{M}$ amplitude and about 10–15 ms decay time constant for dendritic spines; Sabatini et al., 2002). In addition, they may permit downstream Ca^{2+} signaling mechanisms to respond differentially to the activation of various Ca^{2+} sources depending on their location and time course. For cerebellar Purkinje cell dendrites, much higher Ca^{2+} -binding ratios of 900 in 6-day-old rats and 2,000 in 14-day-old rats have been reported (Fierro and Llano, 1996). This demonstrates that the expression of Ca^{2+} -binding proteins is developmentally regulated. These relatively high binding ratios are in agreement with immunocytochemical studies showing that Ca^{2+} -binding proteins such as parvalbumin, calbindin-D28k, and calcineurin are abundant in Purkinje cells (Baimbridge et al., 1992). GABAergic neurons in general display higher expression levels of several types of Ca^{2+} -binding proteins than excitatory neurons (Baimbridge et al., 1992) and, consistently, relatively high Ca^{2+} -binding ratios have been determined for several types of interneurons (Lee et al., 2000a; Kaiser et al., 2001; Aponte et al., 2008). A collection of Ca^{2+} -binding ratios measured in various neuronal cell types can be found in Helmchen and Tank (2011). By affecting the amplitude, time course, and spread of Ca^{2+} , differences in the expression pattern of Ca^{2+} -binding proteins may account for differential synaptic plasticity effects seen across distinct cell types as well as across different brain regions for the same neuronal cell type (Simons et al., 2009).

A promising approach to relate the presence of Ca^{2+} -binding proteins to a specific function is the investigation of mutant mice lacking one or more of these proteins. For example, climbing fiber-evoked dendritic calcium transients in Purkinje neurons of mice lacking calbindin-D28k displayed an initial fast-decaying component, which was absent in wild type mice (Airaksinen et al., 1997). This indicates that calbindin-D28k acts as a fast and high-affinity buffer shaping dendritic calcium transients. A behavioral relevance of this function was indicated by deficits in motor coordination in the mutant mice. In contrast, in parvalbumin null-mutant mice, not the amplitude but the decay time course of dendritic calcium transients was altered, consistent with the slow Ca^{2+} -binding

kinetics of parvalbumin (Schmidt et al., 2003b). Another interesting approach is the combination of whole-cell dialysis through patch pipettes with post-recording immunohistochemistry, which permitted quantification of the endogenous concentration of calbindin-D28k in hippocampal neurons (Müller et al., 2005). Further studies are necessary to resolve the identity, distribution, and functional role of dendritic Ca^{2+} -binding proteins in different cell types. Because of the possible importance of non-equilibrium effects (Markram et al., 1998), the kinetic properties of Ca^{2+} -binding proteins need to be measured and considered as well (Nägerl et al., 2000; Faas and Mody, 2012).

Calcium clearance

Calcium removal mechanisms include extrusion via plasma membrane Ca-ATPases and $\text{Na}^+/\text{Ca}^{2+}$ exchangers, uptake into the endoplasmic reticulum (ER) via endoplasmic Ca-ATPases, and uptake into mitochondria via a uniporter (Fig. 10.1C; Blaustein, 1988). The linear removal rate γ can be estimated from the decay of calcium transients if the Ca^{2+} -binding ratio is known (Equation 10.12). For various cell types values of γ between 100 and 2,000/s have been reported (Helmchen and Tank, 2011). Only a few studies to date have addressed which mechanisms are involved in calcium clearance. In proximal dendrites of neocortical pyramidal neurons, blockers of $\text{Na}^+/\text{Ca}^{2+}$ exchangers and the plasma membrane Ca-ATPases had little effect on the decay time, while blockers of the ER Ca-ATPases significantly prolonged the transients (Markram et al., 1995). Similar results were obtained for dendritic spines (Sabatini et al., 2002). In contrast, fast local extrusion via the $\text{Na}^+/\text{Ca}^{2+}$ exchanger in aspiny dendrites of neocortical fast-spiking interneurons is essential to create highly localized dendritic calcium microdomains (Goldberg and Yuste, 2003). In cerebellar Purkinje cells, the different clearance systems contributed about equally at low $[\text{Ca}^{2+}]_i$, while uptake into the ER and extrusion via the $\text{Na}^+/\text{Ca}^{2+}$ exchanger were more effective at high calcium concentrations (Fierro et al., 1998). Powerful Ca^{2+} extrusion mechanisms along the shaft of Purkinje cell dendrites were shown to keep elevated $[\text{Ca}^{2+}]_i$, caused by prolonged parallel-fiber activation, strictly localized (Schmidt et al., 2012).

Thus, besides extrusion of Ca^{2+} across the plasma membrane, uptake of Ca^{2+} into intracellular stores is a major sink for cytosolic Ca^{2+} , consistent with anatomical studies demonstrating the presence of smooth ER in dendrites and spines (see Chapter 1), and also in agreement with findings that ryanodine-sensitive calcium stores sequester cytosolic Ca^{2+} in hippocampal pyramidal dendrites (Garaschuk et al., 1997). Most notably, these internal stores were partially filled during the resting state, implying that they not only serve as Ca^{2+} sinks but—under appropriate conditions—may function as Ca^{2+} sources by releasing calcium ions from the ER. Besides ER uptake, the uptake of Ca^{2+} into mitochondria in dendrites has also been reported (Pivovarova et al., 2002), probably operating at different levels of $[\text{Ca}^{2+}]_i$ and on a different time scale.

Calcium release

Calcium release from intracellular stores, triggered by activation of IP_3 receptors and potentially amplified by calcium-induced calcium release, is another important source of Ca^{2+} (Verkhratsky, 2005). Several imaging studies have demonstrated highly localized increases in $[\text{Ca}^{2+}]_i$ upon synaptic activation of metabotropic glutamate receptors and a tight relationship of these signals to synaptic plasticity (reviewed in Rose and Konnerth, 2001a; Cavazzini et al., 2005; Segal et al., 2014; Baker et al., 2013). For example, in cerebellar Purkinje cells IP_3 -mediated local calcium signals were shown to be necessary and sufficient for LTD (Miyata et al., 2000). Furthermore, a coincidence mechanism for LTD induction linked to the release of Ca^{2+} from stores has been suggested based on supralinear calcium signals in dendritic spines upon coactivation of climbing fiber input and sparse parallel fiber input (Wang et al., 2000). For dendrites and dendritic spines of pyramidal

neurons, the contribution of Ca^{2+} release to synaptic plasticity and memory function is still under discussion (Baker et al., 2013). A special phenomenon has been discovered in apical dendritic trunks of pyramidal cells, where large mGluR-evoked, IP_3 -mediated calcium signals can propagate in a wave-like fashion (Nakamura et al., 1999; see later). Interestingly, at low levels of mGluR activation the initiation of these waves may be facilitated by Ca^{2+} influx evoked by coincident backpropagating APs. This represents yet another mechanism for coincidence detection mediated by Ca^{2+} as a versatile second messenger.

Other messengers

IP_3

IP_3 causes the release of Ca^{2+} from internal stores and thereby controls a variety of cellular processes (Berridge, 2005). IP_3 is produced by phospholipase C, the activity of which is controlled by a large number of G-protein-linked receptors. It is degraded by the IP_3 3-kinase and 5'-phosphomonoesterase and appears to have a lifetime of a few seconds (Allbritton et al., 1992; Wang et al., 1995). Although IP_3 is not appreciably bound to buffers in cytosolic extracts of *Xenopus* oocytes (Allbritton et al., 1992), it is not clear if high expression levels of IP_3 receptors, for example in dendrites of cerebellar Purkinje cells, may cause significant buffering of IP_3 . IP_3 -mediated Ca^{2+} release is involved in various forms of dendritic Ca^{2+} signals as will be further discussed later in the chapter.

cAMP

cAMP is produced by adenylate cyclase, which is regulated by a variety of G-protein-coupled receptors, and is degraded by phosphodiesterase. The lifetime of cAMP in cells is presumably in the range of seconds to minutes (Bacskaï et al., 1993; Kasai and Petersen, 1994). Increases in cAMP concentration can have both local and more widespread effects. Protein kinase A (PKA) is known to modulate dendritic ion channels; for example it downregulates transient K^+ channels in dendrites of hippocampal neurons, thus altering local dendritic excitability (Hoffman and Johnston, 1998; Magee et al., 1998). On the other hand, cAMP is known to be part of the signaling pathways to the nucleus that cause gene activation (Deisseroth et al., 2003).

The spatio-temporal dynamics of cAMP concentration was first measured by using an optical probe based on FRET between fluorescently labeled subunits of cAMP-dependent PKA (Adams et al., 1991). Application of serotonin onto *Aplysia* sensory neurons evoked large changes in cAMP concentration which were highest in the fine processes, reaching levels of more than 10 μM (Bacskaï et al., 1993). cAMP concentration gradients dissipated with an apparent cAMP diffusion coefficient similar to that in aqueous solutions, indicating negligible cAMP buffering. In another study, afferent stimulation of the lobster stomatogastric ganglion generated local increases of cAMP in fine neurite branches of neurons which eventually spread to the cell body by diffusion (Hempel et al., 1996). Hence these early studies could demonstrate compartmentalization of cAMP signals as well as the ability of cAMP to act as a long-range messenger. The problem of loading the large cAMP indicator protein into cells in brain slices has been alleviated by internal perfusion of patch pipettes (Vincent and Brusciano, 2001). Meanwhile, various fluorescent protein-based probes for cAMP are available (Gorshkov and Zhang, 2014). In the future, these probes will allow much more detailed studies of the spatio-temporal dynamics of cAMP and its role in regulating neuronal function. In addition, the development of genetically encoded fluorescent biosensors for other metabolites, such as NADH, reactive oxygen species, sugars, carbon monoxide, and nitric oxide (Zhang et al., 2013), opens entirely new avenues for investigating signaling dynamics in neurons and their subcellular compartments.

Enzyme activity

In addition to monitoring second messenger concentrations it is desirable to monitor enzyme activity directly. A broad set of fluorescent reporters has been successively designed, based on fluorescent proteins and exploiting FRET changes to visualize the activity of proteins, including proteases, kinases, and Ras GTPases (Zhang et al., 2002; Miyawaki, 2003; Walker and Lockyer, 2004; Yasuda, 2006; Rose et al., 2014). FRET is a mechanism of energy transfer between two molecules, where energy from an excited donor is transferred non-radiatively to an acceptor molecule as a steep function of their physical distance (on the scale of a few nanometers). FRET thus provides a molecular-scale ruler that is sensitive to changes in protein conformation. The FRET signal is read out as a change in fluorescence intensity or lifetime of the donor and acceptor chromophores. Combined with two-photon laser pulses for excitation, Yasuda and co-workers have applied the fluorescence lifetime approach (2pFRET-FLIM) to monitor the time course of activation of several key signaling molecules in dendritic spines after induction of LTP by glutamate uncaging as well as their differential spatial spread into the dendrite (Ras, Harvey et al., 2008; CaMKII, Lee et al., 2009; Rho and Cdc42, Murakoshi et al., 2011).

Degrees of compartmentalization

Depending on the various factors discussed in previous sections, chemical signals can be localized in space and time to different degrees (Fig. 10.3). Using mainly Ca^{2+} signaling as an example, we provide here an overview of the wide range and functional significance of biochemical compartmentalization in dendrites.

Calcium domains

On the smallest scale, ion flux through an open ion channel causes steep concentration gradients in the immediate vicinity of the channel pore (Fig. 10.3A). For calcium-permeable channels, domains of high $[\text{Ca}^{2+}]_i$ develop beneath the plasma membrane, reaching peak concentrations of $>100 \mu\text{M}$ and extending over only a few hundred nanometers (Neher, 1998). Most of the properties of these “calcium domains” have been inferred from theoretical studies (e.g., Naraghi and Neher, 1997) and through experiments on presynaptic structures (Nakamura et al., 2015; Wang and Augustine, 2015). Domains build up and collapse extremely rapidly (within a few tens or hundreds of microseconds) following channel opening and closure, respectively (Roberts, 1994). While immobile buffers have no influence on the steady-state spatial profile of $[\text{Ca}^{2+}]_i$, mobile buffers can effectively narrow the spatial extent of domains and prevent binding to nearby targets (Fig. 10.3A). They cause an exponential term in the spatial profile with a length constant λ_D given by:

$$\lambda_D = \sqrt{D_{\text{Ca}} \tau_D} = \sqrt{D_{\text{Ca}} / (\kappa_{\text{on}} [B])} \quad (10.13)$$

where D_{ca} denotes the diffusion coefficient of free calcium and $\tau_D = (\kappa_{\text{on}} [B])^{-1}$ is the mean time until a calcium ion is captured by a buffer molecule (note again the square-root relationship). As λ_D depends on the association rate, mobile buffers with either slow kinetics (e.g. EGTA) or fast kinetics (e.g. BAPTA) are often used to estimate the distance between intracellular targets and calcium channels.

Besides their importance in controlling neurotransmitter release in presynaptic terminals (Neher, 1998) and at dendro-dendritic synapses (Woolf and Greer, 1994; Yuste and Tank, 1996), calcium domains have been implicated in various other cellular mechanisms that might be

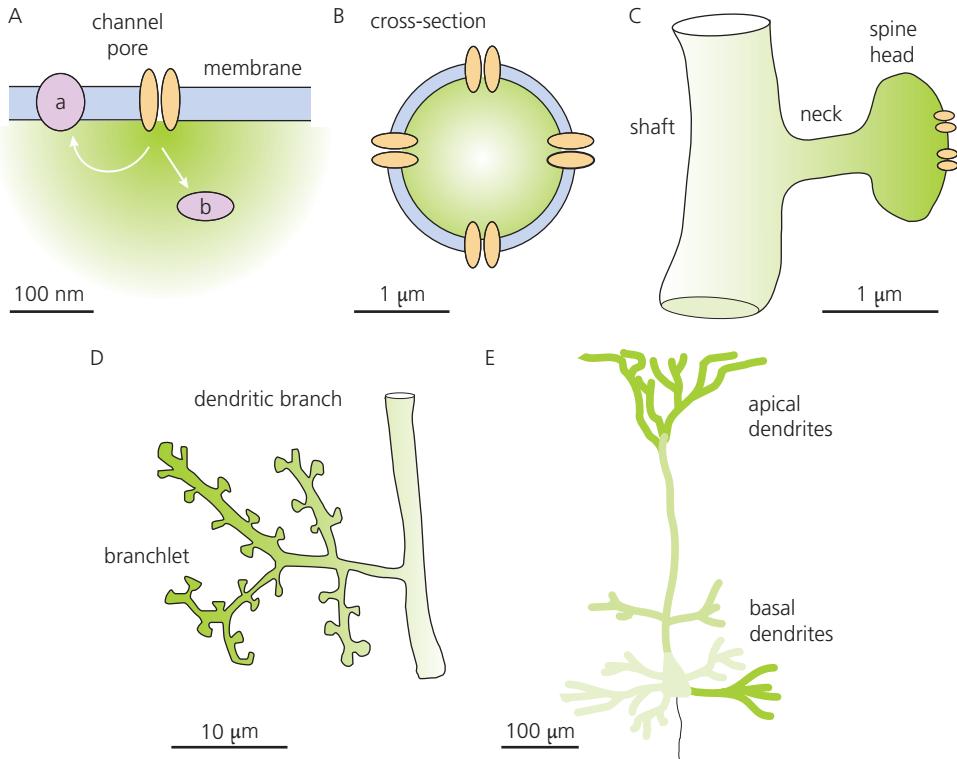


Fig. 10.3 Overview of the degrees of compartmentalization. (A) Domains of high calcium concentrations develop rapidly (<0.1 ms) near the open mouth of a calcium channel. Calcium ions can bind to nearby proteins localized in the membrane (a) or in the cytosol (b). (B) Calcium influx from the extracellular space causes radial concentration gradients that dissipate within milliseconds. (C) Dendritic spines are specialized features which enable cells to confine concentration increases to the synaptic scale. (D) Calcium elevations can be restricted to dendritic branches. In this case the branches can serve as functional units for “chemical integration” of the incoming synaptic input while the soma remains subthreshold. (E) Calcium accumulations may be relatively widespread but restricted to a particular subunit of the entire dendritic tree, for example the distal dendritic tuft or a basal dendritic branch.

important for dendritic function, such as Ca^{2+} -dependent inactivation of calcium channels (Imredy and Yue, 1992) and the activation of Ca^{2+} -activated potassium channels (Roberts, 1994; Marion and Tavalin, 1998). Thus, they may provide a highly localized feedback signal on the electrical membrane properties. Indeed, a dendritic spine imaging study provided evidence for functional microdomains in dendritic spines: influx of Ca^{2+} into a spine through a particular type of voltage-gated Ca^{2+} channels only present in spines selectively activates small-conductance Ca -activated potassium (SK) channels and thereby regulates synaptic signals (Bloodgood and Sabatini, 2007).

Another interesting mechanism is the activation of a third messenger within calcium domains, which subsequently translocates to other parts of the cell. Such a mechanism has been proposed for the phosphorylation of the transcription factor cAMP-response element-binding protein (CREB),

which promotes nuclear gene expression and has been implicated in the formation of long-term memory (Deisseroth et al., 2003). Phosphorylation of CREB in cultured hippocampal neurons specifically depends on Ca^{2+} influx during synaptic activation, and Ca^{2+} was shown to activate calmodulin in a highly localized manner. Calmodulin subsequently translocates to the nucleus where it activates a calcium/calmodulin-dependent protein kinase (CaMK), which then phosphorylates CREB. The advantage of such an activation cascade is that it combines location specificity of initiation with a widespread effect of a chemical signal.

Radial diffusion

Transmembrane Ca^{2+} influx causes radial gradients of $[\text{Ca}^{2+}]_i$ in cell somata (Hernández-Cruz et al., 1990). Radial concentration gradients in dendrites (Fig. 10.3B) have not been demonstrated experimentally so far, because these gradients are at the limit of both the spatial and temporal resolution of current imaging techniques (according to Equation 2, diffusional equilibration in a 1- μm thick dendritic segment occurs within a few milliseconds). Therefore, it is not clear if such gradients are of any significance. Numerical simulations exploring the effect of strategic placement of Ca^{2+} -binding proteins within a dendrite indicate that unequal initial sharing of Ca^{2+} between competing proteins with different kinetics (see the section Binding affinities and kinetics) can be either weakened or enhanced depending on which proteins are placed near the center of the dendrite (Markram et al., 1998). Further exploration of similar effects caused by the specific molecular micro-organization within dendrites awaits more detailed information about the localization and kinetic properties of effector molecules. For many purposes it is justified to neglect radial diffusion and to treat the calcium distribution in dendritic cylinders as a one-dimensional problem (Zador and Koch, 1994; Anwar et al., 2014).

Dendritic spines

Dendritic spines have long been proposed to serve as basic units of neural plasticity, with changes in their morphology representing the structural implementation of memory. Numerous two-photon imaging studies have shown that changes in spine number, turnover, and distribution can be induced by various experimental manipulations *in vitro* and *in vivo* (electrical stimulation of afferent input, glutamate uncaging, altered sensory experience, lesions etc.; reviewed in Holtmaat and Svoboda, 2009), in support of the hypothesis that spines are “memory devices.” Moreover, the spine effects correlate with functional changes at the synaptic and behavioral level. However, their functional impact on synaptic communication has yet to be established. The slow time course of the spine changes are at odds with them playing a significant role as an expression mechanism of LTP measured in acute brain slices. Also, despite the frequently evoked experience-dependent plasticity of spines, it must be noted that their wholesale turnover is a relatively rare phenomenon and that it is remarkable how stable spines actually are in many brain areas. A large fraction of spines can be tracked *in vivo* over many weeks, as shown in the hippocampus (>96%; Gu et al., 2014), visual cortex (>96%; Grutzendler et al., 2002), and barrel cortex (>50%; Trachtenberg et al., 2002).

With their micrometer size and their typical spine head and neck geometry, dendritic spines represent structural specializations well suited for compartmentalization (Zador and Koch, 1993; Fig. 10.3C). Initially suggested by modeling studies (Gamble and Koch, 1987; Wickens, 1988), the confinement of calcium signals to individual spines has been demonstrated directly using imaging techniques. Figure 10.4A shows a synaptically evoked calcium transient that is confined to a single dendritic spine in a hippocampal pyramidal neuron (Yuste and Denk, 1995). Meanwhile, isolated spine calcium signals have been observed in many cell types, including neocortical neurons (Koester and Sakmann, 1998; Nevian and Sakmann, 2004), cerebellar Purkinje neurons (Denk et al., 1995), and neurons in the lateral nucleus of the amygdala (Humeau et al., 2005). Several sources contribute

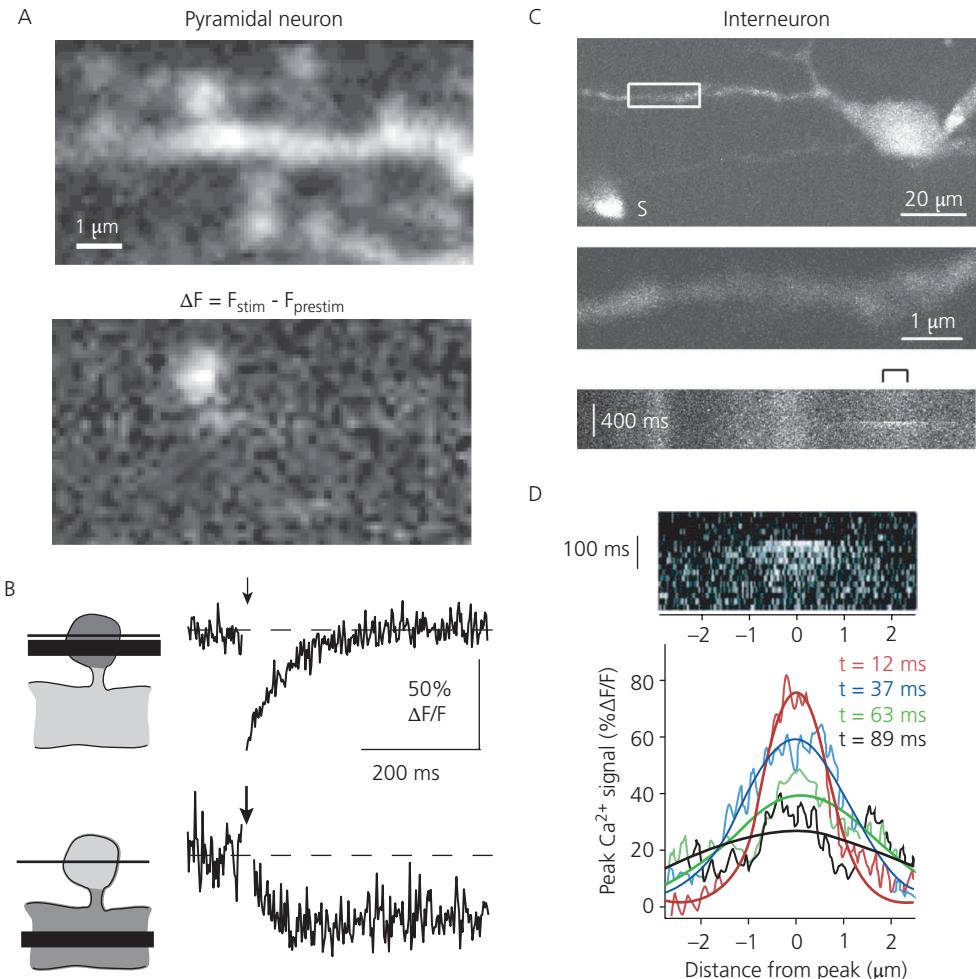


Fig. 10.4 Compartmentalization on the synaptic scale. **(A)** Dendritic spines as biochemical compartments. Two-photon image of a dendritic branch and spines of a hippocampal CA1 pyramidal neuron filled with Calcium Green-1 in a brain slice (top). The difference image ΔF of images taken during and before subthreshold synaptic stimulation (40 Hz, 125 ms) reveals calcium accumulation restricted to an individual spine (bottom). **(B)** Diffusional coupling between a spine and dendritic shaft. A hippocampal CA1 pyramidal neuron was filled with fluorescein dextran ($D \approx 0.04 \mu\text{m}^2/\text{ms}$). Fluorescence was monitored in a dendritic spine head (thin line). Photobleaching was induced with a high-power light exposure for 2 ms (thick line) either in the spine head (top) or in the shaft (bottom). The time course of the relative fluorescence changes ($\Delta F/F$) reflects the diffusion of unbleached (top) or bleached fluorescein (bottom) into the spine. Time constants were in the range of 20 to 90 ms. **(C)** Calcium microdomain in an aspiny interneuron. A parvalbumin-positive interneuron in cortical L2/3 was filled with 100 μM Fluo-4 (top). A stimulation electrode (S) was placed close to the dendrite of interest (white box, expanded in the middle). A single shock delivered by the stimulation electrode caused a localized calcium signal measured using a line scan through the same dendritic segment (bottom). **(D)** Top: expanded $\Delta F/F$ image of the Ca^{2+} signal indicated by the bracket in the lower part of C. Bottom: $\Delta F/F$ is plotted as a function of dendritic space at four points after synaptic activation and overlaid by Gaussian fits. Note how the calcium signal spreads with time.

Part A adapted with permission from Yuste and Denk (1995). Part B adapted with permission from Svoboda et al. (1996). Parts C and D adapted from *Neuron*, 40(4), Jesse H. Goldberg, Gabor Tamas, Dmitriy Aronov, and Rafael Yuste, Calcium microdomains in aspiny dendrites, pp. 807–21, Copyright 2003, Elsevier. With permission from Elsevier.

to single-spine calcium transients, but their relative contribution varies between spine and cell types. In spines of pyramidal cells, NMDA receptors have been identified as most prominent source (Yuste and Denk, 1995; Koester and Sakmann, 1998; Schiller et al., 1998; Kovalchuk et al., 2000) with additional contributions from voltage-dependent calcium channels and release of Ca^{2+} from internal stores (Schiller et al., 1998; Yuste et al., 1999; Rose and Konnerth, 2001b). In cerebellar Purkinje cells, which lack NMDA receptors, IP_3 -mediated Ca^{2+} release following activation of metabotropic glutamate receptors is a major source of calcium in individual spines (Finch and Augustine, 1998; Takechi et al., 1998; Wang et al., 2000).

By using fluorescence recovery after photobleaching (FRAP) or local uncaging, diffusion times between spines and their parent shaft were found to be of the order of 100 ms or longer (Fig. 10.4B; Svoboda et al., 1996). Shorter spines displayed faster exchange, demonstrating the importance of spine neck geometry for chemical isolation. This was also highlighted by a detailed analysis of NMDA receptor current-mediated single-spine transients following focal glutamate uncaging (Noguchi et al., 2005). The exact geometry of dendritic spines thus is a crucial determinant of local Ca^{2+} signals and may be important for the induction of synapse-specific plasticity.

Conversely, tetanic stimulation of afferent inputs or repetitive focal uncaging of glutamate can change spine morphology, leading to an expansion of spine heads (reviewed in Sala and Segal, 2014). While spine enlargement is correlated with increases in synaptic strength, it is not clear whether the structural changes are just a by-product of synaptic plasticity or are in fact necessary for it, for instance to create space or access for new membrane receptors (or other plasticity proteins) to be inserted into potentiated synapses. Moreover, it was directly demonstrated that diffusional coupling across the spine neck can be regulated by neuronal activity (Bloodgood and Sabatini, 2005), and a recent study documented dynamic changes in spine necks after induction of LTP, which may not only have a substantial impact on biochemical compartmentalization but also on the way synaptic potentials are transformed by the electrical resistance imposed by the spine neck (Harnett et al., 2012; Tønnesen et al., 2014) (Fig. 10.5). Hence, spine geometry and

Fig. 10.5 Super-resolution microscopy reveals dynamics of structural and molecular organization of dendritic spines. **(A)** STED image of dendritic spines on YFP-expressing CA1 pyramidal neurons in a mouse hippocampal brain slice. **(B)** Spine neck morphology shapes biochemical compartmentalization of dendritic spines as evidenced by a strong nonlinear and inverse correlation between FRAP diffusional recovery time constants (τ_{YFP}) and dendritic spine diameters. **(C)** The electrical resistance of the spine neck (R_{neck}) varies widely (by more than a factor of 100) based on morphological and diffusion measurements using STED. **(D)** Time-lapse STED imaging records morphological changes in dendritic spines, including the spine neck, after the induction of LTP by two-photon glutamate uncaging. Scale bar = 500 nm. **(E–G)** STORM super-resolution microscopy reveals the relative spatial distribution of the molecular components of the pre- and postsynaptic machinery. **(H), (I)** Single-particle tracking (spt)PALM records the mobility and clustering of glutamate receptors (AMPA type). **(J)** Velocity map of actin dynamics inside a live dendritic spine revealed by sptPALM. Warmer colors represent higher velocities. **(K)** Vectorial velocity map of actin molecules superimposed on a PALM image of polymerized actin density (green) and a widefield image of postsynaptic density protein PSD-95 (red). Scale bar = 250 nm. Vector, 200 nm/s.

Part A reprinted from *Biophysical Journal*, 101(10), Jan Tønnesen, Fabien Nadigny, Katrin I. Willig, Roland Wedlich-Söldner, and U. Valentin Nägerl, Two-color STED microscopy of living synapses using a single laser-beam pair, pp. 2545–2552, Copyright 2011, Elsevier. With permission from Elsevier.

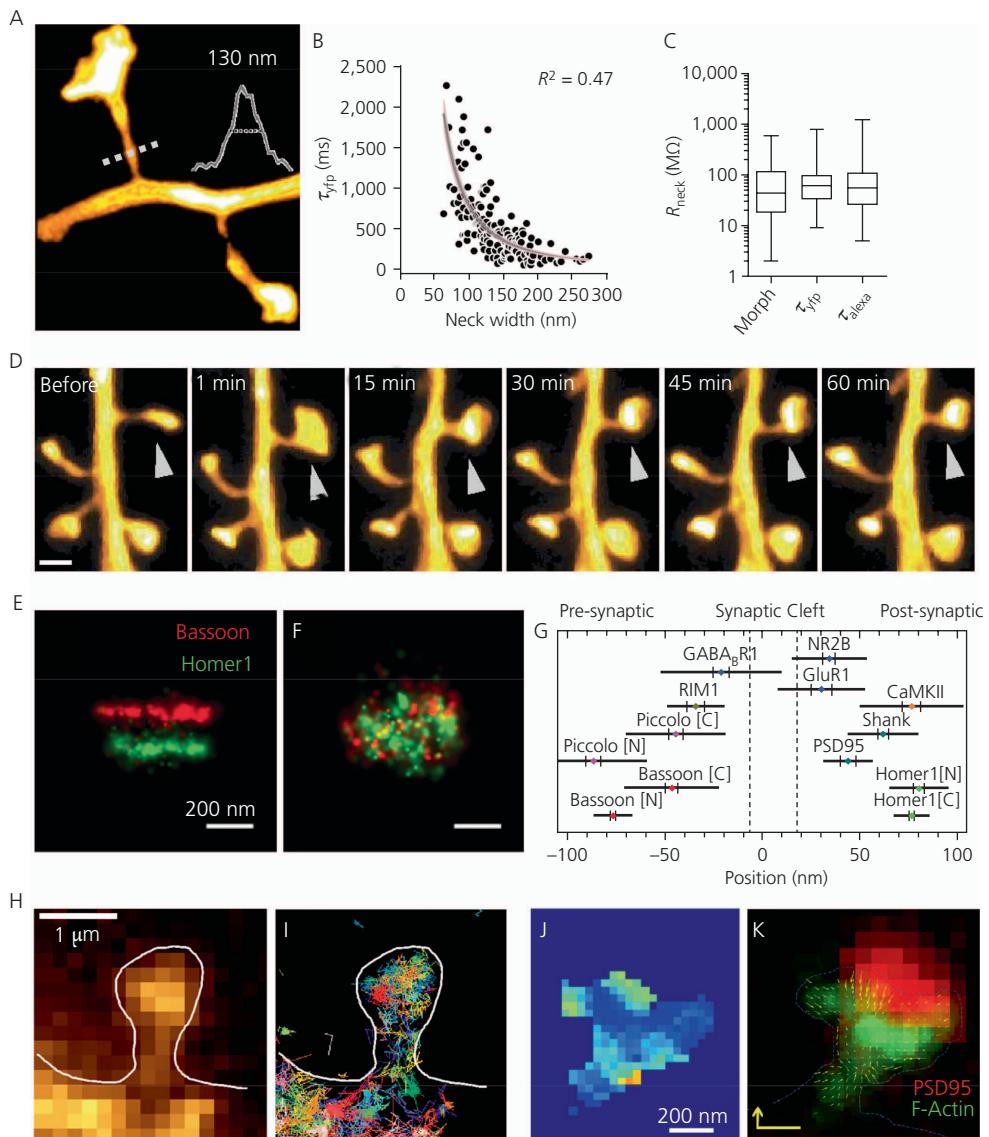


Fig. 10.5 (Continued)

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the electrochemical diffusion barrier between spine and dendrite may turn out to be key adjusting knobs for synaptic function. Moreover, combining stimulated emission depletion microscopy (STED) microscopy with single-molecule tracking techniques (like single-particle tracking photoactivated localization microscopy, spt-PALM; Fig. 10.5) will clarify the contribution of spine morphology to regulating protein trafficking in and out of spines, which is likely an important aspect of synaptic plasticity.

Aspiny interneuron dendrites

Most examples discussed so far concerned pyramidal cell dendrites, which are relatively easily accessible for electrophysiological recording and dye filling. For technical reasons fewer experiments were initially carried out on dendrites of GABAergic interneurons, but these neurons have increasingly shifted into the focus of interest. A clear morphological distinction between pyramidal cells and inhibitory interneurons is that interneuron dendrites are typically aspiny or sparsely spiny. Does this preclude highly localized calcium signals? In fact, this is not the case as has been demonstrated in parvalbumin-positive, soma-targeting interneurons in the neocortex (Goldberg and Yuste, 2003) and in cerebellar stellate cells (Soler-Llavina and Sabatini, 2006). In smooth dendrites of parvalbumin-positive neocortical interneurons, for example, focal synaptic stimulation induced Ca^{2+} microdomains that were fast and spatially restricted to less than a micron (Fig. 10.4C, D). This spine-free Ca^{2+} compartmentalization on the synaptic scale was caused by rapid influx through Ca^{2+} -permeable alpha-amino-3-hydroxy-5-methyl-4-isoazole propionic acid (AMPA) receptors and effective extrusion via strong $\text{Na}^+–\text{Ca}^{2+}$ exchange. Functionally, these fast and localized Ca^{2+} transients presumably reflect the requirement for fast synaptic integration in this particular type of interneuron that mediates fast feedforward inhibition (Goldberg and Yuste, 2003). Different results were obtained in dendrites of bitufted neocortical interneurons, in which synaptically induced local elevations in $[\text{Ca}^{2+}]_i$ were mediated by NMDA receptor channels and restricted to about 10 μm (Kaiser et al., 2004). In view of the large diversity among interneurons, it may not be surprising that variable degrees of Ca^{2+} compartmentalization are found with sub- and suprathreshold stimulation in morphologically and functionally distinct interneuron types (summarized in Goldberg and Yuste, 2005; Camiré and Topolnik, 2012).

Dendritic branches

On the next higher level, $[\text{Ca}^{2+}]_i$ elevations may be restricted to individual dendritic branches including several spines and dendritic branchlets (Fig. 10.3D). This degree of compartmentalization has been observed in early studies on cerebellar Purkinje cells upon subthreshold parallel fiber activation (Miyakawa et al., 1992; Eilers et al., 1995; Hartell, 1996) as well as in hippocampal CA1 pyramidal neurons upon subthreshold Schaffer collateral stimulation (Magee et al., 1995). Localized calcium transients occurred in regions near the synaptic input but were not due to calcium influx through glutamate receptors. Rather, low-voltage-activated calcium channels, opened by the local synaptic potentials, served as the main entry pathway. The generation of localized calcium transients by synaptic potentials, with the soma remaining subthreshold, can be interpreted as a chemical form of synaptic integration, which could serve to modify synapses locally (Eilers et al., 1995). Indeed, local calcium signals in Purkinje cells are capable of inducing parallel fiber LTD (Hartell, 1996; Eilers et al., 1997). Interestingly, Callaway et al. (1995) described local inhibition of widespread calcium increases in Purkinje cell dendrites by IPSPs. This represents the inverse situation to localized $[\text{Ca}^{2+}]_i$ elevations on a low background. Thus, the spatial pattern of $[\text{Ca}^{2+}]_i$ can be highly regulated by the spread of membrane potential changes.

Localized calcium transients in dendritic branchlets of Purkinje cell dendrites were also induced by focal uncaging of IP₃ (Wang and Augustine, 1995; Finch and Augustine, 1998) and by repetitive parallel fiber stimulation (Finch and Augustine, 1998; Takechi et al., 1998; Wang et al., 2000). The peak IP₃ concentration produced by such parallel fiber stimulation was estimated to be of the order of 1 μM. In the meantime, several studies have confirmed that such local increases of IP₃ are involved in spatially restricted parallel-fiber induced LTD (Rose and Konnerth, 2001b).

In the visual system of the fly, compartmentalization at the level of dendritic branches has been directly observed in vivo, with accumulations of [Ca²⁺]_i spatially restricted to those branches that correspond to the topographic projections of the stimulated part of the visual field (Borst and Egelhaaf, 1994; Single and Borst, 1998). For most dendrites, however, it is just beginning to be revealed under what in vivo conditions focal activation of a particular dendritic branch might occur. In tectal neurons of developing *Xenopus* tadpoles the position of visually evoked local dendritic Ca²⁺ signals was found to correlate with stimulus location in visual space (Bollmann and Engert, 2009), indicating a topographical organization of retinotectal inputs onto dendritic trees of tectal neurons. In developing hippocampal dendrites, clustered Ca²⁺ transients corresponding to the coactivation of nearby synapses were reported during spontaneous network activity, indicating that the functional connectivity pattern is topographically mapped onto compartmentalized postsynaptic Ca²⁺ signals in hippocampal neurons (Kleindienst et al., 2011). Furthermore, in neurons of the adult mouse cortex methodological advances with respect to calcium indicators and microscopy technology have enabled mapping of the distribution of activated branches and individual spines upon sensory stimulation throughout the dendritic tree (Jia et al., 2010; Chen et al., 2011, 2013; Hill et al., 2013). The degree of spatial clustering of activated synaptic inputs is an important factor in local dendritic integration, with high cluster densities favoring cooperative effects that may lead to nonlinear processing in branches (see later). In the future, the spatio-temporal patterns of synaptic inputs need to be further investigated under physiological conditions, especially in awake animals during specific behaviors (Grienberger et al., 2015).

Calcium waves

In contrast to spatially confined calcium release events in Purkinje cells, waves of calcium release initiate in the apical dendritic shaft of pyramidal neurons following local production of IP₃ by activation of metabotropic glutamate receptors (Nakamura et al., 1999; Larkum et al., 2003). These regenerative release events can be triggered by repetitive subthreshold synaptic stimulation and are mainly confined to the aspiny dendritic trunk, barely reaching into oblique dendrites (Fig. 10.6). Remarkably, the location of initiation sites correlates with branch points of oblique dendrites, which is consistent with a model assuming localization of IP₃ receptors in the main dendritic shaft and spread of mGluR-mobilized IP₃ from side branches to the apical shaft (Nakamura et al. 2002). Since the regenerative component of calcium waves involves calcium-induced calcium release, Ca²⁺ influx from other sources may facilitate their generation. AP-induced calcium influx through calcium channels or NMDA-receptor mediated Ca²⁺ currents can synergistically enhance calcium release generated by coincident mGluR activation (Nakamura et al., 1999, 2002). Because they spread rather widely, sometimes reaching the soma, calcium waves may be involved in heterosynaptic plasticity and in the activation of nuclear transcription factors (Barbara, 2002). More recently, localized Ca²⁺ release events were found in pyramidal cell dendrites, occurring spontaneously and amenable to modulation by membrane potential changes and synaptic inputs (Miyazaki and Ross, 2013). The physiological function of these events remains elusive.

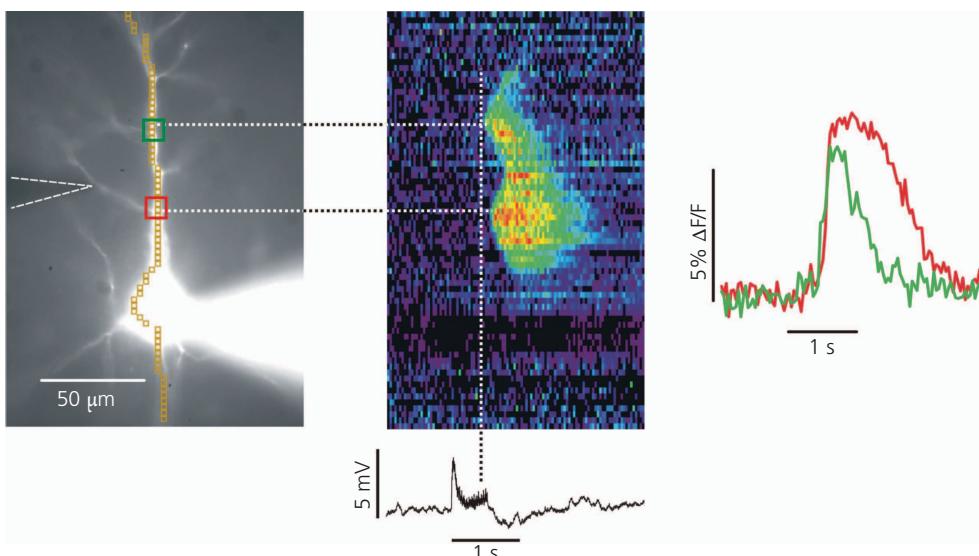


Fig. 10.6 Calcium waves in an apical dendritic shaft of a hippocampal CA1 pyramidal neuron. The pyramidal neuron was filled with 500 μM furaptra, a low-affinity Ca^{2+} indicator. A stimulating electrode was placed near the cell (left). The middle image shows the time course of fluorescence along the line indicated in the left image. In this example subthreshold synaptic stimulation (50 pulses at 100 Hz) initiated two calcium waves in the apical shaft. These large calcium signals are produced by regenerative release of calcium from IP_3 -sensitive stores. The vertical dotted line indicates the onset of the first, more distally initiated, calcium wave. The time course of fluorescence at the initiation points of the two waves is shown on the right. Note that the two waves initiated close to two branch points on the shaft and then came together in the region between them.

Adapted from Spatial segregation and interaction of calcium signalling mechanisms in rat hippocampal CA1 pyramidal neurons, Takeshi Nakamura, Nechama Lasser-Ross, Kyoko Nakamura, and William N. Ross, *The Journal of Physiology*, 543(2), pp. 465–480, Figure 3, Copyright © 2002, John Wiley and Sons.

Functional subunits of dendritic trees

Widespread accumulations of calcium in subunits of the dendritic tree, such as basal or distal dendrites (Fig. 10.3E), are caused by regenerative electrical potentials involving the activation of voltage-gated calcium channels. Backpropagating fast sodium APs (see Chapters 9 and 12) evoke dendritic calcium transients, which may serve as a chemical feedback signal about the output of the cell to part of the dendritic tree (see also the subsection Encoding of firing rate). The spatial extent of these calcium elevations depends on the effectiveness of AP backpropagation. In Purkinje cells, where backpropagation of sodium APs is severely attenuated (Stuart and Häusser, 1994), Ca^{2+} influx during somatic spiking is restricted to the soma and most proximal dendrites (Lev-Ram et al., 1992; Kitamura and Häusser, 2011). In contrast, calcium transients occur through the entire apical dendrite of mitral cells in the olfactory bulb, consistent with non-attenuated propagation of sodium spikes (Charpak et al., 2001). In hippocampal and neocortical pyramidal neurons, which display active but decremental backpropagation, calcium

transients evoked by single backpropagating APs are typically largest in the proximal dendrite and decrease further distally (Häusser et al., 2000; Waters et al., 2005). This spatial profile of calcium transient amplitude can further extend toward the distal dendritic tuft branches during bursts of APs or upon coincident synaptic stimulation (Waters et al., 2003; Waters and Helmchen, 2004; Sjöström and Häusser, 2006). Finally, failure of APs to propagate into subregions of the dendritic tree, for example during frequency-dependent attenuation of backpropagation in hippocampal pyramidal cells, can cause corresponding heterogeneity in the calcium signals in different regions (Spruston et al., 1995). In summary, backpropagating sodium APs in pyramidal cells cause widespread elevations of $[Ca^{2+}]_i$ in proximal parts of the dendritic tree, which decrement toward distal dendrites and are modifiable in their extent by various factors (see also Chapter 12).

While calcium signals evoked by fast APs are usually most prominent in proximal dendritic regions, widespread calcium accumulations can also occur in distal dendrites. Typically, they are associated with local dendritic spikes that cause the opening of voltage-dependent channels. For example, large elevations of $[Ca^{2+}]_i$ in Purkinje cell dendrites are produced by calcium spikes evoked by activation of climbing fibers (Miyakawa et al., 1992). In neocortical layer 5 (L5) pyramidal neurons, the distal apical dendrite near the main bifurcation constitutes a second initiation zone for regenerative calcium potentials (Schiller et al., 1997; Helmchen et al., 1999; Larkum et al., 1999). Because the threshold for these distal calcium spikes is reduced when synaptic activation in L1 is combined with a backpropagating AP, these events represent a cellular correlate for the association of proximal and distal inputs (Larkum et al., 1999; Major et al., 2013). A third type of local dendritic spike that can lead to binary activation of dendritic subunits is the “NMDA spike” (Schiller et al., 2000; Schiller and Schiller, 2001; Larkum et al., 2009; Lavzin et al., 2012). In this case regenerative potentials arise from the voltage dependence of the NMDA receptor channel and are accompanied by localized calcium elevations (Major et al., 2013). As a general concept, local dendritic spikes may form the basis of independent computational dendritic subunits that locally integrate incoming inputs before global summation takes place (Polsky et al., 2004; Larkum et al., 2009). Because of their sensitivity to both presynaptic activation pattern and postsynaptic excitation, local dendritic spikes and the accompanying changes in $[Ca^{2+}]_i$ have been implicated in synaptic plasticity in hippocampal and neocortical pyramidal neurons (Golding et al., 2002; Gordon et al., 2006; Losonczy et al., 2008).

Chemical information processing

In this final section, we address the question of the computational role of biochemical variables in dendritic information processing. Koch (1997) noted that the brain can be thought of as a hybrid computer, operating in both the digital and the analog domain. In a simple view, information is passed digitally from one cell to the other by all-or-none APs. The spatial and temporal pattern of the digital inputs is then converted into graded analog variables such as membrane potential dynamics and biochemical variables like ion concentrations, enzyme activities, and membrane protein composition and modulatory state. As discussed throughout this chapter, these analog variables exhibit complex spatio-temporal dynamics with variable degrees of compartmentalization, influencing dendritic processing and eventually determining the digital output of the particular neuron. Uncovering the computational principles behind this complicated analog integration will be a key part of understanding single-neuron computation. Here we present two examples of how dendritic changes in $[Ca^{2+}]_i$ can encode certain aspects of the digital information transfer between neurons.

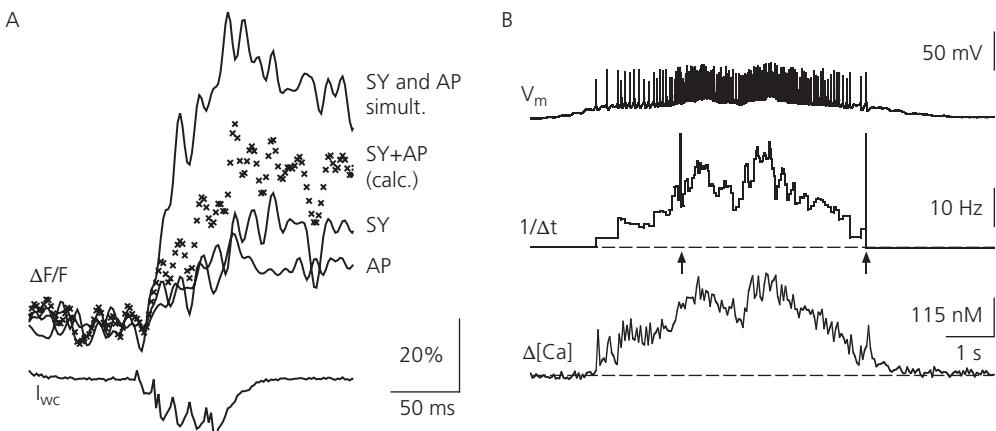


Fig. 10.7 Chemical information processing in dendrites. **(A)** Dendritic spines as coincidence detectors. The relative fluorescence changes ($\Delta F/F$) of Calcium Green-1 in an individual spine of a hippocampal CA1 pyramidal neuron in a brain slice are shown in response to subthreshold synaptic stimulation (SY; five EPSCs at 75 Hz), postsynaptic spikes (AP), and their simultaneous combination (SY and AP). Pairing of synaptic stimulation and postsynaptic action potentials produces supralinear calcium accumulation in spines, since the response to the combination of the stimuli is larger than the calculated sum of the response to each individual stimulus (SY and AP, calc.). **(B)** Mean dendritic calcium levels can encode firing rate. Calcium accumulation ($\Delta[Ca]$) in the proximal dendrite of a L5 pyramidal neuron in a brain slice during action potential firing (V_m) with variable frequency evoked by variable somatic current injection. The neuron was filled with Fura-2. The time course of the instantaneous frequency is shown by plotting the inverse of the interspike intervals ($1/\Delta t$). Two bursts with very short interspike intervals occurred at the times indicated by the arrows (the peaks are truncated)

Part A adapted with permission from Yuste and Denk (1995). Part B reprinted from *Biophysical Journal*, 70(2), F. Helmchen and K. Imoto, and B. Sakmann, T Ca^{2+} buffering and action potential-evoked Ca^{2+} signaling in dendrites of pyramidal neurons, pp. 1069–81, Copyright 1996, Elsevier. With permission from Elsevier.

Coincidence detection

The first example is the detection of coincidence of a pre- and a postsynaptic AP, which is critical in determining synapse-specific changes in synaptic strength (Markram et al., 1997). Several studies have demonstrated that accumulations of $[Ca^{2+}]_i$ in single spines can detect coincidence of a postsynaptic AP with the presynaptic neurotransmitter release onto that spine (Yuste and Denk, 1995; Koester and Sakmann, 1998; Schiller et al., 1998; Yuste et al., 1999). The basic phenomenon is a supralinear summation of changes in $[Ca^{2+}]_i$ caused by pairing of synaptic activation and back-propagating APs (Fig. 10.7A). The amplitude of the calcium transient measured when transmitter release is paired with postsynaptic APs is larger than the arithmetic sum of the calcium transients evoked by each stimulus alone. The predominant mechanism underlying this nonlinearity is a relief of the voltage-dependent Mg^{2+} block of the NMDA receptor by the backpropagating AP, causing additional calcium influx (for review see Sjöström and Nelson, 2002).

Electrophysiological studies have shown that the direction of synaptic changes may be sensitive to the timing of pre- and postsynaptic APs (Sjöström and Nelson, 2002; Feldman, 2012). If

dendritic spines act as coincidence detectors these timing differences should be reflected in the calcium concentrations. Indeed, $[Ca^{2+}]_i$ accumulation in spines critically depends on the relative order of EPSPs and backpropagating APs, switching from sublinear summation when EPSPs come after the APs to supralinear summation when EPSPs are followed by APs (Koester and Sakmann, 1998; Yuste et al., 1999; Nevian and Sakmann, 2004). At first this might seem consistent with earlier suggestions that the absolute levels of postsynaptic $[Ca^{2+}]_i$ determine whether a synapse is potentiated or depressed (Lisman, 1989). However, it is not clear how the average $[Ca^{2+}]_i$ in the spine—which is measured by imaging techniques—should accomplish this task. For example, it is likely that a burst of postsynaptic APs causes similar changes in average $[Ca^{2+}]_i$ in the spine head as during pairing of a single AP with an EPSP. Most likely therefore the average $[Ca^{2+}]_i$ alone is not sufficient to account for the observed differential effects on synaptic changes. The exact source pattern of Ca^{2+} influx on a submicron scale, perhaps also involving AMPA receptors (Holbro et al., 2010), and the localization and kinetics of phosphatases and kinases, for example CaMKII, are important aspects as well (Lisman et al., 2012).

Encoding of firing rate

The second example of chemical information processing is the encoding of spike frequency (i.e., mean firing rate) by the mean $[Ca^{2+}]_i$ level. As described above, each backpropagating AP leads to a relatively widespread calcium transient in dendrites of hippocampal and neocortical pyramidal neurons, characterized by a sudden increase in $[Ca^{2+}]_i$ due to brief influx of Ca^{2+} through voltage-dependent calcium channels and a roughly exponential decay with a time constant of the order of 100 ms. If APs occur at very short intervals compared with this decay time, the amplitude of the calcium transient scales with the number of APs (Svoboda et al., 1997). During slower repetitive firing, however, dendritic calcium accumulation can be thought of as a “leaky integrator,” which is characterized by a steady-state mean calcium level that depends linearly on AP frequency (Helmchen et al., 1996; Helmchen and Tank, 2011). Indeed, the average $[Ca^{2+}]_i$ in proximal dendrites of cortical pyramidal neurons reflects the firing frequency well up to a frequency of 30 Hz (Fig. 10.7B). Changes in the firing frequency can be followed on a time scale of 0.1 s, since the time to reach a steady-state level is given by the decay time constant of the individual transients (Helmchen and Tank, 2011). The effective time constant to reach a plateau $[Ca^{2+}]_i$ level may even be shorter in the presence of spike adaptation, which would make it possible that the mean $[Ca^{2+}]_i$ level follows frequency fluctuations in the 10–100 ms range (Wang, 1998). Given these temporal limitations, one may speak of a “calcium code” for the firing rate (Johnston, 1996). In dendritic spines, with estimated $[Ca^{2+}]_i$ decay time constants of 10–15 ms (Sabatini et al., 2002), a steady-state situation is presumably reached very rapidly but characterized by large-amplitude and fast $[Ca^{2+}]_i$ fluctuations, which, however, may be integrated and thereby decoded by downstream proteins with slower Ca^{2+} -binding kinetics.

A functional role of relatively widespread calcium signals could be to provide the dendrites with a feedback signal to control activity-dependent processes (Wang, 1998). For example, changes in $[Ca^{2+}]_i$ may activate Ca^{2+} -dependent K^+ channels, causing hyperpolarization and reducing the cell’s ability to generate APs. The behavioral relevance of intracellular calcium accumulations acting on a calcium-activated K^+ conductance has been demonstrated for the forward masking effect in omega neurons in the cricket auditory pathway, where a masking input suppresses the neuronal response to a subsequent test input (Sobel and Tank, 1994).

In summary, compartmentalization of chemical signals is an important aspect of dendritic signaling. Different messenger substances may have different spatial and temporal ranges of action,

and even a single messenger ion such as calcium can exert various functions on different spatial scales. More detailed information about the cellular mechanisms governing the diffusional spread of messenger signals as well as about the ultrastructural organization of dendrites is necessary to reveal the functional role of these complex signals. Advances in optical imaging techniques have greatly helped to elucidate the relationship between localized dendritic chemical signals and changes in synaptic efficacy. What is less clear, and needs to be determined in the future, is the behavioral relevance of dendritic biochemical compartments.

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References

- Adams SR, Harootunian AT, Buechler YJ, Taylor SS, Tsien RY (1991) Fluorescence ratio imaging of cyclic AMP in single cells. *Nature*, **349**, 694–697.
- Airaksinen MS, Eilers J, Garaschuk O, Thoenen H, Konnerth A, Meyer M (1997) Ataxia and altered dendritic calcium signaling in mice carrying a targeted null mutation of the calbindin D28k gene. *Proceedings of the National Academy of Sciences of the United States of America* **94**:1488–1493.
- Allbritton NL, Meyer T, Stryer L (1992) Range of messenger action of calcium ion and inositol 1,4,5-triphosphate. *Science* **258**:1812–1815.
- Amatrudo JM, Olson JP, Agarwal HK, Ellis-Davies GC (2015) Caged compounds for multichromic optical interrogation of neural systems. *European Journal of Neuroscience* **1**:5–16.
- Anwar H, Roome CJ, Nedelescu H, Chen W, Kuhn B, De Schutter E (2014) Dendritic diameters affect the spatial variability of intracellular calcium dynamics in computer models. *Frontiers in Cellular Neuroscience* **8**:168.
- Aponte Y, Bischofberger J, Jonas P (2008) Efficient Ca^{2+} buffering in fast-spiking basket cells of rat hippocampus. *Journal of Physiology* **586**:2061–2075.
- Arendt O, Schwaller B, Brown EB, Eilers J, Schmidt H (2013) Restricted diffusion of calretinin in cerebellar granule cell dendrites implies Ca^{2+} -dependent interactions via its EF-hand 5 domain. *Journal of Physiology* **591**:3887–3899.
- Arosio D, Ratto GM (2014) Twenty years of fluorescence imaging of intracellular chloride. *Frontiers in Cellular Neuroscience* **8**:258.
- Bacskaï BJ, Hochner B, Mahaut-Smith M, Adams SR, Kaang B-K, Kandel ER, Tsien RY (1993) Spatially resolved dynamics of cAMP and protein kinase A subunits in *Aplysia* sensory neurons. *Science* **260**:222–226.
- Baimbridge KG, Celio MR, Rogers JH (1992) Calcium-binding proteins in the nervous system. *Trends in Neurosciences* **15**:303–308.
- Baker KD, Edwards TM, Rickard NS (2013) The role of intracellular calcium stores in synaptic plasticity and memory consolidation. *Neuroscience and Biobehavioral Reviews* **37**:1211–1239.
- Barbara J-G (2002) IP_3 -dependent calcium-induced calcium release mediates bi-directional calcium waves in neurons: functional implications for synaptic plasticity. *Biochimica et Biophysica Acta* **1600**:12–18.
- Ben-Ari Y (2006) Seizures beget seizures: the quest for GABA as a key player. *Critical Reviews in Neurobiology* **18**:135–144.
- Bernstein J G, Boyden ES (2011) Optogenetic tools for analyzing the neural circuits of behavior. *Trends in Cognitive Sciences* **15**:592–600.
- Berridge MJ (2005) Unlocking the secrets of cell signaling. *Annual Reviews in Physiology* **67**:1–21.
- Biess A, Korkotian E, Holcman D (2011) Barriers to diffusion in dendrites and estimation of calcium spread following synaptic inputs. *PLoS Computational Biology* **7**:e1002182.

- Blackwell KT (2013) Approaches and tools for modeling signaling pathways and calcium dynamics in neurons. *Journal of Neuroscience Methods* **220**:131–140.
- Blaustein MP (1988) Calcium transport and buffering in neurons. *Trends in Neuroscience* **11**:438–443.
- Bloodgood BL, Sabatini BL (2005) Neuronal activity regulates diffusion across the neck of dendritic spines. *Science* **310**:866–869.
- Bloodgood BL, Sabatini BL (2007) Nonlinear regulation of unitary synaptic signals by CaV(2.3) voltage-sensitive calcium channels located in dendritic spines. *Neuron* **53**:249–260.
- Bollmann JH, Engert F (2009) Subcellular topography of visually driven dendritic activity in the vertebrate visual system. *Neuron* **61**:895–905.
- Borst A, Egelhaaf M (1994) Dendritic processing of synaptic information by sensory interneurons. *Trends in Neurosciences* **17**:257–263.
- Bosch M, Hayashi Y (2012) Structural plasticity of dendritic spines. *Current Opinion in Neurobiology* **22**:383–388.
- Brancq T, Häusser M (2010) The single dendritic branch as a fundamental functional unit in the nervous system. *Current Opinion in Neurobiology* **20**:494–502.
- Callaway JC, Ross WN (1997) Spatial distribution of synaptically activated sodium concentration changes in cerebellar Purkinje neurons. *Journal of Neurophysiology* **77**:145–152.
- Callaway JC, Lasser-Ross N, Ross WN (1995) IPSPs strongly inhibit climbing fiber-activated $[Ca^{2+}]_i$ increases in the dendrites of cerebellar Purkinje neurons. *Journal of Neuroscience* **15**:2777–2787.
- Camiré O, Topolnik L (2012) Functional compartmentalisation and regulation of postsynaptic Ca^{2+} transients in inhibitory interneurons. *Cell Calcium* **52**:339–346.
- Carlisle HJ, Kennedy MB (2005) Spine architecture and synaptic plasticity. *Trends in Neurosciences* **28**:182–187.
- Cavazzini M, Bliss T, Emptage N (2005) Ca^{2+} and synaptic plasticity. *Cell Calcium* **38**:355–367.
- Charpak S, Mertz J, Beaurepaire E, Moreux L, Delaney K (2001) Odor-evoked calcium signals in dendrites of rat mitral cells. *Proceedings of the National Academy of Sciences of the United States of America* **98**:1230–1234.
- Chen X, Leischner U, Rochefort NL, Nelken I, Konnerth A (2011) Functional mapping of single spines in cortical neurons in vivo. *Nature* **475**:501–505.
- Chen TW, Wardill TJ, Sun Y, Pulver SR, Renninger SL, Baohan A, Schreiter ER, Kerr RA, Orger MB, Jayaraman V, Looger LL, Svoboda K, Kim DS (2013) Ultrasensitive fluorescent proteins for imaging neuronal activity. *Nature* **499**:295–300.
- Chesler M (2003) Regulation and modulation of pH in the brain. *Physiological Reviews* **83**:1183–1221.
- Chesler M, Kaila K (1992) Modulation of pH by neuronal activity. *Trends in Neurosciences* **15**:396–402.
- Choquet D, Triller A (2013) The dynamic synapse. *Neuron* **80**:691–703.
- Dani A, Huang B, Bergan J, Dulac C, Zhuang X (2010) Superresolution imaging of chemical synapses in the brain. *Neuron* **68**:843–856.
- Deisseroth K (2011) Optogenetics. *Nature Methods* **8**:26–29.
- Deisseroth K, Mermelstein PG, Xia H, Tsien RW (2003) Signaling from synapse to nucleus: the logic behind the mechanisms. *Current Opinion in Neurobiology* **13**:354–365.
- Denk W, Sugimori M, Llinás R (1995) Two types of calcium response limited to single spines in cerebellar Purkinje cells. *Proceedings of the National Academy of Sciences of the United States of America* **92**:8279–8282.
- De Schutter E, Smolen P (1998) Calcium dynamics in large neuronal models. In: *Methods in Neuronal Modeling: from Ions to Networks*, 2nd edn (Koch C, Segev I, eds), pp. 211–250. Cambridge, MA: MIT Press.
- Douglas RJ, Martin KAC (1998) Neocortex. In: *The Synaptic Organization of the Brain*, 4th edn (Shepherd GM, ed.), pp. 459–510. New York: Oxford University Press.
- Eilers J, Augustine GJ, Konnerth A (1995a) Subthreshold synaptic Ca^{2+} signalling in fine dendrites and spines of cerebellar Purkinje neurons. *Nature* **373**:155–158.

- Eilers J, Takechi H, Finch EA, Augustine GJ, Konnerth A (1997) Local dendritic Ca^{2+} signaling induces cerebellar long-term depression. *Learning and Memory* **3**:159–168.
- Faas GC, Mody I (2012) Measuring the kinetics of calcium binding proteins with flash photolysis. *Biochimica et Biophysica Acta* **1820**:1195–1204.
- Faas GC, Raghavachari S, Lisman JE, Mody I (2011) Calmodulin as a direct detector of Ca^{2+} signals. *Nature Neuroscience* **14**:301–304.
- Falke JJ, Drake SK, Hazard AL, Peersen OB (1994) Molecular tuning of ion binding to calcium signaling proteins. *Quarterly Reviews of Biophysics* **27**:219–290.
- Feldman DE (2012) The spike-timing dependence of plasticity. *Neuron* **75**:556–571.
- Fenno L, Yizhar O, Deisseroth K (2011) The development and application of optogenetics. *Annual Review of Neuroscience* **34**:389–412.
- Fierro L, Llano I (1996) High endogenous calcium buffering in Purkinje cells from rat cerebellar slices. *Journal of Physiology* **496**:617–625.
- Fierro L, DiPolo R, Llano I (1998) Intracellular calcium clearance in Purkinje cell somata from cerebellar slices. *Journal of Physiology* **510**:499–512.
- Finch EA, Augustine G (1998) Local calcium signalling by inositol-1,4,5-trisphosphate in Purkinje cell dendrites. *Nature* **396**:753–756.
- Frost NA, Shroff H, Kong H, Betzig E, Blanpied TA (2010) Single-molecule discrimination of discrete peri-synaptic and distributed sites of actin filament assembly within dendritic spines. *Neuron* **67**:86–99.
- Gabso M, Neher E, Spira ME (1997) Low mobility of the Ca^{2+} buffers in axons of cultured *Aplysia* neurons. *Neuron* **18**:473–481.
- Gamble E, Koch C (1987) The dynamics of free calcium in dendritic spines in response to repetitive synaptic input. *Science* **236**:1311–1315.
- Garaschuk O, Yaari Y, Konnerth A (1997) Release and sequestration of calcium by ryanodine-sensitive stores in rat hippocampal neurones. *Journal of Physiology* **502**:13–30.
- Ghosh A, Greenberg, M. E. (1995) Calcium signaling in neurons: molecular mechanisms and cellular consequences. *Science* **268**:239–247.
- Goldberg JH, Yuste R (2003) Calcium microdomains in aspiny dendrites. *Neuron* **40**:807–821.
- Goldberg JH, Yuste R (2005) Space matters: local and global dendritic Ca^{2+} compartmentalization in cortical interneurons. *Trends in Neurosciences* **28**:158–167.
- Golding NL, Staff NP, Spruston N (2002) Dendritic spikes as a mechanism for cooperative long-term potentiation. *Nature* **418**:326–331.
- Goodman JA, Kroenke CD, Brethorst GL, Ackerman JJ, Neil JJ (2005) Sodium ion apparent diffusion coefficient in living rat brain. *Magnetic Resonance in Medicine* **53**:1040–1045.
- Gordon U, Polksy A, Schiller J (2006) Plasticity compartments in basal dendrites of neocortical pyramidal neurons. *Journal of Neuroscience* **26**:12717–12726.
- Gorshkov K, Zhang J (2014) Visualization of cyclic nucleotide dynamics in neurons. *Frontiers in Cellular Neuroscience* **8**:395.
- Grienberger C, Konnerth A (2012) Imaging calcium in neurons. *Neuron* **73**:862–885.
- Grienberger C, Chen X, Konnerth A (2015) Dendritic function in vivo. *Trends in Neuroscience* **38**:45–54.
- Grutzendler J, Kasthuri N, Gan WB (2002) Long-term dendritic spine stability in the adult cortex. *Nature* **420**:812–816.
- Gu L, Kleiber S, Schmid L, Nebeling F, Chamoun M, Steffen J, Wagner J, Fuhrmann M (2014) Long-term in vivo imaging of dendritic spines in the hippocampus reveals structural plasticity. *Journal of Neuroscience* **34**:13948–13953.
- Hara M, Inoue M, Yasukura T, Ohnishi S, Mikami Y, Inagaki C (1992) Uneven distribution of intracellular Cl^- in rat hippocampal neurons. *Neuroscience Letters* **143**:135–138.
- Hardingham N, Dachtler J, Fox K (2013) The role of nitric oxide in pre-synaptic plasticity and homeostasis. *Frontiers in Cellular Neuroscience* **7**:190.

- Harnett MT, Makara JK, Spruston N, Kath WL, Magee JC (2012) Synaptic amplification by dendritic spines enhances input cooperativity. *Nature* **491**:599–602.
- Hartell N (1996) Strong activation of parallel fibers produce localized calcium transients and a form of LTD that spreads to distant synapses. *Neuron* **16**:601–610.
- Harvey CD, Ehrhardt AG, Cellurale C, Zhong H, Yasuda R, Davis RJ, Svoboda K (2008) A genetically encoded fluorescent sensor of ERK activity. *Proceedings of the National Academy of Sciences of the United States of America* **105**:19264–19269.
- Häusser M, Spruston N, Stuart GJ (2000) Diversity and dynamics of dendritic signaling. *Science* **290**:739–744.
- Hell SW (2007) Far-field optical nanoscopy. *Science* **316**:1153–1158.
- Helmchen F, Denk W (2005) Deep tissue two-photon microscopy. *Nature Methods* **2**:932–940.
- Helmchen F, Tank DW (2011) A single compartment model of calcium dynamics in nerve terminals and dendrites. In: *Imaging in Neuroscience: a Laboratory Manual* (Helmchen F, Konnerth A, eds), Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, pp 355–368.
- Helmchen F, Imoto K, Sakmann B (1996) Ca^{2+} buffering and action potential-evoked Ca^{2+} signaling in dendrites of pyramidal neurons. *Biophysical Journal* **70**:1069–1081.
- Helmchen F, Svoboda K, Denk W, Tank DW (1999) In vivo dendritic calcium dynamics in deep-layer cortical pyramidal neurons. *Nature Neuroscience* **2**:989–996.
- Hempel CM, Vincent P, Adams SR, Tsien RY, Selverston AI (1996) Spatio-temporal dynamics of cyclic AMP signals in an intact neural circuit. *Nature* **384**:166–169.
- Hernández-Cruz A, Sala F, Adams PR (1990) Subcellular calcium transients visualized by confocal microscopy in a voltage-clamped vertebrate neuron. *Science* **247**:858–862.
- Hill DN, Varga Z, Jia H, Sakmann B, Konnerth A (2013) Multibranch activity in basal and tuft dendrites during firing of layer 5 cortical neurons in vivo. *Proceedings of the National Academy of Sciences of the United States of America* **110**:13618–13623.
- Hochbaum DR, Zhao Y, Farhi SL, Klapoetke N, Werley CA, Kapoor V, Zou P, Kralj JM, Maclaurin D, Smedemark-Margulies N, Saulnier JL, Boultong GL, Straub C, Cho YK, Melkonian M, Wong GK, Harrison DJ, Murthy VN, Sabatini BL, Boyden ES, Campbell RE, Cohen AE (2014) All-optical electrophysiology in mammalian neurons using engineered microbial rhodopsins. *Nature Methods* **11**:825–833.
- Hodgkin AL, Keynes RD (1953) The mobility and diffusion coefficient of potassium in giant axons from *Sepia*. *Journal of Physiology* **119**:513–528.
- Hoffman DA, Johnston D (1998) Downregulation of transient K^+ channels in dendrites of hippocampal CA1 pyramidal neurons by activation of PKA and PKC. *Journal of Neuroscience* **18**:3521–3528.
- Holbro N, Grunditz A, Wiegert JS, Oertner TG (2010) AMPA receptors gate spine Ca^{2+} transients and spike-timing-dependent potentiation. *Proceedings of the National Academy of Sciences of the United States of America* **107**:15975–15980.
- Holtmaat A, Svoboda K (2009) Experience-dependent structural synaptic plasticity in the mammalian brain. *Nature Reviews Neuroscience* **10**:647–658.
- Horton AC, Ehlers MD (2004) Secretory trafficking in neuronal dendrites. *Nature Cell Biology* **6**:585–591.
- Humeau Y, Herry C, Kemp N, Shaban H, Fourcaudot E, Bissiere S, Lüthi A (2005) Dendritic spine heterogeneity determines afferent-specific Hebbian plasticity in the amygdala. *Neuron* **45**:119–131.
- Imredy JP, Yue DT (1992) Submicroscopic Ca^{2+} diffusion mediates inhibitory coupling between individual Ca^{2+} channels. *Neuron* **9**:197–207.
- Irving M, Maylie J, Sizto NL, Chandler WK (1990) Intracellular diffusion in the presence of mobile buffers. *Biophysical Journal* **57**:717–721.
- Jia H, Rochefort NL, Chen X, Konnerth A (2010) Dendritic organization of sensory input to cortical neurons in vivo. *Nature* **464**:1307–1312.
- Johnston D (1996) The calcium code. *Biophysical Journal* **70**:1095.

- Johnston D, Christie BR, Frick A, Gray R, Hoffman DA, Schexnayder LK, Watanabe S, Yuan LL (2003) Active dendrites, potassium channels and synaptic plasticity. *Philosophical Transactions of the Royal Society B: Biological Sciences* **358**:667–674.
- Kaila K, Lamsa K, Smirnov S, Taira T, Voipio J (1997) Long-lasting GABA-mediated depolarization evoked by high-frequency stimulation in pyramidal neurons of rat hippocampal slice is attributable to a network-driven, bicarbonate-dependent K^+ transient. *Journal of Neuroscience* **17**:7662–7672.
- Kaiser KM, Zilberman Y, Sakmann B (2001) Back-propagating action potentials mediate calcium signaling in dendrites of bitufted interneurons in layer 2/3 of rat somatosensory cortex. *Journal of Physiology* **535**:17–31.
- Kaiser KM, Lübke J, Zilberman Y, Sakmann B (2004) Postsynaptic calcium influx at single synaptic contacts between pyramidal neurons and bitufted interneurons in layer 2/3 of rat neocortex is enhanced by back-propagating action potentials. *Journal of Neuroscience* **24**:1319–1329.
- Kapitein LC, Hoogenraad CC (2011) Which way to go? Cytoskeletal organization and polarized transport in neurons. *Molecular and Cellular Neuroscience* **46**:9–20.
- Kasai H, Petersen OH (1994) Spatial dynamics of second messengers: IP_3 and cAMP as long-range and associative messengers. *Trends in Neurosciences* **17**:95–101.
- Kasai H, Matsuzaki M, Noguchi J, Yasumatsu N, Nakahara H (2003) Structure–stability–function relationships of dendritic spines. *Trends in Neurosciences* **26**:360–368.
- Kerr JN, Denk W (2008) Imaging in vivo: watching the brain in action. *Nature Reviews Neuroscience* **9**:195–205.
- Kitamura K, Häusser M (2011) Dendritic calcium signaling triggered by spontaneous and sensory-evoked climbing fiber input to cerebellar Purkinje cells in vivo. *Journal of Neuroscience* **31**:10847–10858.
- Kittel RJ, Wichmann C, Rasse TM, Fouquet W, Schmidt M, Schmid A, Wagh DA, Pawlu C, Kellner RR, Willig KI, Hell SW, Buchner E, Heckmann M, Sigrist SJ (2006) Bruchpilot promotes active zone assembly, Ca^{2+} channel clustering, and vesicle release. *Science* **312**:1051–1054.
- Klausberger T (2009) GABAergic interneurons targeting dendrites of pyramidal cells in the CA1 area of the hippocampus. *European Journal of Neuroscience* **30**:947–957.
- Kleindienst T, Winnubst J, Roth-Alpermann C, Bonhoeffer T, Lohmann C (2011) Activity-dependent clustering of functional synaptic inputs on developing hippocampal dendrites. *Neuron* **72**:1012–1024.
- Koch C (1997) Computation and the single neuron. *Nature* **385**:207–210.
- Koch C (1998) Biophysics of computation. *Information Processing in Single Neurons*, pp. 248–279. Oxford: Oxford University Press.
- Koch C, Zador A (1993) The function of dendritic spines: devices subserving biochemical rather than electrical compartmentalization. *Journal of Neuroscience* **13**:413–422.
- Koester H, Sakmann B (1998) Calcium dynamics in single spines during coincident pre- and postsynaptic activity depend on relative timing of back-propagating action potentials and subthreshold excitatory postsynaptic potentials. *Proceedings of the National Academy of Sciences of the United States of America* **95**:9596–9601.
- Kovalchuk Y, Eilers J, Lisman J, Konnerth A (2000) NMDA receptor-mediated subthreshold Ca^{2+} signals in spines of hippocampal neurons. *Journal of Neuroscience* **20**:1791–1799.
- Kuner T, Augustine GJ (2000) A genetically encoded ratiometric indicator for chloride: capturing chloride transients in cultured hippocampal neurons. *Neuron* **27**:447–459.
- Kwon HB, Sabatini BL (2011) Glutamate induces de novo growth of functional spines in developing cortex. *Nature* **474**:100–104.
- Larkum ME, Zhu JJ, Sakmann B (1999) A new cellular mechanism for coupling inputs arriving at different cortical layers. *Nature* **398**:338–341.
- Larkum ME, Watanabe S, Nakamura T, Lasser-Ross N, Ross WN (2003) Synaptically evoked Ca^{2+} waves in layer 2/3 and layer 5 rat neocortical pyramidal neurons. *Journal of Physiology* **549**:471–488.

- Larkum ME, Nevian T, Sandler M, Polsky A, Schiller J (2009) Synaptic integration in tuft dendrites of layer 5 pyramidal neurons: a new unifying principle. *Science* **325**:756–760.
- Lasser-Ross N, Ross WN (1992) Imaging voltage and synaptically activated sodium transients in cerebellar Purkinje cells. *Proceedings of the Royal Society B: Biological Sciences* **247**:35–39.
- Lavzin M, Rapoport S, Polsky A, Garion L, Schiller J (2012) Nonlinear dendritic processing determines angular tuning of barrel cortex neurons in vivo. *Nature* **490**:397–401.
- ledo A, Frade J, Barbosa RM, Laranjinha J (2004) Nitric oxide in brain: diffusion, targets and concentration dynamics in hippocampal subregions. *Molecular Aspects of Medicine* **25**:75–89.
- Lee S-H, Rosenmund C, Schwaller B, Neher E (2000) Differences in Ca^{2+} buffering properties between excitatory and inhibitory hippocampal neurons from the rat. *Journal of Physiology* **525**:405–418.
- Lee SJ, Escobedo-Lozoya Y, Szatmari EM, Yasuda R (2009) Activation of CaMKII in single dendritic spines during long-term potentiation. *Nature* **458**:299–304.
- Lev-Ram V, Miyakawa H, Lasser-Ross N, Ross WN (1992) Calcium transients in cerebellar Purkinje neurons evoked by intracellular stimulation. *Journal of Neurophysiology* **68**:1167–1177.
- Linden DJ, Smeyne M, Connor JA (1993) Induction of cerebellar long-term depression in culture requires postsynaptic action of sodium ions. *Neuron* **11**:1093–1100.
- Lisman J (1989) A mechanism for the Hebb and the anti-Hebb processes underlying learning and memory. *Proceedings of the National Academy of Sciences of the United States of America* **86**:9574–9578.
- Lisman J, Yasuda R, Raghavachari S (2012) Mechanisms of CaMKII action in long-term potentiation. *Nature Reviews Neuroscience* **13**:169–182.
- Looger LL, Griesbeck O (2012) Genetically encoded neural activity indicators. *Current Opinion in Neurobiology* **22**:18–23.
- Losonczy A, Makara JK, Magee JC (2008) Compartmentalized dendritic plasticity and input feature storage in neurons. *Nature* **452**:436–441.
- Magee JC, Christofi G, Miyakawa H, Christie B, Lasser-Ross N, Johnston D (1995) Subthreshold synaptic activation of voltage-gated Ca^{2+} channels mediates a localized Ca^{2+} influx into dendrites of hippocampal pyramidal neurons. *Journal of Neurophysiology* **74**:1335–1342.
- Magee JC, Hoffmann D, Colbert C, Johnston D (1998) Electrical and calcium signaling in dendrites of hippocampal neurons. *Annual Reviews in Physiology* **60**:327–346.
- Maglione M, Sigrist SJ (2013) Seeing the forest tree by tree: super-resolution light microscopy meets the neurosciences. *Nature Neuroscience* **16**:790–797.
- Major G, Larkum ME, Schiller J (2013) Active properties of neocortical pyramidal neuron dendrites. *Annual Review of Neuroscience* **36**:1–24.
- Makino H, Malinow R (2009) AMPA receptor incorporation into synapses during LTP: the role of lateral movement and exocytosis. *Neuron* **64**:381–390.
- Markram H, Helm PJ, Sakmann B (1995) Dendritic calcium transients evoked by single back-propagating action potentials in rat neocortical pyramidal neurons. *Journal of Physiology* **485**:1–20.
- Markram H, Lübke J, Frotscher M, Sakmann B (1997) Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. *Science* **275**:213–215.
- Markram H, Roth A, Helmchen F (1998) Competitive calcium binding: implications for dendritic calcium signaling. *Journal of Computational Neuroscience* **5**:331–348.
- Marrion NV, Tavalin SJ (1998) Selective activation of Ca^{2+} -activated K^+ channels by co-localized Ca^{2+} channels in hippocampal neurons. *Nature* **395**:900–905.
- Matsuzaki M, Honkura N, Ellis-Davies GC, Kasai H (2004) Structural basis of long-term potentiation in single dendritic spines. *Nature* **429**:761–766.
- Matus A (2000) Actin-based plasticity in dendritic spines. *Science* **290**:754–758.
- Matthews EA, Schoch S, Dietrich D (2013) Tuning local calcium availability: cell-type-specific immobile calcium buffer capacity in hippocampal neurons. *Journal of Neuroscience* **33**:14431–14445.

- Miyakawa H, Lev-Ram V, Lasser-Ross N, Ross WN (1992) Calcium transients evoked by climbing fiber and parallel fiber synaptic inputs in guinea pig cerebellar Purkinje neurons. *Journal of Neurophysiology* **68**:1178–1188.
- Miyata M, Finch EA, Khirogi L, Hashimoto K, Hayasaka S, Oda SI, Inouye M, Takagishi Y, Augustine GJ, Kano M (2000) Local calcium release in dendritic spines required for long-term synaptic depression. *Neuron* **28**:233–244.
- Miyawaki A (2003) Visualization of the spatial and temporal dynamics of intracellular signaling. *Developmental Cell* **4**:295–305.
- Miyazaki K, Ross WN (2013) Ca²⁺ sparks and puffs are generated and interact in rat hippocampal CA1 pyramidal neuron dendrites. *Journal of Neuroscience* **33**:17777–17788.
- Müller A, Kukley M, Stausberg P, Beck H, Müller W, Dietrich D (2005) Endogenous Ca²⁺ buffer concentration and Ca²⁺ microdomains in hippocampal neurons. *Journal of Neuroscience* **25**:558–565.
- Murakoshi H, Wang H, Yasuda R (2011) Local, persistent activation of Rho GTPases during plasticity of single dendritic spines. *Nature* **472**:100–104.
- Murthy VN, Sejnowski TJ, Stevens CF (2000) Dynamics of dendritic calcium transients evoked by quantal release at excitatory hippocampal synapses. *Proceedings of the National Academy of Sciences of the United States of America* **97**:901–906.
- Nägerl UV, Novo D, Mody I, Vergara JL (2000) Binding kinetics of calbindin-D(28 k) determined by flash photolysis of caged Ca²⁺. *Biophysical Journal* **79**:3009–3018.
- Nair D, Hosy E, Petersen JD, Constals A, Giannone G, Choquet D, Sibarita JB (2013) Super-resolution imaging reveals that AMPA receptors inside synapses are dynamically organized in nanodomains regulated by PSD95. *Journal of Neuroscience* **33**:13204–13224.
- Nakamura T, Barbara JG, Nakamura K, Ross WN (1999) Synergistic release of Ca²⁺ from IP₃-sensitive stores evoked by synaptic activation of mGluRs paired with backpropagating action potentials. *Neuron* **24**:727–737.
- Nakamura T, Lasser-Ross N, Nakamura K, Ross WN (2002) Spatial segregation and interaction of calcium signalling mechanisms in rat hippocampal CA1 pyramidal neurons. *Journal of Physiology* **543**:465–480.
- Nakamura Y, Harada H, Kamasawa N, Matsui K, Rothman JS, Shigemoto R, Silver RA, DiGregorio DA, Takahashi T (2015) Nanoscale distribution of presynaptic Ca²⁺ channels and its impact on vesicular release during development. *Neuron* **85**:145–158.
- Naraghi M, Neher E (1997) Linearized buffered Ca²⁺ diffusion in microdomains and its implications for calculation of [Ca²⁺] at the mouth of a calcium channel. *Journal of Neuroscience* **17**:6961–6973.
- Neher E (1995) The use of fura-2 for estimating Ca buffers and Ca fluxes. *Neuropharmacology* **34**:1423–1442.
- Neher E (1998) Vesicle pools and Ca²⁺ microdomains: new tools for understanding their roles in neurotransmitter release. *Neuron* **20**:389–399.
- Nerbonne JM (1996) Caged compounds: tools for illuminating neuronal responses and connections. *Current Opinion in Neurobiology* **6**:379–386.
- Nevian T, Sakmann B (2004) Single spine Ca²⁺ signals evoked by coincident EPSPs and backpropagating action potentials in spiny stellate cells of layer 4 in the juvenile rat somatosensory barrel cortex. *Journal of Neuroscience* **24**:1689–1699.
- Noguchi J, Matsuzaki M, Ellis-Davies GCR, Kasai H (2005) Spine-neck geometry determines NMDA receptor-dependent Ca²⁺ signaling in dendrites. *Neuron* **46**:609–622.
- O'Dell TJ, Hawkins RD, Kandel ER, Arancio O (1991) Tests of the roles of two diffusible substances in long-term potentiation: evidence for nitric oxide as a possible early retrograde messenger. *Proceedings of the National Academy of Sciences of the United States of America* **88**:11285–11289.
- Oertner TG (2011) The Lego-logic of optogenetics. *Nature Methods* **8**:1011–1013.
- Oertner TG, Matus A (2005) Calcium regulation of actin dynamics in dendritic spines. *Cell Calcium* **37**:477–482.

- Ohno-Shosaku T, Kano M (2014) Endocannabinoid-mediated retrograde modulation of synaptic transmission. *Current Opinion in Neurobiology* **29**:1–8.
- Palay S, Chan-Palay V (1974) *Cerebellar Cortex*. Berlin: Springer.
- Pivovarova NB, Pozzo-Miller LD, Hongpaisan J, Andrews SB (2002) Correlated calcium uptake and release by mitochondria and endoplasmic reticulum of CA3 hippocampal dendrites after afferent synaptic stimulation. *Journal of Neuroscience* **22**:10653–10661.
- Polksy A, Mel BW, Schiller J (2004) Computational subunits in thin dendrites of pyramidal cells. *Nature Neuroscience* **7**:621–627.
- Regehr W, Tank DW (1994) Dendritic calcium dynamics. *Current Opinion in Neurobiology* **4**:373–382.
- Roberts WM (1994) Localization of calcium signals by a mobile calcium buffer in frog saccular hair cells. *Journal of Neuroscience* **14**:3246–3262.
- Rose CR (2003) High-resolution Na^+ imaging in dendrites and spines. *Pflügers Archiv: European Journal of Physiology* **446**:317–321.
- Rose CR, Konnerth A (2001a) Stores not just for storage: intracellular calcium release and synaptic plasticity. *Neuron* **31**:519–522.
- Rose CR, Konnerth A (2001b) NMDA receptor-mediated Na^+ signals in spines and dendrites. *Journal of Neuroscience* **21**:4207–4214.
- Rose T, Goltstein PM, Portugues R, Griesbeck O (2014) Putting a finishing touch on GECIs. *Frontiers in Molecular Neuroscience* **18**:88.
- Sabatini BL, Oertner TG, Svoboda K (2002) The life cycle of Ca^{2+} ions in dendritic spines. *Neuron* **33**:439–452.
- Sala C, Segal M (2014) Dendritic spines: the locus of structural and functional plasticity. *Physiological Reviews* **94**:141–188.
- Santos RM, Lourenço CF, Gerhardt GA, Cadena E, Laranjinha J, Barbosa RM (2011) Evidence for a pathway that facilitates nitric oxide diffusion in the brain. *Neurochemistry International* **59**:90–96.
- Scelfo B, Strata P, Knöpfel T (2003) Sodium imaging of climbing fiber innervation fields in developing mouse Purkinje cells. *Journal of Neurophysiology* **89**:2555–2563.
- Schiller J, Schiller Y (2001) NMDA receptor-mediated dendritic spikes and coincident signal amplification. *Current Opinion in Neurobiology* **11**:343–348.
- Schiller J, Schiller Y, Stuart G, Sakmann B (1997) Calcium action potentials restricted to distal apical dendrites of rat neocortical pyramidal neurons. *Journal of Physiology* **505**:605–616.
- Schiller J, Schiller Y, Clapham DE (1998) NMDA receptors amplify calcium influx into dendritic spines during associative pre- and postsynaptic activation. *Nature Neuroscience* **1**:114–118.
- Schiller J, Major G, Koester HJ, Schiller Y (2000) NMDA spikes in basal dendrites of cortical pyramidal neurons. *Nature* **404**:285–289.
- Schmidt H, Eilers J (2009) Spine neck geometry determines spino-dendritic cross-talk in the presence of mobile endogenous calcium binding proteins. *Journal of Computational Neuroscience* **27**:229–243.
- Schmidt H, Brown EB, Schwaller B, Eilers J (2003a) Diffusional mobility of parvalbumin in spiny dendrites of cerebellar Purkinje neurons quantified by fluorescence recovery after photobleaching. *Biophysical Journal* **84**:2599–2608.
- Schmidt H, Stiefel KM, Racay P, Schwaller B, Eilers J (2003b) Mutational analysis of dendritic Ca^{2+} kinetics in rodent Purkinje cells: role of parvalbumin and calbindin D28k. *Journal of Physiology* **551**:13–32.
- Schmidt H, Arendt O, Eilers J (2012) Diffusion and extrusion shape standing calcium gradients during ongoing parallel fiber activity in dendrites of Purkinje neurons. *Cerebellum* **11**:694–705.
- Schuman EM, Madison DV (1991) A requirement for the intercellular messenger nitric oxide in long-term potentiation. *Science* **254**:1503–1506.
- Segal M, Vlachos A, Korkotian E (2014) The spine apparatus, synaptopodin, and dendritic spine plasticity. *Neuroscientist* **16**:125–131.

- Sejnowski TJ, Qian N (1992) Synaptic integration by electro-diffusion in dendritic spines. In: *Single Neuron Computation* (McKenna T, Davis J, Zornetzer SF, eds), pp. 117–140. London: Academic Press.
- Simons SB, Escobedo Y, Yasuda R, Dudek SM (2009) Regional differences in hippocampal calcium handling provide a cellular mechanism for limiting plasticity. *Proceedings of the National Academy of Sciences of the United States of America* **106**:14080–14084.
- Single S, Borst A (1998) Dendritic integration and its role in computing image velocity. *Science* **281**:1848–1850.
- Sjöström PJ, Häusser M (2006) A cooperative switch determines the sign of synaptic plasticity in distal dendrites of neocortical pyramidal neurons. *Neuron* **51**:227–238.
- Sjöström PJ, Nelson SB (2002) Spike timing, calcium signals and synaptic plasticity. *Current Opinion in Neurobiology* **12**:305–314.
- Sobel EC, Tank DW (1994) In vivo Ca^{2+} dynamics in a cricket auditory neuron: an example of chemical computation. *Science* **263**:823–826.
- Soler-Llavina GJ, Sabatini BL (2006) Synapse-specific plasticity and compartmentalized signaling in cerebellar stellate cells. *Nature Neuroscience* **9**:798–806.
- Spruston N, Schiller Y, Stuart G, Sakmann B (1995) Activity dependent action potential invasion and calcium influx into hippocampal CA1 dendrites. *Science* **268**:297–300.
- Staley KJ, Soldo BL, Proctor WR (1995) Ionic mechanisms of neuronal excitation by inhibitory GABA A receptors. *Science* **269**:977–981.
- Steward O, Schuman EM (2003) Compartmentalized synthesis and degradation of proteins in neurons. *Neuron* **40**:347–359.
- Stuart G, Häusser M (1994) Initiation and spread of sodium action potentials in cerebellar Purkinje cells. *Neuron* **13**:703–712.
- Svoboda K, Tank DW, Denk W (1996) Direct measurement of coupling between dendritic spines and shaft. *Science* **272**:716–719.
- Svoboda K, Denk W, Kleinfeld D, Tank DW (1997) In vivo dendritic calcium dynamics in neocortical pyramidal neurons. *Nature* **385**:161–165.
- Syková E (2004) Extrasynaptic volume transmission and diffusion parameters of the extracellular space. *Neuroscience* **129**:861–876.
- Takechi H, Eilers J, Konnerth A (1998) A new class of synaptic response involving calcium release in dendritic spines. *Nature* **396**:757–760.
- Tantama M, Hung YP, Yellen G (2012) Optogenetic reporters: fluorescent protein-based genetically encoded indicators of signaling and metabolism in the brain. *Progress in Brain Research* **196**:235–263.
- Tombaugh GC (1998) Intracellular pH buffering shapes activity-dependent Ca^{2+} dynamics in dendrites of CA1 interneurons. *Journal of Neurophysiology* **80**:1702–1712.
- Tønnesen J, Nägerl UV (2013) Superresolution imaging for neuroscience. *Experimental Neurology* **242**:33–40.
- Tønnesen J, Nadirigny F, Willig KI, Wedlich-Söldner R, Nägerl UV (2011) Two-color STED microscopy of living synapses using a single laser-beam pair. *Biophysical Journal* **101**:2545–2552.
- Tønnesen J, Katona G, Rózsa B, Nägerl UV (2014) Spine neck plasticity regulates compartmentalization of synapses. *Nature Neuroscience* **17**:678–685.
- Trachtenberg JT, Chen BE, Knott GW, Feng G, Sanes JR, Welker E, Svoboda K (2002) Long-term in vivo imaging of experience-dependent synaptic plasticity in adult cortex. *Nature* **420**:788–794.
- Tsien RY (1989) Fluorescent probes of cell signaling. *Annual Reviews in Neuroscience* **12**:227–253.
- Verkhratsky A (2005) Physiology and pathophysiology of the calcium store in the endoplasmic reticulum of neurons. *Physiological Reviews* **85**:201–279.
- Vincent P, Brusciano D (2001) Cyclic AMP imaging in neurones in brain slice preparations. *Journal of Neuroscience Methods* **108**:189–198.

- Walker SA, Lockyer PJ (2004) Visualizing Ras signalling in real-time. *Journal of Cell Science* **117**:2879–2886.
- Wang X-J (1998) Calcium coding and adaptive temporal computation in cortical pyramidal neurons. *Journal of Neurophysiology* **79**:1549–1566.
- Wang SS-H, Augustine GJ (1995) Confocal imaging and local photolysis of caged compounds: dual probes of synaptic function. *Neuron* **15**:755–760.
- Wang SS-H, Augustine GJ (1999) Calcium signaling in neurons: a case study in cellular compartmentalization. In: *Calcium in Biological Function* (Carafoli E, Klee CB, eds), New York: Oxford University Press, pp. 545–566.
- Wang LY, Augustine GJ (2015) Presynaptic nanodomains: a tale of two synapses. *Frontiers in Cellular Neuroscience* **8**:455.
- Wang SS-H, Alousi AA, Thompson SH (1995) The lifetime of inositol 1,4,5-trisphosphate in single cells. *Journal of General Physiology* **105**:149–171.
- Wang SS-H, Denk W, Häusser M (2000) Coincidence detection in single dendritic spines mediated by calcium release. *Nature Neuroscience* **3**:1266–1273.
- Waters J, Helmchen F (2004) Boosting of action potential backpropagation by neocortical network activity in vivo. *Journal of Neuroscience* **24**:11127–11136.
- Waters J, Larkum M, Sakmann B, Helmchen F (2003) Supralinear Ca^{2+} influx into dendritic tufts of layer 2/3 neocortical pyramidal neurons in vitro and in vivo. *Journal of Neuroscience* **23**:8558–8567.
- Waters J, Schaefer A, Sakmann B (2005) Backpropagating action potentials in neurones: measurement, mechanisms and potential functions. *Progress in Biophysics and Molecular Biology* **87**:145–170.
- Wickens J (1988) Electrically coupled but chemically isolated synapses: dendritic spines and calcium in a rule for synaptic modification. *Progress in Neurobiology* **31**:507–528.
- Woolf TB, Greer CA (1994) Local communication within dendritic spines: Models of second messenger diffusion in granule cell spines of the mammalian olfactory bulb. *Synapse* **17**:247–267.
- Yasuda R (2006) Imaging spatiotemporal dynamics of neuronal signaling using fluorescence resonance energy transfer and fluorescence lifetime imaging microscopy. *Current Opinion in Neurobiology* **16**:551–561.
- Yu XM, Salter MW (1998) Gain control of NMDA-receptor currents by intracellular sodium. *Nature* **396**:469–474.
- Yuste R, Denk W (1995) Dendritic spines as basic units of neuronal integration. *Nature* **375**:682–684.
- Yuste R, Tank DW (1996) Dendritic integration in mammalian neurons, a century after Cajal. *Neuron* **16**:701–716.
- Yuste R, Majewska A, Cash SS, Denk W (1999) Mechanisms of calcium influx into hippocampal spines: heterogeneity among spines, coincidence detection by NMDA receptors and optical quantal analysis. *Journal of Neuroscience* **19**:1976–1987.
- Yuste R, Majewska A, Holthoff K (2000) From form to function: calcium compartmentalization in dendritic spines. *Nature Neuroscience* **3**:653–659.
- Zador A, Koch C (1994) Linearized models of calcium dynamics: formal equivalence to the cable equation. *Journal of Neuroscience* **14**:4705–4715.
- Zhang J, Campbell RE, Ting AY, Tsien RY (2002) Creating new fluorescent probes for cell biology. *Nature Reviews Molecular Cell Biology* **3**:906–918.
- Zhang C, Wie ZH, Ye BC (2013) Imaging and tracing of intracellular metabolites utilizing genetically encoded fluorescent biosensors. *Biotechnology Journal* **8**:1280–1291.

Chapter 11

Spine calcium signaling

Adam Carter and Bernardo Sabatini

Summary

Spines are specialized cellular compartments found at high density on the dendrites of many neurons. Glutamatergic synapses are made onto spines, which house much of the machinery needed to read out synaptic activity. By providing a diffusionaly isolated signaling compartment, spines allow neighboring synapses to operate independently. Stimulus-evoked increases in spine calcium trigger many forms of synaptic plasticity and can influence local excitability and morphology. This chapter focuses on the mechanisms of calcium handling and signaling within individual spines.

Introduction

Spines are small membrane protrusions found on the dendrites of many excitatory and some inhibitory neurons throughout the brain (Gray, 1959; Palay, 1956; Peters and Kaiserman-Abramof, 1970). As described in Chapter 1, spines consist of a bulbous head (diameter < 1 μm , volume < 1 fL), separated from the dendrite by a narrow neck (diameter < 0.2 μm) (Harris and Kater, 1994; Harris and Stevens, 1988, 1989). In many classes of neurons, excitatory synapses are made primarily onto the spine head, whereas inhibitory and modulatory synapses primarily contact the spine neck and dendrite (Fifkova et al., 1992; Halasy and Somogyi, 1993). Moreover, glutamate receptors are clustered at the spine head, in a region known as the postsynaptic density (PSD), which is located across from the presynaptic terminal (Kennedy, 2000). Both excitatory inputs and postsynaptic action potentials (APs) trigger calcium (Ca^{2+}) signals within spines, via the activation of glutamate receptors, voltage-sensitive Ca^{2+} channels, and internal Ca^{2+} stores. Because of their unique geometry, spines compartmentalize these Ca^{2+} signals for tens to hundreds of milliseconds, even after the initial Ca^{2+} source has terminated. Ultimately, these Ca^{2+} signals influence the function of other receptors, ion channels, enzymes, and cytoskeletal adapter proteins located in the PSD (Husi et al., 2000; Walikonis et al., 2000). Therefore, in many neurons spine Ca^{2+} signals play a central role in controlling local excitability, synaptic plasticity, and spine morphology.

Here we first review multiple aspects of spine Ca^{2+} signals, with a brief description of the tools used to study them. We then describe the sources of these Ca^{2+} signals and the mechanisms that determine their amplitude and time course. Finally, we address the regulation of these Ca^{2+} signals, and their ability to influence diverse neuronal functions. We will focus on pyramidal neurons and Purkinje cells, where spines have been most studied, but introduce data from other types of neurons when possible.

Tools for studying spine Ca^{2+} signals

The study of chemical and electrical signals within spines has presented a formidable technological challenge. The small size of spines means that they have only been accessible to direct electrophysiological recordings in a few cases (Forti et al., 1997). Imaging studies are also difficult because the spatial dimensions of spines are at or below the resolution limits of conventional light microscopy. Thus, significant innovations in both imaging technology and Ca^{2+} indicators have been required in order to study spines.

Early studies used wide-field illumination and charge-coupled device (CCD) cameras to image Ca^{2+} transients in neurons (Connor, 1986; Connor et al., 1986), revealing the presence of Ca^{2+} signals in dendrites (Jaffe et al., 1992; Regehr et al., 1989; Regehr and Tank, 1990) and spines (Guthrie et al., 1991; Muller and Connor, 1991). These studies were made possible by the use of novel Ca^{2+} indicators (Tsien, 1989), namely fluorophore-conjugated Ca^{2+} chelators whose excitation or emission spectra are altered by Ca^{2+} binding. However, the blurring effects of light scattering by brain tissue and fluorescence from out-of-focus structures meant that this approach was limited to studying spines in dissociated neuronal cultures or close to the surface of a brain slice.

The ability to study dendritic and spine Ca^{2+} signals in brain slices was facilitated by the use of laser-scanning confocal microscopy (Alford et al., 1993; Frenguelli et al., 1993; Jaffe et al., 1994; Markram and Sakmann, 1994). This approach uses the resolving power of the objective lens twice: (1) illuminated light is focused to a diffraction-limited spot, and (2) emitted photons are focused onto a pinhole that rejects scattered light and fluorescence from out-of-focus structures. This permits the imaging of spines near the surface of brain slices with higher resolution than is possible with CCD cameras. However, high-resolution imaging deeper within brain slices is not possible with confocal microscopy because scattering of visible photons prevents the formation of a tight focus and causes most emitted photons to be rejected by the pinhole. These inefficiencies necessitate the use of high light intensities that can trigger photodamage and photobleaching and thus destroy fluorescent signals from small structures such as spines.

Imaging from spines located deep within brain slices or in living animals was made possible by the development of two-photon laser scanning microscopy (2PLSM) (Denk et al., 1990, 1996; Denk and Svoboda, 1997). 2PLSM exploits the nonlinear excitation of many fluorophores by the near-simultaneous absorption of two low-energy photons, each of which has too low an energy to excite the fluorophore independently. The quadratic dependence of absorption on light intensity limits two-photon excitation to a small focal volume, thus avoiding excitation of out-of-focus structures. The low efficiency of two-photon excitation necessitates high peak power levels, which are typically attained using “mode-locked” Ti:sapphire lasers with pulse durations of about 100 fs and repetition rates around 100 MHz. Tuning these lasers within the near-infrared range (700–1,100 nm) allows two-photon excitation of many fluorophores, including green fluorescent protein (GFP) and both synthetic Ca^{2+} indicators and genetically encoded Ca^{2+} indicators (GECIs) (Miyawaki et al., 1997; Nagai et al., 2001; Tian et al., 2009; Chen et al., 2011). These long excitation wavelengths have the added advantage of experiencing less scattering in biological tissue, which allows the excitation light to be focused deep within brain tissue. Consequently, 2PLSM can be used to study neuronal morphology and function with high resolution in brain slices (Denk et al., 1995; Yuste and Denk, 1995; Svoboda et al., 1996) as well as in vivo (Svoboda et al., 1997).

An additional use of 2PLSM has been the development of two-photon laser uncaging (2PLU), in which the release of biologically relevant signaling molecules is triggered by the light-induced breakdown of an inert precursor (Denk et al., 1990). 2PLU offers higher spatial resolution and better tissue penetration than one-photon uncaging. Over the past decade, many studies have used

2PLU to map the distribution of glutamate receptors, evoke synaptic plasticity, and study localized spine Ca^{2+} signals in neurons (Matsuzaki et al., 2001; 2004; Smith et al., 2003; Carter and Sabatini, 2004; Tanaka et al., 2005). Importantly, 2PLU permits any spine to be studied in isolation, without the need for extracellular stimulation or presynaptic glutamate release. Finally, recent studies have begun to use novel caged compounds for glutamate and gamma-aminobutyric acid (GABA) that are independently photo-released with two-photon excitation at different wavelengths (Kantvari et al., 2010; Amatrudo et al., 2015).

A complementary approach for studying synaptic Ca^{2+} signals at spines is to combine 2PLSM and optogenetics. Many neurons receive a variety of glutamatergic inputs, which contact different types of spines in the dendrites (Humeau et al., 2005; Richardson et al., 2009). Expressing light-gated channelrhodopsin-2 (ChR2) in presynaptic neurons allows individual inputs to be selectively activated (Boyden et al., 2005). Illumination of presynaptic terminals releases glutamate, which binds to postsynaptic receptors to trigger synaptic Ca^{2+} signals (Zhang and Oertner, 2007). Imaging these signals with 2PLSM identifies the locations and properties of active synapses in the dendrites. For example, this approach has shown that different inputs make unique connections onto spines of pyramidal neurons in the prefrontal cortex and medium spiny neurons in the nucleus accumbens (Little and Carter, 2012, 2013; MacAskill et al., 2012, 2014).

Spines as isolated compartments

Chemical compartments

Spines act as chemical compartments, retarding the diffusion of molecules between the spine head and parent dendrite (Gamble and Koch, 1987; Wickens, 1988; Holmes, 1990; Koch and Zador, 1993). Measurements of the time course of diffusional equilibration across the spine neck have demonstrated that the spine neck acts as a barrier to passively isolate the movement of small molecules and proteins for 0.1–1 s (Axelrod et al., 1976; Svoboda et al., 1996; Majewska et al., 2000b; Korkotian et al., 2004; Noguchi et al., 2005). Interestingly, the degree of biochemical and electrical isolation of the spine head can be regulated by activity (Ngo-Anh et al., 2005; Grunditz et al., 2008; Araya et al., 2014). Thus, chronic changes in activity or membrane potential shift dendritic spines toward greater or lesser degrees of isolation.

It is important to note that the spread of active enzymes out of the spine and through the dendrite is complex and not limited simply by the passive diffusional barrier of the spine neck (see Chapter 10). For example, the spatio-temporal profile of the active molecules depends on the distribution and specific activity of the enzymes that inactivate each signaling cascade, such that even structurally similar synaptically activated enzymes can have vastly different spheres of influence (Murakoshi et al., 2011).

Electrical compartments

The geometry of spines has long been theorized to also allow them to operate as independent electrical compartments (Chang, 1952; Rall, 1970; Koch and Poggio, 1983; Wilson, 1984; Perkel and Perkel, 1985; Koch and Zador, 1993). The small capacitance and high resistance of spines mean that voltage signals from the dendrite can readily invade the spine head. In contrast, high resistance of the spine neck means that synaptic currents can evoke larger voltage signals at the spine than at the dendrite. However, it has been challenging to measure spine neck resistance, with different studies yielding a wide range of values. Early experiments using two-photon bleaching or morphological reconstructions indicated a low average value of 5–150 $\text{M}\Omega$, which would minimally

impact synaptic current flow, and produce voltage drops between the spine and dendrite of only a few millivolts (Harris and Stevens, 1989; Svoboda et al., 1996). Some recent studies have indicated higher average values in the hundreds of megaohms, which would enable larger voltage drops (Bloodgood and Sabatini, 2005; Grunditz et al., 2008; Harnett et al., 2012). Other recent studies have suggested a broader range, with a relatively low average but notably higher outliers (Palmer and Stuart, 2009; Tønnesen et al., 2014). As described below, the ability to evoke large voltage changes in the spine head is important, because it can influence the activation of voltage-sensitive ion channels found in spines.

Ca²⁺ signals in spines

Calcium signals within dendritic spines are thought to activate many cellular pathways that regulate neuronal function, and consequently they have been rigorously studied (Berridge, 1998; Zucker, 1999; West et al., 2002). Spine Ca²⁺ signals arise primarily from AP invasion or the activation of excitatory synaptic input (Fig. 11.1). Here we will discuss the triggers and Ca²⁺ sources

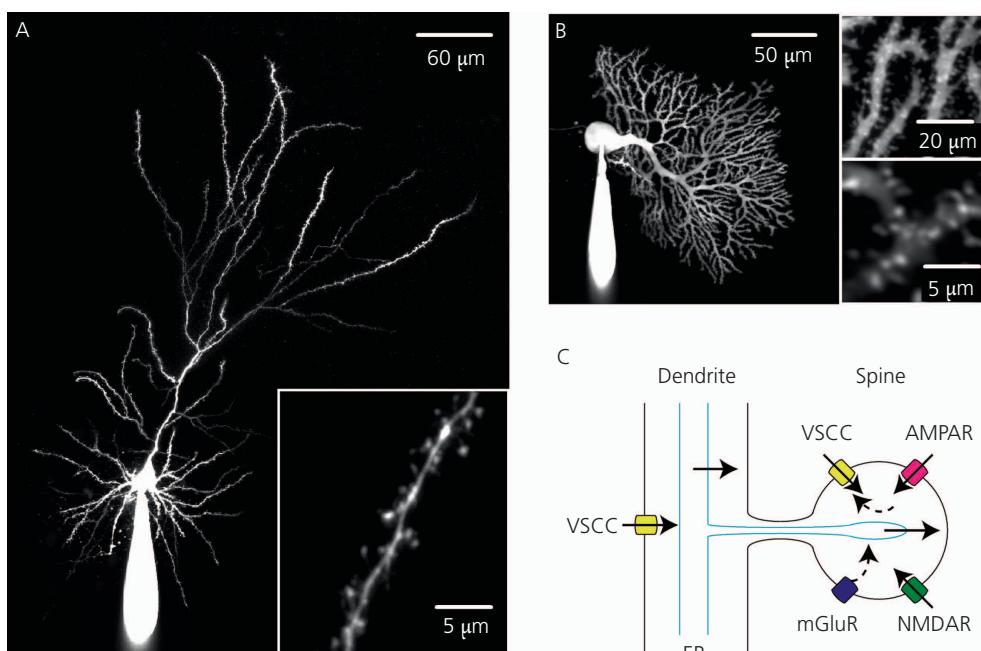


Fig. 11.1 Schematic of Ca²⁺ sources in dendrites and spines. (A) 2PLSM image of a L2/3 cortical pyramidal neuron during a whole-cell recording. The apical and basal dendrites are covered in spines, and the inset shows a section of distal apical dendrite. (B) 2PLSM image of a cerebellar Purkinje cell during a whole-cell recording. The dendrites have a very different morphology, and the insets show a section of spiny dendrite at different magnifications. (C) Schematic showing Ca²⁺ sources in dendrites and spines. Local depolarization can open voltage-sensitive Ca²⁺ channels (VGCCs) in dendrites and spines. Glutamate can bind and open both NMDA and Ca²⁺-permeable AMPA receptors in the spine. AMPA receptors can also generate a local depolarization that opens VGCCs in the spine. Glutamate can also bind and activate metabotropic receptors (mGluRs) located in the spine, which activate IP3Rs to trigger the release of Ca²⁺ from the ER in the dendrite or spine.

responsible for generating spine Ca^{2+} signals, primarily focusing on cerebellar Purkinje cells and cortical and hippocampal pyramidal neurons. When possible, we also discuss spines in other neurons, and it is worth noting that spine morphology, Ca^{2+} channel properties, glutamate receptor composition, and Ca^{2+} handling and efflux mechanisms vary widely among different classes of cells.

Action potential-evoked Ca^{2+} signals

In pyramidal neurons, action potentials (APs) are generated at the axon and backpropagate throughout the proximal dendrites (Stuart and Sakmann, 1994; Spruston et al., 1995). Backpropagating action potentials (bAPs) trigger locally uniform Ca^{2+} signals in dendrites, both *in vitro* (Jaffe et al., 1992; Yuste et al., 1994; Callaway and Ross, 1995; Markram et al., 1995; Schiller et al., 1995; 1997; Helmchen et al., 1996; Magee and Johnston, 1997; Larkum et al., 1999; Waters et al., 2003) and *in vivo* (Svoboda et al., 1997; 1999; Helmchen et al., 1999; Waters and Helmchen, 2004). bAPs also elicit Ca^{2+} signals in dendritic spines (Fig. 11.2) (Jaffe et al., 1994; Segal, 1995; Yuste and Denk, 1995; Koester and Sakmann, 1998; Yuste et al., 1999; Majewska et al., 2000a; Sabatini and Svoboda, 2000). Several studies have indicated that Ca^{2+} signals are larger in spines than dendrites (Jaffe et al., 1994; Segal, 1995; Yuste and Denk, 1995; Koester and Sakmann, 1998; Majewska et al., 2000a), which could be due to different Ca^{2+} sources or handling mechanisms in the two compartments or may be directly related to the high surface-to-volume ratio of the spine head.

In many neurons, the fast rise-time of bAP Ca^{2+} signals in spines indicates that they result from opening of voltage-sensitive Ca^{2+} channels (VSCCs) (Yuste and Denk, 1995). Pharmacological analysis of different Ca^{2+} sources confirms that Ca^{2+} influx through glutamate receptors and internal Ca^{2+} stores make no contribution (Emptage et al., 1999; Mainen et al., 1999; Sabatini and Svoboda, 2000), whereas Ca^{2+} influx through VSCCs dominates in CA1 pyramidal neurons (Sabatini and Svoboda, 2000; Yasuda et al., 2003). Furthermore, bAP Ca^{2+} signals in spines have large trial-to-trial variability, with noise analysis showing that they result from a small number of VSCCs (fewer than 20) that open with low probability (Sabatini and Svoboda, 2000). In addition, the properties of VSCCs in the spine head are not static, with R-type Ca^{2+} channel opening being inhibited following prolonged bAP firing (Yasuda et al., 2003). Moreover, the classes of VSCCs opened in spines by bAPs vary among different cell types, with N-, P/Q-, and R-type channels in cortical pyramidal neurons (Schiller et al., 1998), R-type channels in lateral amygdala projection neurons (Humeau et al., 2005), T-type channels in olfactory bulb granule cells (Egger et al., 2003), and T-, L- and R-type channels in striatal medium spiny neurons (Carter and Sabatini, 2004). These different channels have varying activation and inactivation properties, and are uniquely linked to other channels and signaling cascades.

As APs backpropagate into dendrites and spines, they are strongly influenced by passive and active properties. In many neurons, bAP amplitude decreases with distance from the soma, which in turn leads to a smaller bAP Ca^{2+} signals in more distal spines. This can result from activation of dendritic voltage-sensitive K^+ channels, which increase in density with distance from the soma in pyramidal neurons (Hoffman et al., 1997). Consequently, blocking A-type K^+ channels can enhance bAP Ca^{2+} signals in both dendrites and spines in the distal dendrites of these neurons (Gasparini et al., 2007; Seong et al., 2014). Similarly, blocking small conductance Ca^{2+} -activated K^+ (SK) channels can also enhance bAP Ca^{2+} signals in dendrites and spines of some neurons (Jones and Stuart, 2013). These findings indicate that the properties of bAP Ca^{2+} signals are highly dependent on the unique complement of channels present throughout the dendritic arbor of a given neuron.

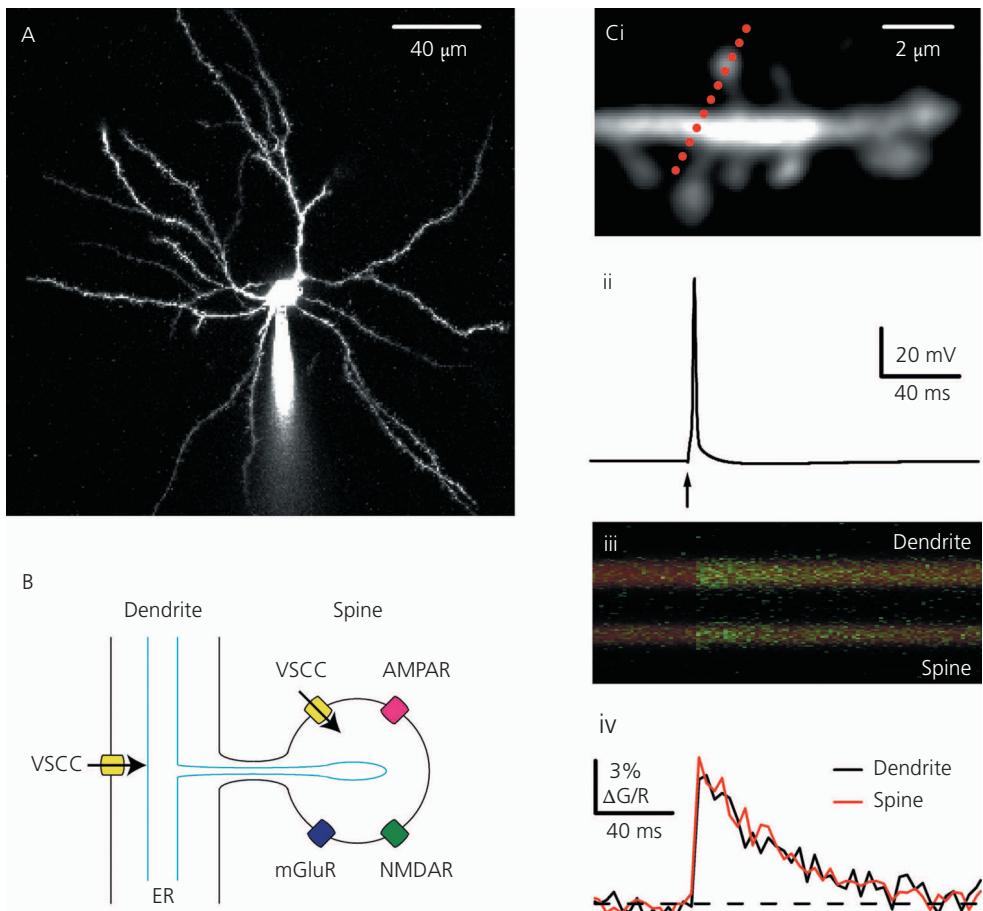


Fig. 11.2 Action potential Ca^{2+} signals in dendrites and spines. **(A)** 2PLSM image of a striatal medium spiny neuron during a whole-cell recording. The radiating dendrites are less extensive than those of pyramidal neurons and Purkinje cells. **(B)** Schematic showing that bAPs open VSCCs in both the spine and dendrite. **(C)** 2PLSM image showing a section of a dendrite in a medium spiny neuron, with the red line indicating the orientation of the line scan through the dendrite and spine (i); an action potential fired in response to current injection (arrow) in a neuron held at -50 mV in whole-cell current-clamp (ii) evokes a Ca^{2+} signal in both the spine and its parent dendrite (iii), which is detected as a change in green over red fluorescence ($\Delta G/R$) (iv).

Synaptically-evoked Ca^{2+} signals

In addition to bAPs, Ca^{2+} signals in spines can be evoked by glutamatergic inputs that often synapse onto the spine head. Synaptic Ca^{2+} signals can be mediated by multiple Ca^{2+} sources, including glutamate receptors, VSCCs, and release from internal Ca^{2+} stores, which couple to different signaling cascades (Fig. 11.3). Studying synaptic Ca^{2+} signals is complicated by the presence of many classes of ion channels in axons, dendrites, and spines, which means that many pharmacological tools affect both presynaptic and postsynaptic function. For example, blocking voltage-sensitive

sodium channels with tetrodotoxin (TTX) abolishes presynaptic APs and prevents evoked glutamate release, making it difficult to assess postsynaptic roles. Similarly, the roles of VSCCs and potassium channels are difficult to identify given their functions in the presynaptic terminal. Understanding synaptic Ca^{2+} signals is further complicated by nonlinear interactions between the many classes of ion channels and Ca^{2+} sources that are present in dendritic spines. Therefore, while it is technically straightforward to monitor the effects of blocking different channels or receptors on spine Ca^{2+} influx, interpreting the experimental results can be a challenge. Fortunately, many of

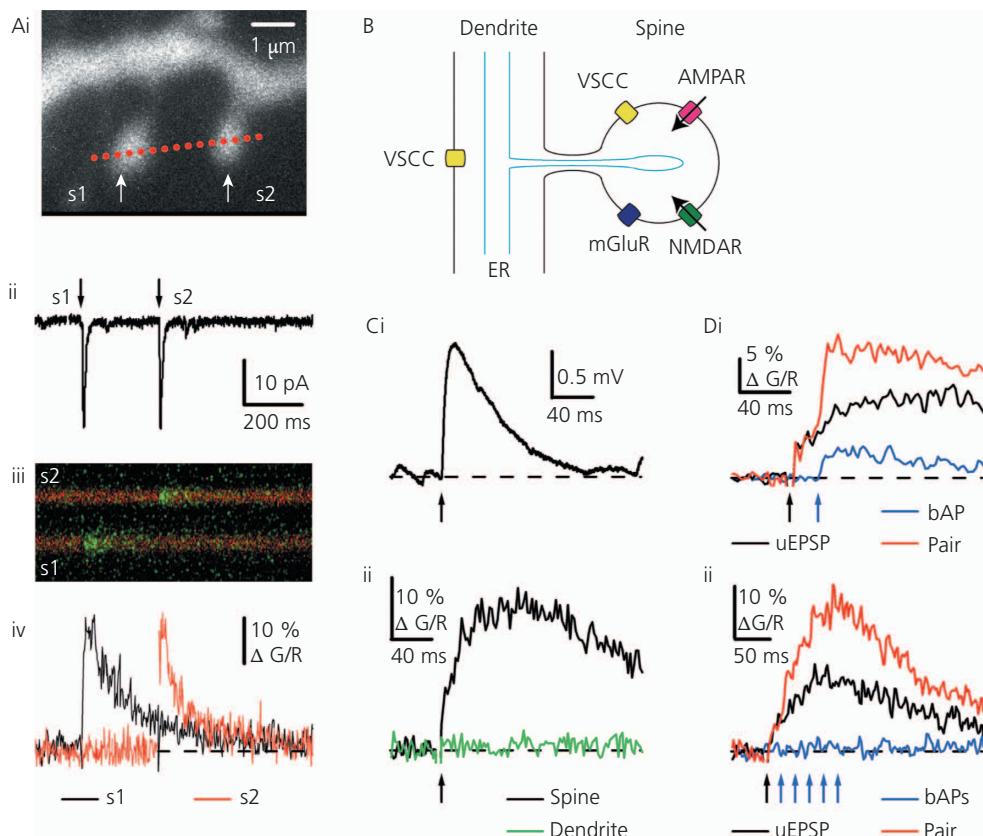


Fig. 11.3 Synaptic Ca^{2+} signals in spines. **(A)** 2PLSM image showing a section of a dendrite in a medium spiny neuron, with the red line indicating the orientation of the line scan through two spines (s1 and s2) (i); 2PLU at the two spines (arrows) evokes EPSCs in a neuron held in whole-cell voltage-clamp (ii) and independent Ca^{2+} signals in the two spines (iii) that are quantified as a change in green over red fluorescence ($\Delta G/R$) (iv). **(B)** Schematic showing that synaptic input allows glutamate to bind and open AMPARs and NMDARs in the spine. **(C)** 2PLU evokes an EPSP in a neuron held at -50 mV in whole-cell current-clamp (i); the corresponding Ca^{2+} signal is mediated by both AMPARs and NMDARs and is restricted to the spine (ii). **(D)** Pairing 2PLU (black arrow) with a bAP (blue arrow) evokes a NMDAR-mediated Ca^{2+} signal (red line) that is larger than the sum of the signals evoked by 2PLU (black line) and the bAP (blue line) alone (i). Sequential bAPs continue to enhance the 2PLU-evoked NMDAR-mediated Ca^{2+} signal even when VSCCs are blocked and the bAPs alone evoke no Ca^{2+} signal (ii).

these complications are overcome by using 2PLU of glutamate to mimic synaptic transmission at individual spines while monitoring evoked Ca^{2+} signals using 2PLSM (Carter and Sabatini, 2004).

Pyramidal neurons

Synaptic Ca^{2+} signals in spines of pyramidal neurons are large and easily monitored with modern tools. Recently, they have even been used to determine the sensory tuning of individual synapses *in vivo* (Chen et al., 2011, 2012, 2013). The mechanisms of these large synaptic Ca^{2+} signals in spines of pyramidal neurons in the hippocampus and cortex have now been extensively studied.

In dissociated cultures, spontaneous excitatory postsynaptic currents (EPSCs) are associated with Ca^{2+} signals restricted to small dendritic regions or individual spines (Murphy et al., 1994, 1995; Murthy et al., 2000). Similarly, in slices, weak electrical stimulation evokes synaptic Ca^{2+} signals that are restricted to individual spines, presumably reflecting the activation of the nearby synapse (Muller and Connor, 1991; Yuste and Denk, 1995; Mainen et al., 1999; Yuste et al., 1999; Kovalchuk et al., 2000; Sabatini et al., 2002; Nimchinsky et al., 2004). Many studies have shown that during synaptic stimulation *N*-methyl-D-aspartic acid (NMDA) receptors (NMDARs) contribute the major fraction of synaptic Ca^{2+} influx into spines (Mainen et al., 1999; Yuste et al., 1999; Kovalchuk et al., 2000; Sabatini et al., 2002). The biophysical properties of NMDARs play a critical role in shaping synaptic Ca^{2+} signals, due to their high Ca^{2+} permeability (Schneggenburger et al., 1993) and blockade by intracellular Mg^{2+} at hyperpolarized potentials (Jahr and Stevens, 1990b). While only a handful of NMDARs are typically recruited by individual glutamatergic inputs (Nimchinsky et al., 2004), the long open time of these receptors prolongs spine Ca^{2+} signals (Sabatini et al., 2002). It is also important to keep in mind that multiple NMDAR subunits are found in pyramidal neurons, the contributions of which can change over development (Monyer et al., 1994). Interestingly, the contributions of the NR2A and NR2B subunits to synaptic Ca^{2+} signals can vary between spines even within the same neuron (Sobczyk et al., 2005).

The contribution of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) to synaptic Ca^{2+} signals in pyramidal neurons has been more controversial. Early studies found that AMPAR blockade had no effect on synaptic Ca^{2+} signals (Mainen et al., 1999), reduced them slightly (Kovalchuk et al., 2000), or abolished them entirely (Emptage et al., 1999). The latter finding suggests that AMPAR-mediated depolarization of the spine relieves the otherwise complete Mg^{2+} block of the NMDAR; however, this interpretation is difficult to reconcile with the finding that substantial Ca^{2+} entry through NMDARs occurs at resting potentials because the block of NMDARs by extracellular Mg^{2+} is “flickering” and the driving force for Ca^{2+} influx is large (Jahr and Stevens, 1990a; Sabatini and Svoboda, 2000). Alternatively, in some cases, Ca^{2+} -permeable AMPARs may contribute to synaptic Ca^{2+} signals (Wang et al., 2002). AMPARs lacking the GluR2 subunit have high Ca^{2+} permeability compared with receptors that contain this subunit (Hollmann et al., 1991; Hume et al., 1991; Schneggenburger et al., 1993; Jonas and Burnashev, 1995). The presence of Ca^{2+} -permeable AMPARs varies between neurons and changes over the course of development, and individual neurons may maintain heterogeneous AMPAR populations within and between different spines (Ogoshi and Weiss, 2003). Ultimately, even in neurons that express GluR2, low numbers of GluR2-lacking, highly Ca^{2+} -permeable AMPARs may be able to endow spines with AMPAR-mediated Ca^{2+} signals.

Some studies also have found that synaptic Ca^{2+} signals can reflect release from internal Ca^{2+} stores (Alford et al., 1993; Emptage et al., 1999). The endoplasmic reticulum (ER) is a prominent internal Ca^{2+} store found in the majority of spines in hippocampal pyramidal neurons (Spacek and Harris, 1997; Bannai et al., 2004), with ryanodine receptors (RyRs) found in both dendrites and spines and inositol 1,4,5-trisphosphate (IP3) receptors (IP3Rs) restricted to dendrites (Sharp

et al., 1993; Berridge, 1998). The contribution of internal stores to synaptic Ca^{2+} signals may depend on both the extent of ER entry into the spine and the presence of the molecular machinery for calcium release. The contribution of internal Ca^{2+} stores to synaptic Ca^{2+} signals may also be influenced by the recording conditions, spine selection, or Ca^{2+} loading of internal Ca^{2+} stores via activity. Finally, the contribution of internal Ca^{2+} stores to synaptic Ca^{2+} signals may depend on the experimental preparation, playing less of a role in acute (Mainen et al., 1999; Kovalchuk et al., 2000; Sabatini et al., 2002) compared with organotypic slices (Emptage et al., 1999).

Purkinje cells

Synaptic Ca^{2+} signals in cerebellar Purkinje cells have also been well studied, but differ remarkably from signals in pyramidal neurons. Purkinje cells receive both parallel fiber (PF) and climbing fiber (CF) inputs, which trigger different physiological responses (Eccles et al., 1967; Llano et al., 1991b). However, mature Purkinje cells lack NMDARs (Perkel et al., 1990; Farrant and Cull-Candy, 1991), and synaptic Ca^{2+} signals are instead mediated by AMPARs and metabotropic glutamate receptors (mGluRs), and are thus very different from pyramidal neurons.

Activation of PF inputs evokes graded EPSPs in Purkinje cells, the size of which depends on the number of presynaptic inputs that are activated. Similarly, PF inputs produce widely different Ca^{2+} signals depending on the strength of activation, from single spines, to branches, to larger dendritic areas (Miyakawa et al., 1992; Hartell, 1996). Weak PF stimulation evokes fast synaptic Ca^{2+} signals that are localized to individual spines and are dependent on AMPAR opening (Denk et al., 1995). Some of these Ca^{2+} signals are mediated by VSCCs; however, other Ca^{2+} signals are mediated by Ca^{2+} -permeable AMPARs. In contrast, stronger PF stimulation generates widespread Ca^{2+} signals in both dendrites and spines. These large Ca^{2+} signals are mediated by VSCCs, which are activated during AMPAR-evoked dendritic spikes (Eilers et al., 1995). Finally, brief trains of subthreshold PF inputs generate Ca^{2+} signals that can be localized to individual spines or spread into dendrites (Finch and Augustine, 1998; Takechi et al., 1998). These Ca^{2+} signals are biphasic, and the late component can be blocked with either mGluR or IP3R antagonists. Conversely, activation of mGluRs and generation of IP3 leads to release of Ca^{2+} from internal stores, generating Ca^{2+} signals similar to the late component (Llano et al., 1991a; Khodakhah and Ogden, 1993). These results suggest that internal Ca^{2+} stores are important for mediating synaptic Ca^{2+} signals in Purkinje cells, which have extensive ER in their dendrites and spines, possess IP3Rs in their dendrites and spines, but have RyRs only in their dendrites (Harris and Stevens, 1988; Satoh et al., 1990; Walton et al., 1991; Berridge, 1998). Thus, depending on the strength and pattern of stimulation, PF inputs can recruit a broad range of Ca^{2+} sources generating diverse synaptic Ca^{2+} signals.

In contrast, activation of the CF input elicits complex spikes in Purkinje cells, generating Ca^{2+} signals across the proximal dendrites (Ross and Werman, 1987). These synaptic Ca^{2+} signals are blocked by AMPAR antagonists, and are mediated by VSCCs (Knopf et al., 1991; Konnerth et al., 1992; Miyakawa et al., 1992). As described later, activation of the CF input also enhances Ca^{2+} signals generated by PF inputs (Wang et al., 2000), which is important for triggering synaptic plasticity.

Other spiny neurons

Synaptic Ca^{2+} signals in several other cell types have also been well studied, including layer 4 (L4) spiny stellate cells (Nevian and Sakmann, 2004), striatal medium spiny neurons (Carter and Sabatini, 2004), olfactory bulb granule cells (Egger et al., 2005), and lateral amygdala projection neurons (Humeau et al., 2005). In the somatosensory cortex, the spines of L4 spiny stellate cells display synaptic Ca^{2+} signals mediated by NMDARs (Nevian and Sakmann, 2004). In contrast,

medium spiny neurons have synaptic Ca^{2+} signals in spines that are mediated by Ca^{2+} -permeable AMPARs and NMDARs (Carter and Sabatini, 2004). In these cells, both AMPAR and NMDAR Ca^{2+} signals are strongly influenced by state transitions, in which the membrane potential shifts from a resting down-state (about -80 mV) to a depolarized up-state (about -50 mV). These transitions decrease the AMPAR Ca^{2+} signal due a decreased driving force for Ca^{2+} entry, and increase the NMDAR Ca^{2+} signal via relief of the Mg^{2+} block. Finally, granule cells in the olfactory bulb have synaptic Ca^{2+} signals at dendro-dendritic synapses mediated by a variety of Ca^{2+} sources, including NMDARs, T-type channels, and release from internal Ca^{2+} stores (Egger et al., 2005).

Nonlinear interactions

Interactions between bAPs and synaptic responses

In many neurons, pairing of presynaptic and postsynaptic activity generates nonlinear spine Ca^{2+} signals, depending on the timing of the activity. Thus, if the synaptic inputs arrive before bAPs, pairing evokes supralinear Ca^{2+} signals, which are larger than the sum of the bAP and synaptic Ca^{2+} signals alone (Fig. 11.3D) (Yuste and Denk, 1995; Magee and Johnston, 1997; Koester and Sakmann, 1998; Schiller et al., 1998; Yuste et al., 1999; Waters et al., 2003; Carter and Sabatini, 2004; Nevian and Sakmann, 2004). These supralinear Ca^{2+} signals can be restricted to small stretches of dendrites or individual spines, depending on the stimulation paradigm. This supralinearity is often due to additional influx of Ca^{2+} through NMDARs, rather than activation of VSCCs, AMPARs, or internal Ca^{2+} stores. Blocking NMDARs eliminates the supralinear Ca^{2+} signal in CA1 and L5 pyramidal cells (Koester and Sakmann, 1998; Yuste et al., 1999), as well as in L4 spiny stellate cells (Nevian and Sakmann, 2004). Several features of NMDARs enable these supralinear responses, including their slow kinetics (Lester et al., 1990) and blockade by extracellular Mg^{2+} (Mayer et al., 1984; Nowak et al., 1984; Ross and Werman, 1987; Vargas-Caballero and Robinson, 2003; Kampa et al., 2004). Reducing extracellular Mg^{2+} greatly reduces the supralinear Ca^{2+} signal, suggesting that it is due to the relief of Mg^{2+} block during bAP depolarization (Carter and Sabatini, 2004; Nevian and Sakmann, 2004). Importantly, the relief of Mg^{2+} block is brief, due to the short duration of the bAP, and generates an additional short-lived influx of Ca^{2+} through NMDARs that contrasts with the normally slow Ca^{2+} signal mediated by these channels. Thus, a burst of bAPs generates a series of brief Ca^{2+} transients, independent of VSCC activation (Carter and Sabatini, 2004). Finally, if the order of activation is reversed, such that the bAP precedes the synaptic input, sublinear summation of the signals is seen (Koester and Sakmann, 1998); however, the mechanism that generates this sublinearity is currently unknown.

Interactions during synaptic responses

The activation of AMPARs and NMDARs in spines enables Ca^{2+} influx and generates EPSPs in the spine head. Direct voltage measurements indicate that single glutamatergic inputs can depolarize spines by tens of millivolts (Tsay and Yuste, 2004; Palmer and Stuart, 2009). Moreover, the high spine neck resistance means that this large EPSP may not be experienced by nearby dendrites (Bloodgood and Sabatini, 2005; Grunditz et al., 2008; Harnett et al., 2012). Recent studies using 2PLU indicate that spine EPSPs engage a variety of voltage-sensitive ion channels that in turn influence synaptic Ca^{2+} signals. For example, blocking AMPARs reduces synaptic Ca^{2+} signals, presumably as smaller EPSPs have less impact on Mg^{2+} block of NMDARs (Grunditz et al., 2008; Bloodgood et al., 2009; Seong et al., 2014). In contrast, augmenting AMPARs promotes synaptic Ca^{2+} signals, as larger EPSPs are better able to relieve this Mg^{2+} block (Grunditz et al.,

2008; Bloodgood et al., 2009). In addition, spine EPSPs can directly activate Ca^{2+} channels, and in some cases blocking these channels reduces synaptic Ca^{2+} signals (Bloodgood and Sabatini, 2007; Grunditz et al., 2008; Seong et al., 2014). Similarly, these EPSPs can directly activate Na^+ channels (Araya et al., 2007; Carter et al., 2012), and blocking these channels also reduces synaptic Ca^{2+} signals (Seong et al., 2014; Bywalek et al., 2015). Finally, these EPSPs can activate K^+ channels, and blocking these channels in some cases enhances synaptic Ca^{2+} signals (Seong et al., 2014). As the density of channels often varies with distance along dendrites, synaptic Ca^{2+} signals are expected to depend on dendritic location. Moreover, the availability of channels depends on the resting membrane potential, which influences synaptic Ca^{2+} signals (Carter and Sabatini, 2004; Seong et al., 2014). Clearly, the roles of different channels will vary between neurons, and this remains an exciting area for future research.

Recent studies have also highlighted how Ca^{2+} signals feed back to strongly influence both Ca^{2+} sources and other ion channels in the spine. For example, intracellular Ca^{2+} modulates inactivation of VSCCs (Eckert and Chad, 1984) and NMDARs (Legendre et al., 1993). Intracellular Ca^{2+} also activates SK channels, which are found in the spines of CA1 pyramidal neurons (Ngo-Anh et al., 2005; Bloodgood and Sabatini, 2007; Bloodgood et al., 2009). Synaptic Ca^{2+} signals can activate SK channels, which in turn trigger a local hyperpolarization of the spine, attenuate the EPSP, and enhance the Mg^{2+} block of NMDARs. Thus, Ca^{2+} influx can trigger a negative feedback loop within the spine head, which limits the induction of synaptic plasticity in CA1 and amygdala pyramidal neurons (Faber et al., 2005; Ngo-Anh et al., 2005). Interestingly, this feedback loop critically depends on influx of Ca^{2+} through R-type VSCCs activated by spine EPSPs (Bloodgood and Sabatini, 2007; Giessel and Sabatini, 2010, 2011).

The above description focuses on the mechanisms of Ca^{2+} handling when a single spine is activated by a synaptic stimulus or when the entire dendritic segment is invaded by a bAP. However, in many situations subsets of clustered spines are activated in spatio-temporal patterns that produce nonlinear effects on Ca^{2+} influx. For example, two nearby active spines can interact sublinearly by reduction of the driving force for Ca^{2+} influx or supralinearly via relief of the Mg^{2+} block of NMDARs, depending on the context of stimulation (Carter et al., 2007). In addition, coactivation of nearby spines on individual dendritic segments can induce local VSCC-dependent Ca^{2+} spikes, as well as Na^+ spikes that alter both dendritic Ca^{2+} influx and somatic potentials (Losonczy and Magee, 2006). Lastly, interactions between the voltage dependence of NMDARs and the geometry of dendritic segments produce non-linearities in Ca^{2+} influx and somatic depolarization that depend on the order of stimulation of synapses (Branco et al., 2010).

Regulation of spine Ca^{2+} signals

Modulation of spine Ca^{2+} signals

In many neurons, Ca^{2+} signals in spines are dynamically regulated by a wide range of G-protein-coupled receptors. Neuromodulator receptors are present throughout the dendrites, and act on many voltage-sensitive ion channels (Hille, 2001). Direct modulation of bAP Ca^{2+} signals occurs via effects on VSCCs. For example, beta-adrenergic receptors enhance L-type Ca^{2+} channels to augment bAP Ca^{2+} signals at spines (Hoogland and Saggau, 2004). In contrast, metabotropic GABA_B receptors inhibit a variety of Ca^{2+} channels to directly suppress bAP Ca^{2+} signals (Sabatini and Svoboda, 2000; Chalifoux and Carter, 2011). Similarly, D2 dopamine receptors inhibit bAP Ca^{2+} signals in striatal medium spiny neurons (Day et al., 2008) by inhibiting R-type Ca^{2+} channels (Higley and Sabatini, 2010). Indirect modulation of bAP Ca^{2+} signals can also occur, via effects on other voltage-sensitive

ion channels in spines and dendrites. For example, Na^+ channels can promote backpropagation to boost bAP Ca^{2+} signals, whereas K^+ channels can inhibit backpropagation to suppress these signals.

Studying the modulation of synaptic Ca^{2+} signals has been more challenging, due to effects on presynaptic release. Using 2PLU circumvents this difficulty, and recent studies have highlighted multifaceted and often unpredictable regulation. For example, NR2B-containing NMDARs can be modulated by serine/threonine phosphatases at spines (Sobczyk and Svoboda, 2007). The Ca^{2+} permeability of NMDARs is also under direct control from PKA-dependent phosphorylation (Skeberdis et al., 2006; Murphy et al., 2014). In addition, GABA_B receptors act via PKA to inhibit synaptic Ca^{2+} signals by reducing influx of Ca^{2+} through NMDARs (Chalifoux and Carter, 2010). Similar modulation occurs at striatal medium spiny neurons, where D2 dopamine receptors and A2 adenosine receptors have opposing influences on Ca^{2+} influx through NMDARs (Higley and Sabatini, 2010). Importantly, modulation can also occur indirectly via effects on a variety of voltage- and Ca^{2+} -sensitive ion channels. For example, muscarinic acetylcholine receptors enhance synaptic Ca^{2+} signals at hippocampal pyramidal neurons by inhibiting SK channels and reducing Mg^{2+} block of NMDARs (Giessel and Sabatini, 2010). Interestingly, in cartwheel cells of the dorsal cochlear nucleus (DCN), these receptors have similar effects, but target Ca^{2+} -activated BK channels (He et al., 2014).

Inhibition of spine Ca^{2+} signals

Inhibitory inputs contact the spine neck and dendritic shaft, and are poised to regulate electrical and biochemical signaling at spines. These inputs can activate both metabotropic GABA_B receptors and ionotropic GABA_A receptors found at dendrites and spines (Isaacson and Scanziani, 2011). Recent results highlight how both types of receptors strongly inhibit local Ca^{2+} signals within the spine head. GABA_B receptors activate K^+ channels and inhibit Ca^{2+} channels to block local spikes in the dendrites (Pérez-Garcí et al., 2006, 2012). They also directly inhibit Ca^{2+} channels to strongly inhibit bAP Ca^{2+} signals at dendrites and spines (Sabatini and Svoboda, 2000; Chalifoux and Carter, 2011). Finally, GABA_B receptors can reduce the Ca^{2+} influx through NMDARs to inhibit synaptic Ca^{2+} signals at spines (Chalifoux and Carter, 2010). In contrast, ionotropic GABA_A receptors are Cl^- channels that generate a local hyperpolarization and conductance increase. This inhibition also strongly suppresses bAP Ca^{2+} signals at dendrites and spines of many neurons (Kanemoto et al., 2011; Hayama et al., 2013). This potent inhibition can be confined to the spine head (Chiu et al., 2013) or spread along dendritic branches (Marlin and Carter, 2014). Interestingly, GABA_B receptors act over tens of milliseconds, whereas GABA_A receptors act on the millisecond time scale. Therefore, dendritic inhibition can strongly influence multiple aspects of spine Ca^{2+} signaling over a broad temporal window.

Recent studies have also highlighted the role of different interneuron populations, which contact unique dendritic domains (Markram et al., 2004; Ascoli et al., 2008; Rudy et al., 2011; DeFelipe et al., 2013). For example, in pyramidal neurons, GABA_A receptor inhibition of bAP Ca^{2+} signals can occur via somatostatin- or 5-HT3a receptor-expressing interneurons (Chiu et al., 2013; Marlin and Carter, 2014). In the future, it will be particularly interesting to assess how interneurons activate GABA_A and GABA_B receptors to influence spine Ca^{2+} signaling during realistic activity patterns.

Technical considerations

The amplitude and kinetics of Ca^{2+} signals help determine their function in a variety of cell types (Berridge, 1998). In spines, these properties are shaped by many factors, including endogenous Ca^{2+} buffers, uptake into the ER or mitochondria, extrusion across the plasma membrane, and

diffusion between spine and dendrite (Emptage et al., 1999; Kovalchuk et al., 2000; Majewska et al., 2000a,b; Holthoff et al., 2002; Sabatini et al., 2002). Ca^{2+} indicators themselves also complicate the interpretation of spine Ca^{2+} signal amplitude and time course. Because spines are small and fluorescent signals are weak, large amounts ($>100 \mu\text{M}$) of high-affinity ($K_d < 1 \mu\text{M}$) Ca^{2+} indicators are used. These indicators buffer Ca^{2+} , often dominating the endogenous Ca^{2+} buffers, and counteract changes in intracellular Ca^{2+} by reducing the amplitude and extending the time course of Ca^{2+} signals. Moreover, these Ca^{2+} indicators are also more mobile than many endogenous Ca^{2+} buffers and therefore tend to accelerate the spread of Ca^{2+} by diffusion some 10- to 100-fold (Allbritton et al., 1992; Zhou and Neher, 1993; Wagner and Keizer, 1994; Jafri and Keizer, 1995).

A theoretical framework for considering the influence of Ca^{2+} indicators on Ca^{2+} signaling has been developed (Neher and Augustine, 1992; Neher, 1995, 1998; Tank et al., 1995), and is discussed fully in Chapter 10. This framework has been applied to many neuronal and non-neuronal cells to measure their endogenous buffer capacity, which is defined as the ratio of buffer-bound Ca^{2+} changes to free Ca^{2+} changes. In pyramidal cell dendrites and spines, the endogenous buffer capacity is low (about 20–200), and a relatively large fraction of total Ca^{2+} exists as free ions (Helmchen et al., 1996; Lee et al., 2000; Maravall et al., 2000; Sabatini et al., 2002). In contrast, the endogenous buffer capacity of Purkinje cells is higher and changes over development, measuring around 900 at postnatal day 6 (P6) and increasing to about 2,000 at P15 (Fierro and Llano, 1996).

Determining the endogenous buffer capacity allows calculation of the amplitude and time course of unperturbed bAP Ca^{2+} signals in dendrites (Helmchen et al., 1996; Maravall et al., 2000) and spines (Sabatini et al., 2002; Carter and Sabatini, 2004). In CA1 pyramidal neurons, bAP Ca^{2+} signals in spines reach $>1 \mu\text{M}$ and decay in about 20 ms in the absence of exogenous Ca^{2+} buffers (Sabatini et al., 2002). In addition, some spine Ca^{2+} signals show bi-exponential decay (Miyakawa et al., 1992; Airaksinen et al., 1997; Maeda et al., 1999; Majewska et al., 2000a), possibly due to different Ca^{2+} buffers or nonlinear Ca^{2+} clearance mechanisms (Schmidt et al., 2003). The main Ca^{2+} clearance mechanisms in spines are extrusion through Ca^{2+} pumps, exchangers, and diffusion to the dendrite. Several Ca^{2+} pumps are expressed in the brain, including those on the plasma membrane (PMCA) (Stauffer et al., 1995) and ER (SERCA) (Miller et al., 1991). PMCA and SERCA blockers can decrease or slow spine Ca^{2+} signals, suggesting that these pumps do exist in spines (Emptage et al., 1999; Kovalchuk et al., 2000; Majewska et al., 2000a; Sabatini et al., 2002; Scheuss et al., 2006). Finally, slow diffusion across the spine neck ($>100 \text{ ms}$) means that the spine head is an isolated compartment for the duration of the Ca^{2+} signal. However, when using a large amount of Ca^{2+} indicator, Ca^{2+} signals are prolonged and diffusion is accelerated, so that the geometry of the spine neck can shape Ca^{2+} clearance (Holthoff et al., 2002; Majewska et al., 2000a; b).

In the absence of Ca^{2+} indicator, NMDAR-mediated Ca^{2+} signals in response to single synaptic inputs are large, reaching about $1 \mu\text{M}$ in spines at resting potentials (Sabatini et al., 2002). However, during tetanic stimulation these signals may become much larger, reaching 20–40 μM in spines (Petrozzino et al., 1995). In contrast to bAP Ca^{2+} signals, these NMDAR-mediated Ca^{2+} signals last for hundreds of milliseconds. This prolonged time course has been measured using small concentrations of low-affinity Ca^{2+} indicators, and is well described by the convolution of the NMDAR EPSC time course and the bAP Ca^{2+} signal impulse response (Sabatini et al., 2002).

Functions of spine Ca^{2+} signals

In many neurons, synaptic Ca^{2+} signals are required for synaptic plasticity induced by pairing protocols and synaptic trains, including both LTP and LTD (Bliss and Collingridge, 1993; Magee and Johnston, 1997; Markram et al., 1997; Zucker, 1999). The source, amplitude, time course, and

location of these Ca^{2+} signals help determine which form of synaptic plasticity is induced by neuronal activity. For example, small Ca^{2+} elevations may favor LTD, whereas larger elevations may bias toward LTP (Cho et al., 2001; Cormier et al., 2001), possibly via differential activation of CaMKII and calcineurin (Lisman, 1989). Because spines act as independent compartments on long time scales, they may physically isolate Ca^{2+} and other signaling molecules to activated synapses, as is required for input-specific synaptic plasticity. Furthermore, the nonlinear spine Ca^{2+} signals generated by pairing synaptic inputs and bAPs may be necessary for “spike-timing dependent plasticity” (STDP) generated by pairing protocols. This form of plasticity has been demonstrated in a variety of neurons including hippocampal (Bi and Poo, 1998; Debanne et al., 1998) and L2/3 (Feldman, 2000; Froemke and Dan, 2002) pyramidal neurons. In general, LTP occurs if the synaptic inputs precede the bAPs by <10 ms, whereas LTD occurs if the bAP precedes the synaptic inputs by less than about 100 ms (Wigström et al., 1979, 1986; Lynch et al., 1983; Magee and Johnston, 1997; Markram et al., 1997; Zhang et al., 1998; Feldman, 2000).

Synaptic Ca^{2+} signals are also required for synaptic plasticity in Purkinje cells. Pairing CF and PF inputs generates LTD at PF inputs (Ito and Kano, 1982; Linden and Connor, 1995), which depends on postsynaptic Ca^{2+} signals (Sakurai, 1990). Pairing CF inputs with weak PF stimulation generates small supralinear Ca^{2+} signals restricted to spines, mediated by mGluRs, IP3Rs, and the release of Ca^{2+} from internal stores (Finch and Augustine, 1998; Wang et al., 2000). This stimulation paradigm also evokes LTD, and is abolished by blocking mGluRs or depleting internal Ca^{2+} stores (Khodakhah and Armstrong, 1997; Finch and Augustine, 1998; Wang et al., 2000). This form of LTD is also eliminated in myosin IVA knock-out mice, in which the spine ER is missing (Miyata et al., 2000). Finally, pairing CF inputs with strong PF stimulation generates large supralinear Ca^{2+} signals in dendrites and spines that depend on VSCCs (Wang et al., 2000). However, both this stimulation paradigm and strong PF stimulation (Hartell, 1996) generate forms of LTD that cannot be abolished with mGluR blockers.

Changes in spine morphology are correlated with both synaptic plasticity and development, and may determine the extent to which spines can compartmentalize chemical and electrical signals (Dunaevsky et al., 1999; Engert and Bonhoeffer, 1999; Maletic-Savatic et al., 1999; Toni et al., 1999; Korkotian and Segal, 2001; Matsuzaki et al., 2004). Spine Ca^{2+} signals may elicit changes in spine morphology by altering the local cytoskeleton. Spines in CA1 pyramidal neurons are rich in actin (Fifkova and Delay, 1982; Matus et al., 1982; Fischer et al., 1998; Capani et al., 2001), but not microfilaments or microtubules (Peters et al., 1991). Consistent with this idea, Ca^{2+} signals generated by AMPAR and NMDAR activation influence spine actin dynamics and spine morphology in hippocampal cultures (Halpain et al., 1998; Fischer et al., 2000; Star et al., 2002). Moreover, mGluR activation and release from internal Ca^{2+} stores also triggers changes in spine morphology (Korkotian and Segal, 1999; Vanderklish and Edelman, 2002). However, the types of Ca^{2+} signals needed to induce these morphological changes remain largely unknown.

Concluding remarks

The past three decades have yielded important insights into how spines function. This progress is largely due to the development of 2PLSM allowing the investigation of Ca^{2+} signals in single spines with high resolution in both space and time. Spine Ca^{2+} signals depend on activation of AMPARs, NMDARs, and mGluRs, as well as VSCCs and release of Ca^{2+} from internal stores. Which Ca^{2+} sources are activated depends on how spines are stimulated and varies among different cell types. The kinetics of Ca^{2+} signals are determined by multiple factors, including endogenous Ca^{2+} buffers, Ca^{2+} pumps and diffusion. Ultimately, spines are able to compartmentalize their Ca^{2+} signals

over long and physiologically relevant time courses, which can allow them to function independently of their nearby dendrites and neighboring spines. Consequently, Ca^{2+} signaling in spines can locally regulate synaptic function, plasticity induction, and spine morphology. In the coming years, we are likely to learn much more about how spine Ca^{2+} signals translate to local function within spines. Moreover, we will continue to learn how activation of many spines generates more complex Ca^{2+} signals in spines and dendrites.

References

- Airaksinen MS, Eilers J, Garaschuk O, Thoenen H, Konnerth A, Meyer M (1997) Ataxia and altered dendritic calcium signaling in mice carrying a targeted null mutation of the calbindin D28k gene. *Proceedings of the National Academy of Sciences of the United States of America* **94**:1488–1493.
- Alford S, Frenguelli BG, Schofield JG, Collingridge GL (1993) Characterization of Ca^{2+} signals induced in hippocampal CA1 neurones by the synaptic activation of NMDA receptors. *Journal of Physiology* **469**:693–716.
- Allbritton NL, Meyer T, Stryer L (1992) Range of messenger action of calcium ion and inositol 1,4,5-trisphosphate. *Science* **258**:1812–1815.
- Amatrudo, J.M., Olson, J.P., Agarwal, H.K., and Ellis-Davies, G.C. (2015) Caged compounds for multichromic optical interrogation of neural systems. *European Journal of Neuroscience* **41**:5–16.
- Araya R, Nikolenko V, Eisenthal KB, Yuste R (2007) Sodium channels amplify spine potentials. *Proceedings of the National Academy of Sciences of the United States of America* **104**:12347–12352.
- Araya R, Vogels TP, Yuste R (2014) Activity-dependent dendritic spine neck changes are correlated with synaptic strength. *Proceedings of the National Academy of Sciences of the United States of America* **111**:E2895–E2904.
- Ascoli GA, et al. (2008) Petilla terminology: nomenclature of features of GABAergic interneurons of the cerebral cortex. *Nature Reviews Neuroscience* **9**:557–568.
- Axelrod D, Koppel DE, Schlessinger J, Elson E, Webb WW (1976) Mobility measurement by analysis of fluorescence photobleaching recovery kinetics. *Biophysical Journal* **16**:1055–1069.
- Bannai, H., Inoue, T., Nakayama, T., Hattori, M., and Mikoshiba, K. (2004) Kinesin dependent, rapid, bi-directional transport of ER sub-compartment in dendrites of hippocampal neurons. *Journal of Cell Science* **117**:163–175.
- Berridge MJ (1998) Neuronal calcium signaling. *Neuron* **21**:13–26.
- Bi GQ, Poo MM (1998) Synaptic modifications in cultured hippocampal neurons: dependence on spike timing, synaptic strength, and postsynaptic cell type. *Journal of Neuroscience* **18**:10464–10472.
- Bliss TV, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* **361**:31–39.
- Bloodgood BL, Sabatini BL (2005) Neuronal activity regulates diffusion across the neck of dendritic spines. *Science* **310**:866–869.
- Bloodgood BL, Sabatini BL (2007) Nonlinear regulation of unitary synaptic signals by $\text{CaV}(2.3)$ voltage-sensitive calcium channels located in dendritic spines. *Neuron* **53**:249–260.
- Bloodgood BL, Giessel AJ, Sabatini BL (2009) Biphasic synaptic Ca influx arising from compartmentalized electrical signals in dendritic spines. *PLoS Biology* **7**:e1000190.
- Boyden ES, Zhang F, Bamberg E, Nagel G, Deisseroth K (2005) Millisecond-timescale, genetically targeted optical control of neural activity. *Nature Neuroscience* **8**:1263–1268.
- Branco T, Clark BA, Häusser M (2010) Dendritic discrimination of temporal input sequences in cortical neurons. *Science* **329**:1671–1675.
- Bywalez WG, Patirniche D, Rupprecht V, Stemmler M, Herz AVM, Pálfi D, Rózsa B, Egger V (2015) Local postsynaptic voltage-gated sodium channel activation in dendritic spines of olfactory bulb granule cells. *Neuron* **85**:590–601.

- Callaway, J.C., and Ross, W.N. (1995) Frequency-dependent propagation of sodium action potentials in dendrites of hippocampal CA1 pyramidal neurons. *Journal of Neurophysiology* **74**:1395–1403.
- Capani F, Martone ME, Deerinck TJ, Ellisman MH (2001) Selective localization of high concentrations of F-actin in subpopulations of dendritic spines in rat central nervous system: a three-dimensional electron microscopic study. *Journal of Comparative Neurology* **435**:156–170.
- Carter AG, Sabatini BL (2004) State-dependent calcium signaling in dendritic spines of striatal medium spiny neurons. *Neuron* **44**:483–493.
- Carter AG, Soler-Llavina GJ, Sabatini BL (2007) Timing and location of synaptic inputs determine modes of subthreshold integration in striatal medium spiny neurons. *Journal of Neuroscience* **27**:8967–8977.
- Carter BC, Giessel AJ, Sabatini BL, Bean BP (2012) Transient sodium current at subthreshold voltages: activation by EPSP waveforms. *Neuron* **75**:1081–1093.
- Chalifoux JR, Carter AG (2010) GABAB receptors modulate NMDA receptor calcium signals in dendritic spines. *Neuron* **66**:101–113.
- Chalifoux JR, Carter AG (2011) GABAB receptor modulation of voltage-sensitive calcium channels in spines and dendrites. *Journal of Neuroscience* **31**:4221–4232.
- Chang HT (1952) Cortical neurons with particular reference to the apical dendrites. *Cold Spring Harbor Symposia on Quantitative Biology* **17**:189–202.
- Chen X, Leischner U, Rochefort NL, Nelken I, Konnerth A (2011) Functional mapping of single spines in cortical neurons in vivo. *Nature* **475**:501–505.
- Chen X, Leischner U, Varga Z, Jia H, Deca D, Rochefort NL, Konnerth A (2012) LOTOS-based two-photon calcium imaging of dendritic spines in vivo. *Nature Protocols* **7**:1818–1829.
- Chen T-W, et al. (2013) Ultrasensitive fluorescent proteins for imaging neuronal activity. *Nature* **499**:295–300.
- Chiu CQ, Lur G, Morse TM, Carnevale NT, Ellis-Davies GCR, Higley MJ (2013) Compartmentalization of GABAergic inhibition by dendritic spines. *Science* **340**:759–762.
- Cho K, Aggleton JP, Brown MW, Bashir ZI (2001) An experimental test of the role of postsynaptic calcium levels in determining synaptic strength using perirhinal cortex of rat. *Journal of Physiology* **532**:459–466.
- Connor JA (1986) Digital imaging of free calcium changes and of spatial gradients in growing processes in single, mammalian central nervous system cells. *Proceedings of the National Academy of Sciences of the United States of America* **83**:6179–6183.
- Connor JA, Kretz R, Shapiro E (1986) Calcium levels measured in a presynaptic neurone of *Aplysia* under conditions that modulate transmitter release. *Journal of Physiology* **375**:625–642.
- Cormier RJ, Greenwood AC, Connor JA (2001) Bidirectional synaptic plasticity correlated with the magnitude of dendritic calcium transients above a threshold. *Journal of Neurophysiology* **85**:399–406.
- Day M, Wokosin D, Plotkin JL, Tian X, Surmeier DJ (2008) Differential excitability and modulation of striatal medium spiny neuron dendrites. *Journal of Neuroscience* **28**:11603–11614.
- Debanne D, Gähwiler BH, Thompson SM (1998) Long-term synaptic plasticity between pairs of individual CA3 pyramidal cells in rat hippocampal slice cultures. *Journal of Physiology* **507**:237–247.
- DeFelipe J, et al. (2013) New insights into the classification and nomenclature of cortical GABAergic interneurons. *Nature Reviews Neuroscience* **14**:202–216.
- Denk W, Svoboda K (1997) Photon upmanship: why multiphoton imaging is more than a gimmick. *Neuron* **18**:351–357.
- Denk W, Strickler JH, Webb WW (1990) Two-photon laser scanning fluorescence microscopy. *Science* **248**:73–76.
- Denk W, Sugimori M, Llinás R (1995) Two types of calcium response limited to single spines in cerebellar Purkinje cells. *Proceedings of the National Academy of Sciences of the United States of America* **92**:8279–8282.
- Denk W, Yuste R, Svoboda K, Tank DW (1996) Imaging calcium dynamics in dendritic spines. *Current Opinion in Neurobiology* **6**:372–378.

- Dunaevsky A, Tashiro A, Majewska A, Mason C, Yuste R (1999) Developmental regulation of spine motility in the mammalian central nervous system. *Proceedings of the National Academy of Sciences of the United States of America* **96**:13438–13443.
- Eccles JC, Ito M, Szentagothai J (1967) *The Cerebellum as a Neuronal Machine*. New York: Springer.
- Eckert R, Chad JE (1984) Inactivation of Ca channels. *Progress in Biophysics and Molecular Biology* **44**:215–267.
- Egger V, Svoboda K, Mainen ZF (2003) Mechanisms of lateral inhibition in the olfactory bulb: efficiency and modulation of spike-evoked calcium influx into granule cells. *Journal of Neuroscience* **23**:7551–7558.
- Egger V, Svoboda K, Mainen ZF (2005) Dendrodendritic synaptic signals in olfactory bulb granule cells: local spine boost and global low-threshold spike. *Journal of Neuroscience* **25**:3521–3530.
- Eilers J, Augustine GJ, Konnerth A (1995) Subthreshold synaptic Ca^{2+} signalling in fine dendrites and spines of cerebellar Purkinje neurons. *Nature* **373**:155–158.
- Emptage N, Bliss TV, Fine A (1999) Single synaptic events evoke NMDA receptor-mediated release of calcium from internal stores in hippocampal dendritic spines. *Neuron* **22**:115–124.
- Engert F, Bonhoeffer T (1999) Dendritic spine changes associated with hippocampal long-term synaptic plasticity. *Nature* **399**:66–70.
- Faber ES, Delaney AJ, Sah P (2005) SK channels regulate excitatory synaptic transmission and plasticity in the lateral amygdala. *Nature Neuroscience* **8**:635–641.
- Farrant M, Cull-Candy SG (1991) Excitatory amino acid receptor-channels in Purkinje cells in thin cerebellar slices. *Proceedings of the Royal Society B: Biological Sciences* **244**:179–184.
- Feldman DE (2000) Timing-based LTP and LTD at vertical inputs to layer II/III pyramidal cells in rat barrel cortex. *Neuron* **27**:45–56.
- Fierro L, Llano I (1996) High endogenous calcium buffering in Purkinje cells from rat cerebellar slices. *Journal of Physiology* **496**:617–625.
- Fifkova E, Delay RJ (1982) Cytoplasmic actin in neuronal processes as a possible mediator of synaptic plasticity. *Journal of Cell Biology* **95**:345–350.
- Fifkova E, Eason H, Schaner P (1992) Inhibitory contacts on dendritic spines of the dentate fascia. *Brain Research* **577**:331–336.
- Finch EA, Augustine GJ (1998) Local calcium signalling by inositol-1,4,5-trisphosphate in Purkinje cell dendrites. *Nature* **396**:753–756.
- Fischer M, Kaech S, Knutti D, Matus A (1998) Rapid actin-based plasticity in dendritic spines. *Neuron* **20**:847–854.
- Fischer M, Kaech S, Wagner U, Brinkhaus H, Matus A (2000) Glutamate receptors regulate actin-based plasticity in dendritic spines. *Nature Neuroscience* **3**:887–894.
- Forti L, Bossi M, Bergamaschi A, Villa A, Malgaroli A (1997) Loose-patch recordings of single quanta at individual hippocampal synapses. *Nature* **388**:874–878.
- Frenguelli BG, Potier B, Slater NT, Alford S, Collingridge GL (1993) Metabotropic glutamate receptors and calcium signalling in dendrites of hippocampal CA1 neurones. *Neuropharmacology* **32**:1229–1237.
- Froemke RC, Dan Y (2002) Spike-timing-dependent synaptic modification induced by natural spike trains. *Nature* **416**:433–438.
- Gamble E, Koch C (1987) The dynamics of free calcium in dendritic spines in response to repetitive synaptic input. *Science* **236**:1311–1315.
- Gasparini S, Losonczy A, Chen X, Johnston D, Magee JC (2007) Associative pairing enhances action potential back-propagation in radial oblique branches of CA1 pyramidal neurons. *Journal of Physiology* **580**:787–800.
- Giessel AJ, Sabatini BL (2010) M1 muscarinic receptors boost synaptic potentials and calcium influx in dendritic spines by inhibiting postsynaptic SK channels. *Neuron* **68**:936–947.
- Giessel AJ, Sabatini BL (2011) Boosting of synaptic potentials and spine Ca transients by the peptide toxin SNX-482 requires alpha-1E-encoded voltage-gated Ca channels. *PLoS ONE* **6**:e20939.

- Gray EG (1959) Electron microscopy of synaptic contacts on dendrite spines of the cerebral cortex. *Nature* **183**:1592–1593.
- Grunditz Å, Holbro N, Tian L, Zuo Y, Oertner TG (2008) Spine neck plasticity controls postsynaptic calcium signals through electrical compartmentalization. *Journal of Neuroscience* **28**:13457–13466.
- Guthrie PB, Segal M, Kater SB (1991) Independent regulation of calcium revealed by imaging dendritic spines. *Nature* **354**:76–80.
- Halasy K, Somogyi P (1993) Distribution of GABAergic synapses and their targets in the dentate gyrus of rat: a quantitative immunoelectron microscopic analysis. *Journal für Hirnforschung* **34**:299–308.
- Halpain S, Hipolito A, Saffer L (1998) Regulation of F-actin stability in dendritic spines by glutamate receptors and calcineurin. *Journal of Neuroscience* **18**:9835–9844.
- Harnett MT, Makara JK, Spruston N, Kath WL, Magee JC (2012) Synaptic amplification by dendritic spines enhances input cooperativity. *Nature* **491**:599–602.
- Harris KM, Kater SB (1994) Dendritic spines: cellular specializations imparting both stability and flexibility to synaptic function. *Annual Review of Neuroscience* **17**:341–371.
- Harris KM, Stevens JK (1988) Dendritic spines of rat cerebellar Purkinje cells: serial electron microscopy with reference to their biophysical characteristics. *Journal of Neuroscience* **8**:4455–4469.
- Harris KM, Stevens JK (1989) Dendritic spines of CA 1 pyramidal cells in the rat hippocampus: serial electron microscopy with reference to their biophysical characteristics. *Journal of Neuroscience* **9**:2982–2997.
- Hartell NA (1996) Strong activation of parallel fibers produces localized calcium transients and a form of LTD that spreads to distant synapses. *Neuron* **16**:601–610.
- Hayama T, Noguchi J, Watanabe S, Takahashi N, Hayashi-Takagi A, Ellis-Davies GCR, Matsuzaki M, Kasai H (2013) GABA promotes the competitive selection of dendritic spines by controlling local Ca^{2+} signaling. *Nature Neuroscience* **16**:1409–1416.
- He S, Wang Y-X, Petralia RS, Brenowitz SD (2014) Cholinergic modulation of large-conductance calcium-activated potassium channels regulates synaptic strength and spine calcium in cartwheel cells of the dorsal cochlear nucleus. *Journal of Neuroscience* **34**:5261–5272.
- Helmchen F, Imoto K, Sakmann B (1996) Ca^{2+} buffering and action potential-evoked Ca^{2+} signaling in dendrites of pyramidal neurons. *Biophysical Journal* **70**:1069–1081.
- Helmchen F, Svoboda K, Denk W, Tank DW (1999) In vivo dendritic calcium dynamics in deep-layer cortical pyramidal neurons. *Nature Neuroscience* **2**:989–996.
- Higley MJ, Sabatini BL (2010) Competitive regulation of synaptic Ca^{2+} influx by D2 dopamine and A2A adenosine receptors. *Nature Neuroscience* **13**:958–966.
- Hille B (2001) *Ion Channels of Excitable Membranes*, 3rd edn. Sunderland, MA: Sinauer Associates.
- Hoffman DA, Magee JC, Colbert CM, Johnston D (1997) K^+ channel regulation of signal propagation in dendrites of hippocampal pyramidal neurons. *Nature* **387**:869–875.
- Hollmann M, Hartley M, Heinemann S (1991) Ca^{2+} permeability of KA-AMPA-gated glutamate receptor channels depends on subunit composition. *Science* **252**:851–853.
- Holmes WR (1990) Is the function of dendritic spines to concentrate calcium? *Brain Research* **519**:338–342.
- Holthoff K, Tsay D, Yuste R (2002) Calcium dynamics of spines depend on their dendritic location. *Neuron* **33**:425–437.
- Hoogland TM, Saggau P (2004) Facilitation of L-type Ca^{2+} channels in dendritic spines by activation of beta2 adrenergic receptors. *Journal of Neuroscience* **24**:8416–8427.
- Hume RI, Dingledine R, Heinemann SF (1991) Identification of a site in glutamate receptor subunits that controls calcium permeability. *Science* **253**:1028–1031.
- Humeau Y, Herry C, Kemp N, Shaban H, Fourcaudot E, Bissiere S, Luthi A (2005) Dendritic spine heterogeneity determines afferent-specific Hebbian plasticity in the amygdala. *Neuron* **45**:119–131.

- Husi H, Ward MA, Choudhary JS, Blackstock WP, Grant SG (2000) Proteomic analysis of NMDA receptor-adhesion protein signaling complexes. *Nature Neuroscience* **3**:661–669.
- Isaacson JS, Scanziani M (2011) How inhibition shapes cortical activity. *Neuron* **72**:231–243.
- Ito M, Kano M (1982) Long-lasting depression of parallel fiber-Purkinje cell transmission induced by conjunctive stimulation of parallel fibers and climbing fibers in the cerebellar cortex. *Neuroscience Letters* **33**:253–258.
- Jaffe DB, Johnston D, Lasser-Ross N, Lisman JE, Miyakawa H, Ross WN (1992) The spread of Na^+ spikes determines the pattern of dendritic Ca^{2+} entry into hippocampal neurons. *Nature* **357**:244–246.
- Jaffe DB, Fisher SA, Brown TH (1994) Confocal laser scanning microscopy reveals voltage-gated calcium signals within hippocampal dendritic spines. *Journal of Neurobiology* **25**:220–233.
- Jafri MS, Keizer J (1995) On the roles of Ca^{2+} diffusion, Ca^{2+} buffers, and the endoplasmic reticulum in IP3-induced Ca^{2+} waves. *Biophysical Journal* **69**:2139–2153.
- Jahr CE, Stevens CF (1990a) A quantitative description of NMDA receptor-channel kinetic behavior. *Journal of Neuroscience* **10**:1830–1837.
- Jahr CE, Stevens CF (1990b) Voltage dependence of NMDA-activated macroscopic conductances predicted by single-channel kinetics. *Journal of Neuroscience* **10**:3178–3182.
- Jonas P, Burnashev N (1995) Molecular mechanisms controlling calcium entry through AMPA-type glutamate receptor channels. *Neuron* **15**:987–990.
- Jones SL, Stuart GJ (2013) Different calcium sources control somatic versus dendritic SK channel activation during action potentials. *Journal of Neuroscience* **33**:19396–19405.
- Kampa BM, Clements J, Jonas P, Stuart GJ (2004) Kinetics of Mg^{2+} unblock of NMDA receptors: implications for spike-timing dependent synaptic plasticity. *Journal of Physiology* **556**:337–345.
- Kanemoto Y, Matsuzaki M, Morita S, Hayama T, Noguchi J, Senda N, Momotake A, Arai T, Kasai H (2011) Spatial distributions of GABA receptors and local inhibition of Ca^{2+} transients studied with GABA uncaging in the dendrites of CA1 pyramidal neurons. *PLoS ONE* **6**:e22652.
- Kantevari S, Matsuzaki M, Kanemoto Y, Kasai H, Ellis-Davies GCR (2010) Two-color, two-photon uncaging of glutamate and GABA. *Nature Methods* **7**:123–125.
- Kennedy MB (2000) Signal-processing machines at the postsynaptic density. *Science* **290**:750–754.
- Khodakhah K, Armstrong CM (1997) Induction of long-term depression and rebound potentiation by inositol trisphosphate in cerebellar Purkinje neurons. *Proceedings of the National Academy of Sciences of the United States of America* **94**:14009–14014.
- Khodakhah K, Ogden D (1993) Functional heterogeneity of calcium release by inositol trisphosphate in single Purkinje neurones, cultured cerebellar astrocytes, and peripheral tissues. *Proceedings of the National Academy of Sciences of the United States of America* **90**:4976–4980.
- Knopfel T, Vranešic I, Staub C, Gähwiler BH (1991) Climbing fibre responses in olivo-cerebellar slice cultures. II. Dynamics of cytosolic calcium in Purkinje cells. *European Journal of Neuroscience* **3**:343–348.
- Koch C, Poggio T (1983) A theoretical analysis of electrical properties of spines. *Proceedings of the Royal Society of London Series B: Biological Sciences* **218**:455–477.
- Koch C, Zador A (1993) The function of dendritic spines: devices subserving biochemical rather than electrical compartmentalization. *Journal of Neuroscience* **13**:413–422.
- Koester HJ, Sakmann B (1998) Calcium dynamics in single spines during coincident pre- and postsynaptic activity depend on relative timing of back-propagating action potentials and subthreshold excitatory postsynaptic potentials. *Proceedings of the National Academy of Sciences of the United States of America* **95**:9596–9601.
- Konnerth A, Dreessen J, Augustine GJ (1992) Brief dendritic calcium signals initiate long-lasting synaptic depression in cerebellar Purkinje cells. *Proceedings of the National Academy of Sciences of the United States of America* **89**:7051–7055.

- Korkotian E, Segal M (1999) Release of calcium from stores alters the morphology of dendritic spines in cultured hippocampal neurons. *Proceedings of the National Academy of Sciences of the United States of America* **96**:12068–12072.
- Korkotian E, Segal M (2001) Spike-associated fast contraction of dendritic spines in cultured hippocampal neurons. *Neuron* **30**:751–758.
- Korkotian E, Holcman D, Segal M (2004) Dynamic regulation of spine-dendrite coupling in cultured hippocampal neurons. *European Journal of Neuroscience* **20**:2649–2663.
- Kovalchuk Y, Eilers J, Lisman J, Konnerth A (2000) NMDA receptor-mediated subthreshold Ca^{2+} signals in spines of hippocampal neurons. *Journal of Neuroscience* **20**:1791–1799.
- Larkum ME, Kaiser KM, Sakmann B (1999) Calcium electrogenesis in distal apical dendrites of layer 5 pyramidal cells at a critical frequency of back-propagating action potentials. *Proceedings of the National Academy of Sciences of the United States of America* **96**:14600–14604.
- Lee SH, Rosenmund C, Schwaller B, Neher E (2000) Differences in Ca^{2+} buffering properties between excitatory and inhibitory hippocampal neurons from the rat. *Journal of Physiology* **525**:405–418.
- Legendre P, Rosenmund C, Westbrook GL (1993) Inactivation of NMDA channels in cultured hippocampal neurons by intracellular calcium. *Journal of Neuroscience* **13**:674–684.
- Lester RA, Clements JD, Westbrook GL, Jahr CE (1990) Channel kinetics determine the time course of NMDA receptor-mediated synaptic currents. *Nature* **346**:565–567.
- Linden DJ, Connor JA (1995) Long-term synaptic depression. *Annual Review of Neuroscience* **18**:319–357.
- Lisman J (1989) A mechanism for the Hebb and the anti-Hebb processes underlying learning and memory. *Proceedings of the National Academy of Sciences of the United States of America* **86**:9574–9578.
- Little JP, Carter AG (2012) Subcellular synaptic connectivity of layer 2 pyramidal neurons in the medial prefrontal cortex. *Journal of Neuroscience* **32**:12808–12819.
- Little JP, Carter AG (2013) Synaptic mechanisms underlying strong reciprocal connectivity between the medial prefrontal cortex and basolateral amygdala. *Journal of Neuroscience* **33**:15333–15342.
- Llano I, Dreessen J, Kano M, Konnerth A (1991a) Intradendritic release of calcium induced by glutamate in cerebellar Purkinje cells. *Neuron* **7**:577–583.
- Llano I, Marty A, Armstrong CM, Konnerth A (1991b) Synaptic- and agonist-induced excitatory currents of Purkinje cells in rat cerebellar slices. *Journal of Physiology* **434**:183–213.
- Losonczy A, Magee JC (2006) Integrative properties of radial oblique dendrites in hippocampal CA1 pyramidal neurons. *Neuron* **50**:291–307.
- Lynch G, Larson J, Kelso S, Barrientos G, Schottler F (1983) Intracellular injections of EGTA block induction of hippocampal long-term potentiation. *Nature* **305**:719–721.
- MacAskill AF, Little JP, Cassel JM, Carter AG (2012) Subcellular connectivity underlies pathway-specific signaling in the nucleus accumbens. *Nature Neuroscience* **15**:1624–1626.
- MacAskill AF, Cassel JM, Carter AG (2014) Cocaine exposure reorganizes cell type—and input-specific connectivity in the nucleus accumbens. *Nature Neuroscience* **17**:1198–1207.
- Maeda H, Ellis-Davies GC, Ito K, Miyashita Y, Kasai H (1999) Supralinear Ca^{2+} signaling by cooperative and mobile Ca^{2+} buffering in Purkinje neurons. *Neuron* **24**:989–1002.
- Magee JC, Johnston D (1997) A synaptically controlled, associative signal for Hebbian plasticity in hippocampal neurons. *Science* **275**:209–213.
- Mainen ZF, Malinow R, Svoboda K (1999) Synaptic calcium transients in single spines indicate that NMDA receptors are not saturated. *Nature* **399**:151–155.
- Majewska A, Brown E, Ross J, Yuste R (2000a) Mechanisms of calcium decay kinetics in hippocampal spines: role of spine calcium pumps and calcium diffusion through the spine neck in biochemical compartmentalization. *Journal of Neuroscience* **20**:1722–1734.
- Majewska A, Tashiro A, Yuste R (2000b) Regulation of spine calcium dynamics by rapid spine motility. *Journal of Neuroscience* **20**:8262–8268.

- Maletic-Savatic M, Malinow R, Svoboda K** (1999) Rapid dendritic morphogenesis in CA1 hippocampal dendrites induced by synaptic activity. *Science* **283**:1923–1927.
- Maravall M, Mainen ZF, Sabatini BL, Svoboda K** (2000) Estimating intracellular calcium concentrations and buffering without wavelength ratioing. *Biophysical Journal* **78**:2655–2667.
- Markram H, Sakmann B** (1994) Calcium transients in dendrites of neocortical neurons evoked by single subthreshold excitatory postsynaptic potentials via low-voltage-activated calcium channels. *Proceedings of the National Academy of Sciences of the United States of America* **91**:5207–5211.
- Markram H, Helm PJ, Sakmann B** (1995) Dendritic calcium transients evoked by single back-propagating action potentials in rat neocortical pyramidal neurons. *Journal of Physiology* **485**:1–20.
- Markram H, Lubke J, Frotscher M, Sakmann B** (1997) Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. *Science* **275**:213–215.
- Markram H, Toledo-Rodriguez M, Wang Y, Gupta A, Silberberg G, Wu C** (2004) Interneurons of the neocortical inhibitory system. *Nature Reviews Neuroscience* **5**:793–807.
- Marlin JJ, Carter AG** (2014) GABA-A receptor inhibition of local calcium signaling in spines and dendrites. *Journal of Neuroscience* **34**:15898–15911.
- Matsuzaki M, Ellis-Davies GC, Nemoto T, Miyashita Y, Iino M, Kasai H** (2001) Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons. *Nature Neuroscience* **4**:1086–1092.
- Matsuzaki M, Honkura N, Ellis-Davies GC, Kasai H** (2004) Structural basis of long-term potentiation in single dendritic spines. *Nature* **429**:761–766.
- Matus A, Ackermann M, Pehling G, Byers HR, Fujiwara K** (1982) High actin concentrations in brain dendritic spines and postsynaptic densities. *Proceedings of the National Academy of Sciences of the United States of America* **79**:7590–7594.
- Mayer ML, Westbrook GL, Guthrie PB** (1984) Voltage-dependent block by Mg²⁺ of NMDA responses in spinal cord neurones. *Nature* **309**:261–263.
- Miller KK, Verma A, Snyder SH, Ross CA** (1991) Localization of an endoplasmic reticulum calcium ATPase mRNA in rat brain by *in situ* hybridization. *Neuroscience* **43**:1–9.
- Miyakawa H, Lev-Ram V, Lasser-Ross N, Ross WN** (1992) Calcium transients evoked by climbing fiber and parallel fiber synaptic inputs in guinea pig cerebellar Purkinje neurons. *Journal of Neurophysiology* **68**:1178–1189.
- Miyata M, Finch EA, Khirog L, Hashimoto K, Hayasaka S, Oda SI, Inouye M, Takagishi Y, Augustine GJ, Kano M** (2000) Local calcium release in dendritic spines required for long-term synaptic depression. *Neuron* **28**:233–244.
- Miyawaki A, Llopis J, Heim R, McCaffery JM, Adams JA, Ikura M, Tsien RY** (1997) Fluorescent indicators for Ca²⁺ based on green fluorescent proteins and calmodulin. *Nature* **388**:882–887.
- Monyer H, Burnashev N, Laurie DJ, Sakmann B, Seeburg PH** (1994) Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. *Neuron* **12**:529–540.
- Muller W, Connor JA** (1991) Dendritic spines as individual neuronal compartments for synaptic Ca²⁺ responses. *Nature* **354**:73–76.
- Murakoshi H, Wang H, Yasuda R** (2011) Local, persistent activation of Rho GTPases during plasticity of single dendritic spines. *Nature* **472**:100–104.
- Murphy TH, Baraban JM, Wier WG, Blatter LA** (1994) Visualization of quantal synaptic transmission by dendritic calcium imaging. *Science* **263**:529–532.
- Murphy TH, Baraban JM, Wier WG** (1995) Mapping miniature synaptic currents to single synapses using calcium imaging reveals heterogeneity in postsynaptic output. *Neuron* **15**:159–168.
- Murphy JA, et al.** (2014) Phosphorylation of Ser1166 on GluN2B by PKA is critical to synaptic NMDA receptor function and Ca²⁺ signaling in spines. *Journal of Neuroscience* **34**:869–879.

- Murthy VN, Sejnowski TJ, Stevens CF (2000) Dynamics of dendritic calcium transients evoked by quantal release at excitatory hippocampal synapses. *Proceedings of the National Academy of Sciences of the United States of America* **97**:901–906.
- Nagai T, Sawano A, Park ES, Miyawaki A (2001) Circularly permuted green fluorescent proteins engineered to sense Ca^{2+} . *Proceedings of the National Academy of Sciences of the United States of America* **98**:3197–3202.
- Neher E (1995) The use of fura-2 for estimating Ca buffers and Ca fluxes. *Neuropharmacology* **34**:1423–1442.
- Neher E (1998) Usefulness and limitations of linear approximations to the understanding of Ca^{2+} signals. *Cell Calcium* **24**:345–357.
- Neher E, Augustine GJ (1992) Calcium gradients and buffers in bovine chromaffin cells. *Journal of Physiology* **450**:273–301.
- Nevian T, Sakmann B (2004) Single spine Ca^{2+} signals evoked by coincident EPSPs and backpropagating action potentials in spiny stellate cells of layer 4 in the juvenile rat somatosensory barrel cortex. *Journal of Neuroscience* **24**:1689–1699.
- Ngo-Anh TJ, Bloodgood BL, Lin M, Sabatini BL, Maylie J, Adelman JP (2005) SK channels and NMDA receptors form a $\text{Ca}(2+)$ -mediated feedback loop in dendritic spines. *Nature Neuroscience* **8**:642–649.
- Nimchinsky EA, Yasuda R, Oertner TG, Svoboda K (2004) The number of glutamate receptors opened by synaptic stimulation in single hippocampal spines. *Journal of Neuroscience* **24**:2054–2064.
- Noguchi J, Matsuzaki M, Ellis-Davies GCR, Kasai H (2005) Spine-neck geometry determines NMDA receptor-dependent Ca^{2+} signaling in dendrites. *Neuron* **46**:609–622.
- Nowak L, Bregestovski P, Ascher P, Herbet A, Prochiantz A (1984) Magnesium gates glutamate-activated channels in mouse central neurones. *Nature* **307**:462–465.
- Ogoshi F, Weiss JH (2003) Heterogeneity of Ca^{2+} -permeable AMPA/kainate channel expression in hippocampal pyramidal neurons: fluorescence imaging and immunocytochemical assessment. *Journal of Neuroscience* **23**:10521–10530.
- Palay SL (1956) Synapses in the central nervous system. *Journal of Biophysical and Biochemical Cytology* **2**:193–202.
- Palmer LM, Stuart GJ (2009) Membrane potential changes in dendritic spines during action potentials and synaptic input. *Journal of Neuroscience* **29**:6897–6903.
- Pérez-Garcí E, Gassmann M, Bettler B, Larkum ME (2006) The GABAB1b isoform mediates long-lasting inhibition of dendritic Ca^{2+} spikes in layer 5 somatosensory pyramidal neurons. *Neuron* **50**:603–616.
- Pérez-Garcí E, Larkum ME, Nevian T (2012) Inhibition of dendritic Ca^{2+} spikes by GABAB receptors in cortical pyramidal neurons is mediated by a direct $\text{Gi}/\text{o}-\beta\gamma$ subunit interaction with Cav1 channels. *Journal of Physiology* **591**:1599–1612.
- Perkel DH, Perkel DJ (1985) Dendritic spines: role of active membrane in modulating synaptic efficacy. *Brain Research* **325**:331–335.
- Perkel DJ, Hestrin S, Sah P, Nicoll RA (1990) Excitatory synaptic currents in Purkinje cells. *Proceedings of the Royal Society B: Biological Sciences* **241**:116–121.
- Peters A, Kaiserman-Abramof IR (1970) The small pyramidal neuron of the rat cerebral cortex. The perikaryon, dendrites and spines. *American Journal of Anatomy* **127**:321–355.
- Peters A, Palay SL, Webster HD (1991) Dendrites. In: *The Fine Structure of the Nervous System: Neurons and Their Supporting Cells*, pp. 70–100. New York: Oxford University Press.
- Petrozzino JJ, Pozzo Miller LD, Connor JA (1995) Micromolar Ca^{2+} transients in dendritic spines of hippocampal pyramidal neurons in brain slice. *Neuron* **14**:1223–1231.
- Rall W (1970) Cable properties of dendrites and effects of synaptic location. In: *Excitatory Synaptic Mechanisms* (Andersen P, Jansen JKS, eds), pp. 175–187. Oslo: Universitetsforlaget.
- Regehr WG, Tank DW (1990) Postsynaptic NMDA receptor-mediated calcium accumulation in hippocampal CA1 pyramidal cell dendrites. *Nature* **345**:807–810.

- Regehr WG, Connor JA, Tank DW (1989) Optical imaging of calcium accumulation in hippocampal pyramidal cells during synaptic activation. *Nature* **341**:533–536.
- Richardson RJ, Blundon JA, Bayazitov IT, Zakharenko SS (2009) Connectivity patterns revealed by mapping of active inputs on dendrites of thalamorecipient neurons in the auditory cortex. *Journal of Neuroscience* **29**:6406–6417.
- Ross WN, Werman R (1987) Mapping calcium transients in the dendrites of Purkinje cells from the guinea-pig cerebellum in vitro. *Journal of Physiology* **389**:319–336.
- Rudy B, Fishell G, Lee S, Hjerling-Leffler J (2011) Three groups of interneurons account for nearly 100% of neocortical GABAergic neurons. *Developmental Neurobiology* **71**:45–61.
- Sabatini BL, Svoboda K (2000) Analysis of calcium channels in single spines using optical fluctuation analysis. *Nature* **408**:589–593.
- Sabatini BL, Oertner TG, Svoboda K (2002) The life cycle of Ca^{2+} ions in dendritic spines. *Neuron* **33**:439–452.
- Sakurai M (1990) Calcium is an intracellular mediator of the climbing fiber in induction of cerebellar long-term depression. *Proceedings of the National Academy of Sciences of the United States of America* **87**:3383–3385.
- Satoh T, Ross CA, Villa A, Supattapone S, Pozzan T, Snyder SH, Meldolesi J (1990) The inositol 1,4,5-trisphosphate receptor in cerebellar Purkinje cells: quantitative immunogold labeling reveals concentration in an ER subcompartment. *Journal of Cell Biology* **111**:615–624.
- Scheuss V, Yasuda R, Sobczyk A, Svoboda K (2006) Nonlinear $[\text{Ca}^{2+}]$ signaling in dendrites and spines caused by activity-dependent depression of Ca^{2+} extrusion. *Journal of Neuroscience* **26**:8183–8194.
- Schiller J, Helmchen F, Sakmann B (1995) Spatial profile of dendritic calcium transients evoked by action potentials in rat neocortical pyramidal neurones. *Journal of Physiology* **487**:583–600.
- Schiller J, Schiller Y, Stuart G, Sakmann B (1997) Calcium action potentials restricted to distal apical dendrites of rat neocortical pyramidal neurons. *Journal of Physiology* **505**:605–616.
- Schiller J, Schiller Y, Clapham DE (1998) NMDA receptors amplify calcium influx into dendritic spines during associative pre- and postsynaptic activation. *Nature Neuroscience* **1**:114–118.
- Schmidt H, Stiefel KM, Racay P, Schwaller B, Eilers J (2003) Mutational analysis of dendritic Ca^{2+} kinetics in rodent Purkinje cells: role of parvalbumin and calbindin D28k. *Journal of Physiology* **551**:13–32.
- Schneggenburger R, Zhou Z, Konnerth A, Neher E (1993) Fractional contribution of calcium to the cation current through glutamate receptor channels. *Neuron* **11**:133–143.
- Segal M (1995) Imaging of calcium variations in living dendritic spines of cultured rat hippocampal neurons. *Journal of Physiology* **486**:283–295.
- Seong HJ, Behnia R, Carter AG (2014) The impact of subthreshold membrane potential on synaptic responses at dendritic spines of layer 5 pyramidal neurons in the prefrontal cortex. *Journal of Neurophysiology* **111**:1960–1972.
- Sharp AH, McPherson PS, Dawson TM, Aoki C, Campbell KP, Snyder SH (1993) Differential immunohistochemical localization of inositol 1,4,5-trisphosphate- and ryanodine-sensitive Ca^{2+} release channels in rat brain. *Journal of Neuroscience* **13**:3051–3063.
- Skeberdis VA, Chevaleyre V, Lau CG, Goldberg JH, Pettit DL, Suadicani SO, Lin Y, Bennett MVL, Yuste R, Castillo PE, Zukin RS (2006) Protein kinase A regulates calcium permeability of NMDA receptors. *Nature Neuroscience* **9**:501–510.
- Smith MA, Ellis-Davies GC, Magee JC (2003) Mechanism of the distance-dependent scaling of Schaffer collateral synapses in rat CA1 pyramidal neurons. *Journal of Physiology* **548**:245–258.
- Sobczyk A, Svoboda K (2007) Activity-dependent plasticity of the NMDA-receptor fractional Ca^{2+} current. *Neuron* **53**:17–24.
- Sobczyk A, Scheuss V, Svoboda K (2005) NMDA receptor subunit-dependent $[\text{Ca}^{2+}]$ signaling in individual hippocampal dendritic spines. *Journal of Neuroscience* **25**:6037–6046.

- Spacek J, Harris KM (1997) Three-dimensional organization of smooth endoplasmic reticulum in hippocampal CA1 dendrites and dendritic spines of the immature and mature rat. *Journal of Neuroscience* **17**:190–203.
- Spruston N, Schiller Y, Stuart G, Sakmann B (1995) Activity-dependent action potential invasion and calcium influx into hippocampal CA1 dendrites. *Science* **268**:297–300.
- Star EN, Kwiatkowski DJ, Murthy VN (2002) Rapid turnover of actin in dendritic spines and its regulation by activity. *Nature Neuroscience* **5**:239–246.
- Stauffer TP, Guerini D, Carafoli E (1995) Tissue distribution of the four gene products of the plasma membrane Ca^{2+} pump. A study using specific antibodies. *Journal of Biological Chemistry* **270**:12184–12190.
- Stuart GJ, Sakmann B (1994) Active propagation of somatic action potentials into neocortical pyramidal cell dendrites. *Nature* **367**:69–72.
- Svoboda K, Tank DW, Denk W (1996) Direct measurement of coupling between dendritic spines and shafts. *Science* **272**:716–719.
- Svoboda K, Denk W, Kleinfeld D, Tank DW (1997) In vivo dendritic calcium dynamics in neocortical pyramidal neurons. *Nature* **385**:161–165.
- Svoboda K, Helmchen F, Denk W, Tank DW (1999) Spread of dendritic excitation in layer 2/3 pyramidal neurons in rat barrel cortex in vivo. *Nature Neuroscience* **2**:65–73.
- Takechi H, Eilers J, Konnerth A (1998) A new class of synaptic response involving calcium release in dendritic spines. *Nature* **396**:757–760.
- Tanaka J, Matsuzaki M, Tarusawa E, Momiyama A, Molnar E, Kasai H, Shigemoto R (2005) Number and density of AMPA receptors in single synapses in immature cerebellum. *Journal of Neuroscience* **25**:799–807.
- Tank DW, Regehr WG, Delaney KR (1995) A quantitative analysis of presynaptic calcium dynamics that contribute to short-term enhancement. *Journal of Neuroscience* **15**:7940–7952.
- Tian L, et al. (2009) Imaging neural activity in worms, flies and mice with improved GCaMP calcium indicators. *Nature Methods* **6**:875–881.
- Toni N, Buchs PA, Nikonenko I, Bron CR, Muller D (1999) LTP promotes formation of multiple spine synapses between a single axon terminal and a dendrite. *Nature* **402**:421–425.
- Tønnesen J, Katona G, Rozsa B, Nagerl UV (2014) Spine neck plasticity regulates compartmentalization of synapses. *Nature Neuroscience* **17**:678–685.
- Tsay D, Yuste R (2004) On the electrical function of dendritic spines. *Trends in Neurosciences* **27**:77–83.
- Tsien RY (1989) Fluorescent probes of cell signaling. *Annual Review of Neuroscience* **12**:227–253.
- Vanderklish PW, Edelman GM (2002) Dendritic spines elongate after stimulation of group 1 metabotropic glutamate receptors in cultured hippocampal neurons. *Proceedings of the National Academy of Sciences of the United States of America* **99**:1639–1644.
- Vargas-Caballero M, Robinson HP (2003) A slow fraction of Mg^{2+} unblock of NMDA receptors limits their contribution to spike generation in cortical pyramidal neurons. *Journal of Neurophysiology* **89**:2778–2783.
- Wagner J, Keizer J (1994) Effects of rapid buffers on Ca^{2+} diffusion and Ca^{2+} oscillations. *Biophysical Journal* **67**:447–456.
- Walikonis RS, Jensen ON, Mann M, Provance DW Jr., Mercer JA, Kennedy MB (2000) Identification of proteins in the postsynaptic density fraction by mass spectrometry. *Journal of Neuroscience* **20**:4069–4080.
- Walton PD, Airey JA, Sutko JL, Beck CF, Mignery GA, Sudhof TC, Deerinck TJ, Ellisman MH (1991) Ryanodine and inositol trisphosphate receptors coexist in avian cerebellar Purkinje neurons. *Journal of Cell Biology* **113**:1145–1157.
- Wang SS, Denk W, Häusser M (2000) Coincidence detection in single dendritic spines mediated by calcium release. *Nature Neuroscience* **3**:1266–1273.

- Wang S, Jia Z, Roder J, Murphy TH (2002) AMPA receptor-mediated miniature synaptic calcium transients in GluR2 null mice. *Journal of Neurophysiology* **88**:29–40.
- Waters J, Helmchen F (2004) Boosting of action potential backpropagation by neocortical network activity in vivo. *Journal of Neuroscience* **24**:11127–11136.
- Waters J, Larkum M, Sakmann B, Helmchen F (2003) Supralinear Ca^{2+} influx into dendritic tufts of layer 2/3 neocortical pyramidal neurons in vitro and in vivo. *Journal of Neuroscience* **23**:8558–8567.
- West AE, Griffith EC, Greenberg ME (2002) Regulation of transcription factors by neuronal activity. *Nature Reviews Neuroscience* **3**:921–931.
- Wickens J (1988) Electrically coupled but chemically isolated synapses: dendritic spines and calcium in a rule for synaptic modification. *Progress in Neurobiology* **31**:507–528.
- Wigström H, Swann JW, Andersen P (1979) Calcium dependency of synaptic long-lasting potentiation in the hippocampal slice. *Acta Physiologica Scandavica* **105**:126–128.
- Wigström H, Gustafsson B, Huang YY, Abraham WC (1986) Hippocampal long-term potentiation is induced by pairing single afferent volleys with intracellularly injected depolarizing current pulses. *Acta Physiologica Scandavica* **126**:317–319.
- Wilson CJ (1984) Passive cable properties of dendritic spines and spiny neurons. *Journal of Neuroscience* **4**:281–297.
- Yasuda R, Sabatini BL, Svoboda K (2003) Plasticity of calcium channels in dendritic spines. *Nature Neuroscience* **6**:948–955.
- Yuste R, Denk W (1995) Dendritic spines as basic functional units of neuronal integration. *Nature* **375**:682–684.
- Yuste R, Gutnick MJ, Saar D, Delaney KR, Tank DW (1994) Ca^{2+} accumulations in dendrites of neocortical pyramidal neurons: an apical band and evidence for two functional compartments. *Neuron* **13**:23–43.
- Yuste R, Majewska A, Cash SS, Denk W (1999) Mechanisms of calcium influx into hippocampal spines: heterogeneity among spines, coincidence detection by NMDA receptors, and optical quantal analysis. *Journal of Neuroscience* **19**:1976–1987.
- Zhang Y-P, Oertner TG (2007) Optical induction of synaptic plasticity using a light-sensitive channel. *Nature Methods* **4**:139–141.
- Zhang LI, Tao HW, Holt CE, Harris WA, Poo M (1998) A critical window for cooperation and competition among developing retinotectal synapses. *Nature* **395**:37–44.
- Zhou Z, Neher E (1993) Mobile and immobile calcium buffers in bovine adrenal chromaffin cells. *Journal of Physiology* **469**:245–273.
- Zucker RS (1999) Calcium- and activity-dependent synaptic plasticity. *Current Opinion in Neurobiology* **9**:305–313.

Chapter 12

Principles of dendritic integration

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Summary

The primary role of neurons is to integrate incoming information conveyed by synaptic input and convert it into an output, usually in the form of action potentials. This process is called synaptic integration. As the vast majority of synaptic input to neurons is made onto their dendrites, the morphology and membrane properties of dendrites play a critical role in this input–output transformation. In this chapter we discuss where action potentials are generated in neurons, as well as the various factors affecting how dendrites integrate synaptic potentials, highlighting the key role of dendritic excitability.

Introduction

Dendrites, as illustrated in previous chapters, are morphologically elaborate structures receiving thousands of presynaptic inputs. A quick glance at the morphology of various neurons (see Figure 1 in the Preface) reveals their dramatic structural differences and hints at their functional specialization. Indeed, the functional heterogeneity suggested by morphology is borne out by experimental analysis of different cell types. Functionally, dendrites are remarkably complex, with a wide variety of neurotransmitter receptors and voltage-activated channels distributed uniquely in different types of neurons. But what impact do these different properties have on dendritic function? And how is dendritic function enriched by the different distributions and properties of synapses and channels found in the dendrites? Significant progress toward answering these questions has been realized in the past twenty years since the development of dendritic patch-clamp and imaging methods. Here we review various aspects of dendritic function, including principles that appear to hold for the majority of neurons studied, as well as examples of functional specialization in the dendrites of neurons in the mammalian central nervous system (CNS).

The action potential is the final output signal of most neurons

Most neurons communicate via action potentials—brief, all-or-none reversals of membrane potential polarity mediated by the opening of voltage-gated Na^+ (Na_v) and K^+ (K_v) channels. Though considerable debate exists regarding the details of information processing in neurons (Shadlen and Newsome, 1994, 1995; Ferster and Spruston, 1995; Softky, 1995), the prevailing view is that in most neurons action potentials are used to produce a kind of digital code, with the state of the nervous system dictated by the rate and timing of action potentials across multiple, interconnected neural networks in the brain.¹

¹ Some axons may also use a hybrid digital–analog code, in which synaptic potentials modulate action potential-mediated release of neurotransmitter from the axon (Alle and Geiger, 2006; Shu et al., 2006).

Most cells fire action potentials only when synaptic excitation sufficiently exceeds inhibition,² allowing depolarization beyond the firing threshold. The simplest view of synaptic integration is that excitatory inputs sum, and if the resulting depolarization is large enough to reach threshold, an action potential is generated. In this simple model, inhibition opposes this depolarization, thus increasing the number of active excitatory inputs required to reach threshold. While this represents a vast over-simplification of how neurons integrate synaptic information, it serves as a useful starting point.

To influence the initiation of action potentials, postsynaptic potentials (PSPs), both excitatory (EPSPs) and inhibitory (IPSPs), must spread from their site of generation to the action potential initiation zone. This propagation is affected by dendritic morphology and the passive cable properties of dendrites, as well as the voltage-gated conductances they contain. Furthermore, even in passive dendrites, excitatory and inhibitory potentials can sum nonlinearly, in a manner determined by their spatial and temporal relationship. Finally, the process of synaptic integration is influenced by ongoing action potential firing, which can shunt synaptic potentials and change the availability of voltage-gated conductances. All of these factors influence synaptic integration in complicated ways.

Action potentials are initiated in the axon

A central issue related to synaptic integration is to determine the final site of action potential initiation. Experiments dating back to the 1950s have addressed this question. Early microelectrode recordings from spinal motoneurons revealed that action potentials consist of two components: an “initial segment spike” (IS spike) and a “somato-dendritic spike” (SD spike). The IS spike always preceded the SD spike, could be evoked in isolation by antidromic stimulation of the axon, and had a lower threshold than the SD spike (Coombs et al., 1957; Fatt, 1957; Fuortes et al., 1957). These data were interpreted to suggest that the action potential begins as a low-threshold IS spike in the axon,³ which subsequently triggers the SD spike in the soma and dendrites. This interpretation was later supported by simultaneous intracellular recording from the soma and dendrites of motoneurons *in vivo* (Terzuolo and Araki, 1961).

In the years that followed these early experiments on motoneurons, a battery of experiments were performed on other types of neurons. Though some studies offered evidence that spikes can be generated in dendrites (see the section Spikes can be generated in dendrites), a large body of evidence suggested that all-or-none events which propagate down the axon to cause transmitter release are initiated in the proximal axon of most neurons. Field potential recordings in the hippocampus indicated that action potentials are earliest and largest in the somatic and axonal fields

² There are some exceptions to this. Some neurons, like Purkinje cells in the cerebellum and dopaminergic cells in the substantia nigra, fire action potentials spontaneously, even when deprived of all synaptic inputs (Häusser et al., 2004). In such neurons, action potential firing is modulated primarily by inhibition, which reduces the firing rate and may synchronize firing across multiple neurons (Yung et al., 1991; Gao et al., 1996; Häusser and Clark, 1997). Other neurons, such as those in the supraoptic nucleus (Bourque and Renaud, 1984), fire spontaneously in rhythmic bursts of action potentials. In these neurons, synaptic inputs can modulate the timing of these rhythmic oscillations.

³ The IS spike was ascribed to the axon initial segment on the basis of its specialized structural features and proximity to the soma. It is worth noting, however, that these early studies provided no direct evidence against the possibility that the IS spike could be preceded by a spike in a more distal region of the axon, such as the first node of Ranvier.

(Jefferys, 1979; Miyakawa and Kato, 1986; Richardson et al., 1987), and comparison of somatic and dendritic microelectrode recordings suggested that the fast spikes mediated by Na_v channels are generated in the axons of hippocampal and neocortical pyramidal neurons and cerebellar Purkinje cells (Llinás and Sugimori 1980a; Benardo et al. 1982; Amitai et al. 1993).

In addition to this experimental work, theoretical studies suggest that the threshold for action potential initiation is lowest in the axon because of a 20- to 1,000-fold higher density of Na_v channels in the axon relative to that found in the soma and dendrites (Dodge and Cooley, 1973; Moore et al., 1983; Mainen et al., 1995; Rapp et al., 1996). Despite early experimental support for a high density of Na_v channels in axons (Conti et al., 1976; Sigworth, 1980; Neumcke and Stämpfli, 1982; Wollner and Catterall, 1986), initial experimental estimates of Na_v channel density in the axon initial segment (AIS) using patch-clamp recording indicated it was similar to that in the soma in neocortical and hippocampal pyramidal neurons (Colbert and Johnston, 1996; Colbert and Pan, 2002). More recent work has challenged these earlier observations, arguing that the Na_v channel density in the AIS of neocortical pyramidal neurons is about 40 times that found at the soma, but is difficult to measure accurately using cell-attached or outside-out patch-clamp recording due to tight coupling of Na_v channels to the cytoskeleton (Kole et al., 2008). Work by others in a range of neuronal cell types has confirmed that the density of Na_v channels in the AIS is significantly higher than at the soma (Hu et al., 2009; Hu and Jonas, 2014), although the magnitude of this difference is a matter of debate and likely to vary across different types of neurons (Schmidt-Hieber et al., 2008; Fleidervish et al., 2010). In addition to a higher Na_v channel density, other factors also contribute to a low threshold for action potential initiation in the axon, including the low capacitance of small-diameter axons (Moore et al., 1983; Mainen et al., 1995; Baranauskas et al., 2013) and the hyperpolarized activation and inactivation voltage dependence of axonal Na_v channels compared with somatic Na_v channels (Rapp et al., 1996; Colbert and Pan, 2002; Kole et al., 2008).

The most direct evidence that action potentials are generated in the axon comes from simultaneous somatic and axonal patch-pipette recordings, as well as optical imaging using voltage-sensitive dyes, which have demonstrated that the action potential occurs first in the axon and later in the soma in a number of types of neurons (Stuart and Häusser, 1994; Stuart and Sakmann, 1994; Colbert and Johnston, 1996; Stuart et al., 1997a; Clark et al., 2005; Palmer and Stuart, 2006; Kole et al., 2007; Shu et al., 2007; Schmidt-Hieber et al., 2008; Palmer et al., 2010; Hu and Jonas, 2014), directly confirming that the action potential is initiated in the axon (Fig. 12.1A–C). Dopaminergic cells in the substantia nigra provide a particularly interesting demonstration of the axonal site of action potential initiation. In about half of these cells, the action potential occurs first at the dendritic recording site during double somatic–dendritic recording; in those cases, however, staining of the cells revealed that the axon emerged from a dendrite near the dendritic recording electrode, again indicating initiation of the action potential at an axonal site (Fig. 12.1D) (Häusser et al., 1995). Recent work in CA1 pyramidal cells has provided another example of this phenomenon, with a significant fraction of pyramidal neurons exhibiting an axon emerging from a basal dendrite (Thome et al., 2014).

Where exactly in the axon is the action potential initiated? In hippocampal pyramidal neurons and cerebellar Purkinje neurons, experiments using either local applications of tetrodotoxin (TTX) or cell-attached recordings initially suggested that the action potential is generated at the first node of Ranvier (Colbert and Johnston, 1996). However, in neocortical layer 5 (L5) pyramidal neurons a variety of methods provide evidence that action potentials are initiated in the axon initial segment (Palmer and Stuart, 2006; Kole et al., 2007; Shu et al., 2007). A range of more recent experiments in other neuronal cell types, including Purkinje cells, also indicate that the most likely site of action potential initiation is in the axon initial segment (Khaliq and Raman, 2006; Schmidt-Hieber et al., 2008; Foust et al., 2010; Palmer et al., 2010; Hu and Jonas, 2014).

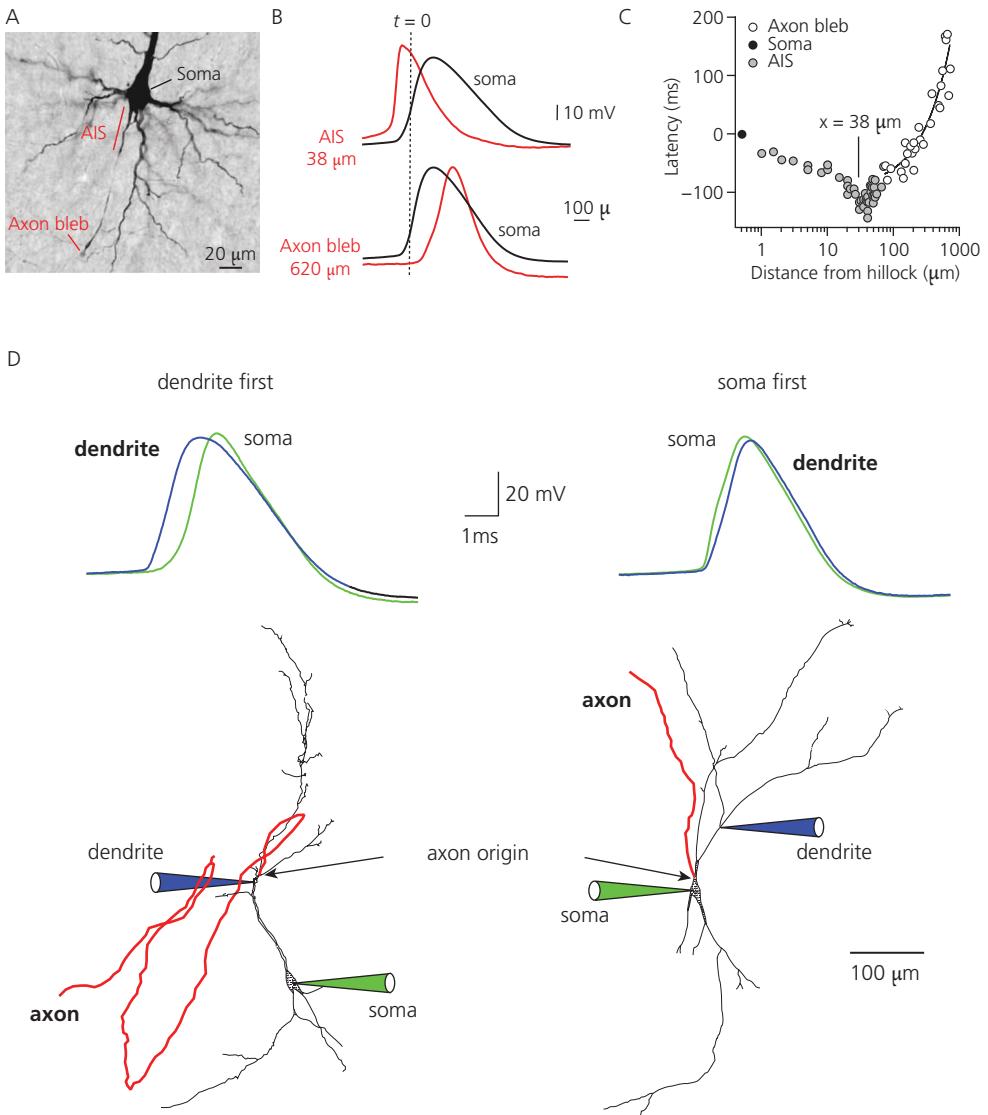


Fig. 12.1 Axonal initiation of action potentials (APs). (A) Biocytin filled L5 neuron with the soma, axon initial segment (AIS), and an axon bleb indicated. (B) APs evoked by somatic current injection and recorded at the soma (black) and the AIS or an axon bleb (red) 38 μm (top) and 620 μm (bottom) from the axon hillock. $t = 0$ marks the time of onset of the somatic AP. (C) Plot of AP latency relative to the soma (black) for axonal APs recorded from the AIS (gray circles, $n = 45$) or axon blebs (open circles, $n = 22$) versus recording distance from the axon hillock. Minimum onset latency occurs about 38 μm from the axon hillock, indicating the site of initiation of the AP in the AIS. (D) Simultaneous dendritic (blue) and somatic (green) recordings (top) from two different substantia nigra dopamine neurons (bottom) during AP generation. The axon originates from a dendrite 215 μm from the soma in the neuron on the left, and at the soma in the neuron on the right. APs are observed first at the recording site closest to the site of axon origin, indicating that they are initiated in the axon of these neurons.

Parts A–C reprinted from *Neuron*, 55(4), Maarten H. P. Kole, Johannes J. Letzkus, and Greg J. Stuart, Axon initial segment Kv1 channels control axonal action potential waveform and synaptic efficacy, pp. 633–47, Copyright 2007, Elsevier. With permission from Elsevier. Part D adapted from *Neuron*, 15(3), Michael Häusser, Greg Stuart, Claudia Racca, and Bert Sakmann, Axonal initiation and active dendritic propagation of action potentials in substantia nigra neurons, pp. 637–47, Copyright 1995, Elsevier. With permission from Elsevier.

The evidence in favor of the axon as the final site of action potential initiation, combined with the fact that the axon usually emerges from the soma in mammalian neurons, has a powerful influence on our understanding of synaptic integration. From this perspective, it follows that neurons integrate their synaptic inputs from a somato-centric point of view as inputs need to pass through the soma to influence the membrane potential in the axon and thereby neuronal output (but see Häusser et al., 1995; Thome et al., 2014). This view is adopted as the default perspective for much of this chapter, while the role of active dendritic spikes is considered later.⁴

The summation and propagation of PSPs depend on dendritic cable properties

The resting potential (V_{rest}) of most neurons is more hyperpolarized than the action potential threshold. Furthermore, unitary PSPs (mediated by one or more contacts between a single presynaptic axon and a postsynaptic dendrite) are usually too small to bridge the gap between the resting potential and action potential threshold. Multiple synaptic inputs must therefore sum to produce firing of action potentials in most neurons.⁵ This section considers the passive electrical structure of dendrites, and their effects on the integration of PSPs. Later we consider how dendritic voltage-activated channels further enrich synaptic integration.

The passive electrical properties of dendrites influence synaptic integration

As already discussed, action potentials are initiated in the axon of most neurons, which usually emerges from the soma. The ability of synaptic inputs to influence the generation of action potentials therefore depends on their initial amplitude and the degree to which they attenuate as they propagate from the dendrites toward the soma and axon. Here, we summarize experimental data on the passive electrical structure of neurons, and illustrate, with examples, how synaptic integration is affected by these properties, the morphology of the cell, the location of a synapse, and the time course of the synaptic current.

Three passive electrical properties contribute to the electrotonic structure of the dendritic tree: the specific membrane resistivity (R_m), the specific membrane capacitance (C_m), and the intracellular resistivity (R_i). High values of R_i and low values of R_m increase the attenuation of synaptic potentials as they propagate passively in dendrites. Attenuation is also greater for brief PSPs compared with the more sustained changes in membrane potential (V_m) (Rall, 1967; Jack et al., 1983; Spruston et al., 1994); this arises as a result of the membrane capacitance, which serves to filter transient changes in V_m . All of these effects are more pronounced for synapses that are located further from the site of initiation of the action potential.

Figure 12.2 illustrates the effects of R_m , R_i , and synapse location on synaptic integration. Panels A–C show the responses of a generic pyramidal neuron model to synaptic input in three different

⁴ The somato-centric view of synaptic integration, while well justified for most mammalian neurons, is not appropriate for most invertebrate neurons, because the soma is often electrotonically remote from both the dendrites and the axon.

⁵ Exceptions to this include, for example, the spherical bushy cells of the ventral cochlear nucleus (Liberman, 1991), neurons in the magnocellular nucleus of the trapezoid body (Borst et al., 1995), and ciliary ganglion neurons (Landmesser and Pilar, 1972). In each of these cases a small number of presynaptic axons form a large calyceal synapse capable of firing the postsynaptic neuron. Another exception is the climbing fiber input to the Purkinje cell, where a single presynaptic fiber reliably generates a stereotyped burst of spikes in the postsynaptic neuron (Llinás and Sugimori, 1980a).

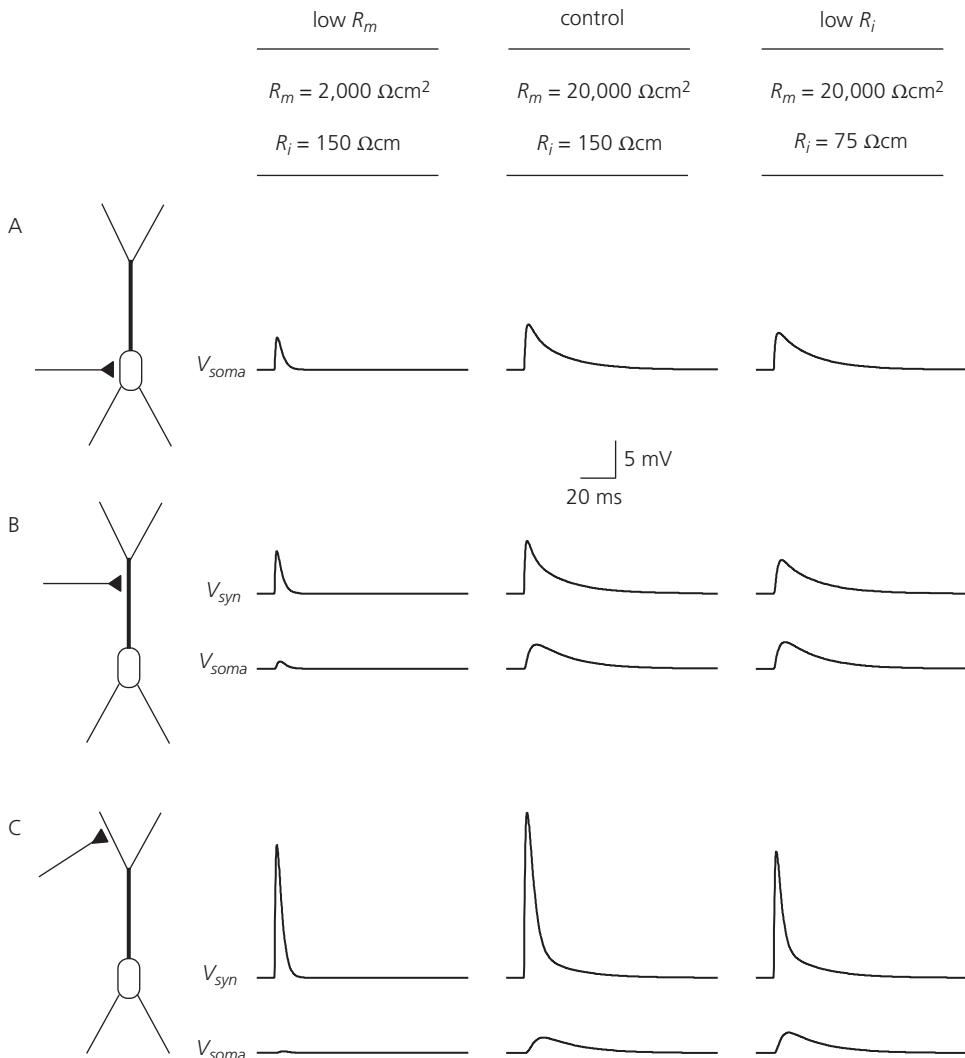


Fig. 12.2 Effects of R_i and R_m on EPSP attenuation. A single excitatory synaptic conductance (g_{syn}) was simulated either at the soma, a mid-apical dendrite, or a distal apical dendrite. Membrane potentials at the soma (V_{soma}) and the synapse (V_{syn}) are shown. The central column illustrates the simulations under control conditions; the left column represents a lower- R_m case; the right column represents a lower- R_i case. See text for discussion of simulation results.

All simulations were performed using NEURON (Hines and Carnevale, 1997). The parameters used in the model were: soma, length $l = 20 \mu\text{m}$, diameter $d = 20 \mu\text{m}$; main apical dendrite, $l = 350 \mu\text{m}$, $d = 2.94 \mu\text{m}$; distal apical and basal dendrites, $l = 250 \mu\text{m}$, $d = 1.5 \mu\text{m}$; $C_m = 1.0 \mu\text{F/cm}^2$; control $R_m = 20,000 \Omega \text{ cm}^2$; control $R_i = 150 \Omega \text{ cm}$. R_m was halved and C_m doubled to account for spines throughout. Using these parameters, the electrotonic lengths (L) of the apical and basal dendrites are 1.0 and 0.5, respectively. The cell had a resting potential (V_{rest}) of -60 mV . Synapses were placed either at the soma (A), 300 μm from the soma on the main apical dendrite (B; electrotonic distance $X = 0.43$), or 550 μm from the soma on a distal apical dendrite (C; apical $X = 0.9$). Synapses were modeled as conductance changes ($g_{max} = 6.38 \text{ nS}$) with a rising exponential (τ_{rise}) of 0.2 ms and a decaying exponential (τ_{decay}) of 2.0 ms and a reversal potential (E_{rev}) of 0 mV. Data and code for this figure are available on the Spruston lab website.

locations. In the control case (Fig. 12.2, central column), moving the synapse from the soma (Fig. 12.2A, center) to a proximal dendrite (Fig. 12.2B, center) results in a smaller somatic EPSP because some of the synaptic charge deposited onto the dendrite capacitance is lost through the membrane resistance as it propagates toward the soma. In this example this results in a nearly two-fold attenuation of the EPSP propagating from the dendrite to the soma. Moving the synapse further out on the dendrite (Fig. 12.2C, center) increases the amplitude of the local synaptic potential in the dendrites, due to the higher input impedance and smaller local capacitance at this dendritic location compared with the soma, but it dramatically increases the dendro-somatic EPSP attenuation (nearly ten-fold attenuation). The result is a net reduction of the somatic EPSP amplitude by a factor of three compared with the somatic input shown in Fig. 12.2A (center).

Reducing R_m by an order of magnitude has only a modest effect on the amplitude of the local synaptic potential, but has a much bigger effect on the amplitude of the somatic EPSP generated by dendritic synapses (Fig. 12.2, left column). For the most distal synaptic input, the EPSP attenuation (synapse to soma) for the low R_m value is almost 100-fold (Fig. 12.2C, left), resulting in a somatic EPSP about 20 times smaller than for the same synapse located at the soma. Such large values of dendro-somatic EPSP attenuation have been suggested from dendritic recordings and modeling of neocortical and hippocampal pyramidal neurons, where channels open at the resting membrane potential have been shown to result in a leaky apical dendrite (Cauller and Connors, 1992; Stuart and Spruston, 1998; Golding et al., 2005; Krueppel et al., 2011). Direct measurement using simultaneous dendritic and somatic patch-clamp recordings has demonstrated a more than 40-fold attenuation of the EPSP from the dendrites to the soma of neocortical L5 pyramidal neurons (Williams and Stuart, 2002; Nevian et al., 2007; Larkum et al., 2009). This remains an underestimate of the maximum EPSP attenuation, as direct recording from the smallest, most distal dendrites is not technically feasible.

The effect of reducing R_i by a factor of two is shown in the right-hand column of Fig. 12.2. For both dendritic synapse locations (Figs 12.2B, C), this change in R_i results in a reduction of the amplitude of the local dendritic EPSP that is greater than that produced by the ten-fold reduction in R_m . This relatively strong effect of R_i occurs because the change in voltage during a brief synaptic current results from charging the membrane capacitance; reductions in R_i increase the radial flow of current away from the synapse, thus reducing the amount of charge deposited on the local capacitance. Because of this increase in radial current flow along the dendrite the attenuation of the EPSP is reduced. The net effect of a change in R_i is therefore determined by its impact on local EPSP amplitude and dendro-somatic attenuation of the EPSP. For the intermediate synapse position shown in Fig. 12.2B, reducing R_i decreases the local EPSP but has only a small effect on the somatic EPSP amplitude. For the more distal synapse shown in Fig. 12.2C, lowering R_i results in a greater decrease in the local EPSP amplitude and an increase in the amplitude of the somatic EPSP, due to reduced attenuation.

Experimental estimates of passive electrical properties

C_m has been widely regarded as a biological constant with a value of approximately $1 \mu\text{F}/\text{cm}^2$. Experimental analysis has provided confirmation of this value for a variety of neurons (Gentet et al., 2000). R_m has been measured for a large number of cell types, revealing a wide range of values for different neurons (see below). R_i in mammalian neurons has been estimated using a variety of methods, yielding values ranging from $70\text{--}500 \Omega\text{cm}$ (Coombs et al., 1959; Rall, 1959; Lux et al., 1970; Barrett and Crill, 1974; Cauller and Connors, 1992; Fromherz and Müller, 1994; Major et al., 1994; Rapp et al., 1994; Thurbon et al., 1994, 1998; Bekkers and Stevens, 1996; Meyer et al., 1997). Simultaneous somatic and dendritic patch-pipette recordings have been used to determine voltage attenuation along the apical dendrites of L5 pyramidal neurons (Stuart and Spruston, 1998),

CA1 pyramidal neurons (Golding et al., 2005), and the primary dendrites of cerebellar Purkinje neurons (Roth and Häusser, 2001). Modeling of these data has indicated a value for R_i of 70–220 Ωcm . These experiments provide the most reliable available estimates of R_i , as the filtering of transient voltage changes by the dendrites, on which these estimates are based, is very sensitive to R_i . Nevertheless, the range of estimates of R_i using this method remains large and it is unclear whether this represents true cell-to-cell variability in this property. In addition, the possibility that R_i might vary in different dendritic domains of the same cell should not be ruled out.

A particularly critical factor affecting PSP summation is the membrane time constant (τ_m), which is given by the product of R_m and C_m . For any change in membrane potential, the slowest component of voltage decay is determined by τ_m . Thus, τ_m defines the time window over which synaptic potentials can sum; for presynaptic inputs separated by more than two to three τ_m , temporal summation becomes diminishingly small.

The membrane time constant can be estimated directly from the slowest exponential component in a multi-exponential fit of the voltage relaxation following current injection. τ_m has now been estimated for several cell types, revealing a tremendous range in the resting membrane properties of different types of neurons. Given that C_m is likely to be a biological constant, variations in τ_m presumably reflect variation in R_m due to differences in the types and densities of ion channels open in the membrane at the resting potential. Hippocampal CA3 pyramidal neurons have among the slowest τ_m values measured—about 50–70 ms in brain slices at physiological temperatures (Spruston and Johnston, 1992; Brown and Randall, 2009; Barnes et al., 2010). Even within the hippocampus, τ_m for other cell types differs from this value: in CA1 pyramidal neurons, τ_m is about half this value—about 30 ms in slices (Spruston and Johnston, 1992). The fastest τ_m values recorded so far are from octopus cells in the ventral cochlear nucleus (Golding et al., 1999a). Patch-pipette recordings from these cells in slices reveal τ_m values of about 0.2 ms. Based on τ_m alone, it can be inferred that in principle CA3 pyramidal neurons will be able to integrate synaptic inputs over a time window about 350-fold longer than in cochlear octopus cells. Differences such as these are certain to be critical for the function of the neuron. For example, cochlear octopus cells *in vivo* phase lock their firing to sound tones of up to 1 kHz (Smith et al., 1993). This kind of precise temporal coding would be difficult or impossible to achieve in a neuron with a long membrane time constant.

Resting membrane properties

Theoretically, τ_m is a purely passive measure, determined only by the membrane capacitance and voltage-independent leak conductances of a neuron. In cells where this assumption has been tested, however, it has proven to be an oversimplification, as measured values of τ_m are voltage dependent and influenced by blockers of voltage-dependent conductances. For example, block of hyperpolarization-activated conductance (I_h) and inward-rectifying K⁺ channels (with CsCl) results in an increase of approximately 50% in the apparent τ_m in CA3 and CA1 pyramidal neurons (Spruston and Johnston, 1992), a two-fold increase in τ_m in neocortical pyramidal neurons (Stuart and Spruston, 1998), and a 20-fold increase in τ_m in cochlear octopus cells (Golding et al., 1999a). Similarly, even small changes in V_m near V_{rest} have been shown to significantly affect estimates of τ_m and input resistance (R_N) (e.g., Spruston and Johnston, 1992; Waters and Helmchen, 2006). These findings suggest that the so-called “passive” membrane properties of most neurons might be more aptly referred to as “resting” membrane properties, since they are actually determined in large part by voltage-dependent channels that are open at V_{rest} . The situation is further complicated by the fact that the resting membrane properties of many neurons are not uniform. Experimental

evidence indicates that many conductances are distributed non-uniformly along dendrites. In neocortical and hippocampal pyramidal neurons, conductances that are open at the resting potential, including I_h and others, are present at higher densities in the distal regions of the apical dendrite (Magee, 1998; Stuart and Spruston, 1998; Williams and Stuart, 2000b; Berger et al., 2001; Lörincz et al., 2002). The net effect of the additional leak in the distal apical dendrites is to increase the electrical isolation of distal synapses (Stuart and Spruston, 1998; Golding et al., 2005).

Synaptic conductances that are on at rest will also lower the effective τ_m by lowering the effective R_m (Bernander et al., 1991; Rapp et al., 1992; Rudolph and Destexhe, 2003). In many brain areas, such as the cerebellar cortex, neurons providing the synaptic input are spontaneously active, thus generating a tonic synaptic conductance that significantly shortens the effective τ_m (Häusser and Clark, 1997). The same is also true in the neocortex, where a reduction of ongoing synaptic activity by local application of TTX has been shown to increase τ_m and input resistance (R_N) substantially, suggesting that synaptic activity reduces both R_N and τ_m (Paré et al., 1998; Destexhe and Paré, 1999). Other work indicates that synaptic activity associated with active network states produces only small changes in R_N and τ_m , in part due to voltage-gated channels, which serve to oppose the decrease in R_m introduced by synaptic activity (Waters and Helmchen, 2006). Earlier estimates of larger changes in τ_m and R_N may also be influenced by higher firing rates, as action potentials can shunt PSPs (Häusser et al., 2001). Thus, the effects of background synaptic activity on the effective R_N and τ_m , and hence the time window for temporal summation, depends on the rate, number, and conductance of activated synapses, as well as active responses produced by synaptic input (see also Chapter 13).

Spatial and temporal integration

R_m , C_m , and R_i are not the only factors that influence summation of synaptic potentials and their propagation to the action potential initiation zone. The structure of the dendritic tree and the position of synapses on dendrites influence synaptic summation in many ways. To illustrate this, Fig. 12.3A shows a simulation of two synapses on a simple isopotential neuron with no dendritic tree. In this system individual EPSPs decay according to τ_m , and summation is dependent on the timing of the two inputs relative to the membrane time constant. In the simulation shown in Fig. 12.3A, τ_m is 20 ms, and the EPSPs sum to a peak depolarization 1.37 times the individual EPSP amplitude when the two inputs are separated by 20 ms. The dashed line shows the subtraction of the first response alone from the paired response. Note that the peak of this subtracted EPSP is slightly smaller than that of the first EPSP (Fig. 12.3A). This occurs because the depolarization associated with the first EPSP produces a slight reduction in driving force for the synaptic current when the second input is activated. Next consider two synapses on a similar soma, but with the addition of apical and basal dendrites (Fig. 12.3B). The synaptic conductances have been scaled up so that the peak of the first EPSP at the soma is the same as in the cell with no dendrites (6 mV in both cases). Note, however, that the EPSPs now rise and decay more quickly, so less summation occurs (Fig. 12.3B; 1.27 times the single EPSP; i.e., less summation than the simulation with no dendrites). This is because only the *final* decay of the EPSP is determined by τ_m ; the early decay of the EPSP is accelerated in this case due to redistribution of charge into the dendrites (see Chapter 15; Rall, 1967; Koch et al., 1996; Geiger et al., 1997; Häusser and Clark, 1997). Now consider moving the synapses from the soma to the apical dendrite (Fig. 12.3C). Again the synaptic conductances have been increased in amplitude so that each input produces a 6 mV EPSP at the soma. The time course of these EPSPs at the soma is slowed, due to the filtering properties of the dendritic membrane between the synapse and the soma (Fig. 12.3C; dashed lines are the simulation from B,

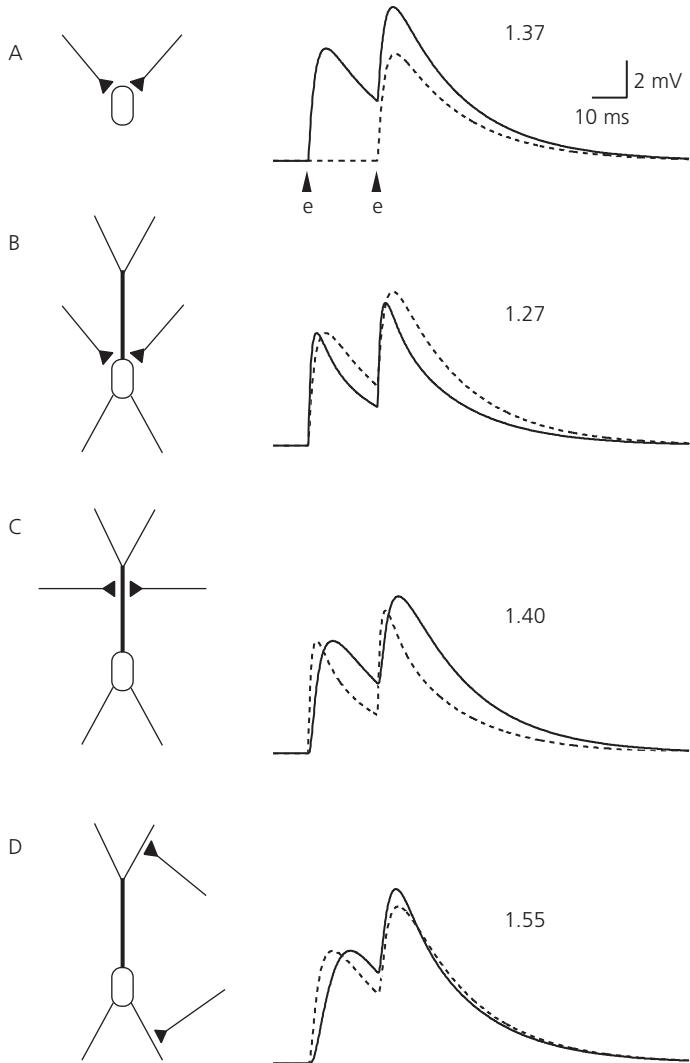


Fig. 12.3 Dendrite structure and synapse location influence EPSP summation. In each panel, the simulation is represented by a schematic diagram with excitatory synapses positioned as shown. In each case the solid line is the simulation of somatic membrane potential following activation of the two excitatory synapses with a 20 ms delay between them. The numbers by each pair of traces represent the ratio of the peak amplitudes of the second EPSP relative to the first. (**A**) Temporal summation in a simple model consisting of just a soma. The dashed line is the subtracted difference between the response to activation of both synapses and just the first (see text). (**B**) Addition of dendrites to the model accelerates the decay of the somatic EPSPs, reducing temporal summation. The dashed line represents the response shown in part A. (**C**) Moving the synapses from the soma to the dendrites slows the somatic EPSP, increasing temporal summation. The dashed line represents the response shown in part B. (**D**) Separating the synapses onto different dendrites maximizes summation (see text). The dashed line represents the response shown in part C.

All simulations use the same model described in Fig. 12.2, except for the isolated soma model. In each case two identical synapses were activated, with a delay of 20 ms. Synapses were located at the soma (A, B), 300 μm from the soma on the main apical dendrite (C; $X = 0.43$), 550 μm from the soma on a distal apical dendrite (D; $X = 0.9$), or 200 μm from the soma on a basal dendrite (D; $X = 0.4$). Synaptic conductances were chosen to yield EPSPs of 6 mV in the soma (A, $g_{\max} = 1.34$ nS each; B, $g_{\max} = 6.38$ nS each; C, $g_{\max} = 13.3$ nS each; D, apical $g_{\max} = 52.5$ nS, basal $g_{\max} = 14.7$ nS). Data and code for this figure are available on the Spruston lab website.

for comparison). As a result, more temporal summation occurs (Fig. 12.3C; 1.40 times the single EPSP). Finally, consider the effect of moving the two synapses to different dendrites. In this case, summation at the soma is maximized (Fig. 12.3D; 1.55 times the single EPSP). This occurs for two reasons: (1) the decay of the first EPSP (apical synapse) is slowed because of its greater electrotonic distance from the soma; (2) the effect of the first EPSP on the driving force of the second synapse is small, because of the greater electrotonic separation of the two synapses. These simulations illustrate three important points regarding summation in passive neurons: (1) the presence of dendrites accelerates the decay of the EPSP near the synapse; (2) cable filtering of dendritic EPSPs slows their time course as measured at the soma, thus increasing temporal summation at the soma; and (3) sublinear summation is expected for synapses located electrotonically close together, but is minimal for electrotonically distant inputs.⁶

Normalization of temporal summation

One consequence of non-uniform channel distributions is that they can equalize temporal summation along the length of the dendrite. As illustrated in Fig. 12.3, uniform passive membrane properties predict that distal inputs will summate to a greater extent in the soma because they are broader there than more proximally generated EPSPs. An increased density of I_h in the apical dendrite compensates for this location-specific dependence of temporal summation (Magee, 1999; Williams and Stuart, 2000b, 2003b; Berger et al., 2001; Day et al. 2005). I_h is capable of normalizing the location-dependence of temporal summation even in neurons that lack a strong somato-dendritic I_h gradient (Bullis et al., 2007; Angelo et al., 2007). Other voltage-gated conductances, including various subtypes of dendritic K_v channels, have also been suggested to contribute to the location independence of temporal summation over a broad range of frequencies (Desjardins et al., 2003; Williams and Stuart, 2003b). Accordingly, modulation of these conductances can influence the extent to which temporal summation is normalized for dendritic location (Takigawa and Alzheimer, 2003; Chen and Johnston, 2005; Day et al., 2005).

Excitation–inhibition interactions in dendrites

Inhibition is another central factor limiting the way excitatory synaptic inputs summate in space and time during synaptic integration. Inhibitory inputs do not simply counter the depolarizing effects of excitation; they are also critical determinants of spike timing. Inhibition can synchronize spiking in a population of neurons, as cells receiving a common inhibitory input can stop spiking and subsequently return to threshold at the same time (Cobb et al., 1995). Inhibition also influences spike timing by limiting the time window for temporal summation of excitatory inputs. Many neural circuits include feed-forward inhibition, which can limit the duration of excitatory inputs to less than a few milliseconds, thus requiring temporally coincident excitatory inputs to trigger action potential firing (Pouille and Scanziani, 2001; Mittmann et al., 2005). Similarly, feedback inhibition does not prevent spiking at the onset of an excitatory stimulus, but can limit the duration of spiking in response to a sustained excitatory stimulus. In the hippocampus, different types of inhibitory interneurons provide feedback inhibition to different dendritic domains with different temporal dynamics (Müller and Remy, 2013). The functional implications of this

⁶ An elegant example of this is found in the medial superior olive (MSO). In this auditory nucleus, binaural processing is optimized by inputs from each side of the brain contacting separate dendrites of MSO neurons in order to minimize nonlinear summation of signals arriving from each ear (Agmon-Snir et al., 1998).

differential dendritic targeting are not understood, but these processes influence dendritic computation in many brain regions, thus highlighting the need to further explore how dendrites influence the integration of excitatory and inhibitory synaptic inputs.

The principles governing dendritic integration of EPSPs apply similarly to IPSPs. The time course of an IPSP at the soma is slowed if the inhibitory synapse is located on the dendrites. In addition, depolarization induced by EPSPs, or hyperpolarization by other IPSPs, will have more effect on the driving force for the inhibitory synaptic current for synapses that are located close together. The latter point is particularly important for IPSPs, as the reversal potential at many inhibitory synapses is close to the resting membrane potential (most notably GABA_A and glycinergic synapses, which activate Cl⁻ channels). Hence, very small changes in V_m can have relatively large effects on the inhibitory synaptic current. This effect of inhibition can be considerable, even when IPSPs generate no change in membrane potential on their own. Figure 12.4A shows the result of activating two excitatory synapses on the soma, either with (solid line) or without (broken line) prior activation of an inhibitory synapse. In these simulations, inhibition is simulated with a reversal potential equal to the resting potential, and hence alone it generates no change in membrane potential. Nevertheless, inhibition results in a 35% reduction of the first EPSP, and about a 13% reduction of the second EPSP (Fig. 12.4A). The relative ineffectiveness of the inhibition on the second EPSP is because the inhibitory synaptic conductance is largely over by the time the second EPSP arrives. This result demonstrates that inhibition is most effective during the inhibitory synaptic conductance change itself. The special case where inhibition occurs without a change in membrane potential is a good illustration of the concept of “shunting inhibition,” which describes the ability of inhibition to be effective even when it produces little or no change in membrane potential on its own, because the effect of the inhibitory conductance change is similar to a transient reduction in R_m which “shunts” the EPSP without an obvious change in membrane potential. Shunting occurs because the depolarization associated with the EPSP increases the driving force for outward current at the inhibitory synapse (Fig. 12.4A, bottom trace).

The ability of an inhibitory synapse to shunt current from excitatory synapses depends on the spatial arrangement of the two inputs (Fig. 12.4B–D). Inhibition placed at the soma has a similar effect on EPSPs arriving from all dendritic locations, whereas inhibition located on particular dendrites can be specific for particular inputs. Figure 12.4B illustrates that somatic inhibition reduces EPSPs originating on different dendrites to similar extents. In fact, somatic inhibition in this case has a slightly more pronounced effect for dendritic excitation than somatic excitation

Fig. 12.4 The spatial relationship between inhibition and excitation influences dendritic integration. In each panel, the simulation is represented by a schematic diagram with excitatory and inhibitory synapses positioned as shown. The inhibitory synapse (\triangle , i) is activated 5 ms before the excitatory synapse (\blacktriangle , e) and has $E_{rev} = V_{rest}$, resulting in no hyperpolarization by activation of the inhibitory synapse at rest. The numbers by each pair of traces represent the peak of the EPSP with inhibition (solid lines) relative to the EPSP without inhibition (dashed lines). **(A)** Two excitatory synapses on the soma are activated (with a 20 ms delay between them, arrowheads indicate timing) either with or without inhibition. The lower traces show the time course of the inhibitory synaptic conductance (thick line, peak = 50 nS) and current (thin line, peak = 109 pA). **(B)** Separate responses to activation of the excitatory synapse on the apical dendrite (top traces) or basal dendrite (bottom traces) with and without somatic inhibition. **(C)** Responses to activation of the same synapses as in part B with and without apical dendritic inhibition. **(D)** Responses to activation of the same excitatory synapses as in parts B and C with and without distal apical inhibition. The plot with the long-dashed line indicates simultaneous activation of the excitatory synapse and inhibition on a different branch (peak = 0.88 of

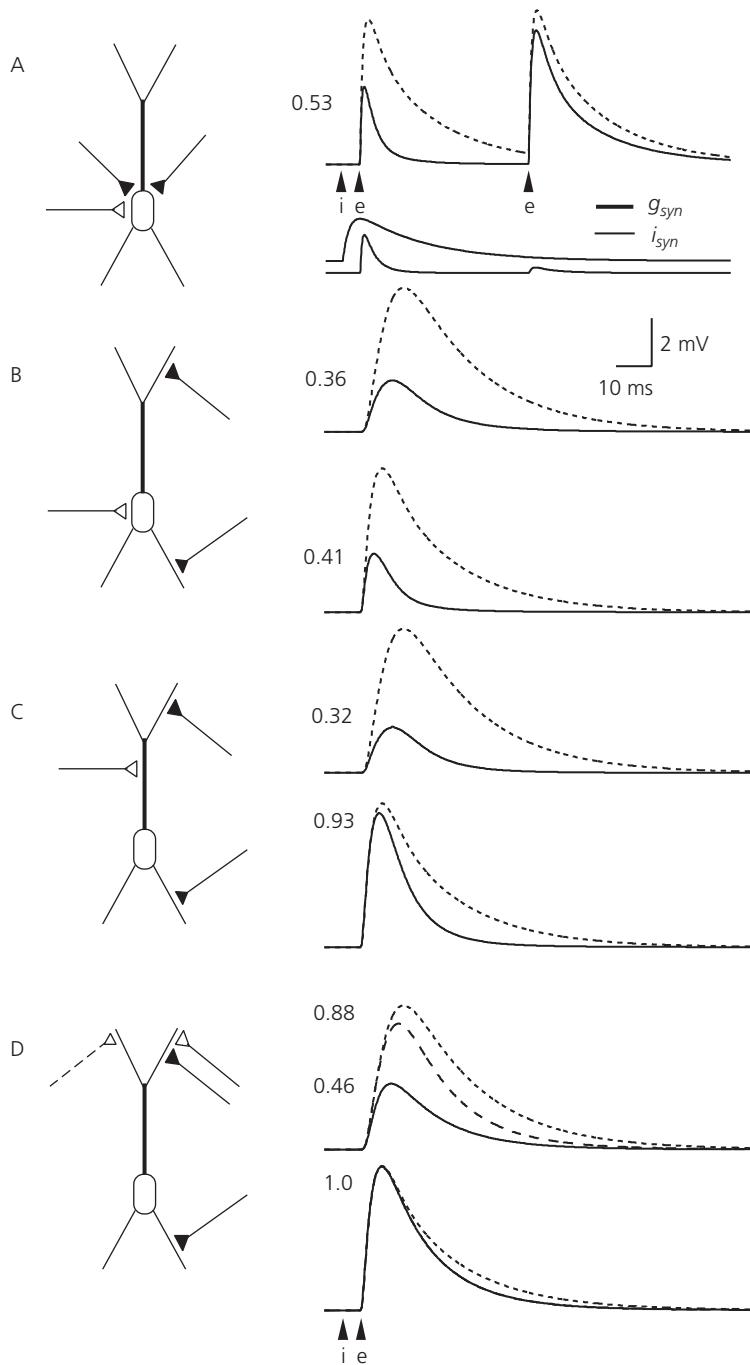


Fig. 12.4 (Continued)

control at the top and 1.0 at the bottom, obscured by the solid line response). Arrowheads indicate the timing of synaptic activation in parts B–D.

Simulation methods are as described in Figs 12.2 and 12.3, including placement and conductance of excitatory synapses for corresponding schematic diagrams. Inhibitory synapses were placed either at the soma (A), 300 μ m from the soma on the main apical dendrite (C; $X = 0.43$), or at the end of a distal apical dendrite (D; $X = 1.0$). Inhibitory synapses were modeled with the following parameters: $\tau_{rise} = 2$ ms; $\tau_{decay} = 20$ ms; $g_{max} = 50$ nS; $E_{rev} = V_{rest} = -60$ mV. Data and code for this figure are available on the Spruston lab website.

(compare Fig. 12.4A and B). When the inhibitory synapse is moved onto a dendrite, the EPSP generated on the same dendrite is preferentially inhibited, leaving the peak of the other EPSP relatively unaffected (Fig. 12.4C). In passive neurons, or when dendritic nonlinearities are not engaged, dendritic inhibition is most effective at limiting somatic EPSPs if the inhibitory synapse is located “on path” between the excitatory synapse and the soma. “Off path” inhibition is only effective if both the excitatory and inhibitory synapses are located near the end of a dendrite; in this case the “off path” inhibition can be almost as effective as the “on path” inhibition (Fig. 12.4D; this effect is also illustrated in Fig. 7.36 of Jack et al., 1983). As shown in Fig. 12.4D, however, distal dendritic inhibition is only effective for excitatory synapses on the same dendritic branch as the inhibitory synapses, and relatively ineffective if located on a different branch.

The location-dependent effects of dendritic inhibition have been explored systematically in a model of a CA1 pyramidal neuron (Hao et al., 2009). This work revealed that inhibition on the apical trunk most effectively shunted (reduced) EPSPs generated at the same dendritic location or more distally; inhibition of the apical dendritic trunk was progressively less effective for excitatory synapses located at increasingly proximal dendritic locations. Furthermore, the effect of dendritic inhibition on apical oblique branches was largely restricted to excitatory synapses on the same branch, with the same location-dependent rules as described above for the apical trunk (Hao et al., 2009).

An interesting example where distal dendritic inhibition may be important has been noted in the CA1 region, where inhibitory interneurons with somata in stratum oriens extend axons to stratum lacunosum-moleculare (thus named O-LM cells) and impinge on the most distal dendrites of CA1 pyramidal neurons (Sik et al., 1995). With this arrangement, inhibition could selectively limit the depolarization from the perforant path, which has excitatory synapses on the distal dendrites of CA1 cells. A similar arrangement exists in neocortical pyramidal neurons, where the apical tuft is selectively inhibited by Martinotti cells (Silberberg and Markram, 2007). The effects of O-LM and Martinotti cells is complicated, however, by the fact that they may also inhibit other interneuron subtypes, leading to disinhibition (Leao et al., 2012; Müller and Remy, 2013; Pfeffer et al., 2013).

The effects of inhibition discussed above provide the basis for understanding the integration of excitatory and inhibitory synaptic inputs in passive dendritic trees or in the subthreshold regime of active dendrites. However, as discussed later and in Chapter 15, the situation is quite different when one considers the effects of inhibition on the initiation and propagation of spikes in active dendrites.

Compensating for dendritic attenuation

The elaborate interactions that occur between excitatory and inhibitory synapses in the dendritic tree indicate that the process of synaptic integration is complex. Ultimately, however, to influence neuronal output synaptic activation must lead to the firing of action potentials in the axon, which usually arises from the soma. One prediction of this somato-centric view of synaptic integration is that the ability of a synapse to contribute to action potential initiation will be diminished for synapses located further from the soma. As already discussed, synaptic potentials generated in distal dendrites may attenuate over 100-fold by the time they reach the soma,⁷ suggesting that distal synapses will be less efficacious (in terms of somatic or axonal depolarization) than more proximal synapses. Two important factors mitigate this seemingly extreme disparity between proximal and

⁷ The long electrotonic location of most synapses from the soma in many neurons also contributes to serious errors associated with the estimation of the amplitude and kinetics of synaptic conductances using somatic voltage clamp (Spruston et al., 1993; Williams and Mitchell, 2008).

distal synapses. First, attenuation of synaptic charge is much less than the attenuation of fast synaptic voltage changes. Second, at least some synapses may scale their conductance to compensate for dendritic distance.

Attenuation and temporal filtering of EPSPs depend not only on the distance of the synapse from the soma but also on the EPSP time course, with faster EPSPs being attenuated and filtered more than slower EPSPs. By contrast, attenuation of synaptic charge depends only on distance from the soma and not on the time course of the charge entry at the synapse (Jack et al., 1983). In addition, the same factors that increase voltage attenuation along small-diameter dendrites (high axial resistance and input impedance) also increase the magnitude of the local synaptic potential (Rall and Rinzel, 1973; Rinzel and Rall, 1974). This was illustrated in Fig. 12.2 (central column), as moving the synapse further from the soma reduced the somatic EPSP but also increased the local EPSP at the synapse. This increase in local EPSP, which is dependent on dendritic morphology (Jaffe and Carnevale, 1999), can result in a significant reduction of the driving force for synaptic charge entry and thereby further decrease somatic EPSP amplitude. On the other hand, it can also result in increased current through *N*-methyl-D-aspartic acid (NMDA) receptors, as a result of greater relief of voltage-dependent Mg²⁺ block of these channels particularly at the ends of small-diameter dendrites where input impedance is high (Branco and Häusser, 2011). Similar effects can be seen in dendritic spines due to spine neck resistance (Gulledge et al., 2012; Harnett et al., 2012), which can reduce the location dependence of EPSP amplitude at the site of synaptic input (Gulledge et al., 2012).

Some synapses may also compensate for dendritic distance by scaling synaptic conductance. In CA1 pyramidal neurons, the Schaffer collateral inputs from CA3 are distributed over about two-thirds (several hundred microns) of the apical dendritic tree, but the average amplitude of somatically recorded EPSPs from these inputs does not vary with the distance of the activated synapses from the soma (Magee and Cook, 2000). This “synaptic scaling” appears to be mediated by a population of synapses with higher alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor density at greater distances from the soma (Andrasfalvy and Magee, 2001; Andrasfalvy et al., 2003; Smith et al., 2003). This distance-dependent increase of AMPA receptors does not continue into the apical tuft dendrites however, where the NMDA/AMPA receptor ratio is higher than at more proximal apical dendrites, suggesting an alternative mechanism by which synaptic efficacy could be scaled in a distance-dependent manner (Bittner et al., 2012). There is also evidence for synaptic scaling in the basal dendrites of CA1 pyramidal neurons, where immunoelectron microscopy suggests distal synapses have more AMPA receptors, but fewer NMDA receptors, compared to proximal synapses on these dendrites (Menon et al., 2013).

Morphological and immunocytochemical analysis of synapses on spines in CA1 apical dendrites suggests that the largest synapses in CA1 correspond to “perforated” synapses, which have large, perforated postsynaptic densities and high AMPA receptor densities; these presumably powerful synapses are most abundant in the region of the distal apical dendrites innervated by Schaffer collaterals. This form of scaling does not appear to extend to the apical tuft dendrites, which are innervated by the perforant path, nor does it apply in the basal dendrites (Nicholson et al., 2006; Menon et al., 2013). Furthermore, synaptic scaling has not been observed in neocortical L5 pyramidal neurons (Williams and Stuart, 2002; Nevian et al., 2007), suggesting that additional mechanisms must exist to allow distal dendritic synapses to contribute to the generation of axonal action potentials in these neurons (Williams and Stuart, 2003a). Collectively, these results suggest that a variety of mechanisms may be used to regulate the distance-dependent effects of synaptic strength, and that these mechanisms may be differentially employed in different dendritic domains and in different cell types. These effects are further influenced by the effects of voltage-gated channels on synaptic integration, which is also dependent on dendritic location and cell type.

Dendritic voltage-gated channels contribute to synaptic integration

Although some evidence supports the view that synaptic potentials are summated linearly or slightly sublinearly (Burke, 1967; Cash and Yuste, 1999; London and Häusser, 2005; Jia et al., 2010; Zhao et al., 2012; Longordo et al., 2013), dendrites are clearly not passive. Though theoretical analysis of the electrical properties of dendrites originally focused largely on passive cable properties,⁸ the importance of active dendrites was considered extensively, and modeled as early as the late 1960s (Rall and Shepherd, 1968; Miller et al., 1985; Perkel and Perkel, 1985; Shepherd et al., 1985).

One of the biggest challenges facing neurophysiologists interested in dendritic function is to determine which types of voltage-gated channels are present in dendrites and how they influence the input–output computations that can be accomplished with synapses on dendrites. Two major obstacles stand in the way of tackling this challenge. First, the small size of dendrites makes them relatively difficult to probe experimentally. Even with the advent of methods for obtaining patch-clamp recordings from dendrites, the smallest dendrites are very difficult to patch and dendritic spines have not yet proved to be accessible to recording with electrodes. Advances in our understanding of voltage-gated channels in dendrites will therefore require a combination of approaches including dendritic patch-clamp recording, imaging using ion- and voltage-sensitive fluorescent dyes, immunocytochemical localization of channels, and genetic manipulation of channels to analyze their expression.

The second problem is that different types of neurons have different channel distributions, reflecting their different functional properties within specialized neural networks. There will be no substitute, therefore, for studying many different cell types using similar methodologies and experimental designs. Furthermore, these properties are likely to change during development, so each cell type will have to be studied at several development stages (i.e., from neonatal to old age), with special consideration paid to key developmental events affecting the system under study (e.g., eye opening for neurons of the visual system).

In addition to tackling these two challenges, an integrated view of each cell type will rely on understanding the interactions between dendritic structure, the types of channels expressed in dendrites, and the synaptic inputs they receive. Progress on all of these issues is reviewed in the following sections, culminating with a discussion of current answers to the question, “What are the functions of dendritic excitability?”

Spikes can be generated in dendrites

Although action potentials are usually generated preferentially at a low-threshold initiation zone in the axon, there is good evidence that regenerative events (“spikes”) can be generated in dendrites under some conditions. The first evidence for dendritic spike⁹ generation came from field potential

⁸ The focus of dendritic cable theory on passive behavior was partially based on the fact that passive systems are more easily treated analytically, whereas simulation of active properties such as Na_v and K_v channels requires numerical approaches. Numerical methods for simulating nonlinear conductances in complex dendritic geometries using compartmental models were introduced later by Rall (1964).

⁹ For convenience, we refer to regenerative events initiated in dendrites as “dendritic spikes.” This offers a semantic way of distinguishing dendritically initiated spikes from the all-or-none action potential initiated in the axon.

recordings in the hippocampus, which indicated an electrogenic response in the apical dendrites of CA1 neurons that preceded the somatic/axonal population spike (Cragg and Hamlyn, 1955; Andersen, 1960; Fujita and Sakata, 1962; Andersen et al., 1966; Herreras, 1990). Around the same time, Eccles and colleagues reported that spikes could be generated in the dendrites of chromatolyzed motoneurons (Eccles et al., 1958) and Spencer and Kandel observed small, spike-like events in intracellular recordings from CA1 neurons *in vivo* (“fast pre-potentials”), which they inferred were generated in the dendrites (Spencer and Kandel, 1961).¹⁰ Similar events, termed “dendritic spikes,” were observed in recordings from neocortical neurons (Purpura, 1967) and cerebellar Purkinje cells (Llinas et al., 1968, 1969; Llinas and Nicholson, 1971). Later, dendritic recordings from hippocampal and neocortical neurons in slices and *in vivo* supported the view that dendrites are capable of generating regenerative spikes mediated by Na_v and/or Ca_v channels (Wong et al., 1979; Turner et al., 1993; Schiller et al., 1997; Seamans et al., 1997; Stuart et al., 1997a; Golding and Spruston, 1998; Kamondi et al., 1998; Smith et al., 2013; Palmer et al., 2014). Early evidence for dendritic spikes has been reviewed elsewhere (Purpura, 1967; Stuart et al., 1997a; Golding and Spruston, 1998; Segev and Rall, 1998; Johnston and Narayanan, 2008). Here we consider some of the most recent findings.

Simultaneous somatic and dendritic patch-pipette recordings have provided direct demonstration of dendritic spike generation. In both L5 and hippocampal pyramidal neurons, dendritic spikes have been recorded either in the absence of somatic spikes or preceding them (Fig. 12.5A). In both of these cell types, the incidence of dendritic spikes is promoted by strong synaptic excitation (Wong et al., 1979; Schiller et al., 1997; Stuart et al., 1997a; Golding and Spruston, 1998; Golding et al., 1999b; Sun et al., 2014; see also Turner et al., 1989, 1991). Dendritic spikes preceding or uncoupled from somatic action potentials have also been observed in hippocampal CA3 and CA2 pyramidal neurons (Kim et al., 2012; Sun et al., 2014), L6 neocortical pyramidal neurons (Ledergerber and Larkum, 2010), cerebellar Purkinje cells (Rancz and Häusser, 2006), hippocampal interneurons (Martina et al., 2000), olfactory mitral cells (Chen et al., 1997, 2002), and retinal ganglion cells (Velte and Masland, 1999; Sivyer and Williams, 2013).

Dendritically initiated spikes can be generated by activation of Na_v channels, voltage-gated Ca^{2+} (Ca_v) channels, or NMDA receptors, leading to sodium spikes (Fig. 12.5A), calcium spikes (Fig. 12.5A), and NMDA spikes (Fig. 12.5B–D). In Purkinje cells, because there are no Na_v channels or NMDA receptors in the dendrites, dendritic spikes are mediated solely by Ca_v channels, which is consistent with the broad width of these spikes (Llinas and Sugimori, 1980a). In pyramidal cells, the situation is more complex, because Na_v , Ca_v , and NMDA receptor channels are all present in the dendrites. In hippocampal CA1 pyramidal neurons, brief dendritic current injections or uncaging of glutamate produces narrow dendritic spikes (with a width at half amplitude of a few milliseconds) that can be blocked by TTX, indicating that they are mediated primarily by Na_v channels (Golding and Spruston, 1998; Gasparini et al., 2004; Gasparini and Magee, 2006; Losonczy and Magee, 2006; Kim et al., 2015). Longer current injections or glutamate uncaging produces broader dendritic spikes that are not blocked by TTX but are sensitive to Ca_v channel blockers (Golding et al., 1999b; Wei et al., 2001). Dendritic spikes elicited by strong synaptic activation are difficult to study pharmacologically (because of presynaptic effects of Na_v and Ca_v channel

¹⁰ Whether these events truly represent dendritic spikes has been a subject of debate (MacVicar and Dudek, 1981; Turner et al., 1993; Valiante et al., 1995; Nedergaard and Hounsgaard, 1996; Epsztain et al., 2010). Nevertheless, direct evidence now supports the occurrence of dendritic spikes in CA1 neurons (see later).

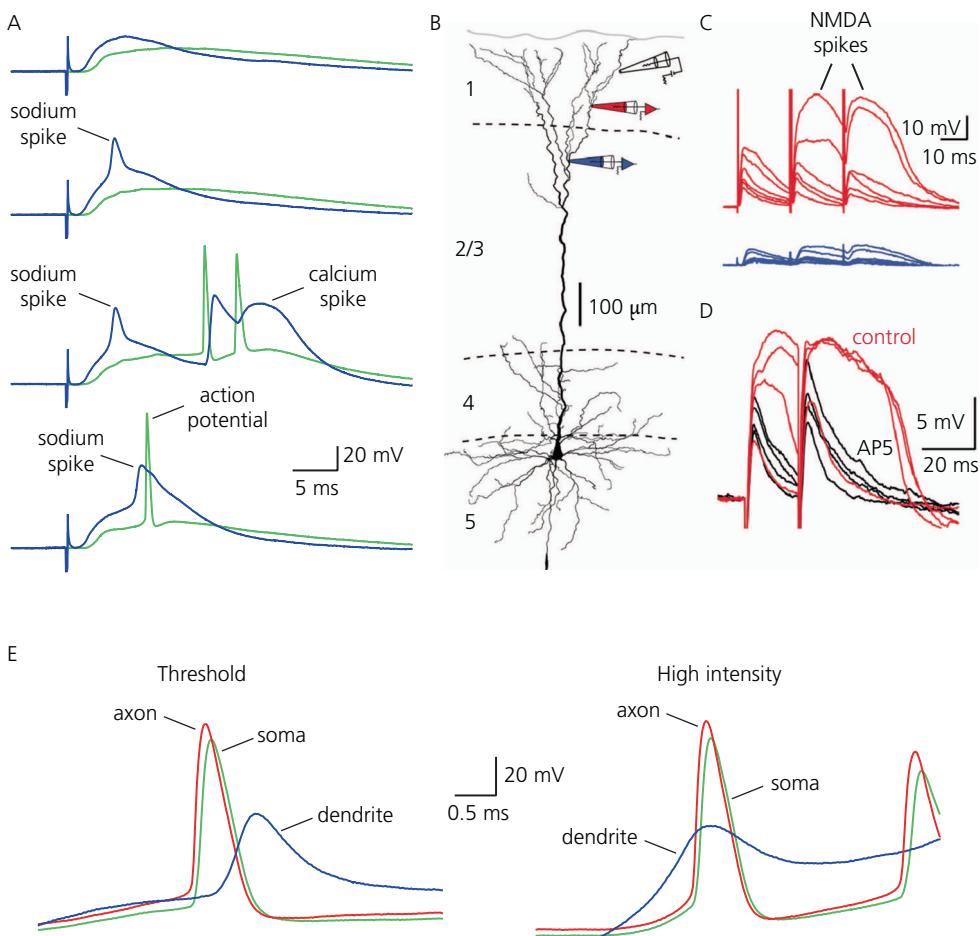


Fig. 12.5 Dendritic spikes and their relation to axonal action potential initiation. (A) Somatic (green traces) and dendritic (blue traces; 440 μm from the soma) recording from a neocortical L5 pyramidal neuron during synaptic stimulation in L2/3. All recordings are from the same cell at a similar stimulation intensity. Top: subthreshold somatic and dendritic EPSPs. Second from the top: initiation of a dendritic sodium spike in the absence of somatic action potentials. Second from the bottom: initiation of a dendritic sodium spike in relative isolation from somatic action potentials which occur in a burst due to generation of a dendritic calcium spike. Bottom: initiation of a dendritic spike just prior to a somatic action potential. (B) L5 pyramidal neuron with dendritic recording pipettes at two locations in the apical tuft (red, 875 μm from the soma; blue, 715 μm from the soma). An extracellular stimulation electrode (black) was positioned about 100 μm distal to the distal recording pipette. (C) Responses to a graded increase in extracellular stimulation (from 4 to 9 μA) recorded at both locations. (D) Distal tuft dendritic recording from a different pyramidal neuron (807 μm from the soma) in control (red) and after the addition of 50 μM AP5 (black). (E) Somatic (green traces), dendritic (blue traces; 300 μm from the soma), and axonal (red traces; 20 μm from the soma) recording from a neocortical L5 pyramidal neuron during synaptic stimulation in L2/3. All recordings are from the same cell. Left: synaptic stimulation in L2/3 at the threshold for somatic action potential initiation. Right: high-intensity synaptic stimulation, which initiated a dendritic spike prior to the somatic action potential.

blockers), but their widths vary considerably, depending on synaptic strength, location of the recording, and presumably the location of activated synapses (Golding et al., 1999a, 2002). This variability in the shape of synaptically evoked dendritic spikes suggests that they are produced by variable contributions from Na_v , Ca_v , and NMDA channels. Similar observations have been made in neocortical L5 pyramidal neurons, where additional evidence suggests that dendritic spikes in relatively proximal locations are mediated mostly by Na_v channels, while more distal dendritic spikes have a larger contribution from voltage-activated Ca_v channels (Schiller et al., 1997; Stuart et al., 1997a; Larkum et al., 1999b, 2001), although it has been suggested that this situation is reversed in the prefrontal cortex (Seamans et al., 1997). Synaptic activation of basal and distal tuft dendrites in L5 pyramidal neurons and CA3 pyramidal neurons has also been shown to elicit NMDA spikes (Schiller et al., 2000; Larkum et al., 2009; Makara and Magee, 2013). Voltage-dependent relief of the block of these channels by Mg^{2+} allows them to mediate regenerative events when present at sufficiently high densities (Rhodes, 2006; Major et al., 2013). NMDA spikes differ fundamentally from spikes mediated by voltage-gated channels because the requirement for glutamate binding dictates that they cannot actively spread beyond the region of synaptic activation.

Taken together, the evidence suggests that although the voltage threshold for action potential initiation is lowest in the axon, under some conditions a higher-threshold dendritic spike initiation zone may be activated first, resulting in a dendritic spike. Because the dendrites are weakly excitable compared with the axon, dendritic spikes can only occur if the local synaptic potential in dendrites is relatively large and fast. The initiation of dendritic spikes is facilitated by the high input impedance of small-diameter branches, which allows a relatively small number of co-activated synapses on the same branch to produce a large local EPSP, and thus evoke a dendritic spike (Losonczy and Magee, 2006; Katz et al., 2009). When a dendritic spike is initiated, and the activated dendrite is sufficiently electrotonically isolated from the axon, voltage attenuation and delay cause the dendritic spike to fail to trigger a somatic action potential (Fig. 12.5A, second from top) or to precede the somatic action potential with a long delay (Fig. 12.5A, third from top), because the lower, axonal threshold is reached well after dendritic spike initiation. In other cases, dendritic spikes immediately precede, and presumably help trigger, action potential initiation (Fig. 12.5A, bottom). Whether a dendritic spike occurs or not, axonal action potentials are followed by an action potential at the soma, which then back-propagates into the dendrites (Fig. 12.5E). Because dendritic spikes in pyramidal neurons only poorly invade the soma, and sometimes fail to trigger axonal action potentials, these dendritic spikes should be regarded as a form of active synaptic integration, where the final site of synaptic integration is in the axon. As discussed in more detail in the next section, the ability of

Fig. 12.5 (Continued)

Part A adapted from Action potential initiation and propagation in rat neocortical pyramidal neurons, Greg Stuart, Jackie Schiller, Bert Sakmann, *The Journal of Physiology*, 505(3), pp. 617–32, Figure 11b and c, Copyright © 1997, John Wiley and Sons. Parts B–D adapted from Matthew E. Larkum, Thomas Nevanian, Maya Sandler, Alon Polksky, and Jackie Schiller, Synaptic integration in tuft dendrites of layer 5 pyramidal neurons: a new unifying principle, *Science*, 325(5941) pp. 756–760, © 2009, The American Association for the Advancement of Science. Reprinted with permission from AAAS. Part E adapted from Action potential initiation and propagation in rat neocortical pyramidal neurons, Greg Stuart, Jackie Schiller, Bert Sakmann, *The Journal of Physiology*, 505(3), pp. 617–32, Figure 11b and c, Copyright © 1997, John Wiley and Sons.

dendritic spikes to propagate to the soma and influence the firing of action potentials in the axon is regulated by many factors.

Propagation of action potentials and dendritic spikes

Action potentials propagate through the dendritic tree in complex ways that are influenced by a variety of factors. Here we consider the effects of dendritic morphology, the properties of dendritic voltage-gated channels, and synaptic inhibition on the propagation of action potentials initiated in the axon and spikes generated in dendrites.

Action potential backpropagation

Following their initiation in the axon, action potentials propagate back into the soma and the dendritic tree. The invasion of the dendrites by so-called “backpropagating action potentials” (bAPs) varies across different cell types. In most neurons where they have been studied, including neocortical and hippocampal pyramidal neurons, hippocampal granule cells and interneurons, dopaminergic and GABAergic neurons in substantia nigra, spinal motoneurons, and mitral cells of the olfactory bulb, action potentials propagate actively back into the dendrites. The amplitude of bAPs in these cell types generally diminishes as the action potential propagates away from the soma, but remains above that expected for passive spread of the action potential (Stuart and Sakmann, 1994; Häusser et al., 1995; Spruston et al., 1995; Larkum et al., 1996; Bischofberger and Jonas, 1997; Chen et al., 1997; Martina et al., 2000; Golding et al., 2001; Waters et al., 2003; Hu et al., 2010; Krueppel et al., 2011; Kim et al., 2012). In pyramidal neurons, bAPs invade not only the primary apical dendrite but also radial oblique and basal dendrites (Frick et al., 2003; Nevian et al., 2007), but they may decay to passive propagation in the apical tuft, at least under some conditions (Golding et al., 2001; Larkum et al., 2001; Stuart and Häusser, 2001; Bernard and Johnston, 2003). Active backpropagation is supported by Na_v channels, which have been shown to be present in the dendrites of several types of neurons (Stuart and Sakmann, 1994; Häusser et al., 1995; Magee and Johnston, 1995; Bischofberger and Jonas, 1997). In most neurons, however, backpropagation is decremental, presumably because the density of Na_v channels is too low (and/or the density of K_v channels too high) to support non-decremental conduction.

There is some variation in dendritic Na_v channel densities between cells. Pyramidal neurons have a low (but relatively constant) density of Na_v channels along the main apical dendrite (Stuart and Sakmann, 1994; Magee et al., 1995), while mitral cells in the olfactory bulb appear to have a higher density of dendritic Na_v channels and support more reliable backpropagation (Bischofberger and Jonas, 1997). Cerebellar Purkinje cells, in contrast, have a very low density of Na_v channels in their dendrites, and do not support active backpropagation of action potentials (Llinás and Sugimori, 1980a,b; Lasser-Ross and Ross, 1992; Stuart and Häusser, 1994). However, the correlation between dendritic Na_v channel density and backpropagation is not a strict one, as dopamine neurons of the substantia nigra have essentially non-decremental backpropagation, even though they have a lower apparent dendritic Na_v channel density than pyramidal cells (Häusser et al., 1995). Even within a class of neuron, cell-to-cell variation in action potential backpropagation can be considerable, as a result of the sensitivity of backpropagation to a variety of factors, including morphology, channel densities, and membrane potential (Golding et al., 2001; Larkum et al., 2001; Bernard and Johnston, 2003; Schaefer et al., 2003). Backpropagation may also be regulated within a given cell as a result of ongoing synaptic activity, neuromodulatory states, or long-term, activity-dependent plasticity (Hoffman and Johnston, 1999; Stuart and Häusser, 2001; Frick et al., 2004).

Effects of morphology on action potential backpropagation

The morphology of the dendritic tree can affect action potential backpropagation in the same way as has been previously shown for propagation of action potentials in axons, where diameter, tapering, and branching are important determinants of action potential propagation (Goldstein and Rall, 1974). Using simplified analytical solutions of action potential propagation, Goldstein and Rall demonstrated that branch points are particularly sensitive regions where action potentials can fail. They quantified branch point geometry using the geometric ratio (GR), defined as:

$$\text{GR} = \sum_i d_j^{3/2} / d_a^{3/2}$$

where d_a is the diameter of the cable along which an action potential is propagating (the “parent” branch) and d_j are the diameters of the i branches into which the action potential propagates (the “daughter” branches; Goldstein and Rall, 1974). This geometric ratio defines the impedance mismatch between the parent and daughter dendrites (Fig. 12.6A). If one assumes that the membrane properties are uniform and the branch is not near a termination point of a cable, the geometric ratio predicts the behavior of the action potential as it propagates across the branch. If $\text{GR} = 1$ (i.e., if the 3/2 power law is obeyed, and therefore the impedance is “matched”), the only effect on propagation is that the velocity decreases due to the smaller diameter of the distal branches. If $\text{GR} < 1$, a favorable impedance mismatch holds, and action potentials propagate efficiently (i.e., with less decrement; Fig. 12.6A1), as in cables with a step decrease in diameter. If $\text{GR} > 1$, the impedance mismatch is unfavorable, and action potentials propagate inefficiently (Fig. 12.6A2), as though they encounter a step increase in diameter, with active propagation failing completely for sufficiently high values of GR. The critical value depends on the density and kinetics of the Na_v and K_v channels in the different branches, as well as the passive membrane properties R_m , R_p , and C_m . Another way of expressing this is that the safety factor for action potential conduction decreases when an action potential propagates into branches that are just slightly smaller than, the same size as, or larger than the parent dendrite (Rall, 1964).

Similar considerations are expected to hold for action potentials propagating in dendritic trees. Because the safety factor for propagation of action potentials in dendrites is low to begin with (recall that backpropagation is decremental in pyramidal neurons), unfavorable impedance mismatches at branch points may result in failure of bAPs or dendritic spikes. Indeed, changes in the shape of bAPs in hippocampal dendrites have been observed (Spruston et al., 1995), which resemble the shape of action potentials propagating close to failure in axons (Lüscher et al., 1994). Furthermore, as discussed earlier, different types of neurons show very different degrees of backpropagation (Stuart et al., 1997b), which may be related to the striking differences in dendritic geometry shown by different cell types. To investigate the contribution of dendritic geometry, one study (Vetter et al., 2001) performed simulations in which the same complement of active and passive properties was inserted into detailed reconstructions of a large variety of cell types, thus isolating morphology as the only variable. Interestingly, the pattern of backpropagation in the different geometries matched the experimental findings, with dopamine neurons of the substantia nigra showing the least attenuation and Purkinje cells the most. Morphological analysis of the dendritic trees revealed that backpropagation was strongly correlated with the way in which membrane area was distributed in the dendritic tree, which is a function of both the number of branch points and the geometric ratio at individual branch points. This study (Vetter et al., 2001) also demonstrated that in very elaborate morphologies, such as Purkinje cells, backpropagation is insensitive to the Na_v channel density over the physiological range, in contrast to pyramidal cells.

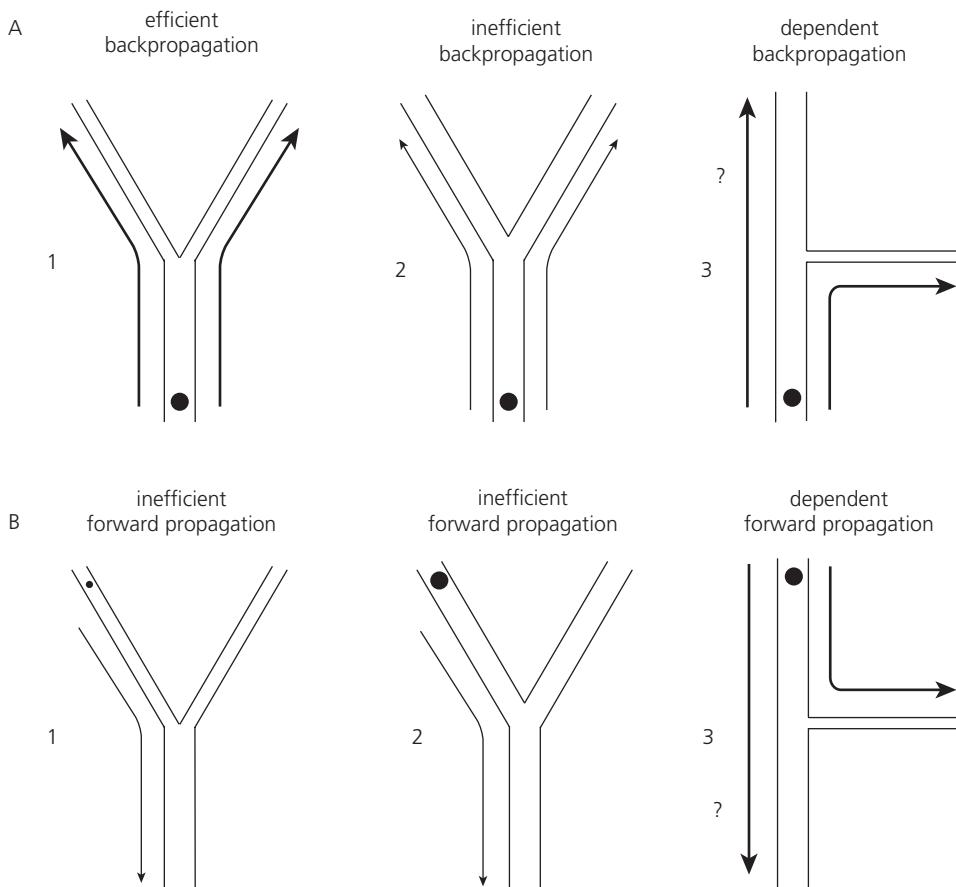


Fig. 12.6 Effects of dendritic branching on spike propagation. **(A)** Action potential backpropagation from a large parent dendrite (black dot) into two smaller daughter dendrites is efficient when $GR \leq 1$ (case A1; see text). Backpropagation is inefficient when the daughter dendrites are large, such that $GR > 1$ (case A2). Backpropagation past oblique branches (case A3) is dependent (denoted by ?) on the geometry and excitability of the oblique branch. If the branch is long and/or relatively inexcitable, it draws current away from the large dendrite, thus reducing backpropagation. If the branch is short and/or relatively excitable, the spike in the oblique branch can return current back to the main dendrite, thus increasing backpropagation. This latter effect is facilitated by synaptic depolarization of the oblique branch. **(B)** Dendritic spikes propagating from a small dendrite (black dot) into a larger one will have a tendency to fail. The smaller black dot indicates that less current is needed to produce a spike in a small-diameter dendrite (case B1). The lower input impedance of the larger branch will cause the membrane potential to drop (possibly below threshold for a spike) at the branch point. Even if a dendritic spike starts in a larger dendrite (case B2), it will tend to fail at branch points, as it is difficult for the current from one branch to bring both branches to threshold for a spike. For both of these cases, dendritic spike propagation will be more efficient if either or both of the downstream branches are depolarized by synaptic input. Propagation of dendritic spikes along a large apical dendrite will be influenced by oblique branches in a way that depends (?) on the excitability of the oblique branch (case B3). Long and/or inexcitable oblique branches will promote propagation failure, whereas shorter and/or more excitable branches will provide return current and promote successful propagation.

where modulation of Na_v or K_v channel density can produce a wide range of dendritic action potential amplitudes.

Other studies have demonstrated that even within a given class of neuron, variations in dendritic geometry can affect action potential backpropagation. For example, in CA1 pyramidal neurons, bAPs in distal dendrites are either relatively large (strong backpropagation) or small (weak backpropagation). The absence of intermediate amplitudes suggests that backpropagation can fail at a critical point in the dendritic tree, about 300 μm from the soma (Golding et al., 2001). Modeling studies indicate that failure of action potential backpropagation is sensitive not only to Na_v and K_v channel density but also to the number of dendritic branches in this region (Golding et al., 2001). Similarly, in neocortical L5 pyramidal neurons, the ability of somatic action potentials to influence the distal dendrites is variable and has been shown to be sensitive to the number of oblique dendrites branching from the main apical dendrite (Larkum et al., 2001; Schaefer et al., 2003). One view is that, depending on whether oblique branches are strongly or weakly excitable and whether or not they are excited or inhibited by synaptic input, they may either facilitate or limit action potential backpropagation (Fig. 12.6A3).

Together, these studies indicate that dendritic morphology, and in particular the branching pattern, is a major determinant of how dendrites will behave functionally, as predicted by Rall (Rall, 1964).

Effects of dendritic voltage-gated channels on action potential backpropagation

Non-uniform distributions of channels, as well as changes in the activation patterns of channels with activity, add a further layer of complexity to our understanding of action potential propagation in dendrites. For example, regional Na_v channel inactivation or non-uniform distributions of dendritic K_v channels can have significant effects on propagation. Hoffman and colleagues have shown that the density of A-type K_v channels in the apical dendrites of CA1 neurons increases as a function of distance from the soma; furthermore, A-type K_v channels in these cells have a lower activation voltage in the distal dendrites than in the soma and proximal dendrites (Hoffman et al., 1997). This channel distribution appears to contribute to a number of physiological features of CA1 neurons, including their relatively high threshold for dendritic spike initiation, and the decremental nature of action potential backpropagation (Hoffman et al., 1997; Frick et al., 2003).

Action potentials backpropagating into CA1 dendrites undergo marked amplitude attenuation during repetitive activity (Andreasen and Lambert, 1995b; Callaway and Ross, 1995; Spruston et al., 1995; Golding et al., 2001). A similar form of activity-dependent action potential backpropagation occurs in the distal regions of the apical dendrites of neocortical pyramidal neurons (Stuart et al., 1997a). This property of action potential backpropagation appears to be largely attributable to the inactivation properties of dendritic Na_v channels (Fig. 12.7A, B). Na^+ currents in cell-attached patches from CA1 pyramidal neurons undergo a form of inactivation that develops rapidly but recovers slowly (Colbert et al., 1997; Jung et al., 1997; Mickus et al., 1999). This prolonged inactivation is particularly pronounced in patches from the apical dendrite. As each action potential invades the dendrites it leaves a fraction of Na_v channels in a long-lived inactivated state, effectively reducing the density of available Na_v channels to support backpropagation of action potentials arriving even several hundred milliseconds later. Because the safety factor for action potential backpropagation is low (Vetter et al., 2001), owing to the relatively low Na_v channel density, high A-type K_v channel density, and extensive branching of CA1 dendrites, inactivation of even a small number of Na_v channels can significantly affect action potential backpropagation.

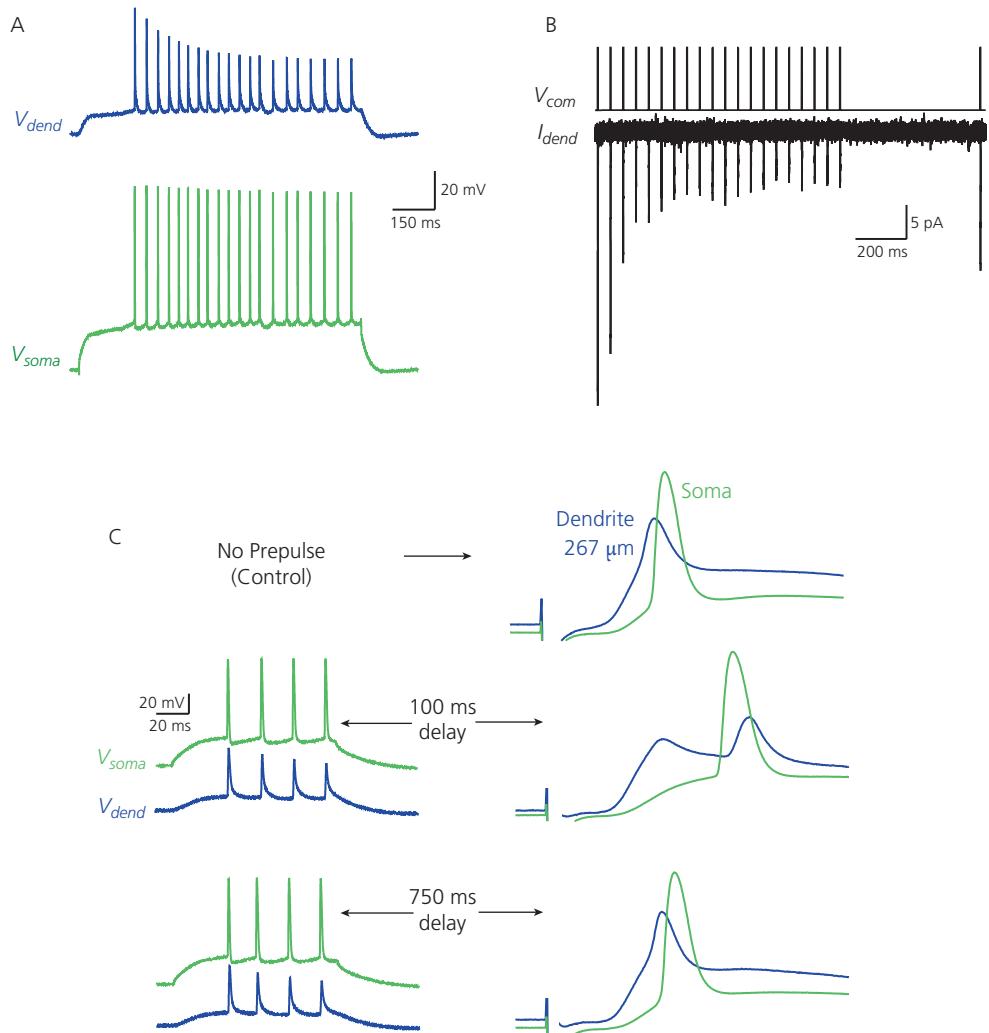


Fig. 12.7 Effects of prolonged Na^+ channel inactivation on bAPs and dendritic spikes in CA1 pyramidal neurons. **(A)** A train of action potentials evoked by somatic current injection (300 pA) and recorded simultaneously in the soma (green traces) and apical dendrite (blue traces, 200 μm from the soma). Repetitive action potential firing results in an activity-dependent decline in the amplitude of bAPs. **(B)** Dendritic Na^+ currents recorded in a cell-attached patch on an apical dendrite 203 μm from the soma. Brief depolarization (2 ms, 70 mV) via a command potential (V_{com} , relative to V_{rest}) delivered to the patch pipette evoked TTX-sensitive inward currents that accumulated in an inactivated state during the train, due to slow recovery from inactivation. A test pulse 500 ms after the train shows only 38% recovery from inactivation. **(C)** A depolarizing pre-pulse in a somatic recording (green traces) evokes four action potentials, which backpropagate into the dendrites, exhibiting activity-dependent amplitude attenuation in a simultaneous dendritic recording (blue traces, 267 μm from the soma). This pre-pulse of bAPs (left) suppressed dendritic spike initiation in response to synaptic stimulation in stratum radiatum when evoked less than 500 ms after the prepulse (right).

Part B adapted from Jung et al. (1997). Part C adapted from *Neuron*, 21(5), Nace L Golding and Nelson Spruston, Dendritic sodium spikes are variable triggers of axonal action potentials in hippocampal CA1 pyramidal neurons, pp.1189–1200, Copyright 1998, Elsevier. With permission from Elsevier.

In this way, prolonged inactivation of dendritic Na_v channels reduces action potential backpropagation and causes an activity-dependent decline in the amplitude of the action potential at a given dendritic recording site. This inactivation, together with unfavorable branching geometry, may also contribute to the failure of bAPs to invade some dendritic branches in CA1 neurons (Spruston et al., 1995).

The amplitude of bAPs is also affected by other factors during repetitive spiking. For example, natural spike trains propagate most reliably into dendrites during periods of high-frequency activity. This frequency-dependent amplification depends on temporal summation of bAPs and activation of distal dendritic Na_v channels (Williams and Stuart, 2000a). These effects are mimicked by depolarization and reversed by hyperpolarization, indicating that synaptic activity will also affect action potential backpropagation.

Effects of synaptic excitation and inhibition on action potential backpropagation

Synaptic excitation and inhibition are other important factors that have been shown to influence action potential backpropagation. In both CA1 and L5 pyramidal neurons, synaptic depolarization of the dendrites has been shown to facilitate the invasion of the apical dendrites by bAPs (Hoffman et al., 1997; Stuart and Häusser, 2001; Watanabe et al., 2002; Sjöström and Häusser, 2006), while synaptic activation of GABAergic inhibitory conductances in the dendrites limits action potential backpropagation (Tsubokawa and Ross, 1996; Perez-Garcia et al., 2006; Müllner et al. 2015). Similarly, in olfactory mitral cells, inhibition limits the spread of action potentials along the lateral dendrites (Lowe, 2002; Xiong and Chen, 2002). The details of synaptic control of bAPs are likely to be complex. For example, in addition to simply inhibiting dendritic excitability, hyperpolarization associated with inhibition could, if appropriately targeted and timed, increase the recovery of dendritic Na_v channels from the slow inactivated state, thus increasing the amplitude of bAPs (Spruston et al., 1995; Colbert et al., 1997; Jung et al., 1997). On the other hand, hyperpolarization could decrease excitability by removing inactivation of A-type K_v channels (Hoffman et al., 1997). Thus, the effects of hyperpolarization may be complex, and dependent on the previous firing history of the neuron.

Dendritic spike propagation

In some neurons, such as mitral cells of the olfactory bulb (Chen et al., 1997; Djurisic et al., 2004; Urban and Castro, 2005) and hippocampal CA2 pyramidal neurons (Sun et al., 2014), dendritic spike propagation is reliable and robustly triggers an action potential in the axon. In other neurons, however, such as the widely studied hippocampal CA1 pyramidal neurons, neocortical L5 pyramidal neurons, and cerebellar Purkinje neurons, the propagation of dendritic spikes is unreliable. Several observations suggest that dendritic spikes propagate poorly in these neurons. First, the amplitude of dendritic spikes is smaller than the somatically recorded action potential, even when the dendritic spike occurs first. Second, dendritic spikes are sometimes observed in isolation from somatic action potentials in both hippocampal and neocortical pyramidal neurons (Fig. 12.5A; Stuart et al., 1997a; Golding and Spruston, 1998; Golding et al., 2002). Third, imaging studies show that Ca^{2+} signals associated with distal calcium spikes can remain localized to their site of origin in pyramidal cells and Purkinje cells, with little or no calcium signal spreading to the soma (Miyakawa et al., 1992; Eilers et al., 1995; Hartell, 1996; Schiller et al., 1997, 2000; Wei et al., 2001; Ariav et al., 2003; Polsky et al., 2004). Fourth, small (<20 mV), spike-like events have been recorded in somata of CA1 pyramidal neurons in response to synaptic stimulation. These events, which are

observed frequently during perisomatic application of TTX to eliminate firing of axonal action potentials, correspond to much larger spikes recorded simultaneously in the dendrites (Wong and Stewart, 1992; Golding et al., 2002; Jarsky et al., 2005). A similar observation has been made in retinal ganglion cells (Oesch et al., 2005). Fifth, local uncaging of glutamate on pyramidal neuron dendrites produces dendritic spikes, as well as nonlinear increases in dendritic calcium and somatic dV/dt , even in the absence of somatic action potentials (Wei et al., 2001; Ariav et al., 2003; Gasparini and Magee, 2006; Losonczy and Magee, 2006). Finally, triple recordings from the axon, soma, and apical dendrite of the same neocortical pyramidal neuron indicate that the axonal action potential always precedes the somatic action potential, even when the dendritic spike precedes the somatic action potential (Fig. 12.5E; Stuart et al., 1997a). These observations suggest that dendritic spikes do not propagate reliably to the soma and axon of neocortical and hippocampal pyramidal neurons. As a consequence, the dendritic spike-mediated depolarization that reaches the soma is sometimes small. In some cases the somatic depolarization due to the EPSP and dendritic spike together are large enough to initiate an axonal action potential, whereas in other cases the EPSP and dendritic spike together produce a subthreshold depolarization in the soma and axon, resulting in an isolated dendritic spike (Fig. 12.5A).

Even though dendritic calcium spikes are typically broader than dendritic sodium spikes, they can also fail as they propagate toward the soma in pyramidal neurons (Schiller et al., 1997; Golding et al., 2002). In fact, calcium spikes are rarely observed in somatic recordings, but they can influence action potential initiation by promoting action potential bursting (Schiller et al., 1997; Golding et al., 1999b; Larkum et al., 1999b; Williams and Stuart, 1999), as originally described in cerebellar Purkinje cells (Llinas and Nicholson, 1971; Llinas and Sugimori, 1980a).

Whether or not dendritic spikes propagate successfully to the soma depends on a number of factors, including dendritic geometry, channel densities, and the spatial and temporal profile of synaptic excitation and inhibition (Segev and Rall, 1998). Dendritic excitability is also affected by previous activity and neuromodulation, thus making the process of dendritic spike initiation and propagation just as complex as action potential backpropagation.

The morphology of the dendritic tree clearly plays an important role in determining this behavior. Small-diameter dendritic branches have higher input impedances than large-diameter branches and may therefore be depolarized to threshold for a dendritic spike by relatively small synaptic conductances (Losonczy and Magee, 2006; Nicholson et al., 2006; Katz et al., 2009). However, the fact that dendritic spike propagation is generally unreliable in pyramidal neurons is also likely to be largely attributable to dendritic geometry, particularly at branch points. As discussed above, spike propagation through branch points depends on the geometric ratio of the parent and daughter dendrite diameters (Goldstein and Rall, 1974; Jack et al., 1983). Spikes that begin in small dendritic branches will have a tendency to fail as they propagate into larger branches (Fig. 12.6B1). Dendritic spikes must frequently traverse such a geometric arrangement as they propagate toward the soma in neurons such as pyramidal cells and Purkinje cells. By contrast, bAPs tend to propagate from large branches into smaller ones (Fig. 12.6A1). Thus, the geometry of the dendritic tree is generally less favorable for forward propagation than for backpropagation.

The reason that dendritic spikes tend to fail as they propagate from small (high-impedance) to large (low-impedance) dendrites is that more current is required to depolarize the larger branch to threshold. Even if a spike begins in a relatively large dendritic branch, it will tend to fail when it must charge two dendrites of similar diameter at a branch point (Fig. 12.6B2). Thus, the propagation of dendritic spikes will be greatly influenced by the synaptic activation of neighboring dendritic branches. Consider, for example, a branch point with one large parent dendrite that gives rise

to two smaller daughter branches. If a spike initiates in one of the daughter branches, it will tend to fail as it propagates into the larger parent dendrite (Fig. 12.6B1). If the spike originates at the same time in both daughter branches, however, forward propagation will be more effective, because there is a larger current source to depolarize the parent dendrite (not illustrated in Fig. 12.6). This mechanism has been suggested to lead to reliable propagation of dendritic spikes in L5 pyramidal neurons and hippocampal CA2 pyramidal neurons, where spikes in multiple apical branches can converge at a proximal branch point (Larkum et al., 2009; Piskorowski and Chevaleyre, 2011; Sun et al., 2014). Even if the spike is restricted to one of the daughter branches, synaptic depolarization of the other daughter branch and/or the parent branch will also increase the likelihood of successful forward propagation; synaptic inhibition, on the other hand, would have the opposite effect (Jarsky et al., 2005).

The effect of radial oblique branches on dendritic spike propagation in pyramidal neurons depends on the origin of the spike and the excitability of the oblique branches. For spikes propagating down the main apical dendrite, oblique branches can act to reduce or facilitate forward propagation of the spike, depending on whether the oblique branch is strongly or weakly excitable (Fig. 12.6B3). Synaptic excitation of the oblique branch would tend to favor propagation, while inhibition would reduce the forward propagating spike. On the other hand, dendritic spikes beginning in oblique branches will tend to fail as they propagate into the large-diameter main apical dendrite (not shown, but similar to Fig. 12.6B1).

Though dendritic geometry may be partly responsible for the poor forward propagation of dendritic spikes, including the occurrence of isolated dendritic spikes (Schiller et al., 1997; Stuart et al., 1997a; Golding and Spruston, 1998), other factors are likely to be important as well. For example, action potential backpropagation may limit the propagation of subsequent dendritic spikes to the soma of CA1 neurons (Golding and Spruston, 1998; Remy et al., 2009), due to inactivation of dendritic Na_v channels by the bAP (Fig. 12.7C). Non-uniform K_v channel distribution may be another important factor. Although the distribution of dendritic A-type K_v channels (Hoffman et al., 1997) probably has the reverse gradient required to explain the poor forward propagation of dendritic spikes, other K_v channels may also be distributed non-uniformly (e.g., Andreasen and Lambert, 1995a). The distributions and effects of a variety of K_v channel subtypes still need further investigation. Interestingly, mitral cells appear to display much better forward propagation than pyramidal cells (Chen et al., 1997) under similar experimental conditions, presumably due to the uniform and unbranching nature of the main apical dendrite in these neurons.

A question of significant importance is whether the propagation of action potentials (back-propagation) and dendritic spikes (forward propagation) *in vivo* is similar to the situation studied *in vitro*. Although this question cannot yet be clearly answered, some evidence is beginning to emerge. This subject is considered in detail in Chapter 13. Furthermore, it should be noted that all of the factors discussed here are subject to modulation by activity, neurotransmitters, and various kinds of plasticity (Tsubokawa and Ross, 1997; Colbert and Johnston, 1998; Hoffman and Johnston, 1999; Williams, 2004; Magee and Johnston, 2005; Johnston and Narayanan, 2008; Losonczy et al., 2008; Makara and Magee, 2013). Even dendritic structure is not static (see Chapter 19), indicating that the effects of morphological changes on action potential and dendritic spike propagation must be considered.

Effects of inhibition on dendritic spikes

Synaptic inhibition has been shown to influence dendritic sodium and calcium spikes. In both hippocampal CA3 and neocortical L5 pyramidal neurons, early work showed that inhibition can prevent, delay, or shorten dendritic spikes, depending on its timing and strength (Kim et al., 1995;

Miles et al., 1996; Larkum et al., 1999b). In hippocampal neurons, the effects of inhibition on spike firing depend on the location of the inhibitory input. Dendritic inputs inhibit dendritic spikes, whereas perisomatic inhibition suppresses repetitive discharge of somatic action potentials (Miles et al., 1996). Computational modeling suggests that the location of dendritic inhibition determines whether it affects dendritic spike initiation or spike amplitude (Jadi et al., 2011). However, experimental work indicates that dendritic inhibition may primarily affect dendritic spike initiation and that the effects of dendritic inhibition can be overcome by activity-dependent increases in dendritic branch excitability (Lovett-Barron et al., 2012; Müller et al., 2012). In L5 pyramidal neurons, distal dendritic inhibition has been shown to inhibit calcium spikes not only through hyperpolarization and shunting, but also by GABA_B receptor-mediated inhibition of dendritic Ca_v channels (Perez-Garci et al., 2006; Murayama et al., 2009; Breton and Stuart, 2012; Palmer et al., 2012). The effects of inhibition on dendritically generated spikes have also been explored in mitral cells of the olfactory bulb, where perisomatic inhibition can prevent dendritic sodium spikes from invading the soma and axon (Chen et al., 1997).

While we have discussed the effects of dendritic inhibition for passive dendrites (or in active dendrites below the threshold for engaging nonlinearities), the effects of dendritic inhibition can be quite different in active dendrites. When an active dendritic “hot spot” exists—either because of clustered excitatory synaptic input (containing NMDA receptors) or clustered Na_v or Ca_v channels—dendritic inhibition more effectively prevents dendritic spike generation if it is distal to the hotspot than if it is proximal to the hotspot (Gidon and Segev, 2012). This effect, which is described in more detail in Chapter 15, results largely from the greater shunting effect of a given inhibitory conductance, relative to the local input conductance, for distal versus proximal inhibition. However, just as inhibition proximal to the soma more effectively shunts somatic EPSPs in passive dendrites, once a spike is generated at a dendritic hotspot, proximal inhibition more effectively reduces the propagation of the spike toward the soma (Gidon and Segev, 2012).

What are the functions of dendritic excitability?

Backpropagating action potentials and dendritic spikes can serve a number of functions. One immediate effect is elevation of intra-dendritic Ca²⁺ concentration, via activation of Ca_v channels and removal of Mg²⁺ block at glutamate-bound NMDA receptors (see Chapter 8). This can in turn result in release of neurotransmitters or other substances from dendrites (see Chapter 21) or induction of synaptic plasticity (see Chapter 18). Another important function of dendritic excitability is to change the way synaptic inputs affect dendritic membrane potential, thus affecting the way that synaptic inputs lead to action potential initiation. This aspect of dendritic excitability is discussed in detail in this section.

Subthreshold amplification of EPSPs by Na_v and Ca_v channels and NMDA receptors

Depending on the types, densities, and distributions of channels in dendrites, PSPs may be amplified and shaped in subtle ways by voltage-gated conductances. For example, Na_v and/or Ca_v channels and NMDA receptors can amplify EPSPs without generating a spike. One of the first indications that voltage-gated conductances may amplify EPSPs in this manner was the observation that a marked increase in the amplitude and integral of EPSPs was observed when pyramidal neurons were held at depolarized potentials (Masukawa and Prince, 1984; Stafstrom et al., 1985; Thomson et al., 1988; Deisz et al., 1991). A similar voltage-dependent amplification was observed

in response to brief somatic current injections used to mimic EPSPs (Deisz et al., 1991). Stuart and Sakmann extended this idea by comparing the voltage dependence of EPSPs and EPSP-like depolarization evoked by injecting current in the shape of an excitatory postsynaptic current (EPSC) through a dendritic recording electrode (Stuart and Sakmann, 1995). They found that the voltage-dependent amplification of both real and “simulated” EPSPs was blocked by application of TTX. Because the activation of Na_v channels occurred in a voltage range below the threshold for action potential initiation, and because the effect of TTX on the EPSP integral was larger than on the peak, the amplification was interpreted to be due to activation of a non-inactivating, or persistent, Na_v conductance. Furthermore, because the amplification of EPSPs was greatest in the soma, and indeed the axon, and selectively blocked by local application of TTX to the soma and axon, it appears that this form of amplification occurs primarily due to activation of persistent Na^+ current in the axon (Stuart and Sakmann, 1995). In CA1 pyramidal neurons, distally generated EPSPs can be amplified by perisomatic persistent Na^+ current (Andreasen and Lambert, 1999), and other experiments also indicate that persistent Na^+ current is generated primarily in the axon (Astman et al., 2006).

Interestingly, IPSPs can also be modulated by axonal persistent Na^+ current in a complementary manner (Stuart, 1999). IPSPs turn off the persistent Na^+ current, producing a net outward current, which increases the amplitude and duration of the IPSPs. At the soma and axon this increases the ability of inhibition to block action potential generation (Stuart, 1999), whereas in the dendrites it could enhance the ability of synaptic inhibition to block dendritic spike generation and propagation. The same mechanism that allows persistent Na^+ current to amplify IPSPs also causes it to enhance the after-hyperpolarization, thus affecting spike rate and timing (Vervaeke et al., 2006).

The voltage-dependent boosting effects described above are generated primarily by axosomatic channels. However, the dendrites of mammalian neurons are richly endowed with voltage-gated channels that also can contribute to boosting effects, particularly for larger and more prolonged synaptic inputs. In the hippocampus, dendritic patch-clamp recordings from CA1 neurons have shown directly that Na_v and Ca_v channels can be activated by EPSPs (Magee et al., 1995). Furthermore, blockers of Na_v and low-threshold Ca_v channels have been shown to reduce the amplitude and duration of EPSPs measured at the soma in CA1 and CA3 pyramidal neurons (Lipowsky et al., 1996; Gillessen and Alzheimer, 1997; Urban et al., 1998). However, the most powerful contribution to dendritic nonlinearities triggered by synaptic input is via the NMDA receptor channels, which are found at most excitatory synapses. The regenerative nature of NMDA current, caused by the voltage-dependent Mg^{2+} block of the channel and the consequent region of negative slope of the I - V relationship, can lead to a number of different effects, depending on the level of depolarization (Schiller and Schiller, 2001; Poirazi et al., 2003a; Major et al., 2013). A mild depolarization can lead to graded inward current, causing boosting of EPSPs; more depolarization can lead to bistability of membrane potential; and strong depolarization can lead to full-blown spikes with a distinct threshold, which have been termed “NMDA spikes” (Schiller et al., 2000; Major et al., 2013). Two-photon glutamate uncaging experiments have allowed the contribution of different voltage-gated channels to subthreshold dendritic amplification of EPSPs to be quantitatively evaluated in neocortical pyramidal cells (Gasparini and Magee, 2006; Losonczy and Magee, 2006; Branco and Häusser, 2010, 2011). This work has shown that while blockers of voltage-gated Na_v and Ca_v channels can reduce amplification of EPSPs, blocking NMDA receptors can entirely abolish amplification. This indicates that the NMDA receptor channel nonlinearity is both necessary and sufficient to trigger regenerative boosting of EPSPs, with the other channel types adding additional regenerative inward current. The recruitment of

the NMDA receptor nonlinearity depends critically on the temporal and spatial properties of the synaptic inputs: when the synaptic input arrives asynchronously or is highly distributed in space, then integration is linear; it is only when input is near-synchronous and clustered that strong nonlinearities are recruited (Gasparini and Magee, 2006). Interestingly, applying the same input at different locations along the same dendrite changes the steepness of the resulting nonlinear input–output curve, which in turn depends on NMDA receptor activation (Branco and Häusser, 2011). This suggests that the degree to which dendritic voltage-gated channels amplify EPSPs depends on the location of the activated synapses, with distal inputs exhibiting a higher gain for amplification than proximal inputs. Ironically, these differences in active properties are a consequence of the passive electrotonic structure of the dendrite, with the passive impedance gradient along the dendrite “helping” to recruit active nonlinearities for inputs made toward the end of a dendrite (Branco and Häusser, 2010, 2011).

Subthreshold attenuation by K_v channels

The effects of K_v channel activation on EPSPs must also be considered. Support for activation of voltage-gated K_v channels in response to subthreshold EPSPs comes from a variety of experiments. Block of K_v channels with 4-aminopyridine (4-AP) increases dendritic EPSP amplitude in hippocampal pyramidal neurons (Hoffman et al., 1997) and can convert sublinear summation to supralinear summation in hippocampal pyramidal neurons (Cash and Yuste, 1998, 1999). Other experiments indicate that the effects of dendritic voltage-gated channels depend on the timing of summated EPSPs (Margulis and Tang, 1998): when two inputs were activated at an interval of less than 10 ms, the second input was amplified by a TTX-sensitive conductance. At slightly longer intervals (15–100 ms) the second input was depressed by a K_v conductance. Experiments using stimulation of perforant path (PP) and mossy fiber (MF) inputs at different time intervals support a similar conclusion regarding K_v channel activation in CA3 neurons (Urban and Barrioñuevo, 1998). In these experiments, activation of MF synapses shunted perforant path EPSPs when MF stimulation preceded PP stimulation by less than 20 ms. This effect was voltage dependent and blocked by intracellular 4-AP, suggesting that the depolarization by the MF EPSP activates A-type K_v channels, which then shunts the PP EPSP propagating from the distal dendrites.

The current view concerning hippocampal pyramidal neurons is that the effect of activation of Na_v and Ca_v channels by subthreshold EPSPs is dampened by activation of K_v channels, particularly the A-type K_v channel which is present at high density in CA1 apical dendrites (Hoffman et al., 1997). However, the generation of dendritic sodium spikes in CA1 neurons suggests that dendritic Na_v channel activation can tip this balance, particularly during synchronous synaptic excitation (Golding and Spruston, 1998; Ariav et al., 2003; Gasparini and Magee, 2006; Losonczi and Magee, 2006).

EPSP shunting by backpropagating action potentials

Backpropagating action potentials can also interact with synaptic potentials. The conductances necessary to generate the action potential are large, and therefore generate a substantial drop in apparent membrane resistance, which is localized largely to the axon and soma. This shunt effectively shortens the membrane time constant in these regions, draining charge from the membrane capacitance. In this way, action potentials can reduce the amplitude of EPSPs and IPSPs, thus providing a mechanism for terminating ongoing synaptic integration. In neocortical L5 pyramidal cells, somatic EPSPs generated by basal synaptic inputs can be attenuated by up to 80% by a

single action potential (Häusser et al., 2001). The degree to which action potentials shunt synaptic potentials depends not only on the magnitude of the local conductances activated by the action potential but also on the location and kinetics of the synaptic conductance. As a consequence, synaptic potentials generated by changes in synaptic conductance with a slow time course (e.g., those mediated by NMDA receptors) are less sensitive to shunting by relatively brief action potentials. In addition, more distal inputs are shunted less, as they are further away from the conductance change generated during the action potential, and shunting of dendritic synaptic events locally is small due to the relatively low density of dendritic conductances activated during action potential backpropagation (Häusser et al., 2001).

Dendritic spikes and synaptic integration

The possibility that dendrites might generate spikes has presented a conundrum for understanding synaptic integration: if spikes can be generated in dendrites, the integrative power of the dendritic tree would appear to be minimized, because many of the spatial and temporal interactions involving excitation and inhibition would be negated by the generation of a dendritic spike in response to a small number of excitatory inputs. A possible solution to this puzzle was presented as early as 1959, when Lorente de Nó suggested that decremental conduction of dendritic spikes might play an important role in dendritic integration in the CNS (Lorente De Nó and Condouris, 1959). In the scenario he envisioned, spikes could be generated in dendrites, but would not propagate reliably to the soma. The effect of dendritic spikes would therefore be to increase the depolarization associated with some synaptic inputs, but would not necessarily trigger an action potential. Jack et al. (1983) also pointed out that an obvious possible function of this kind of restricted dendritic spike would be to amplify synaptic potentials, thus increasing the likelihood that a combination of synapses that evoke a dendritic spike will eventually result in an output from the neuron via generation of an action potential in the axon. As already discussed, in the above section (Propagation of action potentials and dendritic spikes) there is now good experimental evidence in support of this idea. In some cases the additional somatic depolarization associated with the dendritic spike brings the membrane potential above the threshold for a somatic/axonal action potential. Thus, dendritic spikes can contribute to action potential firing, but they are not absolute determinants of it.

One prominent view of the pyramidal neuron is that individual dendritic branches may operate as computational subunits, each of which is capable of generating dendritic spikes. Whether or not these spikes result in the firing of an action potential depends on integration of these subunit responses to determine if a somatic/axonal action potential will be generated. This idea was first advanced on theoretical grounds (Archie and Mel, 2000; Poirazi et al., 2003a), but now has considerable experimental support (see Chapter 16). Dendritic spikes can be triggered by clustered or distributed inputs to a single dendritic branch, but the same number of inputs distributed to multiple branches are less effective (Ariav et al., 2003; Polsky et al., 2004; Gasparini and Magee, 2006; Losonczy and Magee, 2006; Larkum and Nevian, 2008; Branco and Häusser, 2010; Debello et al., 2014) (Fig. 12.8A–D). This view has led to the idea that a pyramidal neuron can be treated as a two-layer network with integration by individual branches serving as one layer and integration of multiple branch responses serving as a second layer (Häusser and Mel, 2003; Poirazi et al., 2003b; Katz et al., 2009; Jadi et al., 2014). This idea could be extended to treat each pyramidal neuron as a multi-layer network, for example with integration by the apical tuft, apical oblique, and basal dendritic branches acting as intermediate layers in this scheme (Spruston and Kath, 2004; London and Häusser, 2005; Larkum et al., 2009) (Fig. 12.8E). Whether this notion accurately captures the

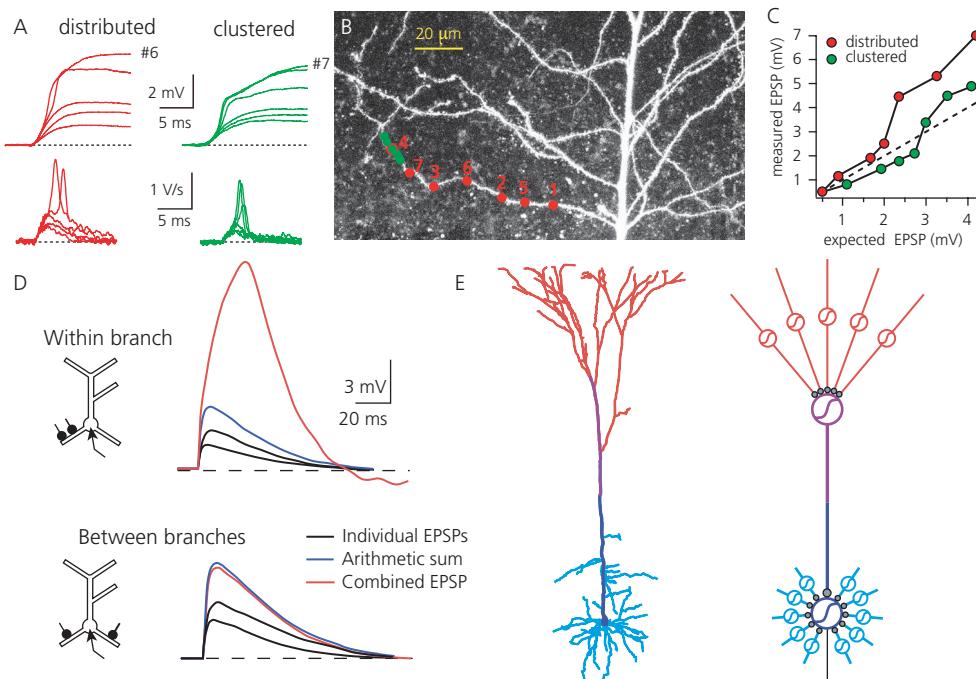


Fig. 12.8 Dendritic spikes in individual dendritic branches suggest a multi-layer model of dendritic integration. **(A)** Somatic voltage recordings in response to uncaging glutamate at a progressively increasing number of locations for distributed (red) and clustered (green) configuration with a 0.1-ms interval. The lower traces represent the corresponding temporal derivatives. Dashed lines across the temporal derivatives indicate the subthreshold dV/dt levels. **(B)** Image of the apical dendritic region of a CA1 pyramidal neuron showing the positions of the seven spines on a radial oblique branch for clustered (green dots) and distributed (red dots) experimental arrangements. Associated numbers indicate the temporal sequence of distributed locations during uncaging with a 0.1-ms interval. **(C)** Plot showing measured versus expected amplitudes of glutamate-evoked potentials for the clustered and distributed recordings shown in A and B. **(D)** Schematic representation of the main finding of Polsky et al. (2004): two multi-synaptic inputs onto a single dendritic branch exhibit supralinear summation of somatic EPSPs (top). Inputs onto separate branches exhibit roughly linear summation (bottom). **(E)** Reconstructed L5 pyramidal neuron (left) and an abstracted three-layer network model (right; based on Häusser and Mel, 2003). Red branches represent the distal apical inputs; light blue branches represent the perisomatic inputs. Together, these inputs constitute the first layer of the network model, each performing supralinear summation of the type shown in part A (indicated by small circles with sigmoids). The outputs of this first layer feed into two integration zones: one near the perisomatic branches (e.g., soma, dark blue) and one near the distal apical branches (e.g., apical spike initiation zone, purple). These integration zones constitute the second layer of the network model (large circles with sigmoids). The third layer (not shown) is the action potential initiation zone in the axon. Gray circles indicate connections between layers.

Parts A–C are adapted from Neuron, 50, Losonczy A and Magee JC, Integrative properties of radial oblique dendrites in hippocampal CA1 pyramidal neurons, 291–307, Copyright (2006), with permission from Elsevier. Parts D and E adapted with permission from Macmillan Publishers Ltd: *Nature Neuroscience*, 7(6), Nelson Spruston and William L Kath, Dendritic arithmetic, pp.567–569, © 2004, Nature Publishing Group.

computational function of neurons remains to be determined, but there is certainly good evidence that synaptic inputs targeting different dendritic compartments can be integrated in interesting ways. For example, in CA1 pyramidal neurons, activation of the perforant-path inputs, which target the distal apical tuft exclusively, can lead to dendritic spikes whose propagation to the soma and axon is facilitated by activation of Schaffer collateral inputs targeting more proximal apical dendrites (Jarsky et al., 2005) (Fig. 12.9A–C).

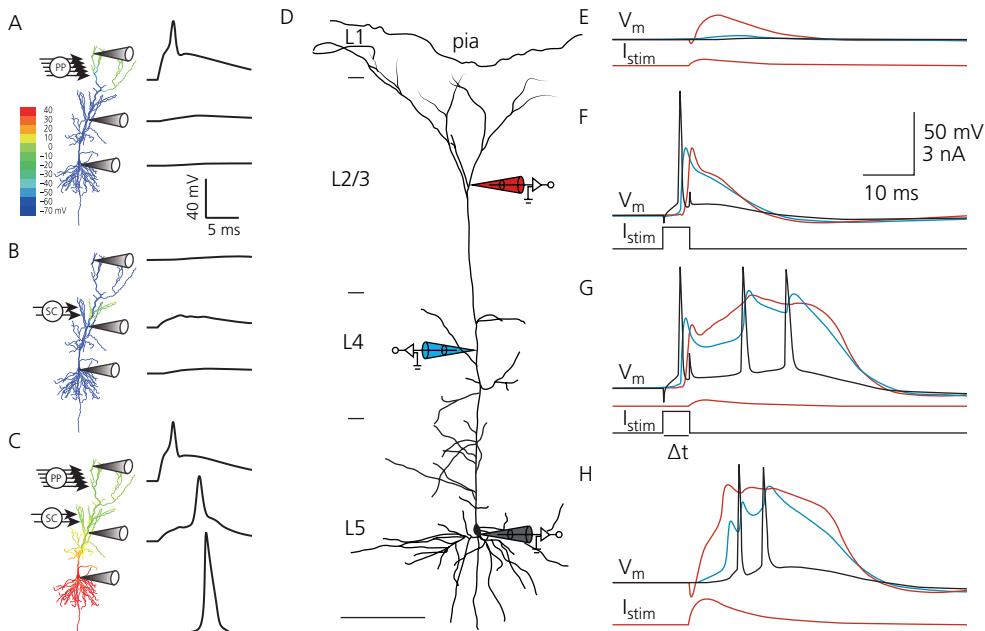


Fig. 12.9 Gating of dendritic spike propagation by synaptic depolarization. (A) Color map of peak depolarization and voltage versus time plots at three dendritic locations for activation of 10% of perforant path (PP) synapses (one trial) in a CA1 pyramidal neuron model with weak dendritic excitability. (B) Response of the same model to activation of 3% of Schaffer collateral (SC) synapses in the upper apical dendrites. (C) Response of the same model to coincident activation of 10% of PP and 3% of SC synapses leading to AP output. (D) Reconstruction of a biocytin-filled pyramidal neuron, with the recording pipette positions shown symbolically (770 μ m from soma in red, 400 μ m from soma in blue, and one at the soma in gray). Cortical layers are indicated on the left. Scale bar = 200 μ m. (E) EPSP-like current injection (I_{stim} , 0.3 nA peak) at the distal pipette (red trace, bottom) produced a subthreshold somatic voltage response (V_m) of only 1.4 mV. Line color indicates the corresponding electrode in the diagram. (F) Threshold current injection at the soma (I_{stim}) evoked an action potential that was reduced in amplitude but increased in width in the dendrite. (G) The combination of the injections of current used in parts E and F separated by an interval of 5 ms (Δt) evoked a burst of action potentials. Scale bars in part C also apply to B and D. (H) A similar dendritic calcium spike could be evoked by injection of a larger (1.2 nA) current alone at the distal dendritic electrode.

Part A–C adapted with permission from Macmillan Publishers Ltd: *Nature Neuroscience*, 8(12), Tim Jarsky, Alex Roxin, William L Kath, and Nelson Spruston, Conditional dendritic spike propagation following distal synaptic activation of hippocampal CA1 pyramidal neurons, pp. 1667–1676, © 2005, Nature Publishing Group. Parts D–H are adapted from Larkum et al. (1999).

Action potential bursting and dendritic excitability

Another way that dendritic excitability can contribute to synaptic integration is by promoting action potential burst firing. Dendritic calcium spikes activated by strong synaptic excitation can lead to burst firing in hippocampal and neocortical pyramidal neurons (Schwartzkroin and Slawsky, 1977; Wong and Stewart, 1992; Schiller et al., 1997; Stuart et al., 1997a; Golding et al., 1999b), thalamo-cortical relay cells (Destexhe et al., 1998), cerebellar Purkinje cells (Llinas and Sugimori, 1980a; Llinas, 1988), and neocortical interneurons (Goldberg et al., 2004). The reason for this is simply that propagation to the soma of the more prolonged inward current generated during dendritic calcium spikes can trigger multiple action potentials in the axon and soma. In addition, however, modeling studies have suggested that the backpropagation of action potentials from the soma into the dendrites can promote bursting in neurons with excitable dendritic trees (Pinsky and Rinzel, 1994; Mainen and Sejnowski, 1996).

Backpropagating action potentials can contribute to bursting in multiple ways. First, a bAP can generate current that flows back to the soma to contribute to an afterdepolarization (ADP), which can promote bursting (Lemon and Turner, 2000). In neocortical L5 pyramidal neurons, such a mechanism depends on the recruitment of dendritic Ca_v channels by bAPs (Williams and Stuart, 1999). In CA1 pyramidal neurons, bursting via this mechanism is limited by A-type and D-type K_v channels in the apical dendrites, as the ADP and bursting are increased following block of these channels (Magee and Carruth, 1999; Metz et al., 2007).

A second mechanism by which bAPs can promote bursting is by triggering calcium spikes in the dendrites, as first suggested in early models of CA3 pyramidal neurons (Traub et al., 1991, 1994). In L5 pyramidal neurons, high-frequency action potential firing alone can trigger dendritic calcium spikes in both apical and basal dendrites (Larkum et al., 1999a; Kampa and Stuart, 2006). bAPs can also facilitate the initiation of distal dendritic calcium spikes by synaptic input, resulting in bursts of action potentials at the soma (Larkum et al., 1999b, 2001, 2004; Stuart and Häusser, 2001) (Fig. 12.9D–H). This interaction between bAPs and dendritic calcium spikes can greatly increase the impact of distal synaptic excitation during ongoing network activity (Williams, 2005) and can also contribute to synaptic plasticity, as dendritic calcium spikes very effectively relieve Mg^{2+} block of dendritic NMDA receptors (Kampa et al., 2006; Letzkus et al., 2006).

Concluding remarks

While it has been clear since the end of the nineteenth century that dendrites come in all shapes and sizes (see Chapter 1), research has shown that this diversity in structure is also associated with diversity in active and passive membrane properties. Voltage-gated channels have been found in the dendrites of all neurons examined to date, with cell-specific differences in the types, properties, and distributions of these channels (Chapter 9). Furthermore, passive membrane properties differ between neuronal types, and even at different locations within single neurons. Together, these differences impart richness to synaptic integration that was unimaginable in the 1950s, when dendrites were regarded by some as more of a nuisance than an asset (see Chapter 14).

The circumstances under which synaptic activation of dendritic voltage-gated channels causes a departure from the passive behavior of dendrites, and thus help define the input–output relation of neurons, remains a matter of intense study, especially *in vivo* (see Chapter 13). Strong evidence indicates that bAPs and dendritically initiated spikes occur in many cell types, where they may mediate functions such as dendritic neurotransmitter release (Chapter 21) and induction of synaptic plasticity (Chapter 18), as well as having a direct influence over the process of synaptic integration,

which is ultimately responsible for the generation of action potentials in the axon. Generalization about the function of dendritic excitability across cell types is made difficult by the wide range of behaviors observed in different neurons. This diversity reflects complexity in the mosaic of active channels and the variety of dendritic morphologies, which is likely related to the different functional roles of individual neurons in their respective networks. Only by studying this diversity and its consequences can we better understand the way in which individual neurons are tuned to perform their particular computational tasks.

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References

- Agmon-Snir H, Carr CE, Rinzel J (1998) The role of dendrites in auditory coincidence detection. *Nature* **393**:268–272.
- Alle H, Geiger JRP (2006) Combined analog and action potential coding in hippocampal mossy fibers. *Science* **311**:1290–1293.
- Amitai Y, Friedman A, Connors BW, Gutnick MJ (1993) Regenerative activity in apical dendrites of pyramidal cells in neocortex. *Cerebral Cortex* **3**:26–38.
- Andersen P (1960) Interhippocampal impulses. II. Apical dendritic activation of CA1 neurons. *Acta Physiologica Scandinavica* **48**:178–208.
- Andersen P, Andersson SA, Lomo T (1966) Mode of activation of hippocampal pyramidal cells by excitatory synapses on dendrites. *Experimental Brain Research* **2**:247–260.
- Andrasfalvy BK, Magee JC (2001) Distance-dependent increase in AMPA receptor number in the dendrites of adult hippocampal CA1 pyramidal neurons. *Journal of Neuroscience* **21**:9151–9159.
- Andrasfalvy BK, Smith MA, Borchardt T, Sprengel R, Magee JC (2003) Impaired regulation of synaptic strength in hippocampal neurons from GluR1-deficient mice. *Journal of Physiology* **552**:35–45.
- Andreasen M, Lambert JD (1995a) The excitability of CA1 pyramidal cell dendrites is modulated by a local Ca(2+)-dependent K(+)-conductance. *Brain Research* **698**:193–203.
- Andreasen M, Lambert JD (1995b) Regenerative properties of pyramidal cell dendrites in area CA1 of the rat hippocampus. *Journal of Physiology* **483**:421–441.
- Andreasen M, Lambert JD (1998) Factors determining the efficacy of distal excitatory synapses in rat hippocampal CA1 pyramidal neurones. *Journal of Physiology* **507**:441–462.
- Andreasen M, Lambert JD (1999) Somatic amplification of distally generated subthreshold EPSPs in rat hippocampal pyramidal neurones. *Journal of Physiology* **519**:85–100.
- Angelo K, London M, Christensen SR, Häusser M (2007) Local and global effects of I(h) distribution in dendrites of mammalian neurons. *Journal of Neuroscience* **27**:8643–8653.
- Archie KA, Mel BW (2000) A model for intradendritic computation of binocular disparity. *Nature Neuroscience* **3**:54–63.
- Ariav G, Polksy A, Schiller J (2003) Submillisecond precision of the input-output transformation function mediated by fast sodium dendritic spikes in basal dendrites of CA1 pyramidal neurons. *Journal of Neuroscience* **23**:7750–7758.
- Astman N, Gutnick MJ, Fleidervish IA (2006) Persistent sodium current in layer 5 neocortical neurons is primarily generated in the proximal axon. *Journal of Neuroscience* **26**:3465–3473.
- Baranauskas G, David Y and Fleidervish IA (2013) Spatial mismatch between the Na⁺ flux and spike initiation in axon initial segment. *Proceedings of the National Academy of Sciences of the United States of America* **110**:4051–4056.

- Barnes SJ, Opitz T, Merkens M, Kelly T, von der Brelie C, Krueppel R, Beck H (2010) Stable mossy fiber long-term potentiation requires calcium influx at the granule cell soma, protein synthesis, and microtubule-dependent axonal transport. *Journal of Neuroscience* **30**:12996–13004.
- Barrett JN, Crill WE (1974) Specific membrane properties of cat motoneurones. *Journal of Physiology* **239**:301–324.
- Bekkers JM, Stevens CF (1996) Cable properties of cultured hippocampal neurons determined from sucrose-evoked miniature EPSCs. *Journal of Neurophysiology* **75**:1250–1255.
- Benardo LS, Masukawa LM, Prince DA (1982) Electrophysiology of isolated hippocampal pyramidal dendrites. *Journal of Neuroscience* **2**:1614–1622.
- Berger T, Larkum ME, Lüscher HR (2001) High $I(h)$ channel density in the distal apical dendrite of layer V pyramidal cells increases bidirectional attenuation of EPSPs. *Journal of Neurophysiology* **85**:855–868.
- Bernander O, Douglas RJ, Martin KaC, Koch C (1991) Synaptic background activity influences spatiotemporal integration in single pyramidal cells. *Proceedings of the National Academy of Sciences of the United States of America* **88**:11569–11573.
- Bernard C, Johnston D (2003) Distance-dependent modifiable threshold for action potential back-propagation in hippocampal dendrites. *Journal of Neurophysiology* **90**:1807–1816.
- Bischofberger J, Jonas P (1997) Action potential propagation into the presynaptic dendrites of rat mitral cells. *Journal of Physiology* **504**:359–365.
- Bittner KC, Andrasfalvy BK, Magee JC (2012) Ion channel gradients in the apical tuft region of CA1 pyramidal neurons. *PLoS ONE* **7**:e46652.
- Borst JG, Helmchen F, Sakmann B (1995) Pre- and postsynaptic whole-cell recordings in the medial nucleus of the trapezoid body of the rat. *Journal of Physiology* **489**:825–840.
- Bourque CW, Renaud LP (1984) Activity patterns and osmosensitivity of rat supraoptic neurones in perfused hypothalamic explants. *Journal of Physiology* **349**:631–642.
- Branco T, Häusser M (2010) The single dendritic branch as a fundamental functional unit in the nervous system. *Current Opinion in Neurobiology* **20**:494–502.
- Branco T, Häusser M (2011) Synaptic integration gradients in single cortical pyramidal cell dendrites. *Neuron* **69**:885–892.
- Breton JD, Stuart GJ (2012) Somatic and dendritic GABA(B) receptors regulate neuronal excitability via different mechanisms. *Journal of Neurophysiology* **108**:2810–2818.
- Brown JT, Randall AD (2009) Activity-dependent depression of the spike after-depolarization generates long-lasting intrinsic plasticity in hippocampal CA3 pyramidal neurons. *Journal of Physiology (London)* **587**:1265–81.
- Bullis JB, Jones TD, Poolos NP (2007) Reversed somatodendritic I_h gradient in a class of hippocampal neurons with pyramidal morphology. *Journal of Physiology* **579**:431–443.
- Burke RE (1967) Composite nature of the monosynaptic excitatory postsynaptic potential. *Journal of Neurophysiology* **30**:1114–1137.
- Callaway JC, Ross WN (1995) Frequency-dependent propagation of sodium action potentials in dendrites of hippocampal CA1 pyramidal neurons. *Journal of Neurophysiology* **74**:1395–1403.
- Cash S, Yuste R (1998) Input summation by cultured pyramidal neurons is linear and position-independent. *Journal of Neuroscience* **18**:10–15.
- Cash S, Yuste R (1999) Linear summation of excitatory inputs by CA1 pyramidal neurons. *Neuron* **22**:383–394.
- Cauller LJ, Connors BW (1992) Function of very distal dendrites: experimental and computational studies of layer I synapses on neocortical pyramidal cells. In: *Single Neuron Computation* (McKenna T, Davis J, Zornetzer SF, eds), pp. 199–229. San Diego, CA: Academic Press.
- Chen X, Johnston D (2005) Constitutively active G-protein-gated inwardly rectifying K⁺ channels in dendrites of hippocampal CA1 pyramidal neurons. *Journal of Neuroscience* **25**:3787–3792.

- Chen WR, Midtgård J, Shepherd GM (1997) Forward and backward propagation of dendritic impulses and their synaptic control in mitral cells. *Science* **278**:463–467.
- Chen WR, Shen GY, Shepherd GM, Hines ML, Midtgård J (2002) Multiple modes of action potential initiation and propagation in mitral cell primary dendrite. *Journal of Neurophysiology* **88**:2755–2764.
- Clark BA, Monsivais P, Branco T, London M, Häusser M (2005) The site of action potential initiation in cerebellar Purkinje neurons. *Nature Neuroscience* **8**:137–139.
- Cobb SR, Buhl EH, Halasy K, Paulsen O, Somogyi P (1995) Synchronization of neuronal activity in hippocampus by individual GABAergic interneurons. *Nature* **378**:75–78.
- Colbert CM, Johnston D (1996) Axonal action-potential initiation and Na^+ channel densities in the soma and axon initial segment of subiculum pyramidal neurons. *Journal of Neuroscience* **16**:6676–6686.
- Colbert CM, Johnston D (1998) Protein kinase C activation decreases activity-dependent attenuation of dendritic Na^+ current in hippocampal CA1 pyramidal neurons. *Journal of Neurophysiology* **79**:491–495.
- Colbert CM, Pan E (2002) Ion channel properties underlying axonal action potential initiation in pyramidal neurons. *Nature Neuroscience* **5**:533–538.
- Colbert CM, Magee JC, Hoffman DA, Johnston D (1997) Slow recovery from inactivation of Na^+ channels underlies the activity-dependent attenuation of dendritic action potentials in hippocampal CA1 pyramidal neurons. *Journal of Neuroscience* **17**:6512–6521.
- Conti F, Hille B, Neumcke B, Nonner W, Stampfli R (1976) Measurement of the conductance of the sodium channel from current fluctuations at the node of Ranvier. *Journal of Physiology* **262**:699–727.
- Coombs JS, Curtis DR, Eccles JC (1957) The interpretation of spike potentials of motoneurones. *Journal of Physiology* **139**:198–231.
- Coombs JS, Curtis DR, Eccles JC (1959) The electrical constants of the motoneurone membrane. *Journal of Physiology* **145**:505–528.
- Cragg BG, Hamlyn LH (1955) Action potentials of the pyramidal neurones in the hippocampus of the rabbit. *Journal of Physiology* **129**:608–627.
- Day M, Carr DB, Ulrich S, Ilijic E, Tkatch, T Surmeier DJ (2005) Dendritic excitability of mouse frontal cortex pyramidal neurons is shaped by the interaction among HCN, Kir2, and K_{leak} channels. *Journal of Neuroscience* **25**:8776–8787.
- Debello WM, McBride TJ, Nichols G, S., Pannoni KE, Sanculi D, Totten DJ (2014) Input clustering and the microscale structure of local circuits. *Frontiers in Neural Circuits* **8**:112.
- Deisz RA, Fortin G, Zieglgänsberger W (1991) Voltage dependence of excitatory postsynaptic potentials of rat neocortical neurons. *Journal of Neurophysiology* **65**:371–382.
- Desjardins AE, Li YX, Reinker S, Miura RM, Neuman RS (2003) The influences of I_h on temporal summation in hippocampal CA1 pyramidal neurons: a modeling study. *Journal of Computational Neuroscience* **15**:131–142.
- Destexhe A, Paré D (1999) Impact of network activity on the integrative properties of neocortical pyramidal neurons *in vivo*. *Journal of Neurophysiology* **81**:1531–1547.
- Destexhe A, Neubig M, Ulrich D, Huguenard J (1998) Dendritic low-threshold calcium currents in thalamic relay cells. *Journal of Neuroscience* **18**:3574–3588.
- Djurisic M, Antic S, Chen WR, Zecevic D (2004) Voltage imaging from dendrites of mitral cells: EPSP attenuation and spike trigger zones. *Journal of Neuroscience* **24**:6703–6714.
- Dodge FA, Cooley JW (1973) Action potential of the motoneuron. *IBM Journal of Research and Development* **17**:219–229.
- Eccles JC, Libet B, Young RR (1958) The behaviour of chromatolyzed motoneurones studied by intracellular recording. *Journal of Physiology* **143**:11–40.
- Eilers J, Augustine GJ, Konnerth A (1995) Subthreshold synaptic Ca^{2+} signalling in fine dendrites and spines of cerebellar Purkinje neurons. *Nature* **373**:155–158.

- Epsztein J, Lee AK, Choref E, Brecht M (2010) Impact of spikelets on hippocampal CA1 pyramidal cell activity during spatial exploration. *Science* **327**:474–477.
- Fatt P (1957) Sequence of events in synaptic activation of a motoneuron. *Journal of Neurophysiology* **20**:61–80.
- Ferster D, Spruston N (1995) Cracking the neuronal code. *Science* **270**:756–757.
- Fleidervish IA, Lasser-Ross N, Gutnick MJ, Ross WN (2010) Na^+ imaging reveals little difference in action potential-evoked Na^+ influx between axon and soma. *Nature Neuroscience* **13**:852–860.
- Foust A, Popovic M, Zecevic D, McCormick DA (2010) Action potentials initiate in the axon initial segment and propagate through axon collaterals reliably in cerebellar Purkinje neurons. *Journal of Neuroscience* **30**:6891–6902.
- Frick A, Magee J, Koester HJ, Migliore M, Johnston D (2003) Normalization of Ca^{2+} signals by small oblique dendrites of CA1 pyramidal neurons. *Journal of Neuroscience* **23**:3243–3250.
- Frick A, Magee J, Johnston D (2004) LTP is accompanied by an enhanced local excitability of pyramidal neuron dendrites. *Nature Neuroscience* **7**:126–135.
- Fromherz P, Müller CO (1994) Cable properties of a straight neurite of a leech neuron probed by a voltage-sensitive dye. *Proceedings of the National Academy of Sciences of the United States of America* **91**:4604–4608.
- Fujita Y, Sakata H (1962) Electrophysiological properties of CA1 and CA2 apical dendrites of rabbit hippocampus. *Journal of Neurophysiology* **25**:209–222.
- Fuortes MG, Frank K, Becker MC (1957) Steps in the production of motoneuron spikes. *Journal of General Physiology* **40**:735–752.
- Gao DM, Hoffman D, Benabid AL (1996) Simultaneous recording of spontaneous activities and nociceptive responses from neurons in the pars compacta of substantia nigra and in the lateral habenula. *European Journal of Neuroscience* **8**:1474–1478.
- Gasparini S, Magee JC (2006) State-dependent dendritic computation in hippocampal CA1 pyramidal neurons. *Journal of Neuroscience* **26**:2088–2100.
- Gasparini S, Migliore M, Magee JC (2004) On the initiation and propagation of dendritic spikes in CA1 pyramidal neurons. *Journal of Neuroscience* **24**:11046–11056.
- Geiger JRP, Lübke J, Roth A, Frotscher M, Jonas P (1997) Submillisecond AMPA receptor-mediated signaling at a principal neuron-interneuron synapse. *Neuron* **18**:1009–1023.
- Gentet LJ, Stuart GJ, Clements JD (2000) Direct measurement of specific membrane capacitance in neurons. *Biophysical Journal* **79**:314–320.
- Gidon A, Segev I (2012) Principles governing the operation of synaptic inhibition in dendrites. *Neuron* **75**:330–341.
- Gillesen T, Alzheimer C (1997) Amplification of EPSPs by low $\text{Ni}^{(2+)}$ - and amiloride-sensitive Ca^{2+} channels in apical dendrites of rat CA1 pyramidal neurons. *Journal of Neurophysiology* **77**:1639–1643.
- Goldberg JH, Lacefield CO, Yuste R (2004) Global dendritic calcium spikes in mouse layer 5 low threshold spiking interneurons: implications for control of pyramidal cell bursting. *Journal of Physiology* **558**:465–478.
- Golding NL, Spruston N (1998) Dendritic sodium spikes are variable triggers of axonal action potentials in hippocampal CA1 pyramidal neurons. *Neuron* **21**:1189–1200.
- Golding NL, Ferragamo MJ, Oertel D (1999a) Role of intrinsic conductances underlying responses to transients in octopus cells of the cochlear nucleus. *Journal of Neuroscience* **19**:2897–2905.
- Golding NL, Jung HY, Mickus T, Spruston N (1999b) Dendritic calcium spike initiation and repolarization are controlled by distinct potassium channel subtypes in CA1 pyramidal neurons. *Journal of Neuroscience* **19**:8789–8798.
- Golding NL, Kath WL, Spruston N (2001) Dichotomy of action-potential backpropagation in CA1 pyramidal neuron dendrites. *Journal of Neurophysiology* **86**:2998–3010.

- Golding NL, Staff NP, Spruston N (2002) Dendritic spikes as a mechanism for cooperative long-term potentiation. *Nature* **418**:326–331.
- Golding NL, Mickus TJ, Katz Y, Kath WL, Spruston N (2005) Factors mediating powerful voltage attenuation along CA1 pyramidal neuron dendrites. *Journal of Physiology* **568**:69–82.
- Goldstein SS, Rall W (1974) Changes of action potential shape and velocity for changing core conductor geometry. *Biophysical Journal* **14**:731–757.
- Gulledge AT, Carnevale NT, Stuart GJ (2012) Electrical advantages of dendritic spines. *PLoS ONE* **7**:e36007.
- Hao J, Wang X-D, Dan Y, Poo M-M, Zhang X-H (2009) An arithmetic rule for spatial summation of excitatory and inhibitory inputs in pyramidal neurons. *Proceedings of the National Academy of Sciences of the United States of America* **106**:21906–21911.
- Harnett MT, Makara JK, Spruston N, Kath WL, Magee JC (2012) Synaptic amplification by dendritic spines enhances input cooperativity. *Nature* **491**:599–602.
- Hartell NA (1996) Strong activation of parallel fibers produces localized calcium transients and a form of LTD that spreads to distant synapses. *Neuron* **16**:601–610.
- Häusser M, Clark BA (1997) Tonic synaptic inhibition modulates neuronal output pattern and spatiotemporal synaptic integration. *Neuron* **19**:665–678.
- Häusser M, Mel B (2003) Dendrites: bug or feature? *Current Opinion in Neurobiology* **13**:372–383.
- Häusser M, Stuart G, Racca C, Sakmann B (1995) Axonal initiation and active dendritic propagation of action potentials in substantia nigra neurons. *Neuron* **15**:637–647.
- Häusser M, Major G, Stuart GJ (2001) Differential shunting of EPSPs by action potentials. *Science* **291**:138–141.
- Häusser M, Raman IM, Otis T, Smith SL, Nelson A, Du Lac S, Loewenstein Y, Mahon S, Pennartz C, Cohen I, Yarom Y (2004) The beat goes on: spontaneous firing in mammalian neuronal microcircuits. *Journal of Neuroscience* **24**:9215–9219.
- Herreras O (1990) Propagating dendritic action potential mediates synaptic transmission in CA1 pyramidal cells *in situ*. *Journal of Neurophysiology* **64**:1429–1441.
- Hines ML, Carnevale NT (1997) The NEURON simulation environment. *Neural Computation* **9**:1179–1209.
- Hoffman DA, Johnston D (1999) Neuromodulation of dendritic action potentials. *Journal of Neurophysiology* **81**:408–411.
- Hoffman DA, Magee JC, Colbert CM, Johnston D (1997) K⁺ channel regulation of signal propagation in dendrites of hippocampal pyramidal neurons. *Nature* **387**:869–875.
- Hu H, Jonas P (2014) A supercritical density of Na(+) channels ensures fast signaling in GABAergic interneuron axons. *Nature Neuroscience* **17**:686–693.
- Hu W, Tian C, Li T, Yang M, Hou H, and Shu Y (2009) Distinct contributions of Na(v)1.6 and Na(v)1.2 in action potential initiation and backpropagation. *Nature Neuroscience* **12**:996–1002.
- Hu H, Martina M, Jonas P (2010) Dendritic mechanisms underlying rapid synaptic activation of fast-spiking hippocampal interneurons. *Science* **327**:52–58.
- Jack JJB, Noble D, Tsien RW (1983) *Electrical Current Flow in Excitable Cells*. Oxford: Oxford University Press.
- Jadi M, Polsky A, Schiller J, Mel BW (2011) Location-dependent effects of inhibition on local spiking in pyramidal neuron dendrites. *PLoS Computational Biology* **8**:e1002550.
- Jadi MP, Behabadi BF, Poleg-Polsky A, Schiller J, Mel BW (2014) An augmented two-layer model captures nonlinear analog spatial integration effects in pyramidal neuron dendrites. *Proceedings of the IEEE* **102**:782–798.
- Jaffe DB, Carnevale NT (1999) Passive normalization of synaptic integration influenced by dendritic architecture. *Journal of Neurophysiology* **82**:3268–3285.

- Jarsky T, Roxin A, Kath WL, Spruston N (2005) Conditional dendritic spike propagation following distal synaptic activation of hippocampal CA1 pyramidal neurons. *Nature Neuroscience* **8**:1667–1676.
- Jefferys JG (1979) Initiation and spread of action potentials in granule cells maintained *in vitro* in slices of guinea-pig hippocampus. *Journal of Physiology* **289**:375–388.
- Jia H, Rochefort NL, Chen X, Konnerth A (2010) Dendritic organization of sensory input to cortical neurons *in vivo*. *Nature* **464**:1307–1312.
- Johnston D, Narayanan R (2008) Active dendrites: colorful wings of the mysterious butterflies. *Trends in Neurosciences* **31**:309–316.
- Jung HY, Mickus T, Spruston N (1997) Prolonged sodium channel inactivation contributes to dendritic action potential attenuation in hippocampal pyramidal neurons. *Journal of Neuroscience* **17**:6639–6646.
- Kamondi A, Acsady L, Buzsáki G (1998) Dendritic spikes are enhanced by cooperative network activity in the intact hippocampus. *Journal of Neuroscience* **18**:3919–3928.
- Kampa BM, Stuart GJ (2006) Calcium spikes in basal dendrites of layer 5 pyramidal neurons during action potential bursts. *Journal of Neuroscience* **26**:7424–7432.
- Kampa BM, Letzkus JJ, Stuart GJ (2006) Requirement of dendritic calcium spikes for induction of spike-timing-dependent synaptic plasticity. *Journal of Physiology* **574**:283–290.
- Katz Y, Menon V, Nicholson DA, Geinisman Y, Kath WL, Spruston N (2009) Synapse distribution suggests a two-stage model of dendritic integration in CA1 pyramidal neurons. *Neuron* **63**:171–177.
- Khaliq ZM, Raman IM (2006) Relative contributions of axonal and somatic Na channels to action potential initiation in cerebellar Purkinje neurons. *Journal of Neuroscience* **26**:1935–1944.
- Kim HG, Beierlein M, Connors BW (1995) Inhibitory control of excitable dendrites in neocortex. *Journal of Neurophysiology* **74**:1810–1814.
- Kim S, Guzman SJ, Hu H, Jonas P (2012) Active dendrites support efficient initiation of dendritic spikes in hippocampal CA3 pyramidal neurons. *Nature Neuroscience* **15**:600–606.
- Kim Y, Hsu CL, Cembrowski MS, Mensh BD, Spruston N. Dendritic sodium spikes are required for long-term potentiation at distal synapses on hippocampal pyramidal neurons. *Elife*. 2015 Aug 6;4. doi: 10.7554/elife.06414. PubMed PMID: 26247712; PubMed Central PMCID: PMC4576155.
- Koch C, Rapp M, Segev I (1996) A brief history of time (constants). *Cerebral Cortex* **6**:93–101.
- Kole MH, Letzkus JJ, Stuart GJ (2007) Axon initial segment Kv1 channels control axonal action potential waveform and synaptic efficacy. *Neuron* **55**:633–647.
- Kole MH, Ilschner SU, Kampa BM, Williams SR, Ruben PC, Stuart GJ (2008) Action potential generation requires a high sodium channel density in the axon initial segment. *Nature Neuroscience* **11**:178–186.
- Krueppel R, Remy S, Beck H (2011) Dendritic integration in hippocampal dentate granule cells. *Neuron* **71**:512–528.
- Landmesser L, Pilar G (1972) The onset and development of transmission in the chick ciliary ganglion. *Journal of Physiology* **222**:691–713.
- Larkum ME, Nevian T (2008) Synaptic clustering by dendritic signalling mechanisms. *Current Opinion in Neurobiology* **18**:321–331.
- Larkum ME, Rioult MG, Lüscher HR (1996) Propagation of action potentials in the dendrites of neurons from rat spinal cord slice cultures. *Journal of Neurophysiology* **75**:154–170.
- Larkum ME, Kaiser KM, Sakmann B (1999a) Calcium electrogenesis in distal apical dendrites of layer 5 pyramidal cells at a critical frequency of back-propagating action potentials. *Proceedings of the National Academy of Sciences of the United States of America* **96**:14600–14604.
- Larkum ME, Zhu JJ, Sakmann B (1999b) A new cellular mechanism for coupling inputs arriving at different cortical layers. *Nature* **398**:338–341.
- Larkum ME, Zhu JJ, Sakmann B (2001) Dendritic mechanisms underlying the coupling of the dendritic with the axonal action potential initiation zone of adult rat layer 5 pyramidal neurons. *Journal of Physiology* **533**:447–466.

- Larkum ME, Senn W, Lüscher HR (2004) Top-down dendritic input increases the gain of layer 5 pyramidal neurons. *Cerebral Cortex* **14**:1059–1070.
- Larkum ME, Nevian T, Sandler M, Polksy A, Schiller J (2009) Synaptic integration in tuft dendrites of layer 5 pyramidal neurons: a new unifying principle. *Science* **325**:756–760.
- Lasser-Ross N, Ross WN (1992) Imaging voltage and synaptically activated sodium transients in cerebellar Purkinje cells. *Proceedings of the Royal Society B: Biological Sciences* **247**:35–39.
- Leao RN, Mikulovic S, Leao KE, Munguba H, Gezelius H, Enjin A, Patra K, Eriksson A, Loew LM, Tort AB, Kullander K (2012) OLM interneurons differentially modulate CA3 and entorhinal inputs to hippocampal CA1 neurons. *Nature Neuroscience* **15**:1524–1530.
- Ledergerber D, Larkum ME (2010) Properties of layer 6 pyramidal neuron apical dendrites. *Journal of Neuroscience* **30**:13031–13044.
- Lemon N, Turner RW (2000) Conditional spike backpropagation generates burst discharge in a sensory neuron. *Journal of Neurophysiology* **84**:1519–1530.
- Letzkus JJ, Kampa BM, Stuart GJ (2006) Learning rules for spike timing-dependent plasticity depend on dendritic synapse location. *Journal of Neuroscience* **26**:10420–10429.
- Liberman MC (1991) Central projections of auditory-nerve fibers of differing spontaneous rate. I. Anteroventral cochlear nucleus. *Journal of Comparative Neurology* **313**:240–258.
- Lipowsky R, Gillessen T, Alzheimer C (1996) Dendritic Na^+ channels amplify EPSPs in hippocampal CA1 pyramidal cells. *Journal of Neurophysiology* **76**:2181–2191.
- Llinás RR (1988) The intrinsic electrophysiological properties of mammalian neurons: insights into central nervous system function. *Science* **242**:1654–1664.
- Llinás R, Nicholson C (1971) Electrophysiological properties of dendrites and somata in alligator Purkinje cells. *Journal of Neurophysiology* **34**:532–551.
- Llinás R, Sugimori M (1980a) Electrophysiological properties of *in vitro* Purkinje cell dendrites in mammalian cerebellar slices. *Journal of Physiology* **305**:197–213.
- Llinás R, Sugimori M (1980b) Electrophysiological properties of *in vitro* Purkinje cell somata in mammalian cerebellar slices. *Journal of Physiology* **305**:171–195.
- Llinás R, Nicholson C, Freeman JA, Hillman DE (1968) Dendritic spikes and their inhibition in alligator Purkinje cells. *Science* **160**:1132–1135.
- Llinás R, Nicholson C, Precht W (1969) Preferred centripetal conduction of dendritic spikes in alligator Purkinje cells. *Science* **163**:184–187.
- London M, Häusser M (2005) Dendritic computation. *Annual Review of Neuroscience* **28**:503–532.
- Longordo F, To M-SS, Ikeda K, Stuart GJ (2013) Sublinear integration underlies binocular processing in primary visual cortex. *Nature Neuroscience* **16**:714–723.
- Lorente de Nó RL, Condouris GA (1959) Decremental conduction in peripheral nerve. Integration of stimuli in the neuron. *Proceedings of the National Academy of Sciences of the United States of America* **45**:592–617.
- Losonczy A, Magee JC (2006) Integrative properties of radial oblique dendrites in hippocampal CA1 pyramidal neurons. *Neuron* **50**:291–307.
- Losonczy A, Makara JK, Magee JC (2008) Compartmentalized dendritic plasticity and input feature storage in neurons. *Nature* **452**:436–441.
- Lörincz A, Notomi T, Tamas G, Shigemoto R, Nusser Z (2002) Polarized and compartment-dependent distribution of HCN1 in pyramidal cell dendrites. *Nature Neuroscience* **5**:1185–1193.
- Lovett-Barron M, Turi GF, Kaifosh P, Lee PH, Bolze F, Sun XH, Nicoud JF, Zemelman BV, Sternson SM, Losonczy A (2012) Regulation of neuronal input transformations by tunable dendritic inhibition. *Nature Neuroscience* **15**:423–430, S1–S3.
- Lowe G (2002) Inhibition of backpropagating action potentials in mitral cell secondary dendrites. *Journal of Neurophysiology* **88**:64–85.

- Lux HD, Schubert P, Kreutzberg GW, Globus A (1970) Excitation and axonal flow: autoradiographic study on motoneurons intracellularly injected with a 3H-amino acid. *Experimental Brain Research* **10**:197–204.
- Lüscher C, Streit J, Quadrini R, Lüscher HR (1994) Action potential propagation through embryonic dorsal root ganglion cells in culture. I. Influence of the cell morphology on propagation properties. *Journal of Neurophysiology* **72**:622–633.
- MacVicar BA, Dudek FE (1981) Electrotonic coupling between pyramidal cells: a direct demonstration in rat hippocampal slices. *Science* **213**:782–785.
- Magee JC (1998) Dendritic hyperpolarization-activated currents modify the integrative properties of hippocampal CA1 pyramidal neurons. *Journal of Neuroscience* **18**:7613–7624.
- Magee JC (1999) Dendritic I_h normalizes temporal summation in hippocampal CA1 neurons. *Nature Neuroscience* **2**:508–514.
- Magee JC, Carruth M (1999) Dendritic voltage-gated ion channels regulate the action potential firing mode of hippocampal CA1 pyramidal neurons. *Journal of Neurophysiology* **82**:1895–1901.
- Magee JC, Cook EP (2000) Somatic EPSP amplitude is independent of synapse location in hippocampal pyramidal neurons. *Nature Neuroscience* **3**:895–903.
- Magee JC, Johnston D (1995) Characterization of single voltage-gated Na^+ and Ca^{2+} channels in apical dendrites of rat CA1 pyramidal neurons. *Journal of Physiology* **487**:67–90.
- Magee JC, Johnston D (2005) Plasticity of dendritic function. *Current Opinion in Neurobiology* **15**:334–342.
- Magee JC, Christofi G, Miyakawa H, Christie B, Lasser-Ross N, Johnston D (1995) Subthreshold synaptic activation of voltage-gated Ca^{2+} channels mediates a localized Ca^{2+} influx into the dendrites of hippocampal pyramidal neurons. *Journal of Neurophysiology* **74**:1335–1342.
- Mainen ZF, Sejnowski TJ (1996) Influence of dendritic structure on firing pattern in model neocortical neurons. *Nature* **382**:363–366.
- Mainen ZF, Joerges J, Huguenard JR, Sejnowski TJ (1995) A model of spike initiation in neocortical pyramidal neurons. *Neuron* **15**:1427–1439.
- Major G, Larkman AU, Jonas P, Sakmann B, Jack JJB (1994) Detailed passive cable models of whole-cell recorded CA3 pyramidal neurons in rat hippocampal slices. *Journal of Neuroscience* **14**:4613–4638.
- Major G, Larkum ME, Schiller J (2013) Active properties of neocortical pyramidal neuron dendrites. *Annual Review of Neuroscience* **36**:1–24.
- Makara JK, Magee JC (2013) Variable dendritic integration in hippocampal CA3 pyramidal neurons. *Neuron* **80**:1438–1450.
- Margulis M, Tang CM (1998) Temporal integration can readily switch between sublinear and supralinear summation. *Journal of Neurophysiology* **79**:2809–2813.
- Martina M, Vida I, Jonas P (2000) Distal initiation and active propagation of action potentials in interneuron dendrites. *Science* **287**:295–300.
- Masukawa LM, Prince DA (1984) Synaptic control of excitability in isolated dendrites of hippocampal neurons. *Journal of Neuroscience* **4**:217–227.
- Menon V, Musial TF, Liu A, Katz Y, Kath WL, Spruston N, Nicholson DA (2013) Balanced synaptic impact via distance-dependent synapse distribution and complementary expression of AMPARs and NMDARs in hippocampal dendrites. *Neuron* **80**:1451–1463.
- Metz AE, Spruston N, Martina M (2007) Dendritic D-type potassium currents inhibit the spike afterdepolarization in rat hippocampal CA1 pyramidal neurons. *Journal of Physiology* **581**:175–187.
- Meyer E, Müller CO, Fromherz P (1997) Cable properties of dendrites in hippocampal neurons of the rat mapped by a voltage-sensitive dye. *European Journal of Neuroscience* **9**:778–785.
- Mickus T, Jung H, Spruston N (1999) Properties of slow, cumulative sodium channel inactivation in rat hippocampal CA1 pyramidal neurons. *Biophysical Journal* **76**:846–860.
- Miles R, Toth K, Gulyas AI, Hajos N, Freund TF (1996) Differences between somatic and dendritic inhibition in the hippocampus. *Neuron* **16**:815–823.

- Miller JP, Rall W, Rinzel J (1985) Synaptic amplification by active membrane in dendritic spines. *Brain Research* **325**:325–330.
- Mittmann W, Koch U, Häusser M (2005) Feed-forward inhibition shapes the spike output of cerebellar Purkinje cells. *Journal of Physiology* **563**:369–378.
- Miyakawa H, Kato H (1986) Active properties of dendritic membrane examined by current source density analysis in hippocampal CA1 pyramidal neurons. *Brain Research* **399**:303–309.
- Miyakawa H, Lev-Ram V, Lasser-Ross N, Ross WN (1992) Calcium transients evoked by climbing fiber and parallel fiber synaptic inputs in guinea pig cerebellar Purkinje neurons. *Journal of Neurophysiology* **68**:1178–1189.
- Moore JW, Stockbridge N, Westerfield M (1983) On the site of impulse initiation in a neurone. *Journal of Physiology* **336**:301–311.
- Müller C, Remy S (2013) Dendritic inhibition mediated by O-LM and bistratified interneurons in the hippocampus. *Frontiers in Synaptic Neuroscience* **6**:23.
- Müller C, Beck H, Coulter D, Remy S (2012) Inhibitory control of linear and supralinear dendritic excitation in CA1 pyramidal neurons. *Neuron* **75**:851–864.
- Müllner FE, Wierenga CJ, Bonhoeffer T (2015) Precision of inhibition: dendritic inhibition by individual GABAergic synapses on hippocampal pyramidal cells is confined in space and time. *Neuron* **87**(3):576–89.
- Murayama M, Perez-Garcia E, Nevian T, Bock T, Senn W, Larkum ME (2009) Dendritic encoding of sensory stimuli controlled by deep cortical interneurons. *Nature* **457**:1137–1141.
- Nedergaard S, Hounsgaard J (1996) Fast Na⁺ spike generation in dendrites of guinea-pig substantia nigra pars compacta neurons. *Neuroscience* **73**:381–396.
- Neumcke B, Stämpfli R (1982) Sodium currents and sodium-current fluctuations in rat myelinated nerve fibres. *Journal of Physiology* **329**:163–184.
- Nevian T, Larkum ME, Polsky A, Schiller J (2007) Properties of basal dendrites of layer 5 pyramidal neurons: a direct patch-clamp recording study. *Nature Neuroscience* **10**:206–214.
- Nicholson DA, Trana R, Katz Y, Kath WL, Spruston N, Geinisman Y (2006) Distance-dependent differences in synapse number and AMPA receptor expression in hippocampal CA1 pyramidal neurons. *Neuron* **50**:431–442.
- Oesch N, Euler T, Taylor WR (2005) Direction-selective dendritic action potentials in rabbit retina. *Neuron* **47**:739–750.
- Palmer LM, Stuart GJ (2006) Site of action potential initiation in layer 5 pyramidal neurons. *Journal of Neuroscience* **26**:1854–1863.
- Palmer LM, Clark BA, Grundemann J, Roth A, Stuart GJ, Häusser M (2010) Initiation of simple and complex spikes in cerebellar Purkinje cells. *Journal of Physiology* **588**:1709–1717.
- Palmer L, Murayama M, Larkum M (2012) Inhibitory regulation of dendritic activity *in vivo*. *Frontiers in Neural Circuits* **6**:26.
- Paré D, Shink E, Gaudreau H, Destexhe A, Lang EJ (1998) Impact of spontaneous synaptic activity on the resting properties of cat neocortical pyramidal neurons *in vivo*. *Journal of Neurophysiology* **79**:1450–1460.
- Perez-Garcia E, Gassmann M, Bettler B, Larkum ME (2006) The GABA_{B1b} isoform mediates long-lasting inhibition of dendritic Ca²⁺ spikes in layer 5 somatosensory pyramidal neurons. *Neuron* **50**:603–616.
- Perkel DH, Perkel DJ (1985) Dendritic spines: role of active membrane in modulating synaptic efficacy. *Brain Research* **325**:331–335.
- Pfeffer CK, Xue M, He M, Huang ZJ, Scanziani M (2013) Inhibition of inhibition in visual cortex: the logic of connections between molecularly distinct interneurons. *Nature Neuroscience* **16**:1068–1076.
- Pinsky PF, Rinzel J (1994) Intrinsic and network rhythrogenesis in a reduced Traub model for CA3 neurons. *Journal of Computational Neuroscience* **1**:39–60.

- Piskorowski RA, Chevaleyre V (2011) Synaptic integration by different dendritic compartments of hippocampal CA1 and CA2 pyramidal neurons. *Cellular and Molecular Life Sciences* **69**:75–88.
- Poirazi P, Brannon T, Mel BW (2003a) Arithmetic of subthreshold synaptic summation in a model CA1 pyramidal cell. *Neuron* **37**:977–987.
- Poirazi P, Brannon T, Mel BW (2003b) Pyramidal neuron as two-layer neural network. *Neuron* **37**:989–999.
- Polksy A, Mel BW, Schiller J (2004) Computational subunits in thin dendrites of pyramidal cells. *Nature Neuroscience* **7**:621–627.
- Pouille F, Scanziani M (2001) Enforcement of temporal fidelity in pyramidal cells by somatic feed-forward inhibition. *Science* **293**:1159–1163.
- Purpura DP (1967) Comparative physiology of dendrites. In: *The Neurosciences: a Study Program* (Quarzon GC, Melnechuk T, Schmitt T, eds), pp. 372–393. New York: Rockefeller University Press.
- Rall W (1959) Branching dendritic trees and motoneuron membrane resistivity. *Experimental Neurology* **1**:491–527.
- Rall W (1964) Theoretical significance of dendritic trees for neuronal input-output relations. In: *Neural Theory and Modeling* (Reiss RF, ed.), pp. 73–97. Stanford, CA: Stanford University Press.
- Rall W (1967) Distinguishing theoretical synaptic potentials computed for different soma-dendritic distributions of synaptic input. *Journal of Neurophysiology* **30**:1138–1168.
- Rall W, Rinzel J (1973) Branch input resistance and steady attenuation for input to one branch of a dendritic neuron model. *Biophysical Journal* **13**:648–687.
- Rall W, Shepherd GM (1968) Theoretical reconstruction of field potentials and dendrodendritic synaptic interactions in olfactory bulb. *Journal of Neurophysiology* **31**:884–915.
- Rancz EA, Häusser M (2006) Dendritic calcium spikes are tunable triggers of cannabinoid release and short-term synaptic plasticity in cerebellar Purkinje neurons. *Journal of Neuroscience* **26**:5428–5437.
- Rapp M, Yarom Y, Segev I (1992) The impact of parallel fiber background activity on the cable properties of cerebellar Purkinje cells. *Neural Computation* **4**:101–118.
- Rapp M, Segev I, Yarom Y (1994) Physiology, morphology and detailed passive models of guinea-pig cerebellar Purkinje cells. *Journal of Physiology* **474**:101–118.
- Rapp M, Yarom Y, Segev I (1996) Modeling back propagating action potential in weakly excitable dendrites of neocortical pyramidal cells. *Proceedings of the National Academy of Sciences of the United States of America* **93**:11985–11990.
- Remy S, Csicsvari J, Beck H (2009) Activity-dependent control of neuronal output by local and global dendritic spike attenuation. *Neuron* **61**:906–916.
- Rhodes P (2006) The properties and implications of NMDA spikes in neocortical pyramidal cells. *Journal of Neuroscience* **26**:6704–6715.
- Richardson TL, Turner RW, Miller JJ (1987) Action-potential discharge in hippocampal CA1 pyramidal neurons: current source-density analysis. *Journal of Neurophysiology* **58**:981–996.
- Rinzel J, Rall W (1974) Transient response in a dendritic neuron model for current injected at one branch. *Biophysical Journal* **14**:759–790.
- Roth A, Häusser M (2001) Compartmental models of rat cerebellar Purkinje cells based on simultaneous somatic and dendritic patch-clamp recordings. *Journal of Physiology* **535**:445–472.
- Rudolph M, Destexhe A (2003) A fast-conducting, stochastic integrative mode for neocortical neurons *in vivo*. *Journal of Neuroscience* **23**:2466–2476.
- Schaefer AT, Larkum ME, Sakmann B, Roth A (2003) Coincidence detection in pyramidal neurons is tuned by their dendritic branching pattern. *Journal of Neurophysiology* **89**:3143–3154.
- Schiller J, Schiller Y (2001) NMDA receptor-mediated dendritic spikes and coincident signal amplification. *Current Opinion in Neurobiology* **11**:343–348.
- Schiller J, Schiller Y, Stuart G, Sakmann B (1997) Calcium action potentials restricted to distal apical dendrites of rat neocortical pyramidal neurons. *Journal of Physiology* **505**:605–616.

- Schiller J, Major G, Koester HJ, Schiller Y (2000) NMDA spikes in basal dendrites of cortical pyramidal neurons. *Nature* **404**:285–289.
- Schmidt-Hieber C, Jonas P, Bischofberger J (2008) Action potential initiation and propagation in hippocampal mossy fibre axons. *Journal of Physiology* **586**:1849–1857.
- Schwartzkroin PA, Slawsky M (1977) Probable calcium spikes in hippocampal neurons. *Brain Research* **135**:157–161.
- Seamans JK, Gorelova NA, Yang CR (1997) Contributions of voltage-gated Ca^{2+} channels in the proximal versus distal dendrites to synaptic integration in prefrontal cortical neurons. *Journal of Neuroscience* **17**:5936–5948.
- Segev I, Rall W (1998) Excitable dendrites and spines: earlier theoretical insights elucidate recent direct observations. *Trends in Neurosciences* **21**:453–460.
- Shadlen MN, Newsome WT (1994) Noise, neural codes and cortical organization. *Current Opinion in Neurobiology* **4**:569–579.
- Shadlen MN, Newsome WT (1995) Is there a signal in the noise? *Current Opinion in Neurobiology* **5**:248–250.
- Shepherd GM, Brayton RK, Miller JP, Segev I, Rinzel J, Rall W (1985) Signal enhancement in distal cortical dendrites by means of interactions between active dendritic spines. *Proceedings of the National Academy of Sciences of the United States of America* **82**:2192–2195.
- Shu Y, Hasenstaub A, Duque A, Yu Y, McCormick DA (2006) Modulation of intracortical synaptic potentials by presynaptic somatic membrane potential. *Nature* **441**:761–765.
- Shu Y, Duque A, Yu Y, Haider B, McCormick DA (2007) Properties of action potential initiation in neocortical pyramidal cells: evidence from whole cell axon recordings. *Journal of Neurophysiology* **97**:746–760.
- Sigworth FJ (1980) The variance of sodium current fluctuations at the node of Ranvier. *Journal of Physiology* **307**:97–129.
- Sik A, Penttonen M, Ylinen A, Buzsáki G (1995) Hippocampal CA1 interneurons: an *in vivo* intracellular labeling study. *Journal of Neuroscience* **15**:6651–6665.
- Silberberg G, Markram H (2007) Disynaptic inhibition between neocortical pyramidal cells mediated by Martinotti cells. *Neuron* **53**:735–746.
- Sivyer B, Williams SR (2013) Direction selectivity is computed by active dendritic integration in retinal ganglion cells. *Nature Neuroscience* **16**:1848–1856.
- Smith PH, Joris PX, Banks MI, Yin TCT (1993) Responses of cochlear nucleus cells and projections of their axons. In: *The Mammalian Cochlear Nuclei: Organization and Function* (Merchán MA, Juiz JM, Godfrey DA, Mugnaini E, eds), pp. 349–360. New York: Springer.
- Smith MA, Ellis-Davies GC, Magee JC (2003) Mechanism of the distance-dependent scaling of Schaffer collateral synapses in rat CA1 pyramidal neurons. *Journal of Physiology* **548**:245–258.
- Softky WR (1995) Simple codes versus efficient codes. *Current Opinion in Neurobiology* **5**:239–247.
- Spencer WA, Kandel ER (1961) Electrophysiology of hippocampal neurons. IV. Fast prepotentials. *Journal of Neurophysiology* **24**:272–285.
- Spruston N, Johnston D (1992) Perforated patch-clamp analysis of the passive membrane properties of three classes of hippocampal neurons. *Journal of Neurophysiology* **67**:508–529.
- Spruston N, Kath WL (2004) Dendritic arithmetic. *Nature Neuroscience* **7**:567–569.
- Spruston N, Jaffe DB, Williams SH, Johnston D (1993) Voltage- and space-clamp errors associated with the measurement of electrotonically remote synaptic events. *Journal of Neurophysiology* **70**:781–802.
- Spruston N, Jaffe DB, Johnston D (1994) Dendritic attenuation of synaptic potentials and currents: the role of passive membrane properties. *Trends in Neurosciences* **17**:161–166.
- Spruston N, Schiller Y, Stuart G, Sakmann B (1995) Activity-dependent action potential invasion and calcium influx into hippocampal CA1 dendrites. *Science* **268**:297–300.

- Stafstrom CE, Schwindt PC, Chubb MC, Crill WE (1985) Properties of persistent sodium conductance and calcium conductance of layer V neurons from cat sensorimotor cortex *in vitro*. *Journal of Neurophysiology* **53**:153–170.
- Stuart G (1999) Voltage-activated sodium channels amplify inhibition in neocortical pyramidal neurons. *Nature Neuroscience* **2**:144–150.
- Stuart G, Häusser M (1994) Initiation and spread of sodium action potentials in cerebellar Purkinje cells. *Neuron* **13**:703–712.
- Stuart GJ, Häusser M (2001) Dendritic coincidence detection of EPSPs and action potentials. *Nature Neuroscience* **4**:63–71.
- Stuart GJ, Sakmann B (1994) Active propagation of somatic action potentials into neocortical pyramidal cell dendrites. *Nature* **367**:69–72.
- Stuart G, Sakmann B (1995) Amplification of EPSPs by axosomatic sodium channels in neocortical pyramidal neurons. *Neuron* **15**:1065–1076.
- Stuart G, Spruston N (1998) Determinants of voltage attenuation in neocortical pyramidal neuron dendrites. *Journal of Neuroscience* **18**:3501–3510.
- Stuart G, Schiller J, Sakmann B (1997a) Action potential initiation and propagation in rat neocortical pyramidal neurons. *Journal of Physiology* **505**:617–632.
- Stuart G, Spruston N, Sakmann B, Häusser M (1997b) Action potential initiation and backpropagation in neurons of the mammalian CNS. *Trends in Neurosciences* **20**:125–131.
- Sun Q, Srinivas KV, Sotayo A, Siegelbaum SA (2014) Dendritic Na^+ spikes enable cortical input to drive action potential output from hippocampal CA2 pyramidal neurons. *eLife*, **3**: doi: 10.7554/eLife.04551.
- Takigawa T, Alzheimer C (2003) Interplay between activation of GIRK current and deactivation of I_h modifies temporal integration of excitatory input in CA1 pyramidal cells. *Journal of Neurophysiology* **89**:2238–2244.
- Terzuolo CA, Araki T (1961) An analysis of intra- versus extracellular potential changes associated with activity of single spinal motoneurons. *Annals of the New York Academy of Sciences* **94**:547–558.
- Thome C, Kelly T, Yanez A, Schultz C, Engelhardt M, Cambridge SB, Both M, Draguhn A, Beck H, Egorov AV (2014) Axon-carrying dendrites convey privileged synaptic input in hippocampal neurons. *Neuron* **83**:1418–1430.
- Thomson AM, Girdlestone D, West DC (1988) Voltage-dependent currents prolong single-axon post-synaptic potentials in layer III pyramidal neurons in rat neocortical slices. *Journal of Neurophysiology* **60**:1896–1907.
- Thurbon D, Field A, Redman S (1994) Electrotonic profiles of interneurons in stratum pyramidale of the CA1 region of rat hippocampus. *Journal of Neurophysiology* **71**:1948–1958.
- Thurbon D, Lüscher HR, Hofstetter T, Redman SJ (1998) Passive electrical properties of ventral horn neurons in rat spinal cord slices. *Journal of Neurophysiology* **80**:2485–2502.
- Traub RD, Wong RK, Miles R, Michelson H (1991) A model of a CA3 hippocampal pyramidal neuron incorporating voltage-clamp data on intrinsic conductances. *Journal of Neurophysiology* **66**:635–650.
- Traub RD, Jefferys JG, Miles R, Whittington MA, Toth K (1994) A branching dendritic model of a rodent CA3 pyramidal neurone. *Journal of Physiology* **481**:79–95.
- Tsubokawa H, Ross WN (1996) IPSPs modulate spike backpropagation and associated $[\text{Ca}^{2+}]_i$ changes in the dendrites of hippocampal CA1 pyramidal neurons. *Journal of Neurophysiology* **76**:2896–2906.
- Tsubokawa H, Ross WN (1997) Muscarinic modulation of spike backpropagation in the apical dendrites of hippocampal CA1 pyramidal neurons. *Journal of Neuroscience* **17**:5782–5791.
- Turner RW, Meyers DE, Barker JL (1989) Localization of tetrodotoxin-sensitive field potentials of CA1 pyramidal cells in the rat hippocampus. *Journal of Neurophysiology* **62**:1375–1387.

- Turner RW, Meyers DE, Richardson TL, Barker JL (1991) The site for initiation of action potential discharge over the somatodendritic axis of rat hippocampal CA1 pyramidal neurons. *Journal of Neuroscience* **11**:2270–2280.
- Turner RW, Meyers DE, Barker JL (1993) Fast pre-potential generation in rat hippocampal CA1 pyramidal neurons. *Neuroscience* **53**:949–959.
- Urban NN, Barrionuevo G (1998) Active summation of excitatory postsynaptic potentials in hippocampal CA3 pyramidal neurons. *Proceedings of the National Academy of Sciences of the United States of America* **95**:11450–11455.
- Urban NN, Castro JB (2005) Tuft calcium spikes in accessory olfactory bulb mitral cells. *Journal of Neuroscience* **25**:5024–5028.
- Urban NN, Henze DA, Barrionuevo G (1998) Amplification of perforant-path EPSPs in CA3 pyramidal cells by LVA calcium and sodium channels. *Journal of Neurophysiology* **80**:1558–1561.
- Valiante TA, Perez-Velazquez JL, Jahromi SS, Carlen PL (1995) Coupling potentials in CA1 neurons during calcium-free-induced field burst activity. *Journal of Neuroscience* **15**:6946–6956.
- Velte TJ, Masland RH (1999) Action potentials in the dendrites of retinal ganglion cells. *Journal of Neurophysiology* **81**:1412–1417.
- Vervaeke K, Hu H, Graham LJ, Storm JF (2006) Contrasting effects of the persistent Na^+ current on neuronal excitability and spike timing. *Neuron* **49**:257–270.
- Vetter P, Roth A, Häusser M (2001) Propagation of action potentials in dendrites depends on dendritic morphology. *Journal of Neurophysiology* **85**:926–937.
- Watanabe S, Hoffman DA, Migliore M, Johnston D (2002) Dendritic K^+ channels contribute to spike-timing dependent long-term potentiation in hippocampal pyramidal neurons. *Proceedings of the National Academy of Sciences of the United States of America* **99**:8366–8371.
- Waters J, Helmchen F (2006) Background synaptic activity is sparse in neocortex. *Journal of Neuroscience* **26**:8267–8277.
- Waters J, Larkum M, Sakmann B, Helmchen F (2003) Supralinear Ca^{2+} influx into dendritic tufts of layer 2/3 neocortical pyramidal neurons *in vitro* and *in vivo*. *Journal of Neuroscience* **23**:8558–8567.
- Wei DS, Mei YA, Bagal A, Kao JP, Thompson SM, Tang CM (2001) Compartmentalized and binary behavior of terminal dendrites in hippocampal pyramidal neurons. *Science* **293**:2272–2275.
- Williams SR (2004) Spatial compartmentalization and functional impact of conductance in pyramidal neurons. *Nature Neuroscience* **7**:961–967.
- Williams SR (2005) Encoding and decoding of dendritic excitation during active states in pyramidal neurons. *Journal of Neuroscience* **25**:5894–5902.
- Williams SR, Mitchell SJ (2008) Direct measurement of somatic voltage clamp errors in central neurons. *Nature Neuroscience* **11**:790–798.
- Williams SR, Stuart GJ (1999) Mechanisms and consequences of action potential burst firing in rat neocortical pyramidal neurons. *Journal of Physiology* **521**:467–482.
- Williams SR, Stuart GJ (2000a) Backpropagation of physiological spike trains in neocortical pyramidal neurons: implications for temporal coding in dendrites. *Journal of Neuroscience* **20**:8238–8246.
- Williams SR, Stuart GJ (2000b) Site independence of EPSP time course is mediated by dendritic $I(h)$ in neocortical pyramidal neurons. *Journal of Neurophysiology* **83**:3177–3182.
- Williams SR, Stuart GJ (2002) Dependence of EPSP efficacy on synapse location in neocortical pyramidal neurons. *Science* **295**:1907–1910.
- Williams SR, Stuart GJ (2003a) Role of dendritic synapse location in the control of action potential output. *Trends in Neurosciences* **26**:147–154.
- Williams SR, Stuart GJ (2003b) Voltage- and site-dependent control of the somatic impact of dendritic IPSPs. *Journal of Neuroscience* **23**:7358–7367.

- Wollner DA, Catterall WA (1986) Localization of sodium channels in axon hillocks and initial segments of retinal ganglion cells. *Proceedings of the National Academy of Sciences of the United States of America* **83**:8424–8428.
- Wong RK, Stewart M (1992) Different firing patterns generated in dendrites and somata of CA1 pyramidal neurones in guinea-pig hippocampus. *Journal of Physiology* **457**:675–687.
- Wong RK, Prince DA, Basbaum AI (1979) Intradendritic recordings from hippocampal neurons. *Proceedings of the National Academy of Sciences of the United States of America* **76**:986–990.
- Xiong W, Chen WR (2002) Dynamic gating of spike propagation in the mitral cell lateral dendrites. *Neuron* **34**:115–126.
- Yung WH, Häusser MA, Jack JJB (1991) Electrophysiology of dopaminergic and non-dopaminergic neurones of the guinea-pig substantia nigra pars compacta *in vitro*. *Journal of Physiology* **436**:643–667.
- Zhao X, Liu M, Cang J (2012) Sublinear binocular integration preserves orientation selectivity in mouse visual cortex. *Nature Communications* **4**:2088.

Chapter 13

Dendritic integration *in vivo*

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Summary

If dendritic integration is important for understanding the input–output function of neurons, then understanding how dendrites integrate information in the behaving organism should be seen as one of the crucial milestones in neuroscience. Most of what we currently know about dendritic integration has been determined using experiments *in vitro*, and we are only just beginning to reveal how dendrites function in the intact organism. This is no easy feat as the properties of dendrites *in vivo* are influenced by various factors such as increased synaptic input, different channel activation states, input arriving from varying information pathways, and neuromodulation. This chapter reviews the influence of these factors on dendritic activity *in vivo* and highlights what has been experimentally determined about dendritic integration in the intact organism to date.

Introduction

The study of dendritic integration *in vivo* can seem overwhelming. In addition to the diversity of dendritic shapes and the cornucopia of channel and receptor types and distributions that make this subject challenging *in vitro*, the dendritic trees of neurons *in vivo* are embedded in a non-static environment where they receive continual changes in ongoing synaptic bombardment, extracellular milieu, neuromodulation, and so on. The traditional methods for recording from dendrites are more difficult to implement *in vivo* due to the normal movement of the tissue and the inability to use microscopy involving transmitted light to visualize the dendrites. It has been argued that the dominance of *in vitro* recordings in determining cellular properties has distorted our view of cortical network dynamics (Steriade, 2001). Nevertheless, because of its scientific importance, more and more laboratories are turning their attention to dendritic integration *in vivo* and there has recently been enormous progress (even since the second edition of this book) with studies being conducted on the function of dendrites during various behavioral tasks from object localization to motor behavior (Fig. 13.1). The inclusion in this third edition of a chapter devoted to dendritic integration *in vivo* is therefore timely. As befits the difficulty of the subject, there are still many open questions and also some controversies. Here we will outline the current state of our knowledge and provide the background to the problems that are still to be unraveled in the field of dendritic integration *in vivo*.

Investigating dendritic properties *in vivo*

Although our knowledge about dendritic properties is quite extensive *in vitro*, it remains unclear whether all the same principles hold true *in vivo*. There are three main differences between *in*

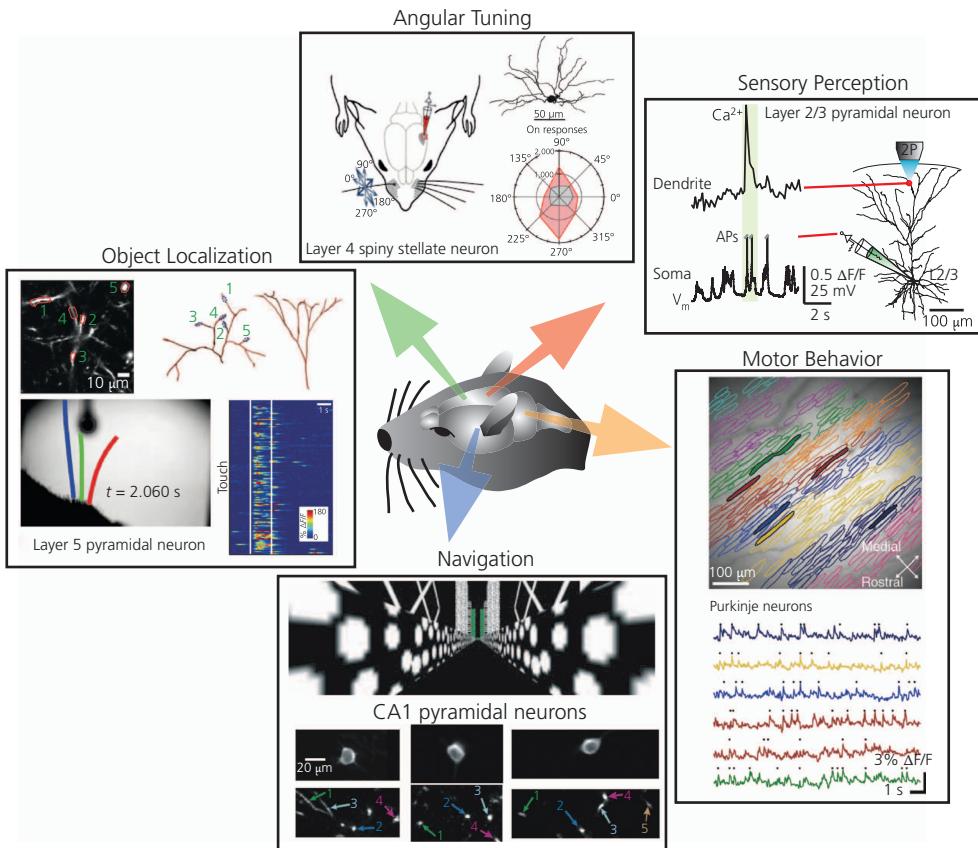


Fig. 13.1 Recent examples of studies on dendritic integration *in vivo*. Clockwise from left. Object localization (touch perception): two-photon imaging of a genetic calcium indicator in L5 pyramidal neurons of the barrel cortex illustrated that global dendritic nonlinearity is involved in the integration of sensory and motor information during a whisker-based object localization task. Angular tuning: whole-cell patch-clamp electrophysiology showed that NMDA-dependent supralinear integration contributes substantially to the angular tuning of L4 spiny stellate neurons in the barrel cortex by preferentially amplifying the preferred angular directions over non-preferred whisker angles. Sensory input: dual patch-clamp electrophysiology and two-photon microscopy showed that local NMDA spikes occur both spontaneously and following sensory input in the distal tuft dendrites of L2/3 pyramidal neurons of the somatosensory cortex, largely influencing the number of output action potentials. Motor behavior: during mouse locomotion, individual cerebellar microzones exhibit large-scale, synchronized calcium spiking in dendrites of Purkinje neurons recorded with a miniature fluorescence microscope. Navigation: two-photon microscopy of soma, dendrites, and axons illustrated that regenerative dendritic events predict the spatial precision and persistence or disappearance of place fields within hippocampal place cells of behaving mice.

Object localization: reprinted by permission from Macmillan Publishers Ltd: *Nature*, 492(7428), J Ning-long Xu, Mark T. Harnett, Stephen R. Williams, Daniel Huber, Daniel H. O'Connor, Karel Svoboda, and Jeffrey C. Magee, Nonlinear dendritic integration of sensory and motor input during an active sensing task, pp. 247–251 © 2012, Nature Publishing Group. Angular tuning: reprinted by permission from Macmillan Publishers Ltd: *Nature*, 492(7420), Maria Lavzin, Sophia Rapoport, Alon Polksky, Liora Garion, and Jackie Schiller, Nonlinear dendritic processing determines angular tuning of barrel cortex neurons *in vivo*,

vivo and in vitro preparations with regard to dendritic properties. Firstly, the fluctuating synaptic bombardment in vivo, which is rarely present in vitro, could in principle lead to large changes in the resting membrane potential (V_{rest}), input resistance (R_N), and the effective membrane time constant (τ_m) of the cell and dendrites (Steriade, 2001). At any one time, it has been suggested that there might be thousands of inputs to the cell (Bernander et al., 1991), which collectively alter the *conductance state* of the membrane, leading to large changes in the integrative properties of the neuron. Some controversy exists over the extent of this influence, however, for the following reasons. (1) There are wildly different estimates of the number and frequency of spiking neurons in the cortex at any given time (Waters and Helmchen, 2006; Kapfer et al., 2007; de Kock and Sakmann, 2008, 2009; Huber et al., 2008; Wolfe et al., 2010; Crochet et al., 2011). (2) While a high-conductance state implies an overall diminishing effect of additional synaptic input on membrane potential and therefore a decreasing influence of further synaptic input on firing, this can theoretically be compensated by the increased variability of the fluctuations in membrane potential (Mainen and Sejnowski, 1995; Hô and Destexhe, 2000; Destexhe et al., 2001). (3) Compensatory mechanisms have been observed that may actually restore the membrane properties to (or even past) their values in resting conditions (Connors et al., 1982; Waters and Helmchen, 2006). Furthermore, there are large errors in the measurement of dendritic synaptic input obtained using somatic voltage clamp experiments (Williams and Mitchell, 2008), which makes direct electrical recordings of dendritic activity even more problematic. Establishing the electrical profile of the dendritic tree in vivo is therefore not a simple task.

Secondly, perhaps the biggest practical problem facing the field is to determine the pattern or distribution of synaptic inputs to the dendritic tree under physiological conditions. There is anatomical evidence that various classes of synaptic inputs have non-uniform distributions over the dendritic trees of some neurons (Colonnier, 1968; Felleman and Van Essen, 1991) which is predicted to have important consequences for cellular properties (Holmes and Woody, 1989). The most detailed recordings to date of synaptic inputs in vivo used two-photon calcium-imaging technology with a technique for very rapid scanning of volumes of tissue. These experiments led to the estimate of between 500 and 1,000 inputs arriving per second at cortical pyramidal neurons during periods of activity (up-states) (Chen et al., 2013b) (Fig. 13.2). This means that these pyramidal neurons may have their integrative properties shaped by the patterns of synaptic bombardment found in vivo (Jia et al., 2010), but it is probably necessary to examine this on a case-by-case basis.

Thirdly, another important difference between in vitro and in vivo recording environments is the extracellular milieu which in vivo is greatly influenced by the ongoing activity of both neurons and glia cells (Zorec et al., 2012). In fact, it is not even easy to accurately measure the typical ion concentrations of the extracellular milieu in vivo (Nicholson et al., 1977; McNay and Sherwin,

Fig. 13.1 (Continued)

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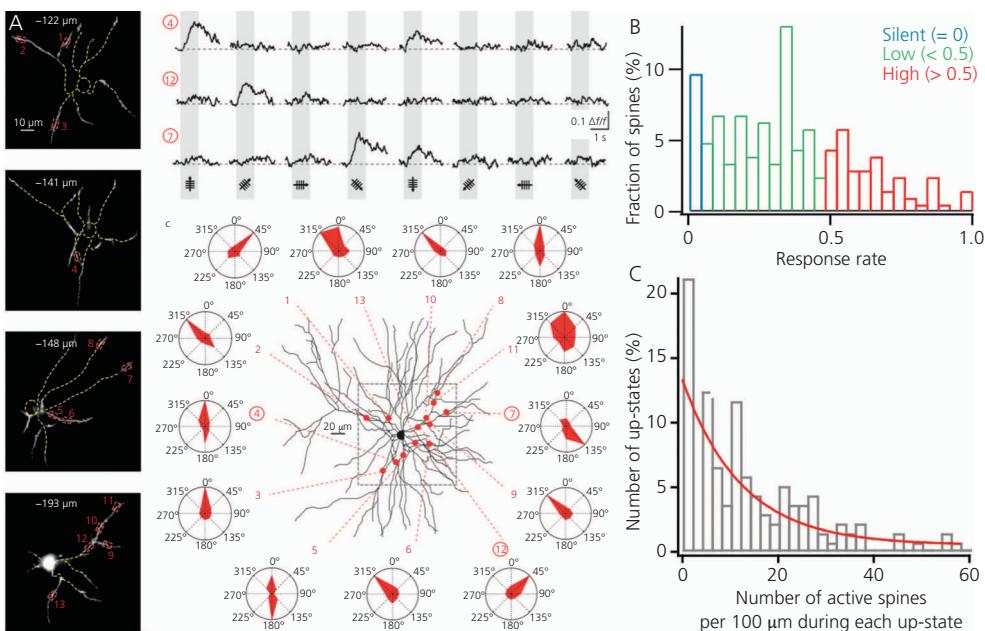


Fig. 13.2 Synaptic input in cortical pyramidal neurons during periods of activity (up-states). **(A)** Left: two-photon images of a L2/3 neuron within the primary visual cortex with red dashed boxes indicating hotspots of local dendritic calcium signaling. Right: (top) local dendritic calcium signals evoked by drifting gratings of different orientations and (bottom) location of hotspots and their orientation tuning on dendritic tree. **(B)** Spine response rates can be classified as “high” (≥ 0.5 Ca²⁺ transients/up-state), “low” (≤ 0.5 Ca²⁺ transients/up-state), or “silent” (no spontaneous Ca²⁺ transients) based on how active they are during spontaneous up-states measured by two-photon microscopy and patch-clamp electrophysiology. **(C)** Distribution of the density of active spines (number of active spines per dendritic length) during up-states as measured by two-photon calcium microscopy from single spines and somatic patch-clamp electrophysiology.

Part A reprinted by permission from Macmillan Publishers Ltd: *Nature*, 464(77293), Hongbo Jia, Nathalie L. Rochefort, Xiaowei Chen, and Arthur Konnerth, Dendritic organization of sensory input to cortical neurons in vivo, pp. 1307–1312, © 2010, Nature Publishing Group. Part B reprinted from *Cell Reports*, 4(1), Xiaowei Chen, Nathalie L. Rochefort, Bert Sakmann, and Arthur Konnerth, Reactivation of the same synapses during spontaneous up states and sensory stimuli, p. 33, Figure 1e., Copyright 2013, with permission from Elsevier. Part C reprinted from *Cell Reports*, 4(1), Xiaowei Chen, Nathalie L. Rochefort, Bert Sakmann, and Arthur Konnerth, Reactivation of the same synapses during spontaneous up states and sensory stimuli, p. 36, Figure 3e., Copyright 2013, with permission from Elsevier.

2004) and it is therefore impossible to correctly replicate the external environment (i.e., artificial cerebrospinal fluid; ACSF) in vitro. In any case, it is standard practice in studies in vitro to deliberately alter the known concentrations of the components of ACSF (Elliott and Jasper, 1949) such as elevating Ca²⁺ and glucose (Edwards et al., 1989). Furthermore, in an active physiological environment, various neurotransmitters, neuromodulators, and other molecules are present that are either absent or at very low concentrations in vitro, such as gamma-aminobutyric acid (GABA), glutamine, ascorbic acid, taurine, lactate, serotonin, etc. (Hajos and Mody, 2009). The differences

between ACSF in vitro and extracellular cerebral fluid (ECF) in vivo do play a role in determining the measured firing properties (Hablitz et al., 1986; Konnerth et al., 1986; Yaari et al., 1986; Watson and Andrew, 1995; Su et al., 2001; An et al., 2008) and plasticity (Kuenzi et al., 2000). There can also be other systematic differences between in vivo and in vitro preparations, such as temperature (Kalmbach and Waters, 2012) and anesthesia (Canal et al., 2005; Potez and Larkum, 2008), which may influence cellular properties, and of course in vitro preparations involve an unavoidable amount of tissue damage versus in vivo preparations.

Given all of these influential differences, it is perhaps surprising that there appears to be a large overlap in the dendritic properties so far measured in vitro and in vivo when measuring properties such as the propagation of action potentials in the dendrites and the activation of dendritic spikes (Larkum and Zhu, 2002; Waters et al., 2003; Xu et al., 2012; Chen et al., 2013b). Therefore, with all of the caveats mentioned already notwithstanding, we focus here largely on the physiological differences such as network activity in vivo in assessing the important issues of synaptic integration in vivo.

New approaches to dendritic integration in vivo

The recent surge in dendritic recordings in vivo derives in part from continual improvements in experimental approaches. Early direct dendritic recordings in vivo used the traditional sharp electrode approach (Buzsáki et al., 1996; Svoboda et al., 1997; Helmchen et al., 1999), whereas later efforts transitioned to patch-clamp recordings (Larkum and Zhu, 2002; Waters and Helmchen, 2004, 2006; Xu et al., 2012; Smith et al., 2013; Palmer et al., 2014). Very quickly, however, deep-tissue two-photon imaging came to dominate the study of dendritic function in vivo (Fig. 13.3). The use of two-photon imaging for exploring dendritic properties was established more or less at the same time as it was introduced as a neuroscientific method (Svoboda et al., 1997; Helmchen et al., 1999). Indeed, the ability to explore dendritic function in vivo was touted as one of the great advantages of this approach from the beginning.

The introduction of “shadow patching” (visualizing target structures using the absence of fluorescence ejected from the patch pipette) provides a method for targeting dendrites with patch electrodes in vivo (Kitamura et al., 2008). Perhaps even more importantly, the ubiquitous use of genetically encoded calcium indicators has made imaging dendritic Ca^{2+} dynamics in vivo with two-photon imaging a reality in the cortex (Gentet et al., 2012; Hill et al., 2012; Xu et al., 2012; Harnett et al., 2013; Palmer et al., 2014), hippocampus (Sheffield and Dombeck, 2015), and cerebellum (Schultz et al. 2009; Najafi et al., 2014). The disadvantage of this method is that multiple neurons are typically fluorescent, making it difficult to identify dendrites stemming from the same neuron (Xu et al., 2012), while the signal-to-noise resolution makes it hard to resolve individual dendritic spikes. For this reason and to simultaneously obtain single-cell imaging combined with patch-clamp recordings, a number of studies have filled individual cells with synthetic calcium indicators by including the dye in the patch pipette (Kitamura et al., 2008; Jia et al., 2010; Kitamura and Häusser, 2011; Varga et al., 2011; Smith et al., 2013; Grienberger et al., 2014; Palmer et al., 2014). In particular, this approach appears to be the method of choice for examining spatially restricted elevations of calcium in dendritic branches and spines. Improvements in the speed of acquisition using resonant scanning mirrors (Leybaert et al., 2005) and acousto-optical devices (Kaplan et al., 2001) for two-photon approaches have also been important for this issue because they increase the coverage of the dendritic tree (Jia et al., 2010) and the signal-to-noise ratio, particularly when using oversampling techniques (Chen et al., 2012). The addition of a piezo- or electric lens-driven objective to a two-photon microscope has become useful for imaging dendrites along the depth

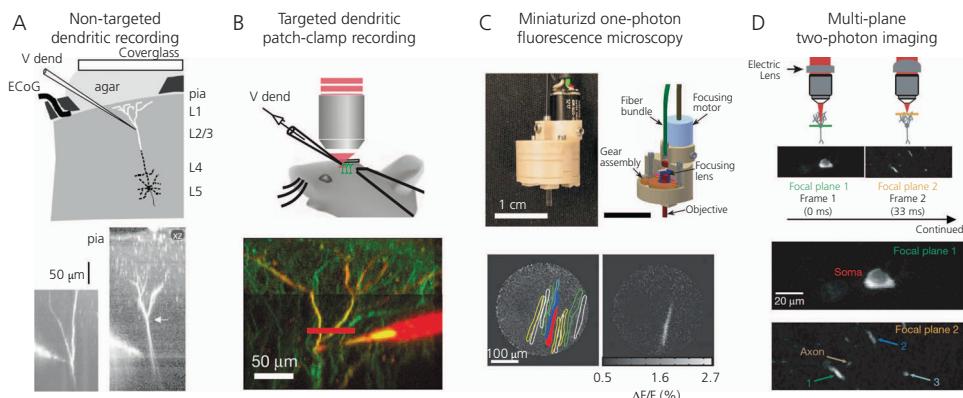


Fig. 13.3 Current methods for exploring dendritic integration *in vivo*. **(A)** Non-targeted “blind” recordings using sharp electrodes can record both the voltage and fluorescence signal from L5 pyramidal dendrites. **(B)** Dendrites with transgenic indicators can be targeted by patch-clamp pipettes for dual electrophysiology and two-photon microscopy. **(C)** A miniaturized one-photon fluorescence microscope can perform high-speed imaging of cerebellar Purkinje cell dendritic calcium activity. **(D)** Two-photon microscopy with an electric lens rapidly switches between two focal planes to generate co-acquired images of soma, dendrites, and axons in hippocampal neurons.

Part A reprinted by permission from Macmillan Publishers Ltd: *Nature Neuroscience*, 2(11), Fritjof Helmchen, Karel Svoboda, Winfried Denk, and David W. Tank, In vivo dendritic calcium dynamics in deep-layer cortical pyramidal neurons, p. 990, Figure 1a and b, Copyright 1999, Nature Publishing Group. Part B reprinted by permission from Macmillan Publishers Ltd: *Nature*, 492(7428), J Ning-long Xu, Mark T. Harnett, Stephen R. Williams, Daniel Huber, Daniel H. O’Connor, Karel Svoboda, and Jeffrey C. Magee, Nonlinear dendritic integration of sensory and motor input during an active sensing task, pp. 247–251, © 2012, Nature Publishing Group. Part C reprinted by permission from Macmillan Publishers Ltd: *Nature Methods*, 5(11), Benjamin A Flusberg, Axel Nimmerjahn, Eric D Cocker, Eran A Mukamel, Robert P J Barreto, Tony H Ko, Laurie D Burns, Juergen C Jung, and Mark J Schnitzer, High-speed, miniaturized fluorescence microscopy in freely moving mice, pp. 935–938, © 2008, Nature Publishing Group. Part D reprinted by permission from Macmillan Publishers Ltd: *Nature*, 517(7533), Mark E. J. Sheffield and Daniel A. Dombeck, Calcium transient prevalence across the dendritic arbor predicts place field properties, pp. 200–204, © 2015 Nature Publishing Group.

axis *in vivo* (Göbel and Helmchen, 2007; Sheffield and Dombeck, 2015). In addition to two-photon approaches, fiberoptic innovations have enabled even awake and freely moving dendritic recordings from populations of dendrites *in vivo* (Murayama et al., 2007; Murayama and Larkum, 2009).

Neuronal and network activity *in vivo*

Action potentials *in vivo*

Ultimately, the question of dendritic computation boils down to how ongoing synaptic inputs are transformed to produce ongoing trains of action potential outputs. It is therefore interesting to look at the kind of action potential firing that occurs *in vivo* at the cell body in as much as it can tell us some interesting facts about the computational processes underlying the generation of action potentials that presumably involve the whole dendritic tree and soma combined. Since the early 1990s it has been clear that action potentials not only travel down the axon but can also invade

dendrites (Stuart and Sakmann, 1994; Stuart and Häusser, 1994; Häusser et al., 1995; Spruston et al., 1995; Larkum et al., 1996, 2008; Bischofberger and Jonas, 1997; Lemon and Turner, 2000; Williams and Stuart, 2000; Waters et al., 2003; Bathellier et al., 2009; Ledergerber and Larkum, 2010; Casale and McCormick, 2011), leading to interesting interactions which affect both the integrative properties of the dendrites and the plasticity of synaptic inputs. Backpropagating action potentials (bAPs) influence the voltage events in the dendrite and have varying waveforms, in stark contrast to the consistent shape of somatic action potentials (Fig. 13.4). bAPs also play an important role in coincidence detection (Markram et al., 1997; Egger et al., 1999; Feldman, 2000; Holmgren and Zilberman, 2001; Sjöström et al., 2001; Stuart and Häusser, 2001; Froemke and Dan, 2002; Letzkus et al., 2006) and are seen as one of the cellular mechanisms behind learning and memory (for a review, see Feldman and Brecht, 2005). The most commonly used method for estimating the spread of bAPs in the dendritic tree is the combined use of two-photon Ca^{2+} imaging and intracellular electrophysiology (Svoboda et al., 1997). It is now relatively well established that bAPs reliably invade the basal dendrites (Hill et al., 2013) but not the distal apical tuft dendrites (Waters et al., 2003; Hill et al., 2013; Palmer et al., 2014) of cortical pyramidal neurons in vivo. For *in vivo* recordings, action potential backpropagation raises issues of interpretation since, as yet, there is no known way to perform dual dendritic/somatic electrical recordings like those that are performed regularly *in vitro*. Furthermore, the calcium signature of bAPs in the dendrites can also be confused with other dendritic events involving Ca^{2+} . Nevertheless, it has been observed that axonal sodium action potentials are typically characterized by a very sharp onset (“kink”) that is indicative of the rapid activation of a high density of sodium channels found in the initial segment (for a review, see Brette, 2012; Kole and Stuart, 2012) and this has been used to distinguish bAPs from other potentials of dendritic origin in direct patch recordings *in vivo* (Smith et al., 2013).

Action potential generation and irregular firing of neurons *in vivo*

As discussed in the Chapter 12, in most neurons (*in vitro*), the site of sodium action potential generation is in the axon (Stuart et al., 1997a; Clark et al., 2005; Palmer and Stuart, 2006). Even though there is no direct evidence for the site of action potential initiation *in vivo*, it is generally assumed to be the same as *in vitro* (Destexhe et al., 1998; Kole and Stuart, 2012). The kinetics and waveform of the generated action potential might be the same as *in vitro*, but the subthreshold state of

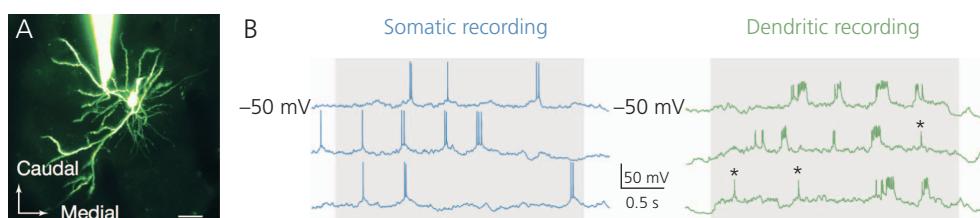


Fig. 13.4 In *vivo* patch-clamp electrophysiological recordings from pyramidal neurons in the visual cortex in response to a square-wave grating visual stimuli. (A) Two-photon microscopy image of a L2/3 pyramidal neuron filled with Alexa Fluor 594 and dendritic patch-clamp recording at 100 μm from the soma. Scale bar = 20 μm . (B) Dendritic recordings have complex and varying waveforms compared with the stereotypical somatic action potential. Asterisks (*) indicate backpropagating action potentials.

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the neuron is drastically different in the presence of network activity. The firing of cells *in vivo* is surprisingly irregular (Renart et al., 2010). It is often assumed that this is due to the fluctuating synaptic bombardment *in vivo*, but the irregularity actually observed *in vivo* is hard to simulate using only synaptic input, particularly in cortical neurons, without invoking active dendritic properties (Softky and Koch, 1993). Generating *in vivo*-like irregularity with noisy current injection into layer 5 (L5) pyramidal neurons in experiments *in vitro* required the activation of dendritic conductances via dendritic current injection (Larkum et al., 2004; Oviedo and Reyes, 2005). Nevertheless, the irregularity of firing seen in cortical neurons could also be explained by other means, such as coordinated, network-level fluctuations (Schölvicck et al., 2015). Furthermore, the conditions for determining the threshold for action potential generation are also fluctuating *in vivo* (Azouz and Gray, 2000). It is therefore not yet possible to assess how much the intricacies of postsynaptic integration versus variable presynaptic input contribute to the irregular spiking of neurons *in vivo*.

The consequences of this irregular activity viewed at both the dendritic and somatic levels are immense; the sensory environment is typically conveyed by a small number of active neurons firing a small number of action potentials (for a review, see Olshausen and Field, 2005; Wolfe et al., 2010). Termed “sparse coding,” this means that complex sensory information is encoded by a surprisingly small number of action potentials and a small fraction of neurons within the brain. Sparse coding has a number of possible advantages including: (1) allowing for dynamic storage of information, (2) maximizing the detectability of weak signals, and (3) conservation of energy. As observed by Barlow et al. (1972), early stage (*i.e.*, lower order) sensory neurons are generally more active than later stage (*higher order*) neurons. Therefore, similar to an energy pyramid, information from the sensory environment appears to be shaped and pruned as it is relayed to higher brain centers. This results in cortical neurons responding to only a specific subset of sensory stimuli, as seen in the visual (Vinje, 2000; Sawinski et al., 2009; Kampa et al., 2011), barrel (Brecht and Sakmann, 2002; Arabzadeh et al., 2005; Waters and Helmchen, 2006; von Heimendahl et al., 2007; Chen et al., 2013a), and somatosensory (Kerr et al., 2005; Gdalyahu et al., 2012; Palmer et al., 2014) cortices. Not only do these neurons only respond to a subset of stimulation paradigms, they also typically only sparsely fire action potentials in response to the relevant stimulation (Brecht and Sakmann, 2002; Tolhurst et al., 2009; Palmer et al., 2014).

Sparse coding of input to the dendritic tree would tend to favor inhomogeneity of input and increase the importance of active dendritic processes. This is indeed true for the generation of dendritic spikes, as illustrated during visual (Smith et al., 2013), whisker (Lavzin et al., 2012), and somatosensory (Palmer et al., 2014) stimulation. Direct dendritic recordings have shown that dendritic spikes can enhance stimulus selectivity during behavior (Lavzin et al., 2012; Xu et al., 2012; Smith et al., 2013) although not in all investigations (Jia et al., 2010; Chen et al., 2011). This leaves open the question as to whether sensory information is normally conveyed from neuron to neuron by the precise spatio-temporal pattern of activity rather than the sheer number of synaptic inputs. Nevertheless, it is still taken for granted that the integration of synaptic input determines the output of the neuron.

Backpropagating action potentials and synaptic integration *in vivo*

The integrative process in neurons is sometimes described in terms of the events leading to the generation of action potential output. This conception of synaptic integration is asymmetric, with the axon as the “final site of integration” (Stuart et al., 1997b). As mentioned above, synaptic integration can also be seen as an ongoing process in which spatio-temporal information conveyed by synaptic inputs is converted into continuous output in the form of spike trains. In this conception of dendritic integration, bAPs may play a vital role in the continuing process of shaping the

input–output function over time. For instance, it was recently demonstrated that bAPs can interact with dendritic Ca^{2+} channels, increasing the total conductance of the cell and therefore influencing the overall frequency of the output train of action potentials (Palmer et al., 2012b). This has the consequence that dendritically targeted inhibition can alter the output of the neuron even when the only input is to the cell body. While this particular situation (exclusively somatic input) is surely not physiological, it illustrates that even axonally generated action potentials have consequences for the ongoing integrative process in the dendrites.

Another possible role for bAPs might involve the association of inputs from different information streams in a process termed “backpropagation-activated coupling” (Larkum, 2013). In this scenario, a neuron fires action potentials (e.g., generated by feedforward input), which then backpropagate into the dendrite and interact with cortical feedback input, halving the current threshold for the generation of dendritic plateau potentials (backpropagation-activated Ca^{2+} spikes, or BAC spikes; Larkum et al., 1999). Since this interaction triggers a burst of multiple action potentials (BAC firing), combining feedforward input (in the form of bAPs) and feedback input (synaptic input onto distal dendrites) generates a robust signal which may be a cellular marker for cortical associations.

Whether or not the precise timing of action potentials is important for relaying information in the brain is still a matter of debate. Network models with large numbers of units (neurons) tend to lead to oscillatory activity that involves many neurons firing in synchrony. Indeed, neurons in some systems reliably fire stereotypical trains of action potentials (Long et al., 2010); however, time and time again, *in vivo* recordings from neurons within the mammalian central nervous system demonstrate irregular firing of principal neurons (reviewed by Softky, 1995; Shadlen and Newsome, 1998). The irregular nature of neuronal firing might convey information in itself. For instance some studies have shown that spike rate is less important than spike timing for conveying information to synaptically coupled neurons (Panzeri et al., 2001; Tiesinga et al., 2008). Therefore, the irregular nature of neuronal firing may in itself be the language of the brain, although the extent of this remains to be determined.

Summation of dendritic input *in vivo*

How dendrites integrate synaptic input largely depends on the number, frequency, strength, sign, and location of synaptic input. However, other factors such as short-term plasticity, fidelity of synaptic input, state of activity of the neuron, availability of postsynaptic ion channels, and activation of inhibitory feedback processes also influence integration. All these conditions vary constantly in the intact brain (unlike in brain slices) so there is unlikely ever to be a single simple summary of integration *in vivo*. Indeed, recent *in vivo* experiments illustrate that dendrites can integrate synaptic input sublinearly (Longordo et al., 2013), linearly (Leger et al., 2005), and supralinearly (Lavzin et al., 2012; Xu et al., 2012; Grienberger et al., 2014; Palmer et al., 2014) (Fig. 13.5). Furthermore, a sufficiently strong dendritic input can evoke local dendritic spikes such as Ca^{2+} spikes (Schiller et al., 1997; Larkum et al., 1999) in the distal apical dendrites of L5 pyramidal neurons, which are mediated primarily by L-type Ca^{2+} channels (Perez-Garcia et al., 2013). Dendritic spikes mediated by Na^+ channels (local Na^+ spikes or spikelets; see Chapter 12) have also been recorded in most regions of the dendritic tree (Larkum and Zhu, 2002; Ariav et al., 2003; Milojkovic et al., 2005; Gasparini and Magee, 2006; Losonczy and Magee, 2006; Larkum et al., 2009). Additionally, the often sub-micron thick dendrites in the basal and apical tuft can generate so-called *N*-methyl-D-aspartic acid (NMDA) spikes in response to local synaptic stimulation (see previous chapters; Polksy et al., 2004; Major et al., 2008; Larkum et al., 2009). These active, regenerative potentials have a large effect both locally and globally, thus influencing both dendritic and neuronal computation

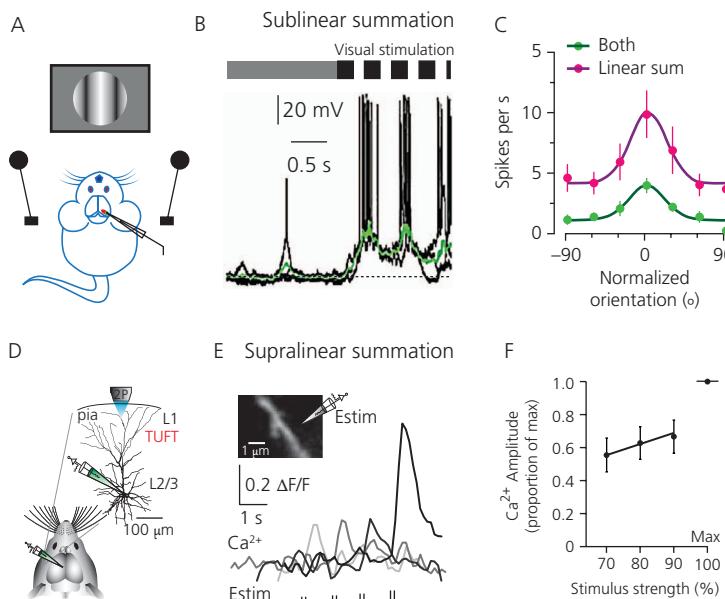


Fig. 13.5 Examples of sublinear (A–C) and supralinear (D–F) summation synaptic inputs. (A) Patch-clamp recordings from L2/3 pyramidal neurons within the primary visual cortex during visual stimulation of the eyes together or in isolation. (B) Patch-clamp recording illustrating the strong electrical response during visual stimulation. (C) Synaptic input during visual stimulation of both eyes sums sublinearly and results in significantly less output than the linear sum of stimulation of each eye in isolation. (D) Dual patch-clamp electrophysiology and two-photon microscopy were performed from L2/3 pyramidal neurons within the primary somatosensory cortex. (E) An extracellular stimulating pipette was placed in close proximity to the dendrite of interest and the stimulating strength (Estim) was linearly increased while recording the calcium response in the dendrite. (F) Supralinear integration occurred at the maximum stimulus strength.

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(for a review see Larkum and Nevian, 2008), as well as the output of the neuron by switching the firing pattern to burst-firing mode (Larkum and Zhu, 2002). Despite their influence on neuronal processing, there are only a few studies that have so far managed to show that dendritic spikes are associated with sensory perception and behavior (Lavzin et al., 2012; Xu et al., 2012; Cichon and Gan, 2015).

The first recordings of dendritic spikes *in vivo* were inferred from calcium imaging with synthetic dyes loaded internally via a patch pipette (Hirsch et al., 1995; Helmchen et al., 1999) or extracellularly (Murayama et al., 2009) as well as with direct dendritic patch recordings (Larkum et al., 2001). More recently, using genetic Ca²⁺ indicators, Xu et al. (2012) showed the existence of Ca²⁺ plateau potentials in the distal dendrites of L5 pyramidal neurons in the barrel cortex

during active whisker sensing. In other studies, NMDA-mediated non-linear dendritic processing was also shown to occur in L4 stellate neurons from the barrel cortex (Lavzin et al., 2012), L2/3 pyramidal neurons from the somatosensory cortex (Palmer et al., 2014), CA1 principal neurons from the hippocampus (Grienberger et al., 2014), and L5 pyramidal neurons of the motor cortex (Cichon and Gan, 2015). Since nonlinear dendritic processing has such a large and direct effect on synaptic integration and neuronal action potential output (Fig. 13.6), the generation of dendritic spikes might eventually be crucial to theories of higher brain functioning and perception (Larkum, 2013). The current trend of *in vivo* recordings from dendrites suggests this is not unlikely. For example, dendritic spikes have been shown to enhance action potential output *in vivo* (Lavzin et al., 2012; Smith et al., 2013; Grienberger et al., 2014; Palmer et al., 2014) and to be associated with orientation tuning of the vibrissal system (Lavzin et al., 2012) and visual cortex (Smith et al., 2013), sensory perception of the hindpaw (Palmer et al., 2014), spatial navigation (Sheffield and Dombeck, 2015), visual direction selectivity (Sivyer and Williams, 2013), and active vibrissal touch (Xu et al., 2012).

Irregular firing in the form of bursting has been attributed to various mechanisms. For instance, the generation of Ca^{2+} spikes in the distal apical dendrite of L5 neocortical pyramidal neurons (Larkum et al., 1999; Williams and Stuart, 1999) and to NMDA spikes in the basal dendrites (Polksy et al., 2009), and even NMDA-dependent Ca^{2+} spikes in basal dendrites of hippocampal pyramidal neurons (Grienberger et al., 2014). However, despite both burst firing and dendritic spikes being observed *in vivo*, it is difficult to attribute burst firing to one particular cause (Larkum and Zhu, 2002; Palmer et al., 2012b; Xu et al., 2012; Smith et al. 2013; Grienberger et al., 2014). Bursts of action potentials could in principle play an extremely important role in neuronal signaling (Lisman, 1997). For example, communication between weakly electric fish is conveyed in bursts of action potentials in the electrosensory lateral-line lobe, whereas behavior associated with prey is conveyed with a single action potential (Doiron et al., 2003). The saliency of action potential trains in terms of influencing a forced choice paradigm in rats is also increased by bursts (Doron et al., 2014). Bursts have also been shown to evoke long-term potentiation to a greater extent than single action potentials (Pike et al., 1999). In these examples, it is not always clear what the underlying mechanisms for bursting are. However, it seems safe to conclude that some proportion of the burst firing normally seen *in vivo* is attributable to active dendritic integrative properties and that they convey important information to synaptic targets.

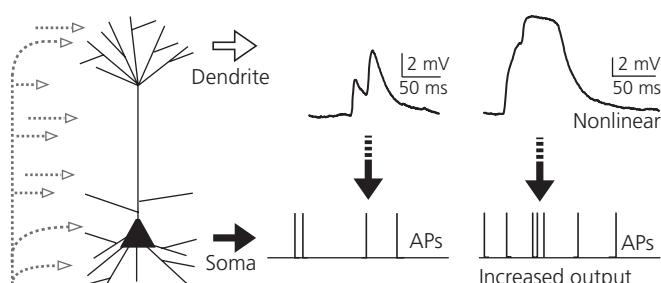


Fig. 13.6 Nonlinear dendritic processing has a large and direct effect on local synaptic integration and neuronal action potential output. Synaptic input pathways can target particular areas of the dendritic tree. Alone, these inputs do not drastically alter neuronal output; however, when combined, the input streams can generate non-linear dendritic plateau potentials which cause increased neuronal output.

Dendritic integration and spatial processing in vivo

Place cells of the hippocampus respond to an animal's location in space (Moser et al., 2008). Interestingly, the subthreshold response properties of place cells can be influenced by postsynaptic current injection (Lee et al., 2012). This suggests that active dendritic processes could control the synaptic integration of place information. Since this influence arises via somatic current injection, it might also be speculated that the active processes are relatively close to the soma (i.e., not in the distal apical tuft dendrite). Two-photon imaging studies from anesthetized mice revealed the presence of NMDA-dependent Ca^{2+} spikes in the basal dendrites of CA1 pyramidal neurons (Grienberger et al., 2014). Basal dendritic Ca^{2+} transients representing branch spikes also correlate with place for head-fixed mice in a virtual environment (Sheffield and Dombeck, 2015). Taken together, there is now good evidence to suggest that the active properties of hippocampal pyramidal dendrites play a major role in the integrative process and therefore spatial processing in vivo.

Dendritic inhibition and neuromodulation in vivo

One cannot discuss dendritic integration in the intact brain without acknowledging the influence of neuromodulation, inhibition, and other dendritic conductances activated by neural networks. The role of dendritic inhibition in controlling neuronal activity has recently received a lot of attention, due largely to the development of reliable markers for labeling different interneuron subtypes (Ascoli et al., 2008). It has long been known that apical and tuft dendrites are directly inhibited by specific subtypes of interneurons (for reviews see Markram et al., 2004; Klausberger, 2009; Kubota, 2014). As we learn more about GABAergic signaling and interneuron activity in vivo (Gentet, 2012; Kaifosh et al., 2013; Lovett-Barron and Losonczy, 2014), the functional relevance of dendritic inhibition during behavior is slowly being realized (Isaacson and Scanziani, 2011; Palmer et al., 2012a; Higley, 2014; Roux and Buzsaki, 2015) (Fig. 13.7). Martinotti neurons

Fig. 13.7 Inhibition of dendritic integration in vivo. (A) Disinhibition (disynaptic) and (B) direct inhibition microcircuits were discovered in the sensorimotor cortex by simultaneous patch-clamp recordings from L5 pyramidal neuron dendrites (black, gray) and either single-bouquet cells (magenta) or elongated neurogliaform cells (green), respectively. Single-bouquet cells promoted whereas elongated neurogliaform cells suppressed the initiation of dendritic complex spikes during both spontaneous and whisker-evoked activity. Recording traces are aligned by spontaneous somatic action potentials or whisker stimulation, and arrowheads indicate the time of interneuron action potentials and dots indicate dendritic complex spikes. (C) Fiberoptically recorded evidence for disynaptic Martinotti cell dendritic inhibition of Ca^{2+} activity in populations of L5 pyramidal cell apical dendrites in vivo. L5 pyramidal neuron dendrites were loaded with a Ca^{2+} indicator and the Ca^{2+} response to hindlimb stimulation (HLS) was measured before (black) and after (orange) application of TTX into L5. Dendritic Ca^{2+} activity increased during TTX application due to the relief of dendritic inhibition by L5 Martinotti cells. (D) Interhemispheric input onto cortical L1 interneurons activates a GABA_B -ergic direct inhibition of L5 pyramidal neuron dendritic activity in vivo. Direct patch-clamp dendritic recordings during hindpaw stimulation (C-HS) illustrate the effective block of dendritic activity by upper layer application of the GABA_B agonist Baclofen (red). (E) Schematic diagram showing the types of dendritic inhibition so far studied experimentally in vivo.

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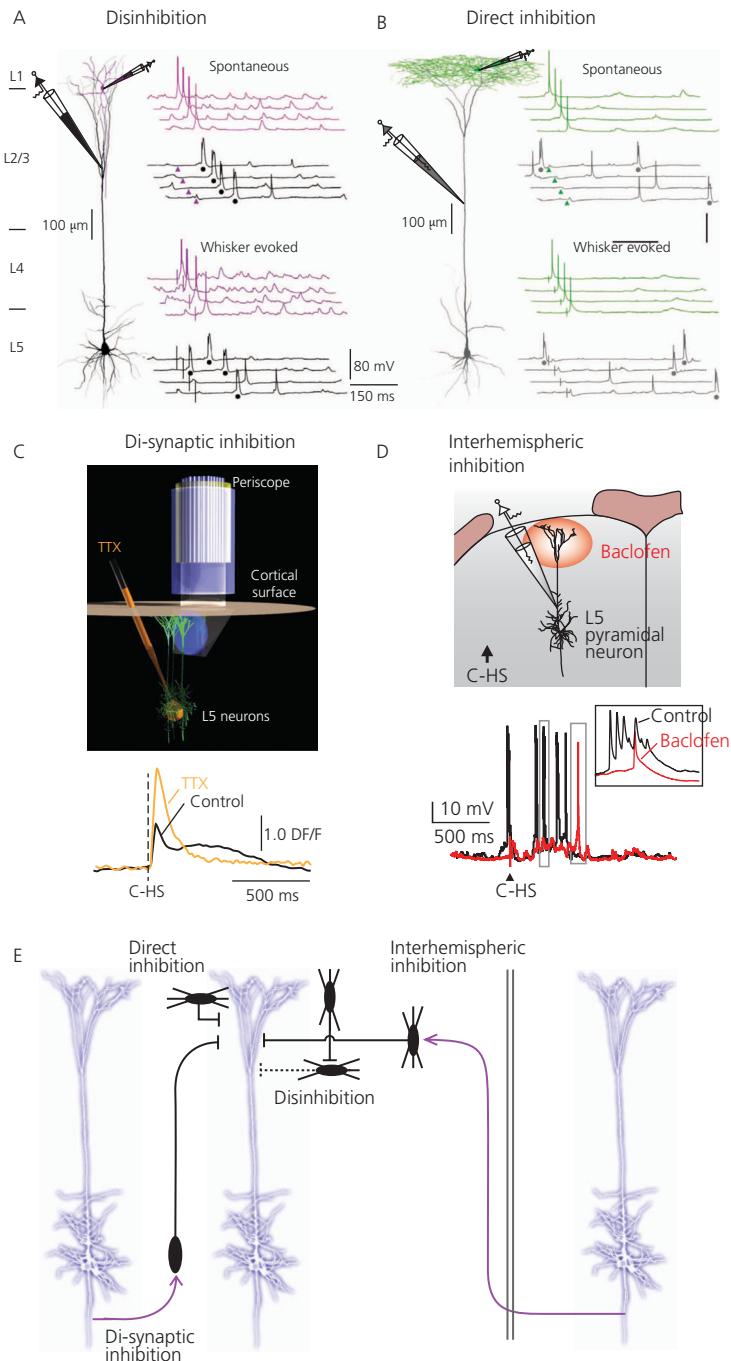


Fig. 13.7 (Continued)

from Macmillan Publishers Ltd: *Nature*, 457(7233), Masanori Murayama, Enrique Pérez-Garcí, Thomas Nevian, Tobias Bock, Walter Senn, and Matthew E. Larkum, Dendritic encoding of sensory stimuli controlled by deep cortical interneurons, pp. 1137–1141, © 2009 Nature Publishing Group. Part D reprinted from Lucy M. Palmer, Jan M. Schulz, Sean C. Murphy, Debora Ledergerber, Masanori Murayama, and Matthew E. Larkum, The cellular basis of GABA_B-mediated interhemispheric inhibition, *Science*, 335(6071) pp. 989–993, © 2012, The American Association for the Advancement of Science. Reprinted with permission from AAAS.

within L5 of the somatosensory cortex provide GABA_A-mediated dendritic inhibition and have been shown to modulate the responses of L5 pyramidal neuron dendrites to sensory stimulation (Murayama et al., 2009). Furthermore, the somatostatin-expressing interneurons in L2/3 of the somatosensory barrel cortex reduce their action potential firing in response to both passive and active whisker sensing (Gentet et al., 2012). This reduction in GABAergic synaptic input during whisker sensing likely results in enhanced distal dendritic excitability during active cortical processing. The opposite was true during fear learning in the hippocampus, where aversive stimuli activated somatostatin-expressing interneurons leading to inhibition of pyramidal cell distal dendrites (Lovett-Barron et al., 2014). Distal dendritic activity of L5 pyramidal neurons in the primary somatosensory cortex is also damped during interhemispheric sensory stimulation by the activation of postsynaptic GABA_B receptors (Palmer et al., 2012b), presumably by neurogliaform cells which provide postsynaptic GABA_B-mediated inhibition (Oláh et al., 2009). This was directly illustrated by Jiang et al. (2013) using dual patch-clamp recordings *in vivo* from cortical pyramidal neuron dendrites and neurogliaform cells. In this study, neurogliaform cells inhibited the initiation of dendritic complex spikes in the majority of L5 pyramidal neurons during both spontaneous and whisker-evoked activity.

In addition to modulating dendritic activity by direct GABAergic input, interneurons can also influence dendritic processing indirectly by providing disinhibition. Single bouquet cells in L1 of the somatosensory cortex directly inhibit L2/3 interneurons during whisker sensing and spontaneous activity, which in turn alleviates dendritic inhibition and leads to complex spike generation (Jiang et al., 2013). Disinhibition also plays important roles in numerous processes, including auditory plasticity (Froemke et al., 2007), motor integration (Lee et al., 2013), and fear learning in both the auditory cortex (Letzkus et al., 2011; Pi et al., 2013) and amygdala (Wolff et al., 2014).

Inhibition is fundamental to neuronal processing (Sillito, 1975; Borg-Graham et al., 1998; Bruno and Simons, 2002; Wehr and Zador, 2003; Rudolph et al., 2007; Cardin et al., 2009; Douglas and Martin, 2009; Runyan et al., 2010; Isaacson and Scanziani, 2011; Letzkus et al., 2011). The sheer diversity of interneurons and whether they contribute to feedforward or feedback processes (Kullmann, 2011) makes it extremely complicated to untangle the microcircuitry that is active during behavior. Taking into account that further computation can be carried out postsynaptically via active dendritic processes (Mel, 1994; Archie and Mel, 2000; Nevian and Sakmann, 2006; Branco and Häusser, 2010; Takahashi et al., 2012), it would also be possible for inhibition to contribute in a complex way to these dendritic processes, for example, silent inhibition, where dendritic inhibition is not effective until dendritic voltage-sensitive channels are sufficiently active (Frégnac et al., 2003; Gidon and Segev, 2012; Palmer et al., 2012b).

Dendritic integration can also be dramatically influenced by other modulatory conductances. For example, voltage-gated potassium channels modulate dendritic excitability and synaptic integration in hippocampal CA1 pyramidal neurons (Cai et al., 2004; Gasparini et al., 2004; Losonczy et al., 2008) and cortical L5 pyramidal neurons (Harnett et al., 2013). *In vivo*, block of voltage-gated potassium channels increased the occurrence, amplitude, and area of dendritic Ca²⁺ signals evoked by whisker-object contact from neurons within the vibrissal cortex (Harnett et al., 2013). Similar effects were seen on dendritic excitability during the block of the hyperpolarization-activated cyclic nucleotide-gated non-selective cation (HCN) channel (Magee, 1999; Berger et al., 2001; Larkum et al., 2009; Harnett et al., 2015). The behavioral consequences of this are still unknown; however, deletion of the *HCN1* gene leads to profound deficits in motor learning (Nolan et al., 2004), which might be a consequence of constraining spatial plasticity at distal CA1 dendrites (Nolan et al., 2004) or altering temporal synchrony (Vaidya and Johnston, 2013). It is hard to predict what influence neuromodulation really has on complex behavior (Marder, 2012) and

ion channels typically work in concert with one another making it difficult to dissect the role one channel plays in isolation.

Synaptic input in vivo

Spatial patterning of synaptic inputs

The spatio-temporal pattern of synaptic input determines the local fluctuation in membrane potential. This can have consequences for dendritic computation, even in passive dendritic trees (Koch et al., 1982). However, it is clearly of great importance when considering active dendrites with thresholds for various intracellular events. The normal condition for a neuron *in vivo* involves a large number of background synaptic inputs even in periods of low activity. During periods of high activity there could be many thousands of inputs activating the dendritic tree simultaneously. It is therefore very important to understand what shapes the spatio-temporal pattern of inputs *in vivo* and what influence this has on local integration. However, actually determining the spatio-temporal pattern of synaptic inputs under particular conditions is also one of the thorniest and most contentious issues currently facing researchers in the field of dendritic integration.

Clustering of synaptic inputs

The simplest kind of spatial patterning is a cluster of inputs, i.e., a group of synaptic terminals located along a small region (a few microns) of the dendritic tree that release their transmitter in a more or less coordinated fashion (Larkum and Nevian, 2008). The exact number or density of synapses that should constitute a cluster is defined loosely in terms of their postsynaptic effect. The theoretical importance of the clusters of inputs on the dendritic trees has long been recognized (Koch et al., 1982; Mel, 1992). One of the most prominent consequences of synaptic clustering that has been observed in pyramidal and spiny stellate neurons of the cortex is the generation of NMDA spikes (Schiller et al., 2000; Antic et al., 2010). These spikes require a minimum of around 10–50 nearby synaptic inputs simultaneously (Larkum et al., 2009; Palmer et al., 2014). The ensuing dendritic spike remains local (i.e., it does not usually propagate more than a few tens of microns) (Polsky et al., 2004), and results in a local current one to two orders of magnitude larger than the equivalent excitatory postsynaptic potential (EPSP) that is generated from alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors at the same synapses (Polsky et al., 2004).

Spatially ordered synaptic input

It has long been proposed that the pattern of inputs to the dendritic tree could drive the higher-order function of neurons *in vivo* (Holmes and Woody, 1989). For example, many theoretical and experimental studies have focused on dendritic mechanisms for realizing direction selectivity in single neurons (Koch et al., 1983; Livingstone, 1998; Anderson et al., 2000; Oesch et al., 2005; Branco et al., 2010; Jia et al., 2010; Smith and Häusser, 2010; Briggman et al., 2011; Smith et al., 2013). It is well established that the spatial patterning of inputs *in vivo* combined with the intrinsic dendritic mechanisms determine the sensitivity of neurons to the direction of movement of light on the retina (Borg-Graham and Grzywacz, 1992; Borst and Egelhaaf, 1994; Single et al., 1997; Borg-Graham, 2001; Euler et al., 2002; Lee and Zhou, 2006; Sivyer and Williams, 2013). Although direction-selective responses often involve active conductances (Branco et al. 2010; Branco and Häusser 2011), even sequential activation of synaptic inputs in one direction along a passive dendrite could preferentially activate the neuron versus the other direction (Rall, 1964; Livingstone,

1998). So far, examples of such an extremely patterned input have yet to be demonstrated *in vivo* except in specialized systems such as auditory neurons (Agmon-Snir et al., 1998).

It has been suggested that a more likely scenario is that high rates of synaptic input lead to activation of many regions of the dendritic tree simultaneously (Farinella et al., 2014). A series of state-of-the-art studies in cortical neurons (carried out by the Konnerth lab) appear to indicate just such a homogeneous “salt-and-pepper” like distribution of synaptic inputs during sensory processing (Jia et al., 2010; Chen et al., 2011; Varga et al., 2011). On the other hand, there is also evidence for clustering of synaptic inputs in various systems in studies from other laboratories (Bollmann and Engert, 2009; Kleindienst et al., 2011; Fu et al., 2012; Takahashi et al., 2012; Druckmann et al., 2014). Although it is generally accepted that the generation of dendritic spikes is dependent on some degree of non-uniform synaptic activation (for review see DeBello et al., 2014), the background synaptic input during periods of high activity may provide the necessary foundation for which only a handful of extra inputs can drive the dendrite to spike threshold. In other words, anatomically clustered input pathways may not be necessary to evoke local spikes in a high-synaptic-input regime (Farinella et al., 2014). Whether synaptic input is clustered or spatially distributed is still debated and may be neuron and/or pathway specific.

Dendritic democracy *in vivo*

Because of the central importance of output action potentials in the integrative process, it is interesting to consider the effect of any given synaptic input in terms of its particular influence on spike generation. This concept was explored explicitly with the notion of “synaptic information efficacy” which outlined a theory for calculating a single number to describe this quantity (London et al., 2002, 2008). Experimental evidence from CA1 pyramidal neurons suggested that the maximum conductances of synaptic inputs might be scaled according to their distance from the cell body (Magee and Cook, 2000). This concept, dubbed “dendritic democracy” (Häusser, 2001), would imply that homeostatic mechanisms might serve to ensure that synaptic inputs are equally effective in producing output spikes regardless of their position on the dendritic tree. However, this experimental finding apparently does not generalize to other neurons (Williams and Stuart, 2002). Furthermore, the results for CA1 were obtained *in vitro* and were subsequently challenged in terms of their applicability to the *in vivo* situation (London and Segev, 2001), serving as a useful demonstration of the difficulties in extrapolating information about processes concerning synaptic integration from *in vitro* data.

Isopotentiality

The general finding that the amplitude of synaptic inputs tends to attenuate drastically over large dendritic lengths (Stuart and Spruston, 1998; Williams and Stuart, 2002; Larkum et al., 2009) suggests that synapse location should play an important role in the computational process. However, in order to reach threshold there must be substantial depolarization above the resting level which, in the absence of dendritic spikes, requires at least several hundreds of simultaneous synaptic inputs (Chen et al., 2013b). As already noted, the seminal work from the Konnerth lab in recent years has provided strong evidence that synaptic activation *in vivo* is often quite homogeneously distributed over the dendritic tree with no particular preference for any given subregion or tree (Jia et al., 2010; Chen et al., 2011; Varga et al., 2011). Under these conditions (large number of inputs homogeneously distributed over the whole dendritic tree), the location of the actual synaptic inputs on the dendritic tree for a given sensory stimulus could be rendered essentially irrelevant. That is, if sensory input typically arrives in barrages of homogeneously distributed inputs, the precise

location of each synaptic input will have only a weak influence on depolarization achieved at the cell body as it will be averaged out in the overall signal. This appears to be the case in the visual cortex where the subthreshold membrane potential envelopes due to different stimulus orientations are largely similar (Jia et al., 2010). Since the resulting action potential firing is extremely tuned to specific orientations, this means that either the subtle differences in the voltage envelopes are crucial (the “iceberg hypothesis;” Rose and Blakemore, 1974; Priebe and Ferster, 2008) or there are other factors at play, for example dendritic spikes (Palmer et al., 2014) or silent inhibition (Palmer et al., 2012a).

However, there is another issue that compromises the importance of synaptic input location in terms of passive integration. The accumulation of enough synaptic inputs to reach threshold can take time; sometimes even longer than the membrane time constant. In this more or less steady-state condition, the total charge from all synaptic inputs will quickly distribute homogeneously throughout the dendritic tree. This result is generally true regardless of the temporal dynamics of the original input, and with enough distributed input, the current at any given location is dominated by the number (not the location) of synaptic inputs. Although counterintuitive, this means that over time scales relevant to the computational process (over tens of milliseconds), the voltage profile due to homogenously distributed synaptic input is likely to be approximately equal everywhere (Fig. 13.8), such that the neuron is approximately isopotential. This prediction has been borne out by dendritic recordings from neurons in very high-activity conditions such as organotypic spinal cord motoneurons and turtle cortex (Larkum et al., 1996, 2008). One consequence of this situation is that the axon hillock, where Na^+ action potentials are typically initiated, will generally have a voltage profile over time that is similar to any other part of the neuron. Another prediction is that up-states are likely to be reflected similarly at all points in the neuron. Although there are not yet any examples of electrical recordings from multiple dendritic sites of cells *in vivo*, distal tuft recordings from L2/3 pyramidal neurons showed up-states with a similar amplitude to somatic up-states (Waters and Helmchen, 2004, 2006) (Fig. 13.8). It remains to be seen whether there are particular circumstances *in vivo* in which the output of the neurons is heavily dependent on non-uniform spatial distributions of synaptic inputs.

Dendritic spikes in a high conductance state

Given the previous discussion, it might seem that the distribution of synaptic inputs is of minor importance; however, the same conditions (large numbers of simultaneous inputs over 10s of ms) that lead to isopotential dendrites also favor the generation and influence of local dendritic spikes. A high-conductance state will tend to depolarize the membrane potential to near threshold values, but at the same time synaptic input simultaneously becomes progressively ineffective (Crochet et al., 2005). This is because EPSPs become progressively more shunted (Thomson and Destexhe, 1999), presynaptic boutons become more depleted of vesicles (Crochet et al., 2005), and sensory stimulation tends to have an effective reversal potential at subthreshold values (Sachidhanandam et al., 2013) because of the balance of excitation and inhibition (Okun and Lampl, 2008). This situation of diminishing returns for synaptic input therefore favors short fluctuations in membrane potential achieved during coordinated barrages of synaptic input where excitation briefly dominates (Douglas and Martin, 1991). For the same reason, dendritic spikes are also predicted to be disproportionately influential during high-conductance states (Thomson and Destexhe, 1999). Importantly however, it is exactly during coordinated barrages of synaptic inputs that the clustered input required for dendritic spikes (Larkum and Nevian, 2008) is most likely to be present. In particular, NMDA-dependent electogenesis is extremely dependent on clustering because of the added requirement for glutamate to bind to NMDA receptors (Larkum and Nevian, 2008; Major

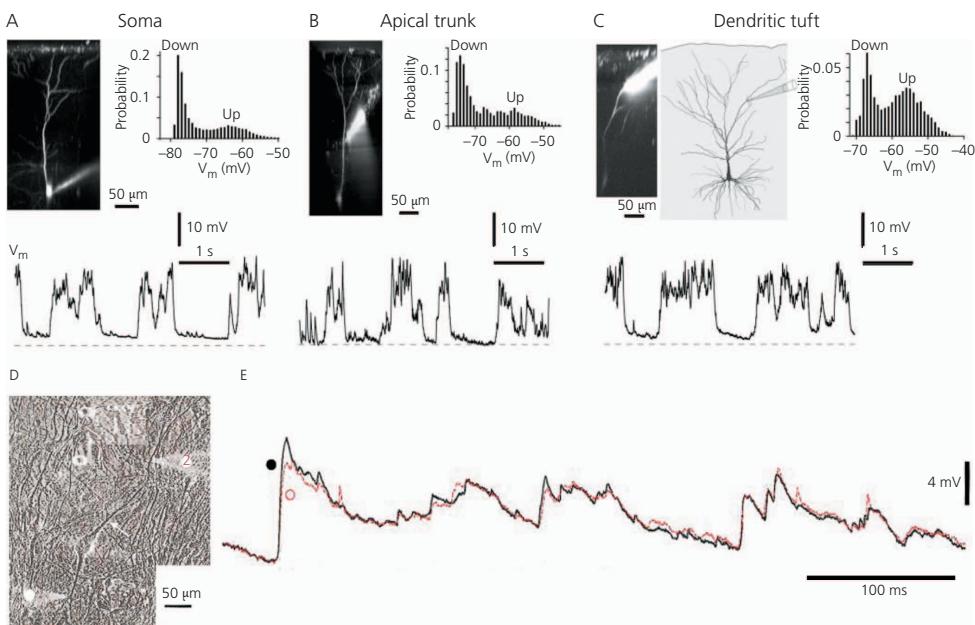


Fig. 13.8 Neurons are approximately isopotential. (A–C) In vivo examples of patch-clamp recordings of up- and down-states in L2/3 pyramidal neurons from anesthetized rats showing the similarity of up-states throughout the dendritic tree. (D–E) Patch-clamp recordings from either side of the cell body on spinal cord motoneuron dendrites in an active slice (organotypic spinal cord co-culture slice) showing isopotentiality on the slow time scale. Note the similarities of the different traces recorded from the two separate recording locations.

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et al., 2013). There is evidence that NMDA spikes might account for nearly two-thirds of the output action potentials in L2/3 neurons during high-conductance up-states (Palmer et al., 2014). Thus, although intracellular blockade of NMDA receptors has no effect on the amplitude of subthreshold up/down-states, it severely reduces action potential output (Chen et al., 2013b; Smith et al., 2013; Palmer et al., 2014).

Dendritic integration in vivo and plasticity

NMDA receptors are also required for many forms of plasticity (Hunt and Castillo, 2012). It has been shown that long-term changes in the excitability of the postsynaptic membrane can be dependent on the details of dendritic integration (Losonczy et al., 2008; Debanne and Poo, 2010). In some cases, postsynaptic plasticity is a function of the local depolarization of the dendritic membrane and is therefore particularly susceptible to local dendritic spikes. Some of these types of processes found in vivo also show NMDA receptor dependence (Bollmann and Engert, 2009),

including the necessity for postsynaptic NMDA-dependent spiking (Gambino et al., 2014; Cichon and Gan, 2015). Spike timing-dependent plasticity may also occur *in vivo* (Richards et al., 2010), and this, as we have already seen, would also depend on the properties of synaptic integration *in vivo*. However, this too is a controversial issue and it has been argued that the complications introduced by *in vivo* conditions will also complicate the rules for spike timing-dependent plasticity (Lisman and Spruston, 2005; Schulz, 2010).

Concluding remarks

Interpreting our environmental surroundings requires the integration of information, which is determined by the activation of multiple senses. At a cellular level, this means receiving and integrating synaptic input, which arrives on the dendrites from different brain regions as well as the periphery. Most of what we currently know about dendritic integration of synaptic input has been gleaned from *in vitro* studies that are better suited to investigate and manipulate dendritic activity precisely. Although this approach has its advantages, it is difficult to gage what the various known dendritic integration rules garnered from *in vitro* studies mean for overall brain function and perception. Advances in recording techniques have recently enabled us to delve into dendritic integration in the intact brain, and the important role these active dendritic processes play in behavior is slowly being realized. Dendritic integrative processes have been shown to be vital for various behaviors ranging from sensory-motor tasks (Lavzin et al., 2012; Palmer et al., 2012b; Xu et al., 2012; Lovett-Barron et al., 2014; Palmer et al., 2014), to visual processing (Longordo et al., 2013; Sivyer and Williams, 2013; Smith et al., 2013), to navigation (Sheffield and Dombeck, 2015). However, before we can digest the implications of these findings, we must first understand neuronal processing in the intact system at the cellular and network levels. We still have much to learn about dendritic integrative properties *in vivo*, but with the rapid advances in recording techniques in the intact brain it becomes reasonable to hope that we will one day be able to describe the true role of dendritic integration during behavior.

References

- Agmon-Snir H, Carr CE, Rinzel J (1998) The role of dendrites in auditory coincidence detection. *Nature* **393**:268–272.
- An JH, Su Y, Radman T, Bikson M (2008) Effects of glucose and glutamine concentration in the formulation of the artificial cerebrospinal fluid (ACSF). *Brain Research* **1218**:77–86.
- Anderson JS, Carandini M, Ferster D (2000) Orientation tuning of input conductance, excitation, and inhibition in cat primary visual cortex. *Journal of Neurophysiology* **84**:909–926.
- Antic SD, Zhou WL, Moore AR, Short SM, Ikonomo KD (2010) The decade of the dendritic NMDA spike. *Journal of Neuroscience Research* **88**:2991–3001.
- Arabzadeh E, Zorzin E, Diamond ME (2005) Neuronal encoding of texture in the whisker sensory pathway. *PLoS Biology* **3**:e17.
- Archie KA, Mel BW (2000) A model for intradendritic computation of binocular disparity. *Nature Neuroscience* **3**:54–63.
- Ariav G, Polsky A, Schiller J (2003) Submillisecond precision of the input-output transformation function mediated by fast sodium dendritic spikes in basal dendrites of CA1 pyramidal neurons. *Journal of Neuroscience* **23**:7750–7758.
- Ascoli GA, et al. (2008) Petilla terminology: nomenclature of features of GABAergic interneurons of the cerebral cortex. *Nature Reviews Neuroscience* **9**:557–568.
- Azouz R and Gray CM (2000) Dynamic spike threshold reveals a mechanism for synaptic coincidence detection in cortical neurons *in vivo*. *Proceedings of the National Academy of Sciences of the United States of America* **97**:8110–8115.

- Barlow HB, Narasimhan R, Rosenfeld A (1972) Visual pattern analysis in machines and animals. *Science* **177**:567–575.
- Bathellier B, Margrie TW, Larkum ME (2009) Properties of piriform cortex pyramidal cell dendrites: implications for olfactory circuit design. *Journal of Neuroscience* **29**:12641–12652.
- Berger T, Larkum ME, Lüscher HR (2001) High I-h channel density in the distal apical dendrite of layer V pyramidal cells increases bidirectional attenuation of EPSPs. *Journal of Neurophysiology* **85**:855–868.
- Bernander O, Douglas RJ, Martin KA, Koch C (1991) Synaptic background activity influences spatiotemporal integration in single pyramidal cells. *Proceedings of the National Academy of Sciences of the United States of America* **88**:11569–11573.
- Bischofberger J, Jonas P (1997) Action potential propagation into the presynaptic dendrites of rat mitral cells. *Journal of Physiology* **504**:359–365.
- Bollmann JH, Engert F (2009) Subcellular topography of visually driven dendritic activity in the vertebrate visual system. *Neuron* **61**:895–905.
- Borg-Graham LJ (2001) The computation of directional selectivity in the retina occurs presynaptic to the ganglion cell. *Nature Neuroscience* **4**:176–183.
- Borg-Graham LJ, Grzywacz NM (1992) A model of the directional selectivity circuit in retina: transformations by neurons singly and in concert. In: *Single Neuron Computation* (McKenna T, Davis J, Zornetzer ZF, eds), pp. 347–376. San Diego, CA: Academic Press.
- Borg-Graham LJ, Monier C, Fregnac Y (1998) Visual input evokes transient and strong shunting inhibition in visual cortical neurons. *Nature* **393**:369–373.
- Borst A, Egelhaaf M (1994) Dendritic processing of synaptic information by sensory interneurons. *Trends in Neurosciences* **17**:257–263.
- Branco T and Häusser M (2010) The single dendritic branch as a fundamental functional unit in the nervous system. *Current Opinion in Neurobiology* **20**:494–502.
- Branco T, Häusser M (2011) Synaptic integration gradients in single cortical pyramidal cell dendrites. *Neuron* **69**:885–892.
- Brech M, Sakmann B (2002) Whisker maps of neuronal subclasses of the rat ventral posterior medial thalamus, identified by whole-cell voltage recording and morphological reconstruction. *Journal of Physiology* **538**:495–515.
- Brette R (2012) Computing with neural synchrony. *PLoS Computational Biology* **8**:e1002561.
- Briggman KL, Helmstaedter M, Denk W (2011) Wiring specificity in the direction-selectivity circuit of the retina. *Nature* **471**:183–188.
- Bruno RM, Simons DJ (2002) Feedforward mechanisms of excitatory and inhibitory cortical receptive fields. *Journal of Neuroscience* **22**:10966–10975.
- Buzsáki G, Penttonen M, Nadasdy Z, Bragin A (1996) Pattern and inhibition-dependent invasion of pyramidal cell dendrites by fast spikes in the hippocampus in vivo. *Proceedings of the National Academy of Sciences of the United States of America* **93**:9921–9925.
- Cai X, Liang CW, Muralidharan S, Kao JP, Tang CM, Thompson SM (2004) Unique roles of SK and Kv4.2 potassium channels in dendritic integration. *Neuron* **44**:351–364.
- Canal CE, McNay EC, Gold PE (2005) Increases in extracellular fluid glucose levels in the rat hippocampus following an anesthetic dose of pentobarbital or ketamine-xylazine: an in vivo microdialysis study. *Physiology and Behavior* **84**:245–250.
- Cardin JA, Carlen M, Meletis K, Knoblich U, Zhang F, Deisseroth K, Tsai LH, Moore CI (2009) Driving fast-spiking cells induces gamma rhythm and controls sensory responses. *Nature* **459**:663–667.
- Casale AE, McCormick DA (2011) Active action potential propagation but not initiation in thalamic interneuron dendrites. *Journal of Neuroscience* **31**:18289–18302.
- Chen X, Leischner U, Rochefort NL, Nelken I, Konnerth A (2011) Functional mapping of single spines in cortical neurons in vivo. *Nature* **475**:501–505.

- Chen X, Leischner U, Varga Z, Jia H, Deca D, Rochefort NL, Konnerth A (2012) LOTOS-based two-photon calcium imaging of dendritic spines in vivo. *Nature Protocols* 7:1818–1829.
- Chen JL, Carta S, Soldado-Magraner J, Schneider BL, Helmchen F (2013a) Behaviour-dependent recruitment of long-range projection neurons in somatosensory cortex. *Nature* 499:336–340.
- Chen X, Rochefort NL, Sakmann B, Konnerth A (2013b) Reactivation of the same synapses during spontaneous up states and sensory stimuli. *Cell Reports* 4:31–39.
- Cichon J, Gan WB (2015) Branch-specific dendritic Ca(2+) spikes cause persistent synaptic plasticity. *Nature* 520: 180–185.
- Clark BA, Monsivais P, Branco T, London M, Häusser M (2005) The site of action potential initiation in cerebellar Purkinje neurons. *Nature Neuroscience* 8:137–139.
- Colonnier M (1968) Synaptic patterns on different cell types in the different laminae of the cat visual cortex. An electron microscope study. *Brain Research* 9:268–287.
- Connors BW, Gutnick MJ, Prince DA (1982) Electrophysiological properties of neocortical neurons in vitro. *Journal of Neurophysiology* 48:1302–1320.
- Crochet S, Chauvette S, Boucetta S, Timofeev I (2005) Modulation of synaptic transmission in neocortex by network activities. *European Journal of Neuroscience* 21:1030–1044.
- Crochet S, Poulet JF, Kremer Y, Petersen CC (2011) Synaptic mechanisms underlying sparse coding of active touch. *Neuron* 69:1160–1175.
- Debanne D, Poo MM (2010) Spike-timing dependent plasticity beyond synapse—pre- and post-synaptic plasticity of intrinsic neuronal excitability. *Frontiers in Synaptic Neuroscience* 2:21.
- DeBello WM, McBride TJ, Nichols GS, Pannoni KE, Sanculi D, Totten DJ (2014) Input clustering and the microscale structure of local circuits. *Frontiers in Neural Circuits* 8:112.
- Destexhe A, Lang EJ, Pare D (1998) Somato-dendritic interactions underlying action potential generation in neocortical pyramidal cells in vivo. In: *Computational Neuroscience: Trends in Research* (Bower J, ed.), pp. 167–172. New York: Plenum Press.
- Destexhe A, Rudolph M, Fellous JM, Sejnowski TJ (2001) Fluctuating synaptic conductances recreate in vivo-like activity in neocortical neurons. *Neuroscience* 107:13–24.
- Doiron B, Noonan L, Lemon N, Turner RW (2003) Persistent Na⁺ current modifies burst discharge by regulating conditional backpropagation of dendritic spikes. *Journal of Neurophysiology* 89:324–337.
- Doron D, von Heimendahl M, Schlattmann P, Houweling AR, Brecht M (2014) Spiking irregularity and frequency modulate the behavioral report of single-neuron stimulation. *Neuron* 89:324–337.
- Douglas RJ, Martin KA (1991) A functional microcircuit for cat visual cortex. *Journal of Physiology* 440:735–769.
- Douglas RJ, Martin KA (2009) Inhibition in cortical circuits. *Current Biology* 19:R398–R402.
- Druckmann S, Feng L, Lee B, Yook C, Zhao T, Magee JC, Kim J (2014) Structured synaptic connectivity between hippocampal regions. *Neuron* 81:629–640.
- Edwards FA, Konnerth A, Sakmann B, Takahashi T (1989) A thin slice preparation for patch clamp recordings from neurones of the mammalian central nervous system. *Pflügers Archiv: European Journal of Physiology* 414:600–612.
- Egger V, Feldmeyer D, Sakmann B (1999) Coincidence detection and changes of synaptic efficacy in spiny stellate neurons in rat barrel cortex. *Nature Neuroscience* 2:1098–1105.
- Elliott KAC, Jasper HH (1949) Physiological salt solutions for brain surgery; studies of local pH and pial vessel reactions to buffered and unbuffered isotonic solutions. *Journal of Neurosurgery* 6:140–152.
- Euler T, Detwiler PB, Denk W (2002) Directionally selective calcium signals in dendrites of starburst amacrine cells. *Nature* 418:845–852.
- Farinella M, Ruedt DT, Gleeson P, Lanore F, Silver RA (2014) Glutamate-bound NMDARs arising from in vivo-like network activity extend spatio-temporal integration in a L5 cortical pyramidal cell model. *PLoS Computational Biology* 10:e1003590.

- Feldman DE (2000) Timing-based LTP and LTD at vertical inputs to layer II/III pyramidal cells in rat barrel cortex. *Neuron* **27**:45–56.
- Feldman DE, Brecht M (2005) Map plasticity in somatosensory cortex. *Science* **310**:810–815.
- Felleman DJ, Van Essen DC (1991) Distributed hierarchical processing in the primate cerebral cortex. *Cerebral Cortex* **1**:1–47.
- Flusberg BA, Nimmerjahn A, Cocker ED, Mukamel EA, Barreto RP, Ko TH, Burns LD, Jung JC, Schnitzer MJ (2008) High-speed, miniaturized fluorescence microscopy in freely moving mice. *Nature Methods* **5**:935–938.
- Frégna Y, Monier C, Chavane F, Baudot P, Graham L (2003) Shunting inhibition, a silent step in visual cortical computation. *Journal of Physiology Paris* **97**:441–451.
- Froemke RC, Dan Y (2002) Spike-timing-dependent synaptic modification induced by natural spike trains. *Nature* **416**:433–438.
- Froemke RC, Merzenich MM, Schreiner CE (2007) A synaptic memory trace for cortical receptive field plasticity. *Nature* **450**:425–429.
- Fu M, Yu X, Lu J, Zuo Y (2012) Repetitive motor learning induces coordinated formation of clustered dendritic spines in vivo. *Nature* **483**:92–95.
- Gambino F, Pages S, Kehayas V, Baptista D, Tatti R, Carleton A, Holtmaat A (2014) Sensory-evoked LTP driven by dendritic plateau potentials in vivo. *Nature* **515**:116–119.
- Gasparini S, Magee JC (2006) State-dependent dendritic computation in hippocampal CA1 pyramidal neurons. *Journal of Neuroscience* **26**:2088–2100.
- Gasparini S, Migliore M, Magee JC (2004) On the initiation and propagation of dendritic spikes in CA1 pyramidal neurons. *Journal of Neuroscience* **24**:11046–11056.
- Gdalyahu A, Tring E, Polack PO, Gruver R, Golshani P, Fanselow MS, Silva AJ, Trachtenberg JT (2012) Associative fear learning enhances sparse network coding in primary sensory cortex. *Neuron* **75**:121–132.
- Gentet LJ (2012) Functional diversity of supragranular GABAergic neurons in the barrel cortex. *Frontiers in Neural Circuits* **6**:52.
- Gentet LJ, Kremer Y, Taniguchi H, Huang ZJ, Staiger JF, Petersen CC (2012) Unique functional properties of somatostatin-expressing GABAergic neurons in mouse barrel cortex. *Nature Neuroscience* **15**:607–612.
- Ghosh KK, Burns LD, Cocker ED, Nimmerjahn A, Ziv Y, Gamal AE, Schnitzer MJ (2011) Miniaturized integration of a fluorescence microscope. *Nature Methods* **8**:871–878.
- Gidon A, Segev I (2012) Principles governing the operation of synaptic inhibition in dendrites. *Neuron* **75**:330–341.
- Grienberger C, Chen X, Konnerth A (2014) NMDA receptor-dependent multidendrite $\text{Ca}^{(2+)}$ spikes required for hippocampal burst firing in vivo. *Neuron* **81**:1274–1281.
- Göbel W, Helmchen F (2007) New angles on neuronal dendrites in vivo. *Journal of Neurophysiology* **98**:3770–3779.
- Hablitz JJ, Heinemann U, Lux HD (1986) Step reductions in extracellular Ca^{2+} activate a transient inward current in chick dorsal root ganglion cells. *Biophysical Journal* **50**:753–757.
- Hajos N, Mody I (2009) Establishing a physiological environment for visualized in vitro brain slice recordings by increasing oxygen supply and modifying aCSF content. *Journal of Neuroscience Methods* **183**:107–113.
- Harnett MT, Xu NL, Magee JC, Williams SR (2013) Potassium channels control the interaction between active dendritic integration compartments in layer 5 cortical pyramidal neurons. *Neuron* **79**:516–529.
- Harnett MT, Magee JC, Williams SR (2015) Distribution and function of HCN channels in the apical dendritic tuft of neocortical pyramidal neurons. *Journal of Neuroscience* **35**:1024–1037.
- Häusser M (2001) Synaptic function: dendritic democracy. *Current Biology* **11**:R10–R12.

- Häusser M, Stuart G, Racca C, Sakmann B (1995) Axonal initiation and active dendritic propagation of action-potentials in substantia-nigra neurons. *Neuron* **15**:637–647.
- von Heimendahl M, Itskov PM, Arabzadeh E, Diamond ME (2007) Neuronal activity in rat barrel cortex underlying texture discrimination. *PLoS Biology* **5**:e305.
- Helmchen F, Svoboda K, Denk W, Tank DW (1999) In vivo dendritic calcium dynamics in deep-layer cortical pyramidal neurons. *Nature Neuroscience* **2**:989–996.
- Higley MJ (2014) Localized GABAergic inhibition of dendritic Ca signalling. *Nature Reviews Neuroscience* **15**:567–572.
- Hill SL, Wang Y, Riachi I, Schurmann F, Markram H (2012) Statistical connectivity provides a sufficient foundation for specific functional connectivity in neocortical neural microcircuits. *Proceedings of the National Academy of Sciences of the United States of America* **109**:E2885–E2894.
- Hill DN, Varga Z, Jia H, Sakmann B, Konnerth A (2013) Multibranch activity in basal and tuft dendrites during firing of layer 5 cortical neurons in vivo. *Proceedings of the National Academy of Sciences of the United States of America* **110**:13618–13623.
- Hirsch JA, Alonso JM, Reid RC (1995) Visually evoked calcium action-potentials in cat striate cortex. *Nature* **378**:612–616.
- Hô N, Destexhe A (2000) Synaptic background activity enhances the responsiveness of neocortical pyramidal neurons. *Journal of Neurophysiology* **84**:1488–1496.
- Holmes WR, Woody CD (1989) Effects of uniform and non-uniform synaptic “activation-distributions” on the cable properties of modeled cortical pyramidal neurons. *Brain Research* **505**:12–22.
- Holmgren CD, Zilberman Y (2001) Coincident spiking activity induces long-term changes in inhibition of neocortical pyramidal cells. *Journal of Neuroscience* **21**:8270–8277.
- Huber D, Petreanu L, Ghitani N, Ranade S, Hromadka T, Mainen Z, Svoboda K (2008) Sparse optical microstimulation in barrel cortex drives learned behaviour in freely moving mice. *Nature* **451**:61–64.
- Hunt DL, Castillo PE (2012) Synaptic plasticity of NMDA receptors: mechanisms and functional implications. *Current Opinion in Neurobiology* **22**:496–508.
- Isaacson JS, Scanziani M (2011) How inhibition shapes cortical activity. *Neuron* **72**:231–243.
- Jia H, Rochefort NL, Chen X, Konnerth A (2010) Dendritic organization of sensory input to cortical neurons in vivo. *Nature* **464**:1307–1312.
- Jiang X, Wang G, Lee AJ, Stornetta RL, Zhu JJ (2013) The organization of two new cortical interneuronal circuits. *Nature Neuroscience* **16**:210–218.
- Kaifosh P, Lovett-Barron M, Turi GF, Reardon TR, Losonczy A (2013) Septo-hippocampal GABAergic signaling across multiple modalities in awake mice. *Nature Neuroscience* **16**:1182–1184.
- Kalmbach AS, Waters J (2012) Brain surface temperature under a craniotomy. *Journal of Neurophysiology* **108**:3138–3146.
- Kampa BM, Roth MM, Gobel W, Helmchen F (2011) Representation of visual scenes by local neuronal populations in layer 2/3 of mouse visual cortex. *Frontiers in Neural Circuits* **5**:18.
- Kapfer C, Glickfeld LL, Atallah BV, Scanziani M (2007) Supralinear increase of recurrent inhibition during sparse activity in the somatosensory cortex. *Nature Neuroscience* **10**:743–753.
- Kaplan A, Friedman N, Davidson N (2001) Acousto-optic lens with very fast focus scanning. *Optics Letters* **26**:1078–1080.
- Kerr JN, Greenberg D, Helmchen F (2005) Imaging input and output of neocortical networks in vivo. *Proceedings of the National Academy of Sciences of the United States of America* **102**:14063–14068.
- Kitamura K, Häusser M (2011) Dendritic calcium signaling triggered by spontaneous and sensory-evoked climbing fiber input to cerebellar Purkinje cells in vivo. *Journal of Neuroscience* **31**(30):10847–10858.
- Kitamura K, Judkewitz B, Kano M, Denk W, Häusser M (2008) Targeted patch-clamp recordings and single-cell electroporation of unlabeled neurons in vivo. *Nature Methods* **5**:61–67.

- Klausberger T (2009) GABAergic interneurons targeting dendrites of pyramidal cells in the CA1 area of the hippocampus. *European Journal of Neuroscience* **30**:947–957.
- Kleindienst T, Winnubst J, Roth-Alpermann C, Bonhoeffer T, Lohmann C (2011) Activity-dependent clustering of functional synaptic inputs on developing hippocampal dendrites. *Neuron* **72**:1012–1024.
- Koch C, Poggio T, Torre V (1982) Retinal ganglion cells: a functional interpretation of dendritic morphology. *Philosophical Transactions of the Royal Society of London Series B: Biological Sciences* **298**:227–263.
- Koch C, Poggio T, Torre V (1983) Nonlinear interactions in a dendritic tree: localization, timing, and role in information processing. *Proceedings of the National Academy of Sciences of the United States of America* **80**L2799–L2802.
- de Kock CP, Sakmann B (2008) High frequency action potential bursts (>or = 100 Hz) in L2/3 and L5B thick tufted neurons in anaesthetized and awake rat primary somatosensory cortex. *Journal of Physiology* **586**:3353–3364.
- de Kock CP, Sakmann B (2009) Spiking in primary somatosensory cortex during natural whisking in awake head-restrained rats is cell-type specific. *Proceedings of the National Academy of Sciences of the United States of America* **106**:16446–16450.
- Kole MH, Stuart GJ (2012) Signal processing in the axon initial segment. *Neuron* **73**:235–247.
- Konnerth A, Heinemann U, Yaari Y (1986) Nonsynaptic epileptogenesis in the mammalian hippocampus in vitro. I. Development of seizure-like activity in low extracellular calcium. *Journal of Neurophysiology* **56**:409–423.
- Kubota Y (2014) Untangling GABAergic wiring in the cortical microcircuit. *Current Opinion in Neurobiology* **26**:7–14.
- Kuenzi FM, Fitzjohn SM, Morton RA, Collingridge GL, Seabrook GR (2000) Reduced long-term potentiation in hippocampal slices prepared using sucrose-based artificial cerebrospinal fluid. *Journal of Neuroscience Methods* **100**:117–122.
- Kullmann DM (2011) Interneuron networks in the hippocampus. *Current Opinion in Neurobiology* **21**:709–716.
- Larkum M (2013) A cellular mechanism for cortical associations: an organizing principle for the cerebral cortex. *Trends in Neurosciences* **36**:141–151.
- Larkum ME, Nevian T (2008) Synaptic clustering by dendritic signalling mechanisms. *Current Opinion in Neurobiology* **18**:321–331.
- Larkum ME, Zhu JJ (2002) Signaling of layer 1 and whisker-evoked Ca^{2+} and Na^+ action potentials in distal and terminal dendrites of rat neocortical pyramidal neurons in vitro and in vivo. *Journal of Neuroscience* **22**:6991–7005.
- Larkum ME, Rioult MG, Lüscher HR (1996) Propagation of action potentials in the dendrites of neurons from rat spinal cord slice cultures. *Journal of Neurophysiology* **75**:154–170.
- Larkum ME, Launey T, Dityatev A, Lüscher HR (1998) Integration of excitatory postsynaptic potentials in dendrites of motoneurons of rat spinal cord slice cultures. *Journal of Neurophysiology* **80**:924–935.
- Larkum ME, Zhu JJ, Sakmann B (1999) A new cellular mechanism for coupling inputs arriving at different cortical layers. *Nature* **398**:338–341.
- Larkum ME, Zhu JJ, Sakmann B (2001) Dendritic mechanisms underlying the coupling of the dendritic with the axonal action potential initiation zone of adult rat layer 5 pyramidal neurons. *Journal of Physiology* **533**:447–466.
- Larkum ME, Senn W, Lüscher HR (2004) Top-down dendritic input increases the gain of layer 5 pyramidal neurons. *Cerebral Cortex* **14**:1059–1070.
- Larkum ME, Watanabe S, Lasser-Ross N, Rhodes P, Ross WN (2008) Dendritic properties of turtle pyramidal neurons. *Journal of Neurophysiology* **99**:683–694.
- Larkum ME, Nevian T, Sandler M, Polksky A, Schiller J (2009) Synaptic integration in tuft dendrites of layer 5 pyramidal neurons: a new unifying principle. *Science* **325**:756–760.

- Lavzin M, Rapoport S, Polksy A, Garion L, Schiller J (2012) Nonlinear dendritic processing determines angular tuning of barrel cortex neurons in vivo. *Nature* **490**:397–401.
- Ledergerber D, Larkum ME (2010) Properties of layer 6 pyramidal neuron apical dendrites. *Journal of Neuroscience* **30**:13031–13044.
- Lee S, Zhou ZJ (2006) The synaptic mechanism of direction selectivity in distal processes of starburst amacrine cells. *Neuron* **51**:787–799.
- Lee D, Lin B-J, Lee AK (2012) Hippocampal place fields emerge upon single-cell manipulation of excitability during behavior. *Science* **337**:849–853.
- Lee S, Kruglikov I, Huang ZJ, Fishell G, Rudy B (2013) A disinhibitory circuit mediates motor integration in the somatosensory cortex. *Nature Neuroscience* **16**:1662–1670.
- Leger JF, Stern EA, Aertsen A, Heck D (2005) Synaptic integration in rat frontal cortex shaped by network activity. *Journal of Neurophysiology* **93**:281–293.
- Lemon N, Turner RW (2000) Conditional spike backpropagation generates burst discharge in a sensory neuron. *Journal of Neurophysiology* **84**:1519–1530.
- Letzkus JJ, Kampa BM, Stuart GJ (2006) Learning rules for spike timing-dependent plasticity depend on dendritic synapse location. *Journal of Neuroscience* **26**:10420–10429.
- Letzkus JJ, Wolff SB, Meyer EM, Tovote P, Courtin J, Herry C, Luthi A (2011) A disinhibitory microcircuit for associative fear learning in the auditory cortex. *Nature* **480**:331–335.
- Leybaert L, de Meyer A, Mabilde C, Sanderson MJ (2005) A simple and practical method to acquire geometrically correct images with resonant scanning-based line scanning in a custom-built video-rate laser scanning microscope. *Journal of Microscopy* **219**:133–140.
- Lisman J, Spruston N (2005) Postsynaptic depolarization requirements for LTP and LTD: a critique of spike timing-dependent plasticity. *Nature Neuroscience* **8**:839–841.
- Lisman JE (1997) Bursts as a unit of neural information: Making unreliable synapses reliable. *Trends in Neuroscience* **20**:38–43.
- Livingstone MS (1998) Mechanisms of direction selectivity in macaque V1. *Neuron* **20**:509–526.
- London M, Segev I (2001) Synaptic scaling in vitro and in vivo. *Nature Neuroscience* **4**:853–855.
- London M, Schreibman A, Häusser M, Larkum ME, Segev I (2002) The information efficacy of a synapse. *Nature Neuroscience* **5**:332–340.
- London M, Larkum ME, Häusser M (2008) Predicting the synaptic information efficacy in cortical layer 5 pyramidal neurons using a minimal integrate-and-fire model. *Biological Cybernetics* **99**:393–401.
- Long MA, Jin DZ, Fee MS (2010) Support for a synaptic chain model of neuronal sequence generation. *Nature* **468**:394–399.
- Longordo F, To MS, Ikeda K, Stuart GJ (2013) Sublinear integration underlies binocular processing in primary visual cortex. *Nature Neuroscience* **16**:714–723.
- Losonczy A, Magee JC (2006) Integrative properties of radial oblique dendrites in hippocampal CA1 pyramidal neurons. *Neuron* **50**:291–307.
- Losonczy A, Makara JK, Magee JC (2008) Compartmentalized dendritic plasticity and input feature storage in neurons. *Nature* **452**:436–441.
- Lovett-Barron M, Losonczy A (2014) Behavioral consequences of GABAergic neuronal diversity. *Current Opinion in Neurobiology* **26**:27–33.
- Lovett-Barron M, Kaifosh P, Kheirbek MA, Danielson N, Zaremba JD, Reardon TR, Turi GF, Hen R, Zemelman BV, Losonczy A (2014) Dendritic inhibition in the hippocampus supports fear learning. *Science* **343**:857–863.
- McNay EC, Sherwin RS (2004) From artificial cerebro-spinal fluid (aCSF) to artificial extracellular fluid (aECF): microdialysis perfuse composition effects on in vivo brain ECF glucose measurements. *Journal of Neuroscience Methods* **132**:35–43.
- Magee JC (1999) Dendritic Ih normalizes temporal summation in hippocampal CA1 neurons. *Nature Neuroscience* **2**:508–514.

- Magee JC, Cook EP (2000) Somatic EPSP amplitude is independent of synapse location in hippocampal pyramidal neurons. *Nature Neuroscience* **3**:895–903.
- Mainen ZF, Sejnowski TJ (1995) Reliability of spike timing in neocortical neurons. *Science* **268**:1503–1506.
- Major G, Polksy A, Denk W, Schiller J, Tank DW (2008) Spatiotemporally graded NMDA spike/plateau potentials in basal dendrites of neocortical pyramidal neurons. *Journal of Neurophysiology* **99**:2584–2601.
- Major G, Larkum ME, Schiller J (2013) Active properties of neocortical pyramidal neuron dendrites. *Annual Review of Neuroscience* **36**:1–24.
- Marder E (2012) Neuromodulation of neuronal circuits: back to the future. *Neuron* **76**:1–11.
- Markram H, Lubke J, Frotscher M, Sakmann B (1997) Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. *Science* **275**:213–215.
- Markram H, Toledo-Rodriguez M, Wang Y, Gupta A, Silberberg G, Wu C (2004) Interneurons of the neocortical inhibitory system. *Nature Reviews Neuroscience* **5**:793–807.
- Mel BW (1992) The clusteron: toward a simple abstraction for a complex neuron. In: *Advances in Neural Information Processing Systems* (Moody J, Hanson S, Lippman R, eds), pp. 35–42. San Mateo, CA: Morgan Kaufmann.
- Mel BW (1994) Information processing in dendritic trees. *Neural Computation* **6**:1031–1085.
- Milojkovic BA, Wuskell JP, Loew LM, Antic SD (2005) Initiation of sodium spikelets in basal dendrites of neocortical pyramidal neurons. *Journal of Membrane Biology* **208**:155–169.
- Moser EI, Kropff E, Moser MB (2008) Place cells, grid cells, and the brain's spatial representation system. *Annual Reviews of Neuroscience* **31**:69–89.
- Murayama M, Larkum ME (2009) Enhanced dendritic activity in awake rats. *Proceedings of the National Academy of Sciences of the United States of America* **106**:20482–20486.
- Murayama M, Perez-Garci E, Luscher HR, Larkum ME (2007) Fiberoptic system for recording dendritic calcium signals in layer 5 neocortical pyramidal cells in freely moving rats. *Journal of Neurophysiology* **98**:1791–1805.
- Murayama M, Perez-Garci E, Nevian T, Bock T, Senn W, Larkum ME (2009) Dendritic encoding of sensory stimuli controlled by deep cortical interneurons. *Nature* **457**:1137–1141.
- Najafi F, Giovannucci A, Wang SS, Medina JF (2014) Sensory-driven enhancement of calcium signals in individual Purkinje cell dendrites of awake mice. *Cell Reports* **6**:792–798.
- Nevian T, Sakmann B (2006) Spine Ca²⁺ signaling in spike-timing-dependent plasticity. *Journal of Neuroscience* **26**:11001–11013.
- Nicholson C, Bruggencate GT, Steinberg R, Stockle H (1977) Calcium modulation in brain extracellular microenvironment demonstrated with ion-selective micropipette. *Proceedings of the National Academy of Sciences of the United States of America* **74**:1287–1290.
- Nolan MF, Malleret G, Dudman JT, Buhl DL, Santoro B, Gibbs E, Vronskaya S, Buzsaki G, Siegelbaum SA, Kandel ER, Morozov A (2004) A behavioral role for dendritic integration: HCN1 channels constrain spatial memory and plasticity at inputs to distal dendrites of CA1 pyramidal neurons. *Cell* **119**:719–732.
- Oesch N, Euler T, Taylor WR (2005) Direction-selective dendritic action potentials in rabbit retina. *Neuron* **47**:739–750.
- Okun M, Lampl I (2008) Instantaneous correlation of excitation and inhibition during ongoing and sensory-evoked activities. *Nature Neuroscience* **11**:535–537.
- Olshausen BA, Field DJ (2005) How close are we to understanding v1? *Neural Computation* **17**:1665–1699.
- Oláh S, Füle M, Komlósi G, Varga C, Báldi R, Barzó P, Tamás G (2009) Regulation of cortical microcircuits by unitary GABA-mediated volume transmission. *Nature* **461**:1278–1281.
- Oviedo H, Reyes AD (2005) Variation of input-output properties along the somatodendritic axis of pyramidal neurons. *Journal of Neuroscience* **25**:4985–4995.

- Palmer LM, Stuart GJ (2006) Site of action potential initiation in layer 5 pyramidal neurons. *Journal of Neuroscience* **26**:1854–1863.
- Palmer L, Murayama M, Larkum M (2012a) Inhibitory regulation of dendritic activity in vivo. *Frontiers in Neural Circuits* **6**:26.
- Palmer LM, Schulz JM, Murphy SC, Ledergerber D, Murayama M, Larkum ME (2012b) The cellular basis of GABAB-mediated interhemispheric inhibition. *Science* **335**:989–993.
- Palmer LM, Shai AS, Reeve JE, Anderson HL, Paulsen O, Larkum ME (2014) NMDA spikes enhance action potential generation during sensory input. *Nature Neuroscience* **17**:383–390.
- Panzeri S, Petersen RS, Schultz SR, Lebedev M, Diamond ME (2001) The role of spike timing in the coding of stimulus location in rat somatosensory cortex. *Neuron* **29**:769–777.
- Perez-Garcia E, Larkum ME, Nevian T (2013) Inhibition of dendritic Ca^{2+} spikes by GABAB receptors in cortical pyramidal neurons is mediated by a direct G_i/o -beta-subunit interaction with Cav1 channels. *Journal of Physiology* **591**:1599–1612.
- Pi HJ, Hangya B, Kvitsiani D, Sanders JI, Huang ZJ, Kepes A (2013) Cortical interneurons that specialize in disinhibitory control. *Nature* **503**:521–524.
- Pike FG, Meredith RM, Olding AW, Paulsen O (1999) Rapid report: postsynaptic bursting is essential for “Hebbian” induction of associative long-term potentiation at excitatory synapses in rat hippocampus. *Journal of Physiology* **518**:571–576.
- Polksy A, Mel BW, Schiller J (2004) Computational subunits in thin dendrites of pyramidal cells. *Nature Neuroscience* **7**:621–627.
- Polksy A, Mel B, Schiller J (2009) Encoding and decoding bursts by NMDA spikes in basal dendrites of layer 5 pyramidal neurons. *Journal of Neuroscience* **29**:11891–11903.
- Potez S, Larkum ME (2008) Effect of common anesthetics on dendritic properties in layer 5 neocortical pyramidal neurons. *Journal of Neurophysiology* **99**:1394–1407.
- Priebe NJ, Ferster D (2008) Inhibition, spike threshold, and stimulus selectivity in primary visual cortex. *Neuron* **57**:482–497.
- Rall W (1964) Theoretical significance of dendritic trees for neuronal input–output relations. In: *Neural Theory and Modeling* (Reis RF, ed.), pp. 72–97. Stanford, CA: Stanford University Press.
- Renart A, de la Rocha J, Bartho P, Hollender L, Parga N, Reyes A, Harris KD (2010) The asynchronous state in cortical circuits. *Science* **327**:587–590.
- Richards BA, Aizenman CD, Akerman CJ (2010) In vivo spike-timing-dependent plasticity in the optic tectum of *Xenopus laevis*. *Frontiers in Synaptic Neuroscience* **2**:7.
- Rose D, Blakemore C (1974) Effects of bicuculline on functions of inhibition in visual cortex. *Nature* **249**:375–377.
- Roux L, Buzsaki G (2015) Tasks for inhibitory interneurons in intact brain circuits. *Neuropharmacology* **88**:10–23.
- Rudolph M, Pospischil M, Timofeev I, Destexhe A (2007) Inhibition determines membrane potential dynamics and controls action potential generation in awake and sleeping cat cortex. *Journal of Neuroscience* **27**:5280–5290.
- Runyan CA, Schummers J, Van Wart A, Kuhlman SJ, Wilson NR, Huang ZJ, Sur M (2010) Response features of parvalbumin-expressing interneurons suggest precise roles for subtypes of inhibition in visual cortex. *Neuron* **67**:847–857.
- Sachidhanandam S, Sreenivasan V, Kyriakatos A, Kremer Y, Petersen CC (2013) Membrane potential correlates of sensory perception in mouse barrel cortex. *Nature Neuroscience* **16**:1671–1677.
- Sawinski J, Wallace DJ, Greenberg DS, Grossmann S, Denk W, Kerr JN (2009) Visually evoked activity in cortical cells imaged in freely moving animals. *Proceedings of the National Academy of Sciences of the United States of America* **106**:19557–19562.

- Schiller J, Schiller Y, Stuart G, Sakmann B (1997) Calcium action potentials restricted to distal apical dendrites of rat neocortical pyramidal neurons. *Journal of Physiology* **505**:605–616.
- Schiller J, Major G, Koester HJ, Schiller Y (2000) NMDA spikes in basal dendrites of cortical pyramidal neurons. *Nature* **404**:285–289.
- Schulz JM (2010) Synaptic plasticity in vivo: more than just spike-timing? *Frontiers in Synaptic Neuroscience* **2**:150.
- Schultz SR, Kitamura K, Post-Uiterweer A, Krupic J, Häusser M (2009) Spatial pattern coding of sensory information by climbing fiber-evoked calcium signals in networks of neighboring cerebellar Purkinje cells. *Journal of Neuroscience* **29**:8005–15.
- Schölvinck ML, Saleem AB, Benucci A, Harris KD, Carandini M (2015) Cortical state determines global variability and correlations in visual cortex. *Journal of Neuroscience* **35**:170–178.
- Shadlen MN, Newsome WT (1998) The variable discharge of cortical neurons: implications for connectivity, computation, and information coding. *Journal of Neuroscience* **18**:3870–3896.
- Sheffield ME, Dombek DA (2015) Calcium transient prevalence across the dendritic arbor predicts place field properties. *Nature* **517**:200–204.
- Sillito AM (1975) The contribution of inhibitory mechanisms to the receptive field properties of neurones in the striate cortex of the cat. *Journal of Physiology* **250**:305–329.
- Single S, Haag J, Borst A (1997) Dendritic computation of direction selectivity and gain control in visual interneurons. *Journal of Neuroscience* **17**:6023–6030.
- Sivyer B, Williams SR (2013) Direction selectivity is computed by active dendritic integration in retinal ganglion cells. *Nature Neuroscience* **16**:1848–1856.
- Sjöström PJ, Turrigiano GG, Nelson SB (2001) Rate, timing, and cooperativity jointly determine cortical synaptic plasticity. *Neuron* **32**:1149–1164.
- Smith SL, Häusser M (2010) Parallel processing of visual space by neighboring neurons in mouse visual cortex. *Nature Neuroscience* **13**:1144–1149.
- Smith SL, Smith IT, Branco T, Häusser M (2013) Dendritic spikes enhance stimulus selectivity in cortical neurons in vivo. *Nature* **503**:115–120.
- Softky WR (1995) Simple codes versus efficient codes. *Current Opinion in Neurobiology* **5**:239–247.
- Softky WR, Koch C (1993) The highly irregular firing of cortical cells is inconsistent with temporal integration of random EPSPs. *Journal of Neuroscience* **13**:334–350.
- Spruston N, Schiller Y, Stuart G, Sakmann B (1995) Activity-dependent action-potential Invasion and calcium influx into hippocampal CA1 dendrites. *Science* **268**:297–300.
- Steriade M (2001) Impact of network activities on neuronal properties in corticothalamic systems. *Journal of Neurophysiology* **86**:1–39.
- Stuart GJ, Häusser M (2001) Dendritic coincidence detection of EPSPs and action potentials. *Nature Neuroscience* **4**:63–71.
- Stuart GJ, Sakmann B (1994) Active propagation of somatic action-potentials into neocortical pyramidal cell dendrites. *Nature* **367**:69–72.
- Stuart G, Spruston N (1998) Determinants of voltage attenuation in neocortical pyramidal neuron dendrites. *Journal of Neuroscience* **18**:3501–3510.
- Stuart G, Schiller J, Sakmann B (1997a) Action potential initiation and propagation in rat neocortical pyramidal neurons. *Journal of Physiology* **505**:617–632.
- Stuart G, Spruston N, Sakmann B, Häusser M (1997b) Action potential initiation and backpropagation in neurons of the mammalian CNS. *Trends in Neurosciences* **20**:125–131.
- Su H, Alroy G, Kirson ED, Yaari Y (2001) Extracellular calcium modulates persistent sodium current-dependent burst-firing in hippocampal pyramidal neurons. *Journal of Neuroscience* **21**:4173–4182.
- Svoboda K, Denk W, Kleinfeld D, Tank DW (1997) In vivo dendritic calcium dynamics in neocortical pyramidal neurons. *Nature* **385**:161–165.

- Takahashi N, Kitamura K, Matsuo N, Mayford M, Kano M, Matsuki N, Ikegaya Y (2012) Locally synchronized synaptic inputs. *Science* **335**:353–356.
- Thomson AM, Destexhe A (1999) Dual intracellular recordings and computational models of slow inhibitory postsynaptic potentials in rat neocortical and hippocampal slices. *Neuroscience* **92**:1193–1215.
- Tiesinga P, Fellous JM, Sejnowski TJ (2008) Regulation of spike timing in visual cortical circuits. *Nature Reviews Neuroscience* **9**:97–107.
- Tolhurst DJ, Smyth D, Thompson ID (2009) The sparseness of neuronal responses in ferret primary visual cortex. *Journal of Neuroscience* **29**:2355–2370.
- Vaidya SP, Johnston D (2013) Temporal synchrony and gamma-to-theta power conversion in the dendrites of CA1 pyramidal neurons. *Nature Neuroscience* **16**:1812–1820.
- Varga Z, Jia H, Sakmann B, Konnerth A (2011) Dendritic coding of multiple sensory inputs in single cortical neurons in vivo. *Proceedings of the National Academy of Sciences of the United States of America* **108**:15420–15425.
- Vinje WE (2000) Sparse coding and decorrelation in primary visual cortex during natural vision. *Science* **287**:1273–1276.
- Waters J, Helmchen F (2004) Boosting of action potential backpropagation by neocortical network activity in vivo. *Journal of Neuroscience* **24**:11127–11136.
- Waters J, Helmchen F (2006) Background synaptic activity is sparse in neocortex. *Journal of Neuroscience* **26**:8267–8277.
- Waters J, Larkum M, Sakmann B, Helmchen F (2003) Supralinear Ca^{2+} influx into dendritic tufts of layer 2/3 neocortical pyramidal neurons in vitro and in vivo. *Journal of Neuroscience* **23**:8558–8567.
- Watson PL, Andrew RD (1995) Effects of micromolar and nanomolar calcium concentrations on non-synaptic bursting in the hippocampal slice. *Brain Research* **700**:227–234.
- Wehr M, Zador AM (2003) Balanced inhibition underlies tuning and sharpens spike timing in auditory cortex. *Nature* **426**:442–446.
- Williams SR, Mitchell SJ (2008) Direct measurement of somatic voltage clamp errors in central neurons. *Nature Neuroscience* **11**:790–798.
- Williams SR, Stuart GJ (1999) Mechanisms and consequences of action potential burst firing in rat neocortical pyramidal neurons. *Journal of Physiology* **521**:467–482.
- Williams SR, Stuart GJ (2000) Action potential backpropagation and somato-dendritic distribution of ion channels in thalamocortical neurons. *Journal of Neuroscience* **20**:1307–1317.
- Williams SR, Stuart GJ (2002) Dependence of EPSP efficacy on synapse location in neocortical pyramidal neurons. *Science* **295**:1907–1910.
- Wolfe J, Houweling AR, Brecht M (2010) Sparse and powerful cortical spikes. *Current Opinion in Neurobiology* **20**:306–312.
- Wolff SB, Grundemann J, Tovote P, Krabbe S, Jacobson GA, Muller C, Herry C, Ehrlich I, Friedrich RW, Letzkus JJ, Luthi A (2014) Amygdala interneuron subtypes control fear learning through disinhibition. *Nature* **509**:453–458.
- Xu NL, Harnett MT, Williams SR, Huber D, O'Connor DH, Svoboda K, Magee JC (2012) Nonlinear dendritic integration of sensory and motor input during an active sensing task. *Nature* **492**:247–251.
- Yaari Y, Konnerth A, Heinemann U (1986) Nonsynaptic epileptogenesis in the mammalian hippocampus in vitro. II. Role of extracellular potassium. *Journal of Neurophysiology* **56**:424–438.
- Zorec R, Araque A, Carmignoto G, Haydon PG, Verkhratsky A, Parpura V (2012) Astroglial excitability and gliotransmission: an appraisal of Ca^{2+} as a signalling route. *ASN Neuro* **4**: doi: 10.1042/AN20110061

Chapter 14

Modeling dendrites: a personal perspective

Wilfrid Rall

Summary

The advent of microelectrode recordings from neurons with branching dendritic trees presented a new problem for neurophysiologists: how to interpret the data obtained from these non-isopotential structures. An important step was the development of mathematical models of dendrites. The theory behind these models is discussed in detail in other chapters in this book. Here I provide a personal view of the development of such models, paying attention to the progression from passive, linear models to the more complex case of neurons with extensive dendritic branching and nonlinear properties.

Introduction

The extensive nature of dendritic branching in most neurons has been established for over 100 years, since the pioneering research of Ramón y Cajal. The need for a mathematical treatment of dendritic cable properties, however, was not recognized until sharp microelectrodes were first used (in the early 1950s) for intracellular recording from cat spinal cord motoneurons. Successful impalement was usually made in the motoneuron soma, rarely in a large dendritic trunk, and never in a small dendritic branch. The goal was to understand what effect the extensively branched dendritic membrane has on voltage transients recorded in the soma, and it was quickly recognized that modeling was essential for the interpretation of the data. As mathematical modeling has evolved over the years, the key to success has been recognition of the important interplay between theory and experiment.

Sharp electrodes and the membrane time constant

In the late 1940s, Graham, Ling, and Gerard at the University of Chicago began to use sharp glass micropipettes for intracellular recording from single muscle fibers. This application to muscle was extended by Hodgkin and Nastuk, and by Fatt and Katz. Similar electrodes were then applied to cat spinal cord motoneurons by Woodbury and Patton, by Brock, Coombs and Eccles, and by Frank and Fuortes in the early 1950s. It is noteworthy that both Woodbury and Frank learned about these electrodes in the physiology labs at the University of Chicago; Eccles heard about them from Dexter Easton, who came to Dunedin, New Zealand as a Fulbright Fellow from Seattle, where he had learned about Woodbury's research.

Although I had done extracellular recording with Brock and Eccles in Dunedin (1949–51), I did not participate in their sharp electrode experiments; my focus then was on completing my PhD research, aimed at matching experiment and theory for a reflex input–output relation, using monosynaptic responses in spinal cord motoneuron populations. When Eccles moved from Dunedin to Canberra in the early 1950s he was succeeded in Dunedin by my good friend Archie McIntyre, who later moved to Monash University in Melbourne. In 1956, I moved from Dunedin to Bethesda, where I met Frank and Fuortes; after 17 months at the Naval Medical Research Institute, I moved to the National Institutes of Health (NIH), where I worked in the mathematical research branch for the next 37 years.

By 1956, two groups (Frank and Fuortes at NIH, and Eccles with collaborators in Canberra) had published estimates of membrane time constants based on their intracellular recordings of voltage transients obtained in response to an electric current that was applied across the soma membrane of the motoneuron by the recording electrode (relative to an extracellular reference electrode). Both groups reported unexpectedly low values for the membrane time constant; their values, between 1 and 2.5 ms, created a puzzling discrepancy with earlier estimates of around 4 or 5 ms, based on the decay of synaptic potentials.

Both groups had analyzed their voltage transients as if they were composed of a single exponential. Although they did not say so, this meant that they had implicitly assumed the neuron was iso-potential, and did not have dendrites. If one does not postulate an explicit biophysical model that includes such an essential complication as the presence of dendritic cables, then one runs the risk of using overly simple data analysis that yields an incorrect value for the membrane time constant.

Data analysis needs models that include dendrites

The error was pointed out in a brief note to *Science* (Rall, 1957), followed by a manuscript with a full explanation and discussion that was submitted to the *Journal of General Physiology* in 1958. Sadly, this manuscript was rejected, at the urging of a negative referee; however, this manuscript did appear in 1959 as a Research Report of the Naval Medical Research Institute (NMRI) in Bethesda, MD. Also, the editors of a new journal, *Experimental Neurology*, encouraged me to publish this material as two papers: the focus of one (Rall, 1959) was on steady-state attenuation and input resistance, that of the other (Rall, 1960) was on transients and a method of estimating membrane time constants.

Frank and Fuortes freely acknowledged their error. Eccles did so only indirectly, by slightly revising his estimates; his postulated residual phase of synaptic current (much illustrated during 1956–58) became reduced in magnitude, and later abandoned. For those readers who may be interested in further details and commentary on the impact of these papers, the following three references are recommended: part of a book of selected reprints with commentaries (Segev et al., 1995, pp. 27–103); part of a book chapter that describes my research “path” (Rall, 1992, pp. 222–225); and part of a handbook chapter (Rall, 1977, pp. 45–58).

In hindsight, we now know that the voltage transients recorded in these early experiments were also made faster by membrane shunting at the electrode penetration site. In 1959, I reported the interesting clue that large electrode tips tended to yield lower estimates of input resistance, but I did not know the magnitude of the leak until much later, when the patch-clamp method was used for whole-cell recording. The significance of this microelectrode shunt was discussed in a multi-authored *Physiological Review* paper (Rall et al., 1992); the mathematical effect of this shunt on the system eigenvalues and time constants has also been solved and discussed (Holmes and Rall, 1992; Major et al., 1993).

Dendritic synaptic input and the shapes of excitatory postsynaptic potentials

Once the cable properties of dendrites had been shown to be important for the interpretation of experimental data, it became clear to me that it was more interesting to investigate the functional implications of synaptic inputs to the dendrites than to pursue refinements in the estimation of time constants. This new objective led to two different mathematical models and to a fruitful research collaboration with a NIH experimental group, consisting of Burke, Smith, Nelson, and Frank in the neurophysiology laboratory.

For idealized dendritic branching, a dendritic tree was shown to be reducible to an equivalent cylinder of finite length. By making the assumption of passive membrane properties, an analytical solution (Rall, 1962a) was used to compute (by slide rule) the first simulations of excitatory postsynaptic potentials (EPSPs) in neuronal models with dendrites. Synaptic input could be applied to one of two regions (such as soma and proximal dendrites, versus more distal dendrites). For a brief synaptic input near the soma, the EPSP rises steeply to an early peak, followed by rapid early decay, and then by slower decay. If the same brief synaptic input is delivered to distal dendrites, the EPSP at the soma shows a delay, and rises more slowly, generating a more rounded peak, and then decays slowly.

In order to compare theoretical EPSP shapes with experimentally recorded EPSP shapes, we invented the shape-index plot (Rall et al., 1967) where each EPSP shape is a point in a two-dimensional plot of EPSP half-width versus time-to-peak, or rise-time. It was an exciting time, because much of the data had already been obtained, but it had not been plotted in this way. Also, to improve the resolution of theoretical EPSP shapes, I introduced a brief input time-course (now known as an alpha function), and represented the soma plus dendritic trees as a chain of ten compartments. It is noteworthy that compartmental modeling of soma and dendrites was first presented at a 1962 symposium; the published chapter (Rall, 1964) has been reprinted in Segev et al. (1995), as has the 1967 modeling paper (Rall et al., 1967).

This research collaboration culminated in a series of five papers published together in a 1967 issue of the *Journal of Neurophysiology*. It can be said that this research persuaded the neurophysiological community that dendritic synaptic input is functional and has predictable characteristics, contrary to what Eccles had maintained. This conclusion was strengthened by confirmatory experimental studies in Oxford by Jack, Redman, and others, and at Harvard by Mendell and Henneman. All of these studies featured a population of EPSP shapes that could be compared with theoretical shape-index loci, but we did not have experimental verification for the presumed proximal and distal dendritic locations of the synaptic inputs. This deficiency was remedied by the remarkable experiments of Redman and Walmsley (1983), which provided strong support for the theoretical relation between EPSP shape and input location.

Passive versus active dendrites

After presenting a seminar in Bern, in 1995, I was asked for a historical perspective on the following question: why had several early experiments that had been offered as evidence of active propagation in motoneuron dendrites (Fatt, 1957; Terzuolo and Araki, 1961) not been widely accepted as compelling evidence? The answer, I believe, lies mainly in the demonstration that the key observation in these experiments, a diphasic extracellular voltage transient during antidromic activation of a single motoneuron soma, was predicted by a computed simulation that assumed passive properties for the dendritic membrane (Rall, 1962b). The computed result was unambiguous, but

it was puzzling to many physiologists. Thus, it is helpful to provide the following physically intuitive explanation.

During the first phase of a somatic action potential (produced by an antidromic axonal impulse) the soma membrane is actively depolarized by inward flow of sodium ions. There is also spread of membrane depolarization from the soma to the dendrites, but the soma remains the most depolarized during this first phase. Thus, the flow of intracellular current is from the soma into the dendrites. As a result the extracellular current flows toward the soma, more or less radially from the distributed multipolar dendritic membrane. It was shown that such current flow in the extracellular volume would produce a field of electric potential that is more negative near the soma and proximal dendrites of a motoneuron than near its distal dendrites and beyond. This explains the initial negative phase of the extracellular voltage transient obtained by an electrode placed near the soma and recording relative to a reference electrode placed near or beyond the distal dendritic branches of a motoneuron during an antidromic action potential.

During the second phase of the somatic action potential, the soma membrane is actively repolarized by outward flow of potassium ions. The direction of current flow is reversed from that in the first phase. During this second phase, the repolarizing somatic membrane becomes less depolarized than the dendritic membrane, because the membrane depolarization that had spread from soma to dendrites during the first phase now decays slowly in the dendrites (because the dendritic membrane is assumed to be passive). The dendritic membrane remains more depolarized than the rapidly repolarizing soma membrane. Thus, the intracellular current flows from the dendrites to soma. As a result the extracellular current flows from the soma, more or less radially outward, to the distal dendritic branches. This radial flow of current in the extracellular volume would produce a reversed extracellular field which is more positive near the soma and proximal dendrites than near or beyond the distal dendritic branches of the motoneuron. The reversed electric field is responsible for the positive second phase of the extracellular voltage transient obtained with an electrode placed near the soma and recording relative to a distant reference electrode. The key point, that others had overlooked, is the rapid reversal of current, due to active repolarization of the soma membrane, described above.

This result based on mathematical modeling was presented in seminars at NIH and elsewhere (in 1960). It was discussed at length with Frank and Nelson, as well as others; they all became convinced. This result was then presented at the first International Biophysics Congress in Stockholm in 1961, and was subsequently published in a special issue of the *Biophysical Journal* (Rall, 1962b); some of the same figures, with discussion, can be found in Rall (1992, pp. 228–231) or Segev et al. (1995, 107–114).

The conclusion was this: these computations do not prove that the dendrites are passive, but they do prove that the observed diphasic extracellular voltage transient during antidromic activation of a single motoneuron can be reproduced theoretically by computations which assume a passive dendritic membrane. Consequently, the claim that such experiments provide evidence for active propagation in the dendrites of motoneurons was abandoned (see also the discussion by Nelson and Frank, 1964).

It is interesting that this issue has not gone away. It still involves careful comparison of theory and experiment. Moreover, with the greatly improved experimental techniques presented elsewhere in this book, the new evidence shows that most neuron types do have significant densities of voltage-dependent ion channels in their dendritic membrane.

Experimental and theoretical analysis of the olfactory bulb

Modeling olfactory bulb field potentials involved several different interactions between theory and experiment. The theory made important use of the insights from modeling the biphasic extracellular voltage transients generated by a single motoneuron described above, but here we found that they also apply to simultaneous antidromic activation of a cortically arranged population of thousands of mitral cells. Gordon Shepherd had studied such field potentials experimentally at Oxford, with Phillips and Powell. Because they were aware of my modeling of extracellular voltage transients from motoneurons, they proposed that Gordon should collaborate with me in an effort to understand the highly reproducible spatio-temporal pattern of the field potentials that they had recorded in the rabbit olfactory bulb, and had correlated with anatomical depths in the bulb. Thus, my recent modeling and their recent experimental data were brought together at NIH (Rall and Shepherd, 1968).

The modeling

We used a compartmental model to simulate antidromic activation of a single mitral cell. We put excitable membrane in the axon and soma compartments, and tested both passive and active membrane in the dendritic compartments. From the extracellular voltage transient that had been recorded at the surface of the bulb, we could deduce that the cortical field was an open field (not closed, as it would be for complete spherical symmetry). We called this “punctured spherical symmetry” and devised a “potential divider” model to take this into account. Using models with both active and passive dendrites we could successfully simulate the contribution of the mitral cell population to early parts of the extracellular field potential; however, it was clear that mitral cell currents could not generate the depth distribution of the field recorded at later times. This forced the conclusion that the potential recorded during the later phase of the extracellular field must be generated by currents produced by the very large population of granule cells. We were then led to postulate that the granule cells received their excitatory synaptic input from the secondary dendrites of the mitral cells, and that the depolarized granule cell dendrites do both of the following: they produce the currents that generate the depth distribution of the field potential, and we postulated that this depolarization might trigger a hypothetical inhibitory synapse back onto the mitral cell dendrites. I had speculated that a single synaptic structure might operate in two modes, first excitatory in one direction and then inhibitory in the other direction. I was proved wrong about the single structure, but I was delighted by what was found.

Tom Reese and Milton Brightman, who worked independently in electron microscopy (EM) at NIH, but who remembered that Gordon had asked about synaptic structures in the external plexiform layer, showed me their new EM images from this layer of the olfactory bulb. These images revealed oppositely oriented synaptic contacts between these two kinds of dendrites and, incredible as it may seem, every synaptic contact from a mitral to a granule cell dendrite was of the excitatory type, while every synaptic contact from a granule to a mitral dendrite was of the inhibitory type; this was later confirmed by several other labs. A four-author manuscript was submitted to *Science* in 1965; apparently the referee could not believe it, and rejected it; it was published in *Experimental Neurology* the following year (Rall et al., 1966). Here we had dendro-dendritic synapses between two kinds of dendrites: smooth mitral dendrites, and spiny dendrites of granule cells (which have no axon). Each of these dendrites both send and receive synaptically: one sends excitation, the other sends inhibition. Some anatomists could not, at first, accept this; they had thought that dendrites could only receive synaptic input; some suggested that if it sends, it must not be called a dendrite. But time heals wounds, and our theoretical interpretation of this remarkable anatomy and physiology is now generally accepted. I cannot close this section without pointing out that the

existence of dendro-dendritic synapses, here and elsewhere in the CNS, opens up a large domain of graded neuronal interactions to challenge network modelers; such interactions are completely missing from the usual network models that ignore the existence of dendrites.

Excitable dendritic spines and asymmetric chain reaction

Apart from motoneurons, most neurons have many dendritic spines. The theoretical implications of assuming an excitable membrane in dendritic spines has been explored by Jack et al. (1975), Perkel and Perkel (1985), Miller et al. (1985), and Shepherd et al. (1985). Purely hypothetical at first, these studies pointed to synaptic amplification, nonlinear sensitivity to several parameters, and the possibility of chain reactions, in which several firing spine heads depolarize the dendritic membrane; the dendritic membrane could be passive, but under favorable conditions, local dendritic depolarization may suffice to bring some adjacent excitable spines to their firing threshold. Would the resulting spike propagate? If so, how far? Segev and I computed answers to such questions in the context of extensive dendritic branching. We learned that this is more likely to happen in distal branches (because their higher input resistance increases the amount of local depolarization). We also learned that such a chain reaction tends to propagate more readily into distal dendritic arbors and less readily toward the soma (in parent branches). Such asymmetry of propagation is related to the asymmetry of passive electrotonic spread in dendritic branches as had been shown in the mid 1970s, in collaboration with Rinzel. It can be understood in terms of contrasting boundary conditions and input resistances: the input resistance into a terminal branch (with a sealed end) is much higher than the input resistance into a parent branch plus cousin branches.

Functional significance of asymmetric chain reaction

The importance of such asymmetry in the chain reaction between excitable spine heads is that it favors nonlinear interactions within subsets of distal branches (i.e., excitable spine clusters in distal arbors); this seems to favor local integration of and discrimination between significantly different synaptic input patterns delivered to the dendrites.

Without this chain-reaction asymmetry, if propagation toward the soma became secure, this would convert the branching system to an all-or-nothing mode and the neuron would lose the richness inherent in nonlinear local dendritic processing (Rall and Segev, 1987; Segev and Rall, 1998). Other chapters in this book have more to say about the rich possibilities that are made apparent by such theoretical studies.

Geometric complexity versus membrane complexity

An important basic principle of prudent research, both theoretical and experimental, is not to tackle too many complications at once. This was my reason for beginning my modeling with a uniform, passive membrane, and with idealized dendritic geometry. Once you solve the reduced problem, you can then begin to deal with some of the complications judged to be functionally important.

Table 14.1 has not previously been published, but these ideas did underlie much of my research perspective. Others may find this table useful; it facilitates an overview of interrelated research problems. Thus, cell A.3 identifies excitable membrane under space-clamp conditions. By combining space clamping (George Marmont) with voltage clamping (K. S. Cole), and adding the key insight of distinguishing ionic conductances for sodium and potassium ions at different clamped membrane potentials, Hodgkin, Huxley, and Katz achieved a major breakthrough in the late 1940s and early 1950s.

Table 14.1 Different combinations of geometric and membrane complications in neuron models

	1. Passive membrane	2. Synaptic membrane	3. Excitable membrane
A. Isopotential membrane area	A.1 Passive soma without dendrites; space-clamped passive membrane	A.2 Nonlinear effects of synaptic excitation and inhibition; no cable spread	A.3 Hodgkin and Huxley equations; voltage-dependent ionic channel properties
B. Uniform cylinder	B.1 Cable equation for passive spread of membrane potential; input resistance and spatial decrements	B.2 Spatial attenuation of EPSPs, IPSPs, and combined; spatio-temporal input patterns contrasted in equivalent cylinder	B.3 Action potential propagation; decrement, block, delay, and reflection
C. Dendritic branching	C.1 Passive cable properties in branched trees; equivalent cylinders; taper and branching	C.2 Synaptic attenuation in dendritic trees; spatio-temporal input patterns in trees and equivalent cylinders	C.3 Compartmental models: any specified pattern of branch geometry, of synaptic input, and of membrane non-uniformity
D. Dendritic spines	D.1 Passive spine dimensions and properties; spine-stem versus dendritic input resistance; asymmetric decrements	D.2 Synaptic attenuation; change of relative synaptic weights; extremely local synaptic inhibition	D.3 Synaptic amplification; nonlinear dependence on many spine parameters; asymmetric spread into distal arbors; rich computation potential

EPSP, excitatory postsynaptic potential; IPSP, inhibitory postsynaptic potential.

Parenthetical note about space, current, and voltage clamping

Some readers may wonder why I mentioned the names of Cole and Marmont in the paragraph above. The answer is that I was there to witness their contributions. During two summers in Woods Hole (1946–7), I assisted Cole and Marmont in testing the clamping (space, current, and voltage) of squid giant axon; indeed, the first long axial intracellular electrode was pulled by me and plated by Marmont in 1947. I was also there when Hodgkin presented a seminar at the University of Chicago in 1948. He reported the important experiments done in the previous summer at Plymouth with Bernard Katz (Hodgkin and Katz, 1949) and thanked Marmont for discussing his space and current clamp, and Cole for favoring the voltage clamp (see Hodgkin, 1992, pp. 281–283). Several relevant 1949 references, and some subsequent references (from 1968, 1976, and 1985) are cited in Rall (1992). I believe that it does not detract from the great achievement of Hodgkin, Huxley, and Katz (experimental, conceptual, and computational), to note the fact that space, current, and voltage clamping of the squid axon were first developed by Cole and Marmont. Here we have an interesting example of the interplay between experimental and theoretical innovation; no one, not even Einstein, can advance science without building on the work of others.

Geometric complexity

Returning now to Table 14.1, if we wish to explore geometric complexity, we first assume a passive membrane (Table 14.1, cells B.1 and C.1). For these cases, it proved possible to find analytical solutions for uniform cylinders of finite length. It was shown that there is a class of dendritic

branching that can be transformed into an equivalent cylinder (Rall, 1962a); analytical solutions were provided for non-uniform synaptic input (two regions), and the effect on EPSP shape was first demonstrated (Rall, 1962a).

By using superposition of mathematical solutions (steady state and transient), Rinzel and I provided analytical solutions for input (injected current) to a single branch of a dendritic tree (branches meet constraints for an equivalent cylinder) (Rall and Rinzel, 1973; Rinzel and Rall, 1974). These papers also discuss physiological implications; they are among the reprints collected in Segev et al. (1995).

In the 1990s complicated analytical solutions for dendritic trees with arbitrary branching patterns were presented and computed (Major et al., 1993), a mathematical tour de force. Also, essentially equivalent solutions for a compartmental representation of arbitrary branching (Holmes et al., 1992) have been presented and computed. Powerful as these two sets of analytical solutions are, it must be pointed out that they are limited by the fact that they depend on the assumption of passive membrane properties (Table 14.1, cells B.1 and C.1).

Compartmental non-uniformity of inputs and nonlinear membranes

Fortunately, it is now possible to use compartmental models to simulate nonlinear membrane properties (Table 14.1, column 3); these nonlinearities can be distributed non-uniformly over the dendritic compartments (Table 14.1, cells C.3 and D.3). Also, synaptic inputs can be distributed non-uniformly, and the branching need not satisfy the equivalent-cylinder constraints.

Reduced compartmental models

Although modern computers allow one to specify thousands of compartments for one dendritic neuron, my own preference is to avoid using so many, even if the branching data may suggest it. I would rather use fewer compartments and put my effort into finding the best compartmental assignment of different ion channel densities and of different synaptic input patterns. An important example is that of Traub et al. (1991). For some purposes, such a model can be reduced even further; Pinsky and Rinzel (1994) reduced it to two compartments, where one compartment collected the ion channels and inputs of the soma membrane (including proximal dendrites) while the other collected the ion channels and inputs of most of the dendritic membrane. Such a reduced model can preserve the most essential non-uniformity of channels and inputs (over soma and dendrites), and can facilitate the construction and the computational exploration of neural networks that are physiologically more realistic than networks composed of point neurons.

Challenge to test the importance of dendrites

With the advent of new methods for obtaining patch-clamp recordings from dendrites, new insights into the functional properties of dendrites are coming at a rapid pace. Some of these advances are discussed in other chapters in this book. For example, Chapter 16 takes a look at how some of these properties may impart functional complexity on the dendritic tree. Such analyses present a new frontier in computational neuroscience—understanding the relationship between dendritic physiology and network function.

To me, it is a useful exercise to look for physiologically interesting network simulations that make use of dendritic compartments with nonlinear membrane properties and inputs that are different from those at the soma (and perhaps also from those at other dendritic compartments),

and then to test the importance of these non-uniformities for generating that interesting behavior. The test is simple: begin with a successful model containing non-uniformities, then perform and compare a new computation where the neuronal non-uniformity is removed, either by lumping the dendritic compartments with the soma, or equivalently, by giving all compartments the same nonlinear membrane properties and the same inputs. Good examples of such test computations provide strong support for the essential functional role of dendrites and dendritic synapses.

References

- Fatt P (1957) Electric potentials occurring around a neurone during its antidromic activation. *Journal of Neurophysiology* **20**:27–60.
- Hodgkin A (1992) *Chance and Design: Reminiscences of Science in Peace and War*. Cambridge: Cambridge University Press.
- Hodgkin AL, Katz B (1949) The effect of sodium ions on the electrical activity of the giant axon of the squid. *Journal of Physiology* **108**:37–77.
- Holmes WR, Rall W (1992) Electrotonic length estimates in neurons with dendritic tapering or somatic shunt. *Journal of Neurophysiology* **68**:1421–1437.
- Holmes WR, Segev I, Rall W (1992) Interpretation of time constant and electrotonic length estimates of multi-cylinder or branched neuronal structures. *Journal of Neurophysiology* **68**:1401–1420.
- Jack JJB, Noble D, Tsien RW (1975) *Electrical Current Flow in Excitable Cells*. Oxford: Oxford University Press.
- Major G, Evans JD, Jack JJB (1993) Solutions for transients in arbitrarily branching cables: I. Voltage recording with a somatic shunt. *Biophysical Journal* **65**:423–449.
- Miller JP, Rall W, Rinzel J (1985) Synaptic amplification by active membrane in dendritic spines. *Brain Research* **325**:325–330.
- Nelson PG, Frank K (1964) Extracellular potential fields of single spinal motoneurons. *Journal of Neurophysiology* **27**:913–927.
- Perkel DH, Perkel DJ (1985) Dendritic spines: role of active membrane in modulating synaptic efficacy. *Brain Research* **325**:331–335.
- Pinski PF, Rinzel J (1994) Intrinsic and network rhythmogenesis in a reduced Traub model for CA3 neurons. *Journal of Computational Neuroscience* **1**:39–40.
- Rall W (1957) Membrane time constant of motoneurons. *Science* **126**:454.
- Rall W (1959) Branching dendritic trees and motoneuron membrane resistivity. *Experimental Neurology* **1**:491–527.
- Rall W (1960) Membrane potential transients and membrane time constant of motoneurons. *Experimental Neurology* **2**:503–532.
- Rall W (1962a) Theory of physiological properties of dendrites. *Annals of the New York Academy of Sciences* **96**:1071–1092.
- Rall W (1962b) Electrophysiology of a dendritic neuron model. *Biophysical Journal* **2**:145–167.
- Rall W (1964) Theoretical significance of dendritic trees for neuronal input-output relations. In: *Neural Theory and Modeling* (Reiss RF, ed.), pp. 73–97 Stanford, CA: Stanford University Press.
- Rall W (1977) Core conductor theory and cable properties of neurons. In *Handbook of Physiology. The Nervous System. Vol. 1, Cellular Biology of Neurons* (Kandel ER, Brookhart JM, Mountcastle VB, eds), pp. 39–97. Bethesda, MD: American Physiological Society.
- Rall W (1992) Path to biophysical insights about dendrites and synaptic function. In: *The Neurosciences: Paths of Discovery II* (Samson F, Adelman G, eds), pp. 215–240. Boston, MA: Birkhauser.
- Rall W, Rinzel J (1973) Branch input resistance and steady attenuation for input to one branch of a dendritic neuron model. *Biophysical Journal* **13**:648–688.

- Rall W, Segev I (1987) Functional possibilities for synapses on dendrites and dendritic spines. In: *Synaptic Function* (Edelman GM, Gall WE, Cowan WM, eds), pp. 605–636. New York: Wiley.
- Rall W, Shepherd GM (1968) Theoretical reconstruction of field potentials and dendrodendritic synaptic interactions in olfactory bulb. *Journal of Neurophysiology* **31**:884–915.
- Rall W, Shepherd GM, Reese TS, Brightman MW (1966) Dendro-dendritic synaptic pathway for inhibition in the olfactory bulb. *Experimental Neurology* **14**:44–56.
- Rall W, Burke RE, Smith TG, Nelson PG, Frank K (1967) Dendritic location of synapses and possible mechanisms for the monosynaptic EPSP in motoneurons. *Journal of Neurophysiology* **30**:1169–1193.
- Rall W, Burke RE, Holmes WR, Jack JJB, Redman SJ, Segev I (1992) Matching dendritic neuron models to experimental data. *Physiological Reviews* **72**:S159–S186.
- Redman S, Walmsley B (1983) The time course of synaptic potentials evoked in cat spinal motoneurones at identified group Ia synapses. *Journal of Physiology* **343**:117–133.
- Rinzel J, Rall W (1974) Transient response in a dendritic neuron model for current injected at one branch. *Biophysical Journal* **14**:759–790.
- Segev I, Rall W (1998) Excitable dendrites and spines: earlier theoretical insights elucidate recent direct observations. *Trends in Neurosciences* **21**:453–460.
- Segev I, Rinzel J, Shepherd GM (eds) (1995) *The Theoretical Foundation of Dendritic Function: Selected Papers of Wilfrid Rall With Commentaries*. Cambridge, MA: MIT Press.
- Shepherd GM, Brayton RK, Miller JP, Segev I, Rinzel J, Rall W (1985) Signal enhancement in distal cortical dendrites by means of interactions between active dendritic spines. *Proceedings of the National Academy of Science of the United States of America* **82**:2192–2195.
- Terzuolo CA, Araki T (1961) An analysis of intra- vs. extracellular potential changes associated with activity of single spinal motoneurons. *Annals of the New York Academy of Sciences* **94**:547–558.
- Traub RD, Wong RKS, Miles R, Michelson H (1991) A model of a CA3 hippocampal pyramidal neuron incorporating voltage clamp data on intrinsic conductances. *Journal of Neurophysiology* **66**:635–660.

Chapter 15

A theoretical view of the neuron as an input–output computing device

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Summary

Wilfrid Rall developed the theoretical foundations for understanding dendritic function almost 50 years ago. Since then his theory has been intensely employed and extended in order to explore the biophysical principles that link the structure, physiology, and function of dendrites. In this chapter we highlight some of the main insights that were gained from this theory. Based on these biophysical insights we next highlight several key computations that electrically distributed dendrites (as opposed to a “point” neuron) could implement. This theoretical study of neurons as computation devices often involves an interplay between two main themes: (1) obtaining a mathematical representation of the neuron and (2) utilizing realistic models to explore the consequences of specific dendritic properties for the computations that are likely to be carried out by the neuron. In the second half of the this chapter we demonstrate the first theme by reviewing a new experimentally constrained approach to modeling dendritic/somatic nonlinearities, based on the multiple objective optimization method combined with an evolutionary algorithm. We then demonstrate the computational aspect by reviewing recent insights about the role of dendritic inhibition obtained using theoretical and modeling approaches. Finally we highlight additional emerging themes that we believe will become central in the near future to the theoretical study of dendrites. We are confident that models of dendrites and their synapses are essential for creating the bridge between the single-neuron level and the systems level.

Introduction

Our brains are mostly composed of dendrites, a term that was coined by the Swiss scientist William His in 1889. Around that time, the “neuron doctrine” started to take form, driven by the leading anatomist the Spaniard Santiago Ramón y Cajal, who assumed that dendrites serve as the input region for other neurons and that the signal then flows from dendrites to the soma and axon—“the theory of dynamic polarization” (Shepherd, 1991). But it was only in the late 1960s the biophysical nature of dendrites and their role in signal processing became the focus of direct experimental research, following Wilfrid Rall’s groundbreaking theoretical framework for modeling electrical current flow in dendritic trees. His linear cable theory for branching dendrites handled the passive case analytically (Rall, 1959a, 1977; Jack et al., 1983) and his compartmental modeling method could treat the case of nonlinear (excitable) dendrites (Rall, 1964) numerically. This theoretical foundation for modeling dendrites has guided experimental studies for the last 50 years and has dramatically advanced our understanding of signal processing in dendrites (see Chapter 14 for a historical perspective on these developments).

Recent advances in diverse technologies are setting the stage for major breakthroughs in understanding how dendrites and their synapses subserve overall brain function. With new converging optical, anatomical, physiological, and computational technologies (including differential interference contrast video microscopy, two-photon microscopy, molecular probes, voltage-dependent dyes, and powerful parallel computers), the fine structure of neurons and their connectivity to other neurons are beginning to be unraveled. Rules that govern the plastic changes that neurons and dendrites undergo are now better understood, and new ideas regarding the role of dendrites in neuronal computation are emerging (Koch and Segev, 2000; Häusser and Mel, 2003). But it is imperative that we incorporate the quickly accumulating experimental information on synapses, dendrites, and neurons into Rall's theoretical framework. Examples of this are provided in the present chapter. Indeed, we believe that key theoretical insights that will be obtained from studying neuronal processing and plasticity at the single-cell level will be essential for the development of a large-scale theory that can explain how the brain works as a whole and how it orchestrates the complex and dynamic interactions of an organism with its environment. This is probably one of the greatest scientific challenges of the third millennium.

Models of dendrites—the foundation

Until recently, most of the stable electrical recordings from neurons were made from the relatively large soma. It is useful to record from the soma because the soma is electrically close to the axon, where in most neurons in the mammalian central nervous system the output—a train of action potentials—is produced. However, most of the membrane area of central neurons is in their dendrites and it is there where synaptic input contacts the neuron. Can one learn about what happens locally at synaptic sites in the dendrites from recordings made at the soma?

This question motivated Rall to formulate his cable theory for dendrites (Rall, 1959a), followed by his compartmental modeling approach (Rall, 1964). These groundbreaking studies have provided the theoretical framework for understanding how electrical current (both passive and active) flows in complicated dendritic trees. Details of this theoretical framework can be found in Jack et al. (1983), Segev et al. (1995), Rall and Agmon-Snir (1998), and Koch (1999).

Briefly, the one-dimensional cable theory for dendrites decomposes the dendritic tree into a set of interconnected cylindrical cables, each representing a small section of the dendritic tree. At any point along this cable the axial current (which flows along the axial resistance, r_i) may continue to either flow longitudinally or to flow into the membrane (either charging the membrane capacitance, c_m , or crossing the membrane resistance, r_m). As a first approximation, Rall assumed that the dendrites are passive. Namely, that all the electrical parameters are constant (i.e., independent of time and voltage). The flow of current in a passive cable can then be described mathematically by the one-dimensional passive cable equation:

$$\left(\frac{r_m}{r_i}\right)\frac{\partial^2 V(x,t)}{\partial x^2} - r_m c_m \frac{\partial V(x,t)}{\partial t} - V(x,t) = 0 \quad (15.1)$$

where V is the transmembrane potential, and r_i (in Ω/cm), r_m (in $\Omega \cdot \text{cm}$), and c_m (in F/cm) are all per unit length. The general solution for this equation can be expressed as an infinite sum of decaying exponentials (Rall, 1969):

$$V(x,t) = \sum_{i=0}^{\infty} C_i e^{-t/\tau_i} \quad (15.2)$$

where, for a given tree, the coefficients C_i are independent of time, t , but they do depend on the point of observation, x , and on the input current (the initial conditions). In contrast, for a given passive tree, the values for the (“equalizing”) time constants, τ_i , are independent of input location or input current (Rall, 1969) (Note that, for passive cable structures with sealed ends and with uniform membrane properties, $\tau_0 = \tau_m$, the membrane time constant.) Rall showed that, for any passive tree, there is an analytic solution for the cable equation (namely, C_i and τ_i can be written explicitly). Thus, it became possible to combine the anatomical and physiological knowledge about a given dendritic tree and (assuming the tree is passive) to analytically describe how the input current flows from any input site to any other dendritic location, and specifically the soma (Rall, 1959a; Segev et al., 1995; Rall and Agmon-Snir, 1998). Important insights were obtained when these analytic solutions were applied to both synthetic and to real dendritic trees (see below). However, when a strong synaptic input (a transient conductance change, rather than a current input) or a significant nonlinearities exist in the dendritic tree, such as voltage-dependent membrane conductance, numerical (compartmental) methods for solving the corresponding (nonlinear) cable equation should be employed (Rall, 1964; Segev et al., 1992).

In the compartmental approach, the continuous cable equation is discretized into a finite set of compartments, each representing a small section of the dendritic tree. The membrane properties of this dendritic section are grouped into a single RC element (where the membrane resistance, r_m , may depend on time and/or voltage, whereas c_m , the membrane capacitance, is typically assumed to be constant). The resistivity of the dendritic cytoplasm is lumped into a single (typically passive) axial resistivity, r_i . Thus, in an unbranched neuron, the current flowing in compartment j is:

$$\frac{d_j}{4r_{i,j}} \frac{V_{j+1} - 2V_j + V_{j-1}}{\Delta x^2} - c_{m,j} \frac{dV_j}{dt} - i_{ion,j} = 0 \quad (15.3)$$

where d_j , $r_{i,j}$ and $c_{m,j}$ are respectively the compartment’s diameter, axial resistivity, and membrane capacitance; $i_{ion,j}$ includes all currents flowing through ionic channel conductances within the compartment, and Δx is the length of the compartment.

It can be shown that if the length of this dendritic section, Δx , is sufficiently small, the solution for the compartmental model converges to that of the corresponding continuous cable model (Rall, 1964; Segev and Burke, 1998). These two complementary approaches, the cable and compartmental models, have been utilized very successfully and have become even more popular due to their implementations in user-friendly software packages, such as NEURON (Hines and Carnevale, 1997) and GENESIS (Bower and Beeman, 1998).

Broadly speaking, three levels of questioning are explored using cable and compartmental modeling of dendritic neurons:

- 1 *Estimation of electrical properties of dendrites and their synapses.* By reconciling model predictions and experimental results, models can be used to estimate the electrical properties of remote dendritic arbors and distal dendritic synapses (including the time course and location of the synaptic input) from measurements made at the soma (and currently also at specific dendritic sites). The models have also suggested further experiments for refining these estimates. Important examples can be found in the works of Rall et al. (1966, 1967, 1992), Redman and Walmsley (1983a,b), Stratford et al. (1989), Major (1994), Rapp et al. (1994), Häusser and Roth (1997), and Schiller et al. (1997).
- 2 *Identifying key biophysical parameters that determine the input-output characteristics of the dendritic tree.* For passive dendritic models these parameters are: (a) the specific membrane

capacitance (C_m , in F/cm^2) and resistance (R_m , in $\Omega \text{ cm}^2$); (b) the specific axial resistivity (R_i , in $\Omega \text{ cm}$); (c) the input resistance of the neuron (R_N , in Ω); (d) the electrotonic length of the dendritic tree, $L = l/\lambda$ (in dimensionless units), where l is the physical length of the dendrites, $\lambda = \sqrt{(d/4)(R_m/R_i)}$ is the space constant (in cm), and d is the diameter of the cylindrical cable; and (e) the membrane time constant ($\tau_m = R_m C_m$, in s). For nonlinear models, the properties (reversal potential, time course and magnitude) of synaptic conductance and excitable membrane ion channels (type, density, kinetics) at different dendritic locations must be specified, in addition to the passive properties already mentioned.

- 3 *Exploring the functional and plastic capabilities of dendrites and their synapses.* These models can be used in a more abstract fashion in order to study the type of functions that could (in principle) be implemented by a single neuron having an extended dendritic tree (Koch et al., 1982; Segev et al., 1995; Koch and Segev, 2000; Loewenstein and Sompolinsky, 2003; London and Häusser, 2005).

Passive and excitable cable models for dendrites—main insights

The dendritic membrane is endowed with transmitter- and voltage-dependent ion channels that are inherently nonlinear. This may lead to the erroneous (and increasingly popular) notion that passive models of neurons are irrelevant. As we will show later, the insights that were gained from passive models are essential for understanding the behavior of nonlinear signals in dendrites. In fact, the passive model should be viewed as a framework on top of which further (nonlinear) complications are added. We will now summarize the basic insights that were gained from passive models of dendrites and add some of their extensions for excitable trees.

Dendrites are electrically distributed devices

Dendrites tend to ramify and often diverge into several large and elaborate trees. They are thin processes, starting with a diameter of a few microns near the soma, with a branch diameter typically falling to below 1 μm as they successively divide. Many (but not all) types of dendrites are studded with abundant tiny appendages called dendritic spines (the typical length of a spine is $\sim 1 \mu\text{m}$ and its area is about $1.5 \mu\text{m}^2$). When the actual geometry of dendrites is taken into account, together with the specific membrane and axial resistivity (typical values for R_m are between 5,000 and 100,000 $\Omega \text{ cm}^2$ and for R_i between 70 and 250 $\Omega \text{ cm}$), the electrotonic length (L , in units of λ) of the dendritic tree is calculated to be of the order of unity (a total of one space constant from the soma to the distal dendritic tips). In other words, neurons are not isopotential units and, consequently, a large (ten-fold or more) voltage difference (voltage attenuation) may exist between different regions of the dendritic tree (and the soma) following a local synaptic input to the dendrites (Rall and Rinzel, 1973).

A significant portion of the synaptic charge reaches the soma even for distal inputs

Because the electrical resistance of the dendritic membrane per unit length is much larger than that of the cytoplasm, the synaptic input current (charge) tends to flow longitudinally along the dendrites rather than being lost through membrane leakage to the extracellular space. Consequently, a significant portion of synaptic current reaches the soma from dendritic input sites. In other words, distal dendritic excitatory synapses make a significant contribution to the depolarizing charge that reaches the soma and axon membrane.

Another consequence of the relatively large membrane resistance and the small dimensions of dendrites is that large input impedance ($\sim 10^3 \text{ M}\Omega$ for DC inputs) is expected at distal dendritic locations. As a result, even the small amount of current that is produced by a single excitatory synapse can generate a large local (e.g., in a distal spine head) excitatory postsynaptic potential (EPSP) of 10–20 mV and more. The same input would produce a much smaller peak depolarization when placed directly on the soma, where the input resistance could be 10- to 100-fold smaller (Rall and Rinzel, 1973; Rinzel and Rall, 1974). Therefore, one expects large voltage fluctuations in distal dendritic arbors with a large input impedance at that location (see later). Indeed, excitatory input at a distal site may depolarize this region sufficiently to ignite a local Na^+ , Ca^{2+} , and N-methyl-D-aspartic acid (NMDA) spikes (see Chapter 12).

Voltage attenuation and active propagation in the dendritic tree is asymmetrical

When current flows from thin distal arbors toward the soma it encounters a large conductance load (“sink”); the boundary conditions are very different when the current flows in the reverse direction (from soma to dendrites). Because the sink for current is much larger in the dendrite-to-soma direction, the attenuation of voltage in this direction is significantly greater than in the reverse direction. In other words, the dendritic tree is electrically more compact (voltage is distributed more uniformly) when the input is at (or near) the soma compared with an input given locally at distal dendritic arbors (Rall and Rinzel, 1973; Zador et al., 1995). For exactly the same reason, the conditions enabling a somatic spike to backpropagate into the dendrites are much more favorable than those for a dendritic spike to propagate forward, toward the soma (Stuart and Sakmann, 1994; Rapp et al., 1996; Vetter et al., 2001). Indeed, the morphology of the dendritic tree strongly affects the preferred direction and degree of active propagation in the tree (Stuart and Sakmann, 1994; Vetter et al., 2001; Williams, 2004).

Dendrites are significant time delay lines for synaptic potentials as well as for dendritic spikes

As a result of the capacitive properties of the dendritic membrane, a significant delay in peak time is expected when the synaptic potential spreads from distal dendritic locations to the soma. The velocity of synaptic potentials in the dendritic tree (when a passive membrane is assumed) is of the order of $2\lambda/\tau_m$, where τ_m is the passive membrane time constant (Jack et al., 1983; Agmon-Snir and Segev, 1993a; Zador et al., 1995). With τ_m of, say, 20 ms and λ of 1 mm, the velocity of the EPSP is of the order of 0.1 mm/ms, which is rather slow. Thus, the time delay from distal dendritic input sites (sometimes located more than 1 mm away from the soma) to the soma is expected to be of the order of 10–20 ms. Indeed, such a range of delays was found experimentally (Nicoll et al., 1993; Larkum et al., 1998; Stuart and Spruston, 1998; Berger et al., 2001; Letzkus et al., 2006; Sjöström and Häusser, 2006). It is noteworthy that the backpropagating action potential (bAP) recorded in the apical dendrite of layer 5 (L5) cortical pyramidal neurons also propagates rather slowly, at around 0.15 mm/ms (Stuart et al., 1997a). As a matter of fact, this signal is only weakly active and so is strongly influenced by the passive characteristics of the dendritic membrane.

Another consequence of the membrane capacitance is the slowing in the time course of somatic EPSPs with distance of the input from the soma (Rall et al., 1967; Rinzel and Rall, 1974). The dependence of time-to-peak and half-width of the somatic EPSP on input location can serve as the basis for estimating the electrotonic distance, X ($X = x/\lambda$) of the dendritic synapses from the soma (Rall et al., 1967; Redman and Walmsley, 1983a). The “dendritic delay” and the change in the shape

of the EPSP for distal versus proximal inputs have important consequences for neuronal processing and computations (Rall, 1964; Segev, 1992; Segev et al., 1995; Zador et al., 1995).

The time-window for the summation of synaptic inputs at the soma is approximately τ_m

For brief synaptic inputs, the duration (time course) of EPSPs at the soma is of the order of τ_m . During this time window, synaptic inputs arriving at the soma can temporally summate (integrate) with each other. Indeed, τ_m sets the upper limit for the temporal resolution (the requirement for input synchronization) of neurons (Agmon-Snir and Segev, 1993b; Koch et al., 1996). However, this limit does not hold for certain non-passive cases, in which the decay time of the synaptic conductance substantially exceeds τ_m , such as NMDA synaptic conductances (Forsythe and Redman, 1988), as well as for active conductances that can prolong the effective summation window well beyond τ_m , such as the persistent Na conductance (French et al., 1990). Feedforward inhibition is an additional extrinsic factor that could selectively determine the size of the somatic window of summation (Pouille and Scanziani, 2001).

The temporal resolution at distal dendritic sites (and spines) can be as short as 0.1 τ_m

Locally, at the dendritic input site, the time course of the EPSP is determined primarily by the fast flow (loss) of the input current to other regions of the dendritic tree (due to the low axial resistance), rather than by τ_m . In particular, the morphology of the dendritic tree, rather than the membrane properties, determines to a large extent how fast an input current is lost from the input site. Consequently, the local synaptic potential at the input site is briefer than the resulting EPSP at the soma. As a result, the effective time window for the summation of synaptic potentials locally at distal dendritic sites can be an order of magnitude smaller than τ_m (see also Softky, 1994). Thus, the temporal resolution for summation of synaptic inputs locally at distal dendritic locations is up to ten times finer than in the soma. A more complete discussion of the functional significance of τ_m is given by Agmon-Snir and Segev (1993a), Zador et al. (1995), and Koch et al. (1996).

Dendritic spines are favorable sites for plastic changes and for nonlinear boosting of their synaptic inputs

The special morphology of dendritic spines (see Chapter 1), consisting of a small spherical head (with a volume ranging from 0.005 to $0.3 \mu\text{m}^3$) connected to a thin ($0.05\text{--}0.2 \mu\text{m}$) and often long ($1\text{--}2 \mu\text{m}$) cylindrical neck, provides a favorable site for modifying the efficacy of synaptic input (typically excitatory) impinging on the spine head. Rall (1974, 1978) and others (Rinzel, 1981; Koch and Poggio, 1983; Rall and Segev, 1988) have shown in modeling studies that, for an appropriate range of spine parameters, a small change in the dimensions of the spine neck may result in a large change in the amount of charge that the spiny synapse generates. Actual spine neck resistance has been shown experimentally to be $< 150 \text{ M}\Omega$ (Svoboda et al., 1996), although recent findings indicate that, at least in culture, some spine necks have a much larger resistance (Bloodgood and Sabatini, 2005); most recently, Rafael Yuste's lab. has demonstrated that spine input summates linearly, indicating that spines may indeed serve as electrical isolators limiting the extent of electrical interaction between nearby synapses (Araya et al., 2006; Bywalek et al., 2015). In addition, the minute volume of the spine head compartment implies a significant change in intracellular ion concentrations due to the flow of a relatively small number of ions into the spine head (e.g., following the activation of the spiny synapse), suggesting that spines may subserve biochemical compartmentalization (Koch and Zador,

1993). This concentration change may trigger a cascade of intracellular processes that can lead to highly specific activity-dependent long-term changes in the efficacy of spine synapses (Shepherd, 1996; Yuste and Majewska, 2001; Carlisle and Kennedy, 2005).

Furthermore, the spine head with its large input impedance and voltage-gated ion channels could act as a specialized site for boosting excitatory synaptic inputs. This boosting effect was demonstrated in theoretical studies (Jack et al., 1983; Baer and Rinzel, 1991; Segev and Rall, 1998) and has been experimentally assessed by several groups (Yuste and Denk, 1995; Caldwell et al., 2000; Bywalez et al., 2015).

Why consider passive models when dendrites have active properties?

The insights summarized in the previous section were primarily based on passive cable models of dendrites. However, synaptic input, which is in reality a conductance change rather than a simple current input, as well as voltage-dependent ion currents render the dendrite an inherently nonlinear device. For example, dendritic spikes are observed in some types of neurons (Nicholson and Llinas, 1971; Chen et al., 1997; Golding and Spruston, 1998; Larkum et al., 1999b; Schiller et al., 2000; Ariav et al., 2003) (see other chapters in this book for further discussion). These findings may lead to the erroneous notion that passive models of neurons are essentially irrelevant.

Perhaps the best way to point out the problem with this view is to consider the propagation of the action potential (AP) in the axon. Axons are highly nonlinear electrical elements but, nevertheless, the propagation of an AP is strongly affected by the passive properties of the axon. In fact, the propagation velocity of an AP is directly proportional to the passive space constant, λ (Jack et al., 1983). The intuitive reason is that the “foot” of the AP is essentially a passive signal and so current that spreads “in front” of the AP and activates the more distal regions of the axon is strongly affected by the passive properties of the axon.

Significant spatial averaging takes place in the dendritic tree when input is delivered locally (London et al., 1999). Because the input current flows rapidly from the local (synaptic) input site to other dendritic regions, the electrical properties of these not-yet-activated regions have a marked effect on the behavior of the signal at the input site. In other words, the passive properties of the dendritic tree shape, to a large degree, the local voltage response at its site of origin even when active channels are involved in the generation of the input signal.

The passive model should, therefore, be viewed as a framework upon which active properties can be added. One cannot fully understand active phenomena in neurons without understanding the interplay between the underlying passive and active mechanisms. As we have shown, important insights were gained by a careful analysis of the passive case. These insights should be refined and expanded depending on the variety of active currents (assuming their properties are known) which are integrated into the passive model.

Computing with dendrites and their synapses

The models described above focused on understanding how the neuron’s substrate, dendrites, synapses, and membrane ion channels affect the processing of synaptic inputs in neurons. The next question is how the dendritic “substrate” might give rise to specific computations? In particular, one may ask how does the electrically distributed dendritic structure endow neurons with the potential for performing elementary computations, beyond those that a “point” neuron could implement? Indeed, one may view the dendritic tree as composed of a system of semi-independent functional

subunits (subtrees) where local computations may take place almost independently from other subunits. The result of this local nonlinear computation may (or may not) be then delivered to the soma–axon region (Mel, 1993; Archie and Mel, 2000; Fantana et al., 2008; Gollo et al., 2009).

Some of the key computations that could be performed by dendrites are as follows (see also Koch and Segev, 2000; London and Häusser, 2005; Herz et al., 2006):

- 1 *Detection of direction of motion.* This operation is of particular importance for the survival of any living/moving creature. Indeed, directional-selective neurons are already found in the retina of both vertebrates and invertebrates. Possible mechanism for implementing this computation are suggested in Rall (1964), Koch et al. (1983), Anderson et al. (1999), Borg-Graham (2001), Euler et al. (2002), and Branco et al. (2010).
- 2 *Collision avoidance.* In the lobula giant movement detector (LGMD) of locusts two inputs (one excitatory, one inhibitory) converge onto the LGMD dendritic tree, where they are multiplied. This operation underlies the response of the LGMD to looming visual stimuli (Gabbiani et al., 2002; Fotowat and Gabbiani, 2011).
- 3 *Storage and classification of multiple input features.* Models show that mapping of excitatory synapses onto specific dendritic branches, combined with local nonlinearities in these branches, enhances the input classification capacity of neurons (Mel, 1992; Losonczy et al., 2008).
- 4 *Calculation and memory of position variables.* This requires temporal integration of velocity signals—an operation that is essential for maintaining a particular posture and for navigation. It is argued that time-dependent position representation could be implemented by calcium dynamics in the dendrites of single neurons (Loewenstein and Sompolinsky, 2003).
- 5 *Recovering input signals in the presence of strong noise.* The mechanism suggested and experimentally tested in the optic neuropile of the blowfly is that temporal summation of corrupted signals arriving from different dendritic branches averages out local fluctuations, thus giving rise to a faithful (smooth) representation in the axon (output) of the visual input (Single and Borst, 1998).
- 6 *Enhancing temporal resolution for coincidence detection.* The short effective time constant (see above) in dendrites and the strong voltage saturation of the synaptic input in dendrites (due to high local input resistance), combined with dendritic nonlinearities, could equip the neuron with a temporal resolution in the submillisecond range—which is faster than its membrane time constant or the typical time constants of its synapses (Agmon-Snir et al., 1998; Mathews et al., 2010).
- 7 *Allowing different temporal codings in the same neurons.* In vitro experiments in dendrites of L2/3 cortical pyramidal cells have shown that different coding schemes could be implemented in different parts of the same dendrite. Due to stronger activation of the slow NMDA receptors at distal sites, the summation of synaptic inputs in these sites is less sensitive to their exact timing (i.e., they follow a “rate code”), whereas inputs to more proximal sites (where there is less activation of NMDA receptors) are more dependent on temporal precision (i.e., they follow a “temporal code”) (Branco et al., 2010).

Thanks to the advance of new optical and anatomical technologies, the direct involvement of dendrites in specific computations has been experimentally demonstrated *in vivo* (Single and Borst, 1998). Probably the most complete example to date is the computation of direction of visual motion in retinal ganglion cells (GC). This computation requires some source of asymmetry whereby the synaptic input impinging on a GC for one (e.g., the preferred) direction of visual motion differs from the synaptic input to the GC for movement in the opposite (null) direction. Indeed, theoretical

ideas regarding computation of directional selectivity in dendrites were put forward some 50 years ago (Rall, 1964; Koch et al., 1983; Anderson et al., 1999; Segev and Rall, 1998) and in vitro experiments have confirmed some of these predictions (Borg-Graham, 2001; Euler et al., 2002; Branco et al., 2010). With the recent convergence of several new technologies—optical imaging from dendrites *in vivo* (see also Jia et al., 2010) and large-scale reconstruction of synaptic connections onto the GCs using electron microscopy—significant progress has been made in this long-standing problem (Briggman et al., 2011; Sterling, 2002; Demb, 2007; Taylor and Vaney, 2003).

Modeling neurons with active dendritic properties

The dendrites of cortical neurons exhibit numerous types of ion channels, some of which are distributed non-uniformly, giving rise to complex dendritic excitability. Active dendritic properties of pyramidal cells, in particular the thick-tufted cells (TTCs) in L5, have been studied extensively over the past few decades. The apical dendrites of TTCs backpropagate the AP from the soma via dendritic Na^+ channels (Stuart et al., 1997b), and can generate dendritic Ca^{2+} spikes in response to intense dendritic stimulation or a high frequency of somatic action potentials (Amitai et al., 1993; Larkum et al., 1999a; Larkum and Zhu, 2002; Schiller et al. 1997). In vitro studies also show that coincidence of the bAP and distal dendritic EPSPs can trigger a dendritic Ca^{2+} spike, which in turn triggers one or more axo-somatic APs as a high-frequency spike burst (Larkum et al., 1999b). This bAP-activated Ca^{2+} spike (BAC) firing demonstrates the interplay between the two firing zones of these pyramidal cells, the axo-somatic (Na^+ spikes) and the dendritic (Ca^{2+} spikes), and is thought to be involved in coincidence detection of EPSPs and APs (Stuart and Häusser, 2001), spike timing-dependent plasticity (Markram et al., 1997; Bar Ilan et al., 2011), and neural computations such as cortical association (Larkum, 2013). Recent studies demonstrated that Ca^{2+} spikes are triggered by coincident sensory inputs *in vivo* (Takahashi and Magee, 2009; Xu et al., 2012) and can modulate the frequency–current (f – I) gain of these cells (Larkum et al., 2004; Murayama and Larkum, 2009; Palmer et al., 2012).

Constraining multi-compartment models of complex cells such as TTCs with their axo-somatic firing pattern (Le Bé et al., 2007) and an increasing number of known active dendritic properties is a daunting task if done manually, since it requires finding the densities of numerous types of ion channels with nonlinear kinetics, some of which are distributed non-uniformly over the dendrites. In recent years the multi-objective optimization with evolutionary algorithms (Deb et al., 2002) has been shown to be useful in neuronal modeling (Keren et al., 2005; Druckmann et al., 2007). In this section we will look at optimizing models for TTCs with the somatic and dendritic firing repertoire using such algorithms (Hay et al., 2011).

Multi-objective optimization with an evolutionary algorithm

Key constraints for TTC models include features of the firing at the soma, firing at the dendrites, and the attenuation of bAPs, interconnecting the two firing zones. These experimental features serve as the objectives for optimization, and their statistics are extracted from recorded voltage traces or taken from the experimental literature. For example, voltage traces recorded during BAC firing show that the interspike interval during the burst of APs is 9.9 ± 0.9 ms (Larkum et al., 1999b). As another example, we can derive from the experimental literature that the amplitude of the bAP is 45 ± 10 mV at $600 \mu\text{m}$ from the soma and 36 ± 9 mV at $800 \mu\text{m}$ (Larkum et al., 2001). These features are used to constrain model parameters.

The unknown parameters found by the optimization procedure are the densities of conductance mechanisms (K^+ , Na^+ and Ca^{2+} ion channels) expressed at the soma and distributed throughout

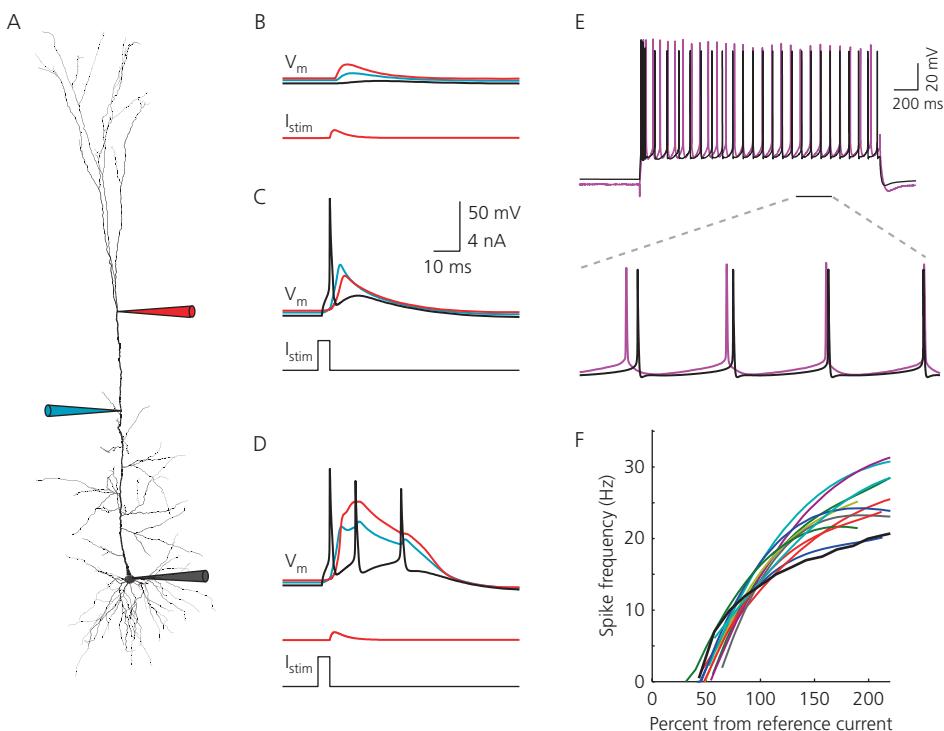


Fig. 15.1 TTC model reproducing active dendritic and somatic firing properties. (A) Reconstructed morphology of a L5 thick-tufted neocortical pyramidal cell at postnatal day 36, used for the fitting and simulations. Recording and stimulation sites are indicated by schematic electrodes at the soma (black), proximal apical dendrites (400 μm from the soma, blue), and distal apical site (620 μm from the soma, red). (B–D) Model simulation of BAC firing (scale bar in C applies to B and D as well): (B) EPSPs attenuated from the distal dendrites to the soma; (C) APs backpropagated from the soma to the distal apical dendrites with attenuation as observed experimentally; (D) coincidence of bAP and local dendritic EPSP evoked Ca^{2+} spike at the main apical bifurcation, which in turn depolarized the soma and triggered a burst of two additional somatic APs. (E) The model's response (black) to depolarizing step current injected at the soma was within the statistics measured experimentally in TTCS (magenta, example voltage trace recorded *in vitro* in a TTC). (F) The model's f - I relationship (black) was within the range seen experimentally in TTCS (color curves derived from recordings in 11 cells).

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the dendrites of a multi-compartmental 3D reconstruction of a TTC (Fig. 15.1A). The distribution of channel densities over the dendrites is based on previous findings. For example, *in vitro* studies indicate the existence of a “hot” zone around the main bifurcation of the apical dendrites, a region of low threshold for triggering dendritic Ca^{2+} spikes (Larkum and Zhu, 2002). This low threshold can be mediated by a difference in the balance between Ca^{2+} and K^+ currents at the main bifurcation, either via a higher density of Ca^{2+} channels or, as more recent experiments indicate, a lower density of Ca^{2+} -activated K^+ channels (Almog and Korngreen, 2014). Other parameters that are

better known from previous studies, such as ion reversal potentials, the membrane time constant, and capacitance, or the distribution of I_h channels in the apical dendrites (Kole et al., 2006) can be set as fixed rather than searched for.

In each iteration of the algorithm, a set of candidate models that best fit the experimental features is generated using an elitist non-dominated sorting evolutionary algorithm (Deb et al., 2002). Non-dominated candidate models, each fitting at least one objective but not necessarily all better than any other model, are selected to generate the set for the next iteration. Generating the new set involves random perturbations of the parameter values (“mutations”) and mixing between models (“crossovers”). After a few hundred to thousands of iterations, the firing features in many of the candidate models are within the experimentally observed statistics, giving a set of acceptable models, namely non-unique solutions (Druckmann et al., 2007; Hay et al., 2011).

An example of a model that reproduces the axo-somatic and dendritic firing within the experimental statistics is shown in Fig. 15.1. Distal apical EPSPs attenuate along the dendrites, so that a local dendritic depolarization of 14 mV is reflected as a 2.5 mV depolarization at the soma (Fig. 15.1B). In the other direction, APs backpropagate from the soma to the distal apical dendrites with their amplitude attenuated as seen experimentally (Fig. 15.1C). The coincidence of a bAP and an EPSP at the distal dendrites triggers BAC firing (Fig. 15.1D), as described above. The model’s dendritic Ca^{2+} spike amplitude and width, as well as the somatic AP burst frequency are also within the range found experimentally in TTCs. Additional objectives including features of the typical somatic firing response to a step current injection (e.g., spike rate and adaptation) as well as the f - I relationship are also reproduced by the model in agreement with the experimental statistics measured in TTCs (Fig. 15.1E, F).

The TTC conductance-based model reproduces other somatic–dendritic firing properties

We have seen that the model reproduces the multiple objectives that were used to constrain it. The next important step in assessing the quality of the model is to test it on stimuli with which it was not constrained (model generalization). In vitro experiments show that when a train of APs at the soma passes a critical frequency, the summated bAPs at the distal apical dendrites trigger a dendritic Ca^{2+} spike (Larkum et al., 1999a). We injected the model soma with a train of brief suprathreshold pulses. At low frequencies (e.g., 70 Hz) the bAPs at the dendrites do not elicit a Ca^{2+} spike (Fig. 15.2A, left). However, at higher frequencies (e.g., 120 Hz) the summation of bAPs in the apical dendrite is sufficiently strong to trigger a dendritic Ca^{2+} spike (Fig. 15.2A, right). A series of such simulations with different AP train frequencies reveals a sharp transition around 100 Hz from absence to presence of dendritic Ca^{2+} spikes (Fig. 15.2B), in agreement with experiments (Larkum et al., 1999a).

Sets of models delineate the necessary ranges of ion channel densities

As we have described, the evolutionary algorithm assesses hundreds to thousands of possible models in each iteration and yields a set of solutions rather than a single one. Each solution is a different combination of ion channel densities, and some models may differ in their response features (within the experimentally measured statistics). Previous experiments show that different combinations of ion channel densities can yield similar electrophysiological properties (Goaillard et al., 2009). In addition, cortical cells such as TTCs are non-homogeneous in terms of their active properties. The generation of a variable set of models is therefore advantageous, considering the biological variability in terms of ion channel densities and response features.

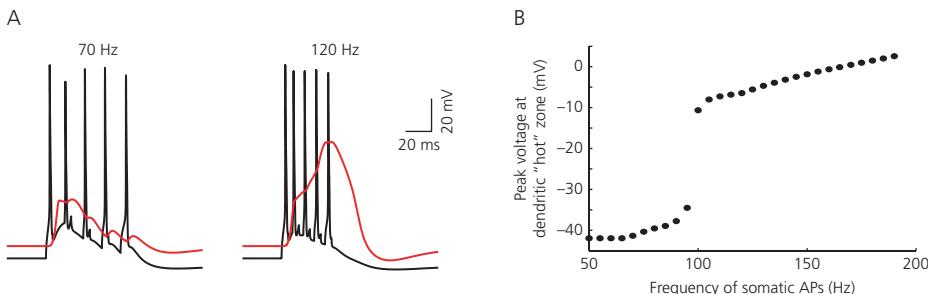


Fig. 15.2 Ca²⁺ electrogenesis in distal apical dendrites in response to a critical frequency of somatic stimulation. **(A)** The model neuron shown in Fig. 15.1 was stimulated at the soma with a train of five brief suprathreshold depolarizing pulses, resulting in a train of somatic APs (black). Left: the train at 70 Hz did not elicit a Ca²⁺ spike in the distal apical dendrites (red, measured 830 μm from the soma), whereas at 120 Hz (right) it did. **(B)** Peak voltage response in the distal apical dendrites as a function of the frequency of the somatic train of APs. At a “critical frequency” around 100 Hz an “all or none” Ca²⁺ spike was generated in the dendrites.

Reproduced from Etay Hay, Sean Hill, Felix Schürmann, Henry Markram, and Idan Segev, Models of neocortical layer 5b pyramidal cells capturing a wide range of dendritic and perisomatic active properties, *PLoS Computational Biology*, 7(7): e1002107, Figure 5, © 2011, The Authors.

TTCs, as with most neuron types, also vary in their dendritic morphology despite sharing some unique morphological features (Le Bé et al., 2007). Generating sets of models using different reconstructed morphologies can reveal differences in the ranges of ion channel densities required to reproduce the experimental features for neurons with different morphological features. When repeating the optimization of active dendritic and somatic properties using a different morphology that had a larger transfer impedance between the soma and the main apical bifurcation, the range of apical Na⁺ density in the resulting models was lower (84–96 versus 101–134 pS/μm²). This agrees with previous studies showing that the dendritic Na⁺ channel density necessary to reproduce attenuation of bAPs is inversely related to the transfer impedance between soma and dendrites (Vetter et al., 2001). This case demonstrates the facilitated analysis made possible by generating sets of different models and comparing the ranges of ion channel densities they delineate. The order of magnitude of apical Na⁺ channel density in the models also agrees with estimates based on experimental recordings in TTCs (Stuart and Sakmann, 1994).

In summary, the multi-objective optimization evolutionary algorithm is a powerful tool for reconciling experimental responses (dendritic and somatic firing) and the behavior of a corresponding conductance-based model. As in biology, the resultant model is not unique—different combinations of ion channel densities may yield similar firing properties (Goaillard et al., 2009). The automated nature of the optimization facilitates future refinement of models with new experimental findings, such as the distribution of certain dendritic ion channels (Almog and Korngreen, 2014). The TTC model reproducing key features of both somatic and dendritic firing is highly realistic, and is therefore an attractive tool for theoretical studies. This and similar realistic models for other cell types such as different inhibitory neurons (Druckmann et al., 2007) already serve as the building blocks for large-scale *in silico* neuronal networks (e.g., in the Blue Brain Project), allowing direct assessment of questions about the contribution of various neuronal dynamics, such as active dendritic properties, to the overall network states. A recent study simulating small networks (microcircuits) of the TTC models showed that bAPs and BACs-firing are important factors in

the amplification of thalamic inputs by cortical microcircuits and the maintenance of input selectivity (Hay and Segev, 2014). The TTC model can be found in ModelDB (Hines et al., 2004).

Synaptic inhibition in dendrites

Synaptic inhibition is one of the fundamental mechanisms that shape the dynamics of neuronal systems. Like excitatory synapses, inhibitory synapses are distributed over the whole dendritic tree of their target neuron and, surprisingly, are found even at very distal dendritic branches far from the output axonal region.

In aiming to provide a theoretical understanding for dendritic inhibition, a few (by now classical) analytical studies have utilized Rall's cable theory (Rall, 1964; Koch et al., 1982; Jack et al., 1983). Two fundamental insights were provided by these studies. First, that the "visibility" of the inhibitory conductance perturbation is highly local. Namely, that the effect of local conductance perturbation in dendrites decays very steeply with distance from the inhibitory synaptic contact (Koch et al., 1990; Williams, 2004). Second, that "on-path" inhibition, located between the excitatory synapse and the soma, is more effective than the respective "off-path" inhibition in dampening the excitatory current that reaches the soma ("on-path theorem;" Koch and Segev, 1998).

These theoretical studies were limited in several ways. Firstly, they adopted a "somato-centric" viewpoint, whereby the impact of dendritic inhibition is considered solely at the soma and/or axon. Secondly, the dendrites were assumed to be passive. Thirdly, inhibition was modeled (in most of the cases) as forming a single synaptic contact onto the dendrites rather than multiple synapses contacting multiple dendritic branches as is the biological case. Indeed, a large number of contacts per synaptically connected pair of neurons (more than ten) and dendritic domain-specific innervation of specific axonal afferents are common to neurons in the cerebral cortex (see review by Markram et al., 2004), the hippocampus (Buhl et al., 1994; Miles et al., 1996), and other brain regions (e.g., the striatum; Tepper et al., 2004). This commonality may suggest a similar underlying functional principle.

Another important feature of dendrites is that they are decorated by an abundance of voltage-gated excitable ion channels such as NMDA, Ca^{2+} , and Na^+ inward currents which, in some cases, trigger a dendritic spike and operate in a highly nonlinear fashion (see Magee, 2007; Larkum et al., 2009; Lavzin et al., 2012; Palmer et al., 2012; Major et al., 2013). Therefore, multiple inhibitory contacts in a restricted dendritic domain can specifically influence regional dendritic nonlinear phenomena.

Here we describe an experimentally based extension of the classical theoretical framework that incorporates a "dendro-centric" viewpoint under realistic conditions, whereby multiple synaptic contacts impinge on a dendritic tree, thus providing several (sometimes counterintuitive) principles that govern the operation of dendritic inhibition. By introducing a measure (the "shunt level") for the impact of inhibitory conductance perturbation, we explain how a relatively small number of strategically placed inhibitory synapses effectively counterbalance the effect of the larger number of excitatory synapses found in many central neurons such as cortical (DeFelipe et al., 2002) and hippocampal (Megías et al., 2001) pyramidal cells.

The shunt level—a tractable measure for the impact of dendritic inhibition

When an inhibitory synapse is activated at a dendritic location, i , a local conductance perturbation g_i (a shunt) is induced in the dendritic membrane. Depending on the reversal potential of that synapse, either an inhibitory postsynaptic potential (IPSP) is also generated or no potential change is observed (a "shunting" or "silent" inhibition; Koch and Poggio, 1985).

Although the membrane shunt due to the activation of the inhibitory synapses at x_i is highly local, its effect spreads to (i.e., it is visible at) other dendritic locations (Rall, 1967; Koch et al., 1990; Williams, 2004). Indeed, this spatial spread is reflected by a change in input resistance, ΔR_N , at location x_d . We define the shunt level at location x_d , $SL(x_d)$ as:

$$SL(x_d) = \Delta R_N(x_d) / R_N(x_d) \quad (15.4)$$

where R_N is the input resistance in location x_d prior to the activation of g_i . SL is thus the relative drop in R_N at location x_d due to the activation of single (or multiple) steady conductance perturbations at arbitrary dendritic locations. For a single g_i , using the solution for conductance change visibility (Koch et al., 1990) together with the transfer resistance, SL becomes:

$$SL(x_d) = \left[\frac{g_i(x_i) R_N(x_i)}{1 + g_i(x_i) R_N(x_i)} \right] A(x_i, x_d) \times A(x_d, x_i) \quad (15.5)$$

where $A(x_i, x_d)$ is the degree of attenuation from location x_i to location x_d , i.e., $V(x_d)/V(x_i)$ due to current injection at location x_i (see Gidon and Segev, 2012, for generalization to the transient case). The square bracket denotes the amplitude of SL at the input location i . The attenuation of SL is $SL(x_i, x_d) = A(x_i, x_d) \times A(x_d, x_i)$ and thus $SL(x_i, x_d) = SL(x_d, x_i)$, i.e. attenuation of SL is symmetrical. The value of SL ranges from 0 (no shunt) to 1 (infinite shunt) and depends on the strength and dendritic distribution of g_i s. For example, $SL(x_d) = 0.2$ implies that the inhibitory synapse reduced the input resistance at location x_d by 20%, which is also the relative drop in the steady voltage at x_d due to the inhibition following the injection of steady current at location x_d . Note that the SL measure is also applicable for assessing the change in input resistance due to excitatory synapses, which, like inhibition, exert a local membrane conductance change.

Distal dendritic inhibition effectively controls excitability at proximal regions

We started with a geometrically simple case, whereby a single inhibitory synapse impinges on a dendritic cylinder that has a sealed end and is coupled to an isopotential excitable soma at the other end (Fig. 15.3A). The dendritic cylinder comprises hotspot (Magee and Johnston, 1995; Schiller et al., 1997, 2000; Larkum et al., 1999a; Antic et al., 2010) which is modeled by a cluster of 20 NMDA synapses, each randomly activated at 20 Hz (Fig. 15.3A). We then search for the strategic placement of the inhibitory synapse that would effectively dampen this local dendritic hotspot.

Using the numerical simulations (the NEURON simulation environment; Hines and Carnevale, 1997) for the nonlinear cable model described above (Fig. 15.3B), we found that when the inhibitory conductance perturbation, g_i , was placed distally (“off-path”) to the hotspot, the rate of the soma APs was reduced more effectively than when the same inhibitory synapse was placed proximally (“on-path”) at the same distance from the hotspot (Fig. 15.3B). The asymmetry of the impact of proximal versus distal inhibition for dampening local dendritic hotspot holds regardless of the distance from the hotspot (Fig. 15.3C). When we increase the distance (in a given cable length) of both the distal and the proximal synapses from the hotspot, the relative advantage of distal inhibition also increases (Fig. 15.3C).

In order to provide an explanation for this result, we analytically computed the value for SL at the hotspot (x_h) and thus assessed the impact of inhibition at this location (Fig. 15.3D). In the corresponding passive case, SL at the hotspot due to the inhibitory conductance perturbation g_i at location x_i can be expressed as the product of the amplitude of SL at location x_i and the attenuation of SL from x_i to x_h , i.e., $SL(x_h) = SL(x_i) \times SL(x_i, x_h)$. The asymmetry of the impact of distal versus proximal inhibition at x_h (the hotspot) results from the difference in the model’s boundary

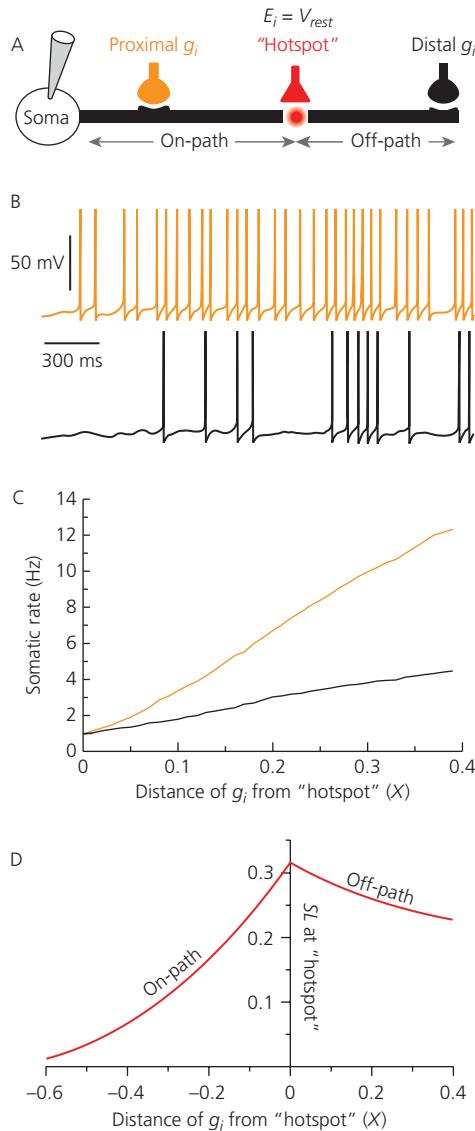


Fig. 15.3 Distal ("off-path") inhibition is more effective than the corresponding proximal ("on-path") inhibition in dampening a local dendritic hotspot. **(A)** A model of a cylindrical cable (sealed end at $L = 1$) coupled to an isopotential excitable soma. Twenty NMDA synapses ($g_{\max} = 0.5 \text{ nS}$; Sarid et al., 2007) are clustered at the hotspot located at $X = 0.6$ (red synapse); each synapse is randomly activated at 20 Hz. A single inhibitory synapse ($g_i = 1 \text{ nS}$) is placed either distally or proximally at the same electrotonic distance ($|X| = 0.4$) from the hotspot. **(B)** Inhibition of the somatic Na^+ spikes is more effective when inhibition is placed distally to the hotspot (black synapse and corresponding black somatic spikes, compared with the orange synapse and the corresponding orange somatic spikes). **(C)** Somatic rate as a function of the distance between the synapse and the hotspot. Black and orange lines correspond to the on-path and off-path synaptic locations, respectively. **(D)** SL at the hotspot in the modeled cell depicted in (A) as a function of the distance of the inhibitory synapse from the hotspot. Off-path inhibition attenuates less steeply compared with the respective on-path inhibition. Model parameters: $R_a = 100 \Omega \text{ cm}$; $R_m = 20 \text{ k}\Omega \text{ cm}^2$; $C_m = 1 \mu\text{F}/\text{cm}^2$; cable diameter = 1 μm ; somatic $R_N = 0.1 \times$ dendritic terminal R_N when disconnected. Na^+ and K^+ channels are as in Traub et al. (1991) with activation and inactivation functions shifted by +15 mV.

Reproduced from Gidon and Segev, 2012.

conditions, namely, a sealed-end boundary at the distal end and an isopotential soma at the proximal end. This difference implies that the input resistance and $SL(x_i)$ (in cases of a fixed g_i) also increase monotonically with distance from the soma. Thus, $SL(x_i)$ at the distal site is larger than that at the corresponding proximal site. Additionally, the overall attenuation of $SL(x_p, x_h)$ from the inhibitory synapses to the hotspot is shallower for the distal synapse than for the proximal synapse, because the latter is more affected by the somatic current sink. The product of these two effects—the initially larger $SL(x_i)$ at the distal synapse and the shallower attenuation, $SL(x_p, x_h)$, from the distal synapse to the hotspot—implies that SL at the hotspot is larger for this synapse. The advantage of distal versus proximal inhibition is amplified for voltage-dependent (nonlinear) hotspots because inhibition at the hotspot increases the threshold for the activation of regenerative inward currents (Jadi et al., 2012). In fact, the powerful impact of off-path inhibition on somatic firing (Fig. 15.3) is a secondary outcome of the reduction of the inward current at the excitable hotspot itself. Thus our results do not contradict the “on-path theorem” (Koch and Segev, 1998) which holds for passive dendrites and synapses.

Inhibitory shunt spreads centripetally in dendrites encircled by multiple inhibitory synapses

Next, we examine the implications of multiple inhibitory synapses for SL in dendrites, using the model of a reconstructed CA1 neuron (Golding et al., 2005) depicted in Fig. 15.4. This modeled neuron receives inhibition at three distinct dendritic subdomains: the basal, the apical, and the oblique dendrites. In CA1, these morphological domains are indeed innervated by inhibitory synapses arising from different classes of inhibitory interneurons (e.g., the axon of bistratified cells target the basal and the oblique dendrites, while the apical dendrite is targeted by the oriens lacunosum-moleculare cells; Klausberger and Somogyi, 2008). We assume that each domain receives a cluster of five inhibitory contacts (white dots).

The color-coded value of SL induced by the activation of these 15 inhibitory synapses is shown in Fig. 15.4A and B, superimposed on the modeled cell. SL spreads poorly (it attenuates steeply) in the direction of the dendritic terminals (Fig. 15.4A, blue dendrites) but, surprisingly, it spreads effectively (Fig. 15.4A, red region) hundreds of micrometers centripetally to the region delineated by the contact sites themselves. Even more surprising was that SL became larger in regions lacking inhibitory synapses compared with SL at the synaptic sites themselves (Fig. 15.4B). This is in contrast to the prevailing view that the maximal effect of inhibition is always at the synaptic site itself (Jack et al., 1983).

The effective spread of SL into the dendritic region surrounded by multiple inhibitory synapses (Fig. 15.4) leads to a spatially extended shunted dendritic domain beyond the anatomical domain demarcated by these synapses. Therefore, the spatial extent of SL could serve as a way to assess the spatial specificity of the *impact* of different classes of interneurons on their postsynaptic targets (“functional subdomain”) rather than relying on the anatomical connectivity alone. Additionally, the global spread of the inhibitory shunt implies that the functional subdomain would be less specific than the anatomical subdomain. Namely, in order to dampen excitatory/excitatory dendritic currents, it is not necessary to match each excitatory synapse with a corresponding adjacent inhibitory synapse. Rather, by surrounding a dendritic region with a few inhibitory contacts it is possible to effectively dampen the excitatory/excitatory current that would be generated in this region (Figs 15.4 and 15.5) and thereby effectively control the neuron’s output. This may explain why in the neocortex and the hippocampus, only about 20% of the synapses are inhibitory (DeFelipe and Fariñas, 1992; Megías et al., 2001; Merchán-Pérez et al., 2009). In contrast, SL spreads poorly

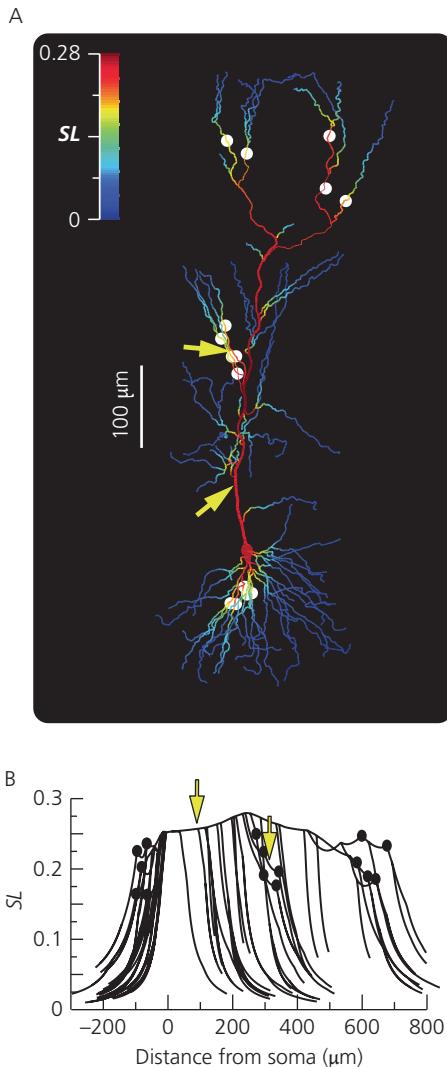


Fig. 15.4 Global spread of inhibitory shunt in trees with multiple inhibitory synapses. (A) SL (color-coded) in a passive model of a reconstructed CA1 pyramidal neuron (Golding et al., 2005; Ascoli et al., 2007) receiving a total of 15 inhibitory synapses (white dots; $g_i = 0.5$ nS each, at steady state) targeting distinct dendritic subdomains (basal, oblique, and apical dendrites). At the oblique branch (top arrow) $SL = 0.2$, whereas at the apical trunk (bottom arrow), far from any one of the inhibitory synapses, $SL = 0.25$. (B) SL as a function of distance from the soma for the model shown in (A). Inhibitory synapses are marked by black dots and yellow arrows correspond to the arrows in (A) (passive model parameters as in Fig. 15.3 but $R_m = 15,000 \Omega \text{ cm}^2$).

Reproduced from Gidon and Segev, 2012.

to thin distal branches (Fig. 15.4), even for dendritic synapses. Therefore, in order to control non-linear processes in distal dendritic branches, inhibitory synapses should directly target the distal end of these branches.

Maximal inhibition may occur in dendritic domains lacking inhibitory synapses

To analytically explain the counterintuitive result that inhibition accumulates centrally at the synapses which encircle the somatic regions (Fig. 15.4) we constructed a symmetrical starburst-like dendritic model (Fig. 15.5) consisting of multiple identical branches stemming from a common junction ($X = 0$). Each of these branches received an identical g_i at a fixed distance ($X = 0.4$) from

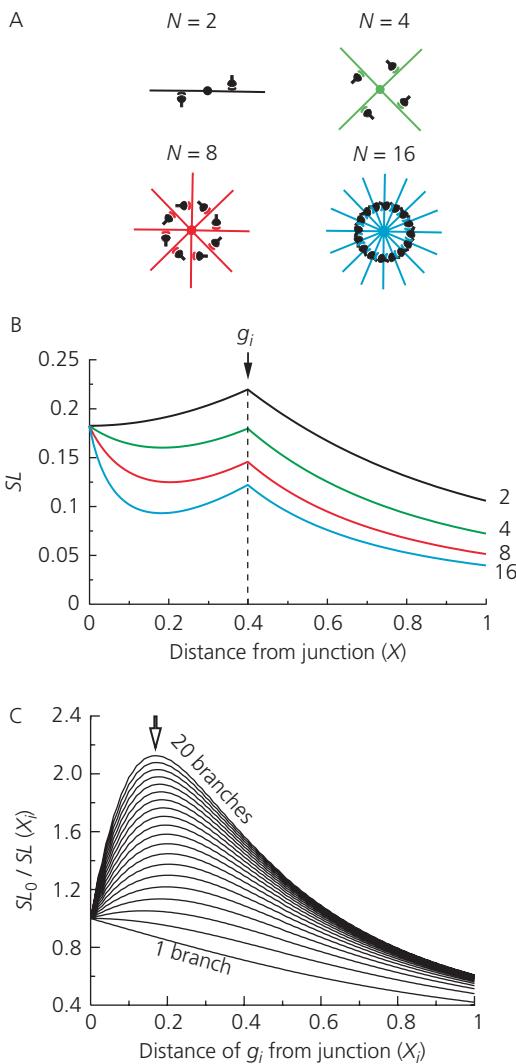


Fig. 15.5 Enhanced centripetal accumulation of inhibitory shunt in trees with multiple inhibitory synapses. **(A)** Symmetrical starburst-like dendritic models consisting of multiple ($N = 2, 4, 8, 16$) identical branches ($L = 1$) stemming from a common junction ($X = 0$). **(B)** SL for the corresponding models depicted in (A). Each branch receives a single g_i (1 nS) at $X = 0.4$ (dashed line). For $N = 8$ (red) and $N = 16$ (blue) SL at the junction (lacking synapses) is larger than SL at the site of the inhibitory synapse. **(C)** Ratio between SL at the junction (SL_0) and at the synaptic sites ($SL(X_i)$) as a function of the distance of the synapses from the junction. Models as in (A) but with varying number of branches (1–20) each with one synapse. $SL_0/SL(X_i)$ increases with the number of branches (with a maximum of about 2.2 for a large number of synapses and branches; arrow).

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the junction (Fig. 15.5A). One way to solve for SL in a symmetrical starburst-like dendritic model consisting of N identical branches with N synapses is to use an equivalent model consisting of two connected branches with two synapses. Following Rall's cable theory (Rall, 1959b) it is straightforward to show that, in such a structure, SL at the junction remains constant, independent of the number of stem branches (Fig. 15.5B; all curves converge at $X = 0$). However, increasing the number of branches (each with an additional inhibitory synapse) has two consequences. First, the local input resistance at each synapse is reduced, and therefore $SL(X_i)$ at these sites is also reduced (Fig. 15.5B, arrow). Second, the input resistance at the junction is reduced following the increase in the number of branches. Consequently, the attenuation of SL from the junction to all the synaptic sites increases. Namely, the synapses have a progressively smaller shunting impact on each other as the number of branches increases. Together, these results imply that when the number of branches

is large enough, SL at the junction (lacking synapses) may become larger than SL at each of the synaptic sites. Interestingly, such elevation in centripetal inhibition requires at least three inhibitory synapses encircling a dendritic region consisting of at least three branches (for further analysis see Gidon and Segev, 2012). Figure 15.5C summarizes SL as a function of the distance of g_i from the soma for different numbers of branches in the starburst-like model. The enhanced centripetal impact of SL becomes more prominent as the number of branches increases.

In summary, SL is a simple, intuitive, and analytically tractable measure for assessing the impact of inhibitory conductance perturbation on dendritic trees. Using SL in arbitrary passive dendritic trees receiving multiple inhibitory contacts has provided several surprising results: (1) distal dendritic sites are effective in dampening more proximal nonlinear events (“off-path” inhibition); (2) with multiple synapses, SL spreads very effectively toward dendritic regions encircled by these synapses; and (3) SL may become larger in these centripetal regions than at the synaptic loci themselves.

Going beyond the present work, we highlight several open questions, which we believe are central to the understanding of the inhibitory system. (1) Why do GABAergic interneurons innervate the entire dendritic surface of their target neurons (i.e., what is the functional significance of such a widespread dendritic coverage by inhibition)? (2) Why do the axons of each class of inhibitory interneuron target a specific subdomain of the dendritic tree of their target neurons? (3) Why are the numbers of both inhibitory interneurons and inhibitory synapses so much smaller (10–20%) than that of the excitatory neurons synapses? (4) Why is the population of GABAergic interneurons, despite their smaller number, more diverse in their morphology and their firing repertoire than the rather homogeneous excitatory population? (5) Why do inhibitory axons contact their dendritic targets with multiple (10–20) synapses per connection? (6) Why, unlike the excitatory synapses, do inhibitory synapses predominantly contact dendritic shafts? (7) What is the role of the inhibitory synapses in regulating the plasticity of the excitatory synapses? This is particularly important because inhibition can regulate the amplitude of the postsynaptic potential and calcium influx into the dendrite in a locally dependent manner (Bar Ilan et al., 2013; Paille et al., 2013; Lovett-Barron et al., 2014).

Although the inhibitory and the excitatory systems counter balance each other, they are not (anti)symmetric in their anatomical and physiological properties as one might naïvely expect, but markedly differ in structural and functional organization. Inspired by the design of the inhibitory microcircuit, our work advocates a “dendro-centric” viewpoint for understanding how the neuron’s output is first and foremost shaped in the dendrite.

Concluding remarks

In recent years it has become increasingly clear that the complexity of dendrites, apparent in their morphology and physiology as well as in the properties of their synaptic input, allows neurons to behave as highly nonlinear input–output devices. A modeling framework is required in order to understand how these devices transform the large number of synaptic inputs they receive into a meaningful output in the axon (which may then also travel back into the dendrites). As shown in this chapter, Rall’s cable theory provides a very versatile framework, allowing one to incorporate the very rapid increase in morphological and physiological information about dendrites (as documented in other chapters in this book).

Important insights into the relation between structure and function of dendrites have been gained using these models. However, many fundamental questions still remain. What is the functional role of the unique structure of dendrites? Why do some dendrites have pyramidal-like

morphologies while others (e.g., cerebellar Purkinje cells) have very different characteristics? Why do some dendrites have excitable Na^+ channels in their dendrites while other cell types do not? But most importantly, what is the functional role of dendrites in the normal operation of the nervous system (e.g., as we write this chapter, or as you read it)?

The more we learn about dendrites the more we realize that they are potentially very powerful both as a functional and as a plastic device. But whether individual neurons actually enrich the functional capabilities of the community of neurons can only be answered in the context of the whole network. In other words, the functional meaning of the individual neuron can only be understood within the broader perspective of the specific “language” that the neuronal network uses. We do not yet understand how biological neuronal networks work. It thus remains one of the biggest challenges of the twenty-first century to connect the single-neuron level to the level of global operation of the network. It is hoped that this chapter will provide a small step in this direction.

Acknowledgments

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References

- Agmon-Snir H, Carr CE, Rinzel J (1998) The role of dendrites in auditory coincidence detection. *Nature* **393**:268–272.
- Agmon-Snir H, Segev I (1993a) Signal delay in passive dendritic trees. In: *Computation and Neuronal Systems* (Eeckman FH, Bower JM, eds), pp 73–78. Dordrecht: Kluwer Academic Publishers.
- Agmon-Snir H, Segev I (1993b) Signal delay and input synchronization in passive dendritic trees. *Journal of Neurophysiology* **70**:2066–2085.
- Almog M, Korngreen A (2014) A quantitative description of dendritic conductances and its application to dendritic excitation in layer 5 pyramidal neurons. *Journal of Neuroscience* **34**:182–196.
- Amitai Y, Friedman A, Connors BW, Gutnick MJ (1993) Regenerative electrical activity in apical dendrites of pyramidal cells in neocortex. *Cerebral Cortex* **3**:26–38.
- Anderson JC, Binzegger T, Kahana O, Martin KA, Segev I (1999) Dendritic asymmetry cannot account for directional responses of neurons in visual cortex. *Nature Neuroscience* **2**:820–824.
- Antic SD, Zhou WL, Moore AR, Short SM, Ikonomou KD (2010) The decade of the dendritic NMDA spike. *Journal of Neuroscience Research* **88**:2991–3001.
- Araya R, Jiang J, Eisenthal KB, Yuste R (2006) The spine neck filters membrane potentials. *Proceedings of the National Academy of Sciences of the United States of America* **103**:17961–17966.
- Archie KA, Mel BW (2000) A model for intradendritic computation of binocular disparity. *Nature Neuroscience* **3**:54–63.
- Ariav G, Polksy A, Schiller J (2003) Submillisecond precision of the input-output transformation function mediated by fast sodium dendritic spikes in basal dendrites of CA1 pyramidal neurons. *Journal of Neuroscience* **23**:7750–7758.
- Ascoli GA, Donohue DE, Halavi M (2007) NeuroMorpho.Org: A central resource for neuronal morphologies. *Journal of Neuroscience* **27**:9247–9251.
- Baer SM, Rinzel J (1991) Propagation of dendritic spikes mediated by excitable spines: a continuum theory. *Journal of Neurophysiology* **65**:874–890.
- Bar Ilan L, Gidon A, Segev I (2011) Interregional synaptic competition in neurons with multiple STDP-inducing signals. *Journal of Neurophysiology* **105**:989–998.

- Bar Ilan L, Gidon A, Segev I (2013) The role of dendritic inhibition in shaping the plasticity of excitatory synapses. *Frontiers in Neural Circuits* **6** (2013): 118.
- Berger T, Larkum ME, Luscher HR (2001) High I(h) channel density in the distal apical dendrite of layer V pyramidal cells increases bidirectional attenuation of EPSPs. *Journal of Neurophysiology* **85**:855–868.
- Bloodgood BL, Sabatini BL (2005) Neuronal activity regulates diffusion across the neck of dendritic spines. *Science* **310**:866–869.
- Borg-Graham LJ (2001) The computation of directional selectivity in the retina occurs presynaptic to the ganglion cell. *Nature Neuroscience* **4**:176–183.
- Bower J, Beeman D (1998) *The Book of GENESIS: Exploring Realistic Neural Models with the General NEural SImulation System*. Berlin: TELOS/Springer.
- Branco T, Clark BA, Häusser M (2010) Dendritic discrimination of temporal input sequences in cortical neurons. *Science* **329**:1671–1675.
- Briggman KL, Helmstaedter M, Denk W (2011) Wiring specificity in the direction-selectivity circuit of the retina. *Nature* **471**:183–188.
- Buhl EH, Halasy K, Somogyi P (1994) Diverse sources of hippocampal unitary inhibitory postsynaptic potentials and the number of synaptic release sites. *Nature* **368**:823–828.
- Bywalek WG, Patirniche D, Rupprecht V, Stemmler M, Herz AV, Palfi D, Rozsa B, Egger V (2015) Local postsynaptic voltage-gated sodium channel activation in dendritic spines of olfactory bulb granule cells. *Neuron* **85**:590–601.
- Caldwell JH, Schaller KL, Lasher RS, Peles E, Levinson SR (2000) Sodium channel Na(v)1.6 is localized at nodes of Ranvier, dendrites, and synapses. *Proceedings of the National Academy of Sciences of the United States of America* **97**:5616–5620.
- Carlisle HJ, Kennedy MB (2005) Spine architecture and synaptic plasticity. *Trends in Neurosciences* **28**:182–187.
- Chen WR, Midtgård J, Shepherd GM (1997) Forward and backward propagation of dendritic impulses and their synaptic control in mitral cells. *Science* **278**:463–467.
- Deb K, Pratap A, Agarwal S, Meyarivan T (2002) A fast and elitist multiobjective genetic algorithm: NSGA-II. *IEEE Transactions on Evolutionary Computing* **6**:182–197.
- DeFelipe J, Fariñas I (1992) The pyramidal neuron of the cerebral cortex: morphological and chemical characteristics of the synaptic inputs. *Progress in Neurobiology* **39**:563–607.
- DeFelipe J, Alonso-Nanclares L, Arellano JI (2002) Microstructure of the neocortex: comparative aspects. *Journal of Neurocytology* **31**:299–316.
- Demb JB (2007) Cellular mechanisms for direction selectivity in the retina. *Neuron* **55**:179–186.
- Druckmann S, Banitt Y, Gidon A, Schürmann F, Markram H, Segev I (2007) A novel multiple objective optimization framework for constraining conductance-based neuron models by experimental data. *Frontiers in Neuroscience* **1**:7–18.
- Euler T, Detwiler PB, Denk W (2002) Directionally selective calcium signals in dendrites of starburst amacrine cells. *Nature* **418**:845–852.
- Fantana AL, Soucy ER, Meister M (2008) Rat olfactory bulb mitral cells receive sparse glomerular inputs. *Neuron* **59**:802–814.
- Forsythe ID, Redman SJ (1988) The dependence of motoneurone membrane potential on extracellular ion concentrations studied in isolated rat spinal cord. *Journal of Physiology* **404**:83–99.
- Fotowat H, Gabbiani F (2011) Collision detection as a model for sensory-motor integration. *Annual Review of Neuroscience* **34**:1–19.
- French CR, Sah P, Buckett KJ, Gage PW (1990) A voltage-dependent persistent sodium current in mammalian hippocampal neurons. *Journal of General Physiology* **95**:1139–1157.
- Gabbiani F, Krapp HG, Koch C, Laurent G (2002) Multiplicative computation in a visual neuron sensitive to looming. *Nature* **420**:320–324.

- Gidon A, Segev I (2012) Principles governing the operation of synaptic inhibition in dendrites. *Neuron* 75:330–341.
- Goaillard J-M, Taylor AL, Schulz DJ, Marder E (2009) Functional consequences of animal-to-animal variation in circuit parameters. *Nature Neuroscience* 12:1424–1430.
- Golding NL, Spruston N (1998) Dendritic sodium spikes are variable triggers of axonal action potentials in hippocampal CA1 pyramidal neurons. *Neuron* 21:1189–1200.
- Golding NL, Mickus TJ, Katz Y, Kath WL, Spruston N (2005) Factors mediating powerful voltage attenuation along CA1 pyramidal neuron dendrites. *Journal of Physiology* 568:69–82.
- Gollo LL, Kinouchi O, Copelli M (2009) Active dendrites enhance neuronal dynamic range. *PLoS Computational Biology* 5:e1000402.
- Häusser M, Mel B (2003) Dendrites: bug or feature? *Current Opinion in Neurobiology* 13:372–383.
- Häusser M, Roth A (1997) Estimating the time course of the excitatory synaptic conductance in neocortical pyramidal cells using a novel voltage jump method. *Journal of Neuroscience* 7:1–21.
- Hay E, Hill S, Schürmann F, Markram H, Segev I (2011) Models of neocortical layer 5b pyramidal cells capturing a wide range of dendritic and perisomatic active properties. *PLoS Computational Biology* 7:e1002107.
- Hay E, Segev I (2014) Dendritic excitability and gain control in recurrent cortical microcircuits. *Cerebral Cortex* doi: 10.1093/cercor/bhu200.
- Herz AV, Gollisch T, Machens CK, Jaeger D (2006) Modeling single-neuron dynamics and computations: a balance of detail and abstraction. *Science* 314:80–85.
- Hines ML, Carnevale NT (1997) The NEURON simulation environment. *Neural Computation* 9:1179–1209.
- Hines ML, Morse T, Migliore M, Carnevale NT, Shepherd GM (2004) ModelDB: a database to support computational neuroscience. *Journal of Computational Neuroscience* 17:7–11.
- Jack JJB, Noble D, Tsien RW (1983) *Electrical Current Flow in Excitable Cells*. Oxford: Oxford University Press.
- Jadi M, Polksy A, Schiller J, Mel BW (2012) Location-dependent effects of inhibition on local spiking in pyramidal neuron dendrites. *PLoS Computational Biology* 8:e1002550.
- Jia H, Rochefort NL, Chen X, Konnerth A (2010) Dendritic organization of sensory input to cortical neurons in vivo. *Nature* 464:1307–1312.
- Keren N, Peled N, Korngreen A (2005) Constraining compartmental models using multiple voltage recordings and genetic algorithms. *Journal of Neurophysiology* 94:3730–3742.
- Klausberger T, Somogyi P (2008) Neuronal diversity and temporal dynamics: the unity of hippocampal circuit operations. *Science* 321:53–57.
- Koch C (1999) *Biophysics of Computation: Information Processing in Single Neurons*. New York: Oxford University Press.
- Koch C, Poggio T (1983) A theoretical analysis of electrical properties of spines. *Proceedings of the Royal Society of London Series B: Biological Sciences* 218:455–477.
- Koch C, Poggio T (1985) A simple algorithm for solving the cable equation in dendritic trees of arbitrary geometry. *Journal of Neuroscience Methods* 12:303–315.
- Koch C, Segev I (1998) *Methods in Neuronal Modeling: From Ions to Networks*, 2nd edn. Cambridge, MA: MIT Press.
- Koch C, Segev I (2000) The role of single neurons in information processing. *Nature Neuroscience* 3(Suppl.):1171–1177.
- Koch C, Zador A (1993) The function of dendritic spines: devices subserving biochemical rather than electrical compartmentalization. *Journal of Neuroscience* 13:413–422.
- Koch C, Poggio T, Torre V (1982) Retinal ganglion cells: a functional interpretation of dendritic morphology. *Philosophical Transactions of the Royal Society of London Series B: Biological Sciences* 298:227–263.

- Koch C, Poggio T, Torre V (1983) Nonlinear interactions in a dendritic tree: Localization, timing, and role in information processing. *Proceedings of the National Academy of Sciences of the United States of America* **80**:2799–2802.
- Koch C, Douglas R, Wehmeier U (1990) Visibility of synaptically induced conductance changes: theory and simulations of anatomically characterized cortical pyramidal cells. *Journal of Neuroscience* **10**:1728–1744.
- Koch C, Rapp M, Segev I (1996) A brief history of time (constants). *Cerebral Cortex* **6**:93–101.
- Kole MHP, Hallermann S, Stuart GJ (2006) Single Ih channels in pyramidal neuron dendrites: properties, distribution, and impact on action potential output. *Journal of Neuroscience* **26**:1677–1687.
- Larkum M (2013) A cellular mechanism for cortical associations: an organizing principle for the cerebral cortex. *Trends in Neurosciences* **36**:141–151.
- Larkum ME, Zhu JJ (2002) Signaling of layer 1 and whisker-evoked Ca^{2+} and Na^+ action potentials in distal and terminal dendrites of rat neocortical pyramidal neurons *in vitro* and *in vivo*. *Journal of Neuroscience* **22**:6991–7005.
- Larkum ME, Launey T, Dityatev A, Luscher HR (1998) Integration of excitatory postsynaptic potentials in dendrites of motoneurons of rat spinal cord slice cultures. *Journal of Neurophysiology* **80**:924–935.
- Larkum ME, Kaiser KM, Sakmann B (1999a) Calcium electrogenesis in distal apical dendrites of layer 5 pyramidal cells at a critical frequency of back-propagating action potentials. *Proceedings of the National Academy of Sciences of the United States of America* **96**:14600–14604.
- Larkum ME, Zhu JJ, Sakmann B (1999b) A new cellular mechanism for coupling inputs arriving at different cortical layers. *Nature* **398**:338–341.
- Larkum ME, Zhu JJ, Sakmann B (2001) Dendritic mechanisms underlying the coupling of the dendritic with the axonal action potential initiation zone of adult rat layer 5 pyramidal neurons. *Journal of Physiology* **533**:447–466.
- Larkum ME, Senn W, Lüscher H-R (2004) Top-down dendritic input increases the gain of layer 5 pyramidal neurons. *Cerebral Cortex* **14**:1059–1070.
- Larkum ME, Nevian T, Sandler M, Polksy A, Schiller J (2009) Synaptic integration in tuft dendrites of layer 5 pyramidal neurons: a new unifying principle. *Science* **325**:756–760.
- Lavzin M, Rapoport S, Polksy A, Garion L, Schiller J (2012) Nonlinear dendritic processing determines angular tuning of barrel cortex neurons *in vivo*. *Nature* **490**:397–401.
- Le Bé J-V, Silberberg G, Wang Y, Markram H (2007) Morphological, electrophysiological, and synaptic properties of corticocortical pyramidal cells in the neonatal rat neocortex. *Cerebral Cortex* **17**:2204–2213.
- Letzkus JJ, Kampa BM, Stuart GJ (2006) Learning rules for spike timing-dependent plasticity depend on dendritic synapse location. *Journal of Neuroscience* **26**:10420–10429.
- Loewenstein Y, Sompolsky H (2003) Temporal integration by calcium dynamics in a model neuron. *Nature Neuroscience* **6**:961–967.
- London M, Häusser M (2005) Dendritic computation. *Annual Review of Neuroscience* **28**:503–532.
- London M, Meunier C, Segev I (1999) Signal transfer in passive dendrites with nonuniform membrane conductance. *Journal of Neuroscience* **19**:8219–8233.
- Losonczy A, Makara JK, Magee JC (2008) Compartmentalized dendritic plasticity and input feature storage in neurons. *Nature* **452**:436–441.
- Lovett-Barron M, Kaifosh P, Kheirbek MA, Danielson N, Zaremba JD, Reardon TR, Turi GF, Hen R, Zemelman BV, Losonczy A (2014) Dendritic inhibition in the hippocampus supports fear learning. *Science* **343**:857–863.
- Magee JC (2007) Voltage-gated ion channels in dendrites. In: *Dendrites* (Stuart G, Spruston N, Häusser M, eds), pp 139–160. Oxford: Oxford University Press.
- Magee JC, Johnston D (1995) Characterization of single voltage-gated Na^+ and Ca^{2+} channels in apical dendrites of rat CA1 pyramidal neurons. *Journal of Physiology* **487**:67–90.

- Major G** (1994) Detailed passive cable models of whole-cell recorded CA3 pyramidal neurons in rat hippocampal slices. *Journal of Neuroscience* **14**:4613–4638.
- Major G, Larkum ME, Schiller J** (2013) Active properties of neocortical pyramidal neuron dendrites. *Annual Review of Neuroscience* **36**:1–24.
- Markram H, Lubke J, Frotscher M, Sakmann B** (1997) Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. *Science* **275**:213–215.
- Markram H, Toledo-Rodriguez M, Wang Y, Gupta A, Silberberg G, Wu C** (2004) Interneurons of the neocortical inhibitory system. *Nature Reviews Neuroscience* **5**:793–807.
- Mathews PJ, Jercog PE, Rinzel J, Scott LL, Golding NL** (2010) Control of submillisecond synaptic timing in binaural coincidence detectors by K(v)1 channels. *Nature Neuroscience* **13**:601–609.
- Megías M, Emri Z, Freund TF, Gulyás AI** (2001) Total number and distribution of inhibitory and excitatory synapses on hippocampal CA1 pyramidal cells. *Neuroscience* **102**:527–540.
- Mel BW** (1992) NMDA-based pattern discrimination in a modeled cortical neuron. *Neural Computation* **4**:502–516.
- Mel BW** (1993) Synaptic integration in an excitable dendritic tree. *Journal of Neurophysiology* **70**:1086–1101.
- Merchán-Pérez A, Rodriguez J-R, Alonso-Nanclares L, Schertel A, Defelipe J** (2009) Counting synapses using FIB/SEM microscopy: a true revolution for ultrastructural volume reconstruction. *Frontiers in Neuroanatomy* **3**:18.
- Miles R, Toth K, Gulyas AI, Hajos N, Freund TF** (1996) Differences between somatic and dendritic inhibition in the hippocampus. *Neuron* **16**:815–823.
- Murayama M, Larkum ME** (2009) Enhanced dendritic activity in awake rats. *Proceedings of the National Academy of Sciences of the United States of America* **106**:20482–20486.
- Nicholson C, Llinas R** (1971) Field potentials in the alligator cerebellum and theory of their relationship to Purkinje cell dendritic spikes. *Journal of Neurophysiology* **34**:509–531.
- Nicoll A, Larkman A, Blakemore C** (1993) Modulation of EPSP shape and efficacy by intrinsic membrane conductances in rat neocortical pyramidal neurons in vitro. *Journal of Physiology* **468**:693–710.
- Paille V, Fino E, Du K, Morera-Herreras T, Perez S, Kotasinski JH, and Venance L** (2013) GABAergic circuits control spike-timing-dependent plasticity. *The Journal of Neuroscience* **33**: 9353–9363.
- Palmer LM, Schulz JM, Murphy SC, Ledergerber D, Murayama M, Larkum ME** (2012) The cellular basis of GABA(B)-mediated interhemispheric inhibition. *Science* **335**:989–993.
- Pouille F, Scanziani M** (2001) Enforcement of temporal fidelity in pyramidal cells by somatic feed-forward inhibition. *Science* **293**:1159–1163.
- Rall W** (1959a) Branching dendritic trees and motoneuron membrane resistivity. *Experimental Neurology* **1**:491–527.
- Rall W** (1959b) *Dendritic Current Distribution and Whole Neuron Properties*. Naval Medical Research Institute Research Report NM 0105.01.02,479–525. Bethesda, MD; Naval Medical Research Institute.
- Rall W** (1964) Theoretical significance of dendritic trees for neuronal input–output relations. In: *Neural Theory and Modeling* (Reiss R, ed.), pp. 73–97. Stanford, CA: Stanford University Press.
- Rall W** (1967) Distinguishing theoretical synaptic potentials computed for different soma-dendritic distributions of synaptic input. *Journal of Neurophysiology* **30**:1138–1168.
- Rall W** (1969) Time constants and electrotonic length of membrane cylinders and neurons. *Biophysical Journal* **9**:1483–1508.
- Rall W** (1974) Dendritic spines, synaptic potency and neuronal plasticity. In: *Cellular Mechanisms Subserving Changes in Neuronal Activity* (Woody CD, Brown KA, Crow TJ, Knispel JD, eds), pp. 13–21. Los Angeles, CA: Brain Information Service/Brain Research Institute UCLA.
- Rall W** (1977) Core conductor theory and cable properties of neurons. In *Handbook of Physiology. The Nervous System. Vol. 1, Cellular Biology of Neurons* (Kandel ER, Brookhart JM, Mountcastle VB, eds), pp. 39–97. Bethesda, MD: American Physiological Society.

- Rall W (1978) Dendritic spines and synaptic potency. In: *Studies in Neurophysiology* (Porter R, ed.), pp. 203–209. Cambridge: Cambridge University Press.
- Rall W, Agmon-Snir H (1998) Cable theory for dendritic neurons. In: *Methods in Neuronal Modeling: From Ions to Networks*, 2nd edn (Koch C, Segev I, eds), pp 27–92. Cambridge, MA: MIT Press.
- Rall W, Rinzel J (1973) Branch input resistance and steady attenuation for input to one branch of a dendritic neuron model. *Biophysical Journal* **13**:648–688.
- Rall W, Segev I (1988) Dendritic spine synapses, excitable spine clusters, and plasticity. In: *Cellular Mechanisms of Conditioning and Behavioral Plasticity* (Woody CD, Alkon DL, McGaugh JL, eds), pp. 221–236. New York: Plenum.
- Rall W, Shepherd GM, Reese TS, Brightman MW (1966) Dendrodendritic synaptic pathway for inhibition in the olfactory bulb. *Experimental Neurology* **14**:44–56.
- Rall W, Burke RE, Smith TG, Nelson PG, Frank K (1967) Dendritic location of synapses and possible mechanisms for the monosynaptic EPSP in motoneurons. *Journal of Neurophysiology* **30**:1169–1193.
- Rall W, Burke RE, Holmes WR, Jack JJ, Redman SJ, Segev I (1992) Matching dendritic neuron models to experimental data. *Physiological Review* **72**:S159–S186.
- Rapp M, Segev I, Yarom Y (1994) Physiology, morphology and detailed passive models of cerebellar Purkinje cells. *Journal of Physiology* **474**:101–118.
- Rapp M, Yarom Y, Segev I (1996) Modeling back propagating action potential in weakly excitable dendrites of neocortical pyramidal cells. *Proc. Natl Acad Sci U S A*,**93** (21):11985–90.
- Redman S, Walmsley B (1983a) Amplitude fluctuations in synaptic potentials evoked in cat spinal motoneurons at identified group Ia synapses. *Journal of Physiology* **343**:135–145.
- Redman S, Walmsley B (1983b) The time course of synaptic potentials evoked in cat spinal motoneurones at identified group Ia synapses. *Journal of Physiology* **343**:117–133.
- Rinzel J (1981) Models in neurobiology. *Lectures in Applied Mathematics* **19**:281–297.
- Rinzel J, Rall W (1974) Transient response in a dendritic neuron model for current injected at one branch. *Biophysical Journal* **14**:759–790.
- Sarid L, Bruno R, Sakmann B, Segev I, Feldmeyer D (2007) Modeling a layer 4-to-layer 2/3 module of a single column in rat neocortex: interweaving in vitro and in vivo experimental observations. *Proceedings of the National Academy of Sciences of the United States of America* **104**: 16353–16358.
- Schiller J, Schiller Y, Stuart G, Sakmann B (1997) Calcium action potentials restricted to distal apical dendrites of rat neocortical pyramidal neurons. *Journal of Physiology* **505**:605–616.
- Schiller J, Major G, Koester HJ, Schiller Y (2000) NMDA spikes in basal dendrites of cortical pyramidal neurons. *Nature* **404**:285–289.
- Segev I (1992) Single neurone models: oversimple, complex and reduced. *Trends in Neurosciences* **15**:414–421.
- Segev I, Burke RE (1998) Compartmental models of complex neurons. In: *Methods in Neuronal Modeling: From Ions to Networks*, 2nd edn (Koch C, Segev I, eds), pp. 93–136. Cambridge, MA: MIT Press, Massachusetts.
- Segev I, Rall W (1998) Excitable dendrites and spines: earlier theoretical insights elucidate recent direct observations. *Trends in Neurosciences* **21**:453–460.
- Segev I, Rapp M, Manor Y, Yarom Y (1992) Analog and digital processing in single nerve cells: dendritic integration and axonal propagation. In: *Single Neuron Computation* (McKenna T, Davis J, Zornetzer SF, eds), pp. 173–198. New York: Academic Press.
- Segev I, Rinzel J, Shepherd G (eds) (1995) *The Theoretical Foundation of Dendritic Function*. Cambridge, MA: MIT Press.
- Shepherd GM (1991) Foundations of the neuron doctrine. New York: Oxford University Press.
- Shepherd GM (1996) The dendritic spine: a multifunctional integrative unit. *Journal of Neurophysiology* **75**:2197–2210.

- Single S, Borst A (1998) Dendritic integration and its role in computing image velocity. *Science* **281**:1848–1850.
- Sjöström PJ, Häusser M (2006) A cooperative switch determines the sign of synaptic plasticity in distal dendrites of neocortical pyramidal neurons. *Neuron* **51**:227–238.
- Softky W (1994) Sub-millisecond coincidence detection in active dendritic trees. *Neuroscience* **58**:13–41.
- Sterling P (2002) Neuroscience: how neurons compute direction. *Nature* **420**:375–376.
- Stratford RD, Mason AJR, Larkman AU, Major G, Jack JJB (1989) The modeling of pyramidal neurons in the visual cortex. In: *The Computing Neuron* (Durbin R, Miall C, Mitchson C, eds), pp. 296–321. Reading, MA: Addison-Wesley.
- Stuart GJ, Häusser M (2001) Dendritic coincidence detection of EPSPs and action potentials. *Nature Neuroscience* **4**:63–71.
- Stuart GJ, Sakmann B (1994) Active propagation of somatic action potentials into neocortical pyramidal cell dendrites. *Nature* **367**:69–72.
- Stuart G, Spruston N (1998) Determinants of voltage attenuation in neocortical pyramidal neuron dendrites. *Journal of Neuroscience* **18**:3501–3510.
- Stuart G, Schiller J, Sakmann B (1997a) Action potential initiation and propagation in rat neocortical pyramidal neurons. *Journal of Physiology* **505**:617–632.
- Stuart G, Spruston N, Sakmann B, Häusser M (1997b) Action potential initiation and backpropagation in neurons of the mammalian CNS. *Trends in Neurosciences* **20**:125–131.
- Svoboda K, Tank DW, Denk W (1996) Direct measurement of coupling between dendritic spines and shafts. *Science* **272**:716–719.
- Takahashi H, Magee JC (2009) Pathway interactions and synaptic plasticity in the dendritic tuft regions of CA1 pyramidal neurons. *Neuron* **62**:102–111.
- Taylor WR, Vaney DI (2003) New directions in retinal research. *Trends in Neurosciences* **26**:379–385.
- Tepper JM, Koos T, Wilson CJ (2004) GABAergic microcircuits in the neostriatum. *Trends in Neurosciences* **27**:662–669.
- Traub RD, Wong RK, Miles R, Michelson H (1991) A model of a CA3 hippocampal pyramidal neuron incorporating voltage-clamp data on intrinsic conductances. *Journal of Neurophysiology* **66**: 635–650.
- Vetter P, Roth A, Häusser M (2001) Propagation of action potentials in dendrites depends on dendritic morphology. *Journal of Neurophysiology* **85**:926–937.
- Williams SR (2004) Spatial compartmentalization and functional impact of conductance in pyramidal neurons. *Nature Neuroscience* **7**:961–967.
- Xu N-I, Harnett MT, Williams SR, Huber D, O'Connor DH, Svoboda K, Magee JC (2012) Nonlinear dendritic integration of sensory and motor input during an active sensing task. *Nature* **492**:247–251.
- Yuste R, Denk W (1995) Dendritic spines as basic functional units of neuronal integration. *Nature* **375**:682–684.
- Yuste R, Majewska A (2001) On the function of dendritic spines. *Neuroscientist* **7**:387–395.
- Zador A, Agmon-Snir H, Segev I (1995) The morphoelectrot tonic transform: a graphical approach to dendritic function. *Journal of Neuroscience* **15**:1669–1682.

Chapter 16

Toward a simplified model of an active dendritic tree

Bartlett W. Mel

Summary

The ability to compartmentalize voltage signals and to generate local spikes suggests that the spatially extended dendritic trees of neurons in the central nervous system may allow a single cell to carry out computations that go well beyond the ability to “integrate and fire.” This chapter reviews the history of ideas regarding the computing capabilities of dendritic trees, focusing on “cluster sensitive” spatial interactions between excitatory and inhibitory synapses within the dendritic trees of neocortical and hippocampal pyramidal neurons. The chapter culminates with a recently proposed layered model of a pyramidal neuron, whose thin dendrites use their analog spatial processing capabilities to compute low-dimensional sigmoidal functions of their inputs, before passing their results onto a traditional integrate and fire operation at the soma.

Introduction

In this chapter we consider the question of how best to describe the computing functions of dendrites in neurons of the central nervous system (CNS). Before tackling this fascinating question, though, it is useful to address two background issues. First, could dendrites exist for reasons other than to enhance a cell’s computing capabilities? If we focus on their spatial extent, we might infer that dendrites exist to increase the receptive surface area of a neuron, which they do by at least 20-fold (e.g., Fox and Barnard, 1957; Ulvhake and Kellerth, 1981; Routh et al., 2009). This makes room for a much larger number of synaptic connections between neurons, which seems advantageous. If we focus on their highly branched morphology, we might infer that dendrites are optimally shaped (per unit length) to extract information-bearing signals from the surrounding neuropil, analogous to root systems that are optimized for the extraction of water and nutrients from soil (Bejan, 2000; Chklovskii et al., 2002; Cuntz et al., 2007). If we focus on their anatomically distinct subregions (e.g., apical versus basal subtrees), we might infer that dendrites exist so that different input pathways can be physically segregated on the surface of the cell (Shepherd, 1998; Petreanu et al., 2009; Piskorowski and Chevaleyre 2012; Harris and Shepherd, 2015). This could allow different classes of inputs to be targeted by different neurotransmitters or modulators (Patil et al., 1998), to be influenced by different classes of inhibitory interneurons (McBain and Fisahn, 2001; Jadi et al., 2012; Fino et al., 2013), or to be subjected to different synaptic learning rules (Golding et al., 2002; Froemke et al., 2005; Gordon et al., 2006). Thus, apart from their computing functions per se, dendritic trees, by virtue of their three-dimensional physical structures, are likely to play a variety of “logistical” roles in neural circuits, helping to collect, route, and manage the information-bearing signals that a neuron is supposed to process.

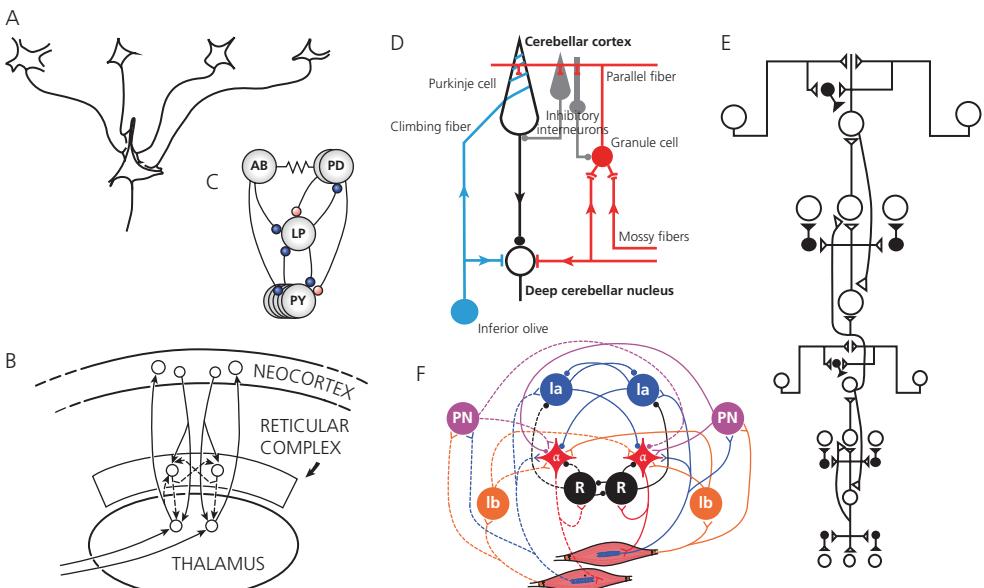


Fig. 16.1 Examples of influential neural circuit models constructed from “point neurons” which contain a single integrative node. **(A)** Model of a “simple” cell in visual cortex (Hubel and Wiesel, 1962). **(B)** “Searchlight” model of attention (Crick, 1984). **(C)** Subset of the stomatogastric ganglion circuit (Marder and Eisen, 1984). **(D)** Cerebellar circuit (Raymond et al., 1996). **(E)** Circuit in the visual cortex (Grossberg et al., 1997). **(F)** Spinal cord circuit (Raphael et al., 2010).

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The second background issue pertains to the computing operations carried out within a single neuron. Before considering the role that dendrites might play, it is useful to clarify our expectations as to what a neuron lacking dendrites should be able to compute; this can serve as a baseline against which to measure the added value, if any, provided by a spatially extended dendritic tree. Historically, it has generally been assumed that a neuron combines its excitatory and inhibitory inputs from across the cell in a relatively simple way, and the result of this process of “synaptic integration” determines the cell’s overall firing rate and/or pattern. When expressed in mathematical terms, a neuron’s integrative function has most often been modeled as a simple thresholded linear (TL) operation, that is, a weighted sum of its excitatory and inhibitory inputs followed by a thresholding nonlinearity (Rosenblatt, 1962; Hopfield, 1982; Rumelhart et al., 1986):

$$y = g\left(\sum_i w_i x_i\right) \quad (16.1)$$

where x_i is the firing rate of the i th presynaptic axon, w_i is the efficacy of the i th synapse (positive or negative), and $g()$ is a function associated with the cell’s output spiking mechanism—essentially the cell’s f - I curve. In situations where a more biophysically realistic model of a neuron has been required, especially one that includes temporal dynamics, the “integrate and fire” (I&F) neuron

has been the model of choice, consisting of a single-compartment RC circuit with an explicit spike-generating mechanism superimposed (see Abbott, 1999). Given that TL and I&F neurons each contain only a single integrative node, and therefore lack any representation of dendritic space, they are examples of “point neuron” models (Fig. 16.1). Point neurons are typically depicted in diagrams as circles or other simple shapes with multiple synaptic inputs and a single output line representing the axon. Despite various conceptual challenges, to be detailed later, the point neuron hypothesis (PNH) has proven to be remarkably durable by virtue of its simplicity, and has remained the default view of the neuron for most experimental and computationally oriented neuroscientists for more than a 100 years. Given that it provides a reasonable answer to the question, “What should a neuron lacking dendrites be able to compute?,” the PNH can also serve as our reference as we contemplate what the more exotic computing functions of dendritic trees may be.

Challenges to the point neuron hypothesis

Problem 1: dendrites are active

Scratching just below the surface, the PNH with its underlying linear summation operation seems seriously underpowered to describe the behavior of most CNS neurons with large dendritic trees. In particular, a survey of the neurophysiological literature from the 1960s onward reveals that the dendritic trees of many types of CNS neurons are far from linear devices, at least at the component level (see Mel (1994) and Johnston et al. (1996) for reviews of the early “active dendrite” literature). They contain many types of voltage-dependent channels, including Na^+ , Ca^{2+} , and N -methyl-D-aspartic acid (NMDA) currents capable of boosting synaptic inputs, and even of generating full-blown spikes that remain localized within the dendritic tree (see Chapters 12 and 13; Antic et al., 2010; Branco et al., 2010; Major et al., 2013). It is difficult to accept that this panoply of nonlinear membrane mechanisms does not somehow endow a neuron with more powerful computing capabilities (London and Häusser, 2005; Spruston, 2008; Branco and Häusser, 2010; Silver, 2010; Jadi et al., 2014).

There are reasons to be cautious, however, before dismissing a theory as parsimonious as the PNH: the presence of voltage dependent channels in dendrites does not automatically invalidate the point neuron idea. In fact, it is possible that certain biophysical properties of neurons may appear to introduce hard-to-model nonlinearities, but may in fact exist to simplify or regularize the overall input–output behavior of the cell. The issue of “synaptic democracy” is illustrative: according to passive cable theory, a dendritic tree could create such large location-dependent disparities among synapses, in terms of both their ability to influence the cell body (Rall and Rinzel, 1973; Zador et al., 1995) and of their signal delay and temporal integration characteristics (Rall, 1964; Agmon-Snir and Segev, 1993), that the PNH would be stretched to breaking point. In mitigation, voltage-dependent currents in the dendrites could exist in part to boost and/or normalize the effects of distal synapses as seen at the cell body, and/or to help counteract classical synaptic sublinearities—leading in the end to a more linear, more point-neuron-like cell (Spencer and Kandel, 1961; Shepherd et al., 1985; Bernander et al., 1994; Caulier and Connors, 1994; De Schutter and Bower, 1994; Cook and Johnston, 1997; Magee, 1998; Cash and Yuste, 1999; Williams and Stuart, 2000). Similar arguments have been made to explain location-dependent scaling of synaptic efficacies (Magee and Cook, 2000; London and Segev, 2001; London and Häusser, 2005; Shipman et al., 2013). Continuing in the pro-PNH vein, the voltage-dependent Na^+ channels that support backpropagating action potentials (bAPs) could help to collapse a large dendritic tree down to a virtual point neuron by broadcasting a trace of somatic firing activity to distal regions

of the cell that would not be reached by passive voltage spread alone (Stuart and Sakmann, 1994; Schiller et al., 1997; Koester and Sakmann, 1998; Larkum, Zhu et al. 1999). By extending the reach of the somatic action potential (AP) into the dendrites, bAPs could, for example, allow Hebb-type learning rules—which generally require that synapses “know” when the postsynaptic neuron has fired—to function in a large non-isopotential neuron with widely scattered synaptic inputs (Magee and Johnston, 1997; Markram et al., 1997; Bi and Poo, 1998; Dan and Poo, 2004; Behabadi and Mel, 2014).

Problem 2: dendrites compartmentalize voltage signals

A second type of finding, initially from modeling studies and more recently from neurophysiological recordings, presents a different sort of problem for the PNH. Given the technical difficulties involved in recording voltage signals from dendrites, and of targeting multiple, specific synaptic sites for activation, much of our understanding of the integrative functions of spatially extended dendritic trees derives from computer modeling studies. Modeling studies beginning in the 1980s had already mounted a challenge to the PNH, demonstrating that dendrites are capable of supporting spatially articulated (i.e., multi-subunit) information-processing tasks (Koch et al., 1983; Rall and Segev, 1987; Shepherd and Brayton, 1987; Mel, 1992a,b, 1993; Mel et al., 1998; Archie and Mel, 2000). Rall and Rinzel (1973) provided a seminal early demonstration of the highly non-uniform voltage environment that is expected in a spatially extended dendritic tree. The effect derives from the severe attenuation that voltage signals experience moving from regions of the cell with high input resistance to those with low input resistance—such as when signals propagate from thin distal branches toward the main trunks and cell body (Zador et al., 1995; Stuart and Spruston, 1998; Golding et al., 2005) (see Chapters 12 and 15). In the first modeling study that explicitly addressed the compartmentalization of synaptic interactions, Koch et al. (1982) found that owing to its passive cable structure, a single large retinal ganglion cell could contain dozens of separate voltage compartments, or “subunits,” defined by strong within-subunit voltage interactions and weak between-subunit interactions (Fig. 16.2A). Woolf et al. (1991) came to similar conclusions using models of reconstructed olfactory cells. These early studies focused on voltage communication—or the lack thereof—between one stimulus and one recording site in a passive dendritic tree. Subsequent studies have confirmed that a dendritic tree can maintain a significant degree of voltage compartmentalization (Golding et al., 2005; Milojkovic et al., 2005; Losonczy et al., 2008; Larkum et al., 2009; Sivyer and Williams, 2013), even when synapses are activated at multiple sites around the dendritic tree (Koch et al., 1983; Rall and Segev, 1987; Borg-Graham and Grzywacz, 1992; Mel, 1992a,b, 1993; Poirazi and Mel, 2003b; Polksy et al., 2004; Palmer et al., 2014; Behabadi and Mel, 2014).

In summary, it is true that active channels in the dendrites of CNS neurons may help to equalize the effects of individual synaptic inputs independent of location, and may also help signals propagate more effectively from the soma outward; both effects are compatible with the PNH as with other theories. But two clear messages have emerged from compartmental modeling studies from their earliest days, and more recently from neurophysiological studies. First, the dendrites of many types of neuron, by virtue of their passive cable properties, have a natural tendency to subdivide the cell into multiple quasi-independent integrative compartments. Second, neurons with active dendrites can produce local regenerative voltage responses. Taken together, these two observations suggest that CNS neurons are more powerful information-processing devices than point neurons. But if the PNH is inadequate to describe a neuron, what more elaborate model should we adopt to describe the input–output behavior of a single nerve cell?

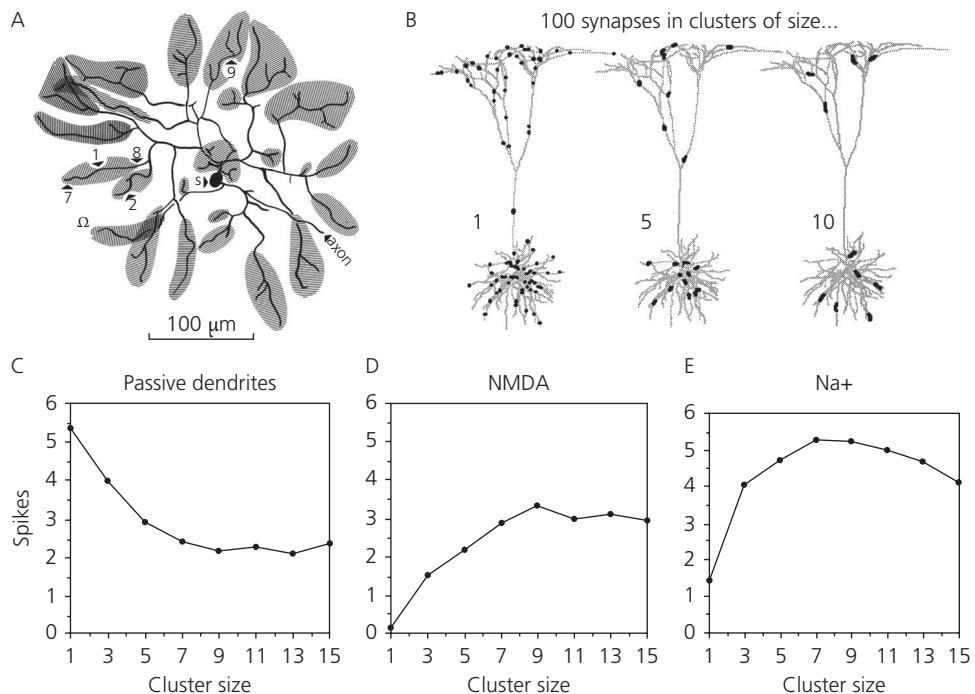


Fig. 16.2 Early compartmental modeling studies indicated that dendrites are well suited to spatially compartmentalize voltage signaling. (A) Koch et al. (1982) showed that a single retinal ganglion cell contains multiple independent voltage “subunits.” (B) Mel (1993) quantified the responses of a L5 pyramidal neuron to 100 excitatory synaptic inputs distributed randomly in clusters of increasing size (clusters of size 1, 5, and 10 are shown). Passive dendrites responded maximally to unclustered inputs (C), whereas dendritic trees containing NMDA channels (D) or voltage-dependent Na⁺ channels (E) responded maximally when synapses were activated in clusters.

In the remainder of this chapter, we will trace a series of simplified models for the computing function(s) of a dendritic tree, with a focus on spatial processing in pyramidal neurons of the hippocampus and neocortex.

In search of simple abstractions for complex neurons

There have been a variety of ideas about dendritic spatial computation (for a detailed review of the early literature see Mel, 1994). In the 1980s, dendritic trees were proposed to implement Boolean logic networks (Koch et al., 1982; Shepherd and Brayton, 1987; Zador et al., 1991), to compute sums of products (Mel, 1987; Durbin and Rumelhart, 1989), to function as multiplexers (Mel, 1986; Anderson and Van Essen, 1987), and to support complex analog/digital interactions between spines in nearby terminal branches (Rall and Segev, 1987).

Common to most of these early models of nonlinear dendritic integration was the idea that in a first “layer” of processing, a set of nonlinear terms is computed locally within a set of computational subunits, where each subunit corresponds to a spatially limited dendritic subregion such as a branch or subtree. Then in a second layer of processing, the subunit responses are combined at the

soma and passed through a function that transforms the total dendritic response into the cell's output firing rate. By judicious assignment of parameters, the subunit nonlinearity could, for example, implement a logical AND operation while the second layer provides an OR operation, leading to a two-layer Boolean logic network. Or, if subunits compute products of their inputs, the cell may exhibit a polynomial (sigma-pi) input-output function, and so on. In all of these early proposals, a key idea was that synaptic inputs are grouped on the basis of proximity in the dendritic tree, and interact with each other through nonlinear membrane mechanisms that implement conventional logical or arithmetic computing operations.

Active dendrites lead to “cluster sensitivity”

An alternative to this explicit logical/mathematical view was to consider synaptic integration from a more statistical perspective. In particular, we carried out compartmental modeling studies to determine what types of spatial activation patterns would produce the strongest responses in neurons containing excitatory nonlinear membrane mechanisms, including NMDA, Na^+ , Ca^{2+} , and anomalous rectifying K^+ channels (Mel 1992a,b, 1993). We found that in dendrites containing various combinations of active channels, a fixed number of excitatory synaptic inputs generated the largest postsynaptic response when the inputs were activated in randomly distributed clusters of intermediate size, as opposed to either (1) dispersing the synaptic excitation diffusely about the dendritic tree, which made it difficult for the synapses to interact through local voltage signals, or (2) concentrating the activated synapses at a small number of dendritic sites, which led to saturation effects (Fig. 16.2B-E).

The finding of “cluster sensitivity” in active dendrites (Gasparini et al., 2004; Polsky et al., 2009; Katona et al., 2011; Makara and Magee, 2013) was in stark contrast to the behavior of a passive dendritic tree, where a diffuse pattern of excitation was known to be optimal (Rall, 1964). To explore its computational capabilities, the behavior of a cluster-sensitive dendritic tree was studied using an abstract model neuron called a “clusteron” (Mel, 1992b). In this abstraction, unlike the discrete subunits defined by Koch et al. (1982), or discrete tuples of inputs feeding into logic functions or product terms in other previous proposals, the clusteron defined a sliding window of nonlinear interaction between synaptic inputs: any two inputs i and j separated by less than a distance $D/2$ gave rise to a multiplicative interaction term $x_i x_j$ (Fig. 16.3). These product terms were then summed across the dendritic tree as a whole, so that the clusteron was in effect a constrained sigma-pi unit with product terms for every pair of nearby synapses (Fig. 16.3).

The clusteron abstraction was useful in two ways. First, it provided a specific, if simplistic, proposal as to how the cell's nonlinear computing operations are physically mapped onto the dendritic tree. The model could therefore be used to predict the responses of a given neuron to arbitrary

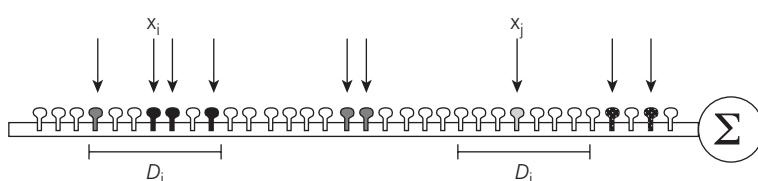


Fig. 16.3 The clusteron was an early model for active dendritic integration (Mel, 1992b). The output of the neuron was a sum of pairwise product terms, one for every synapse and each of its neighbors lying within a radius $D/2$. Arrows indicate which synapses are active.

spatial patterns of excitation. Second, the clusteron facilitated the exploration of structural synaptic plasticity rules capable of storing information through structured addressing of synaptic contacts onto the dendritic tree (Mel 1992a,b; for related work see Poirazi and Mel, 2001; Legenstein and Maass, 2011).

Toward an explicit two-layer model of a neuron

The clusteron had significant limitations, however, in that it only dealt with the integration of excitatory inputs, it provided no guidance regarding the effects of dendritic branching morphology on synaptic integration, and could only be used to predict firing rates of biophysically detailed neuron models in a crude, semi-quantitative way (Mel, 1992b). Reflecting its over-simplified treatment of dendritic morphology, the clusteron included a stereotypical, binary, distance-dependent cluster membership criterion, making no allowances for the effects of varying dendritic diameters or proximity to branch points or the soma, which according to passive cable theory would be expected to warp and/or soften the distance metric used to determine cluster membership. The clusteron also took no account of the fact that when more than one site is activated on the same dendrite, the response caused by a distal cluster must travel through the site where a more proximal cluster of synapses is activated in order to reach the more central parts of the cell. This would seem to lead to voltage interactions between stimulated sites that are not accounted for in the basic clusteron model.

Properly handling these morphological subtleties within a simple, conceptually transparent scheme is challenging, however. As additional details are incorporated, and the “simple” model begins to approach the complexity of the actual neuron, its utility as an abstraction declines. In an attempt to avoid this pitfall, we pursued a different type of single-neuron abstraction that retained the same basic sum-of-nonlinear-subunits form, but whose morphology reflected only the most salient shape features of commonly encountered dendritic trees.

In particular, the “two-layer model” (2LM) (Fig. 16.4) represented a dendritic tree as a set of separately thresholded dendritic subunits in a stellate configuration, where each subunit corresponded to an unbranched thin terminal dendrite emanating directly from the soma or major dendritic trunk (Archie and Mel 2000; Poirazi et al. 2003a,b). The input–output rule of the 2LM was as follows:

$$d_j = g\left(\sum_i w_{ij} x_i\right) \quad (16.2)$$

$$r = fI\left(\sum_i W_j d_j\right) \quad (16.3)$$

where x_i is the activation level of the i th input, w_i is its synaptic weight, g is the subunit nonlinearity (typically a sigmoid function), d_j is the j th subunit output, W_j is a weight that represents the potency of the branch subunit owing to its size or proximity to the soma, and the function fI represents the frequency–current relationship (i.e., the f – I curve) associated with the cell’s output spike-generating mechanism.

Rationale for the simplifying assumptions of the 2LM

The 2LM depends on three assumptions. First the pure stellate morphology assumed by the 2LM ignores higher-order branching patterns, but captures the fact that in certain classes of CNS neurons, including pyramidal neurons, the majority of excitatory synapses lie on relatively long, unbranched terminal sections (Beaulieu and Colonnier, 1985; Elston and Rosa, 1998; Megias et al., 2001).

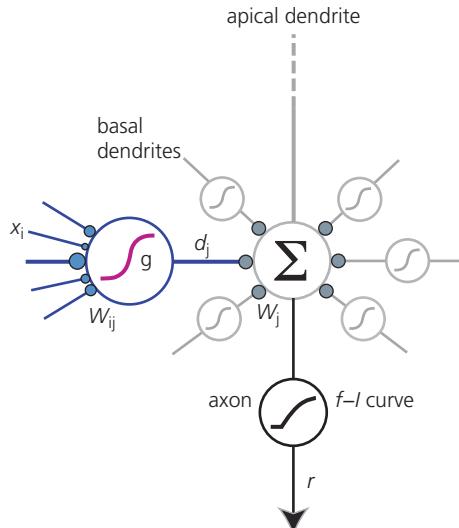


Fig. 16.4 Basic two-layer model of a pyramidal neuron dendritic subtree. Each dendritic “subunit” computes a weighted sum of its inputs (weights are shown as blue circles), and then applies a nonlinear subunit function g to produce the dendritic output d . These outputs are then summed with weights and fed into the global f - I curve to produce the neuron’s response r . A model of this form was used to predict average spike rates produced by a biophysically detailed compartmental model (Poirazi et al., 2003b). The depiction here is intended to suggest a basal subtree; apical oblique, and apical tuft dendrites are thought to behave similarly (Mel et al., 1998; Larkum et al., 2009).

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Second, the assumption that a subunit in the 2LM corresponds to an entire thin dendrite, within which the locations of activated synapses do not matter, is not valid in general (as discussed below in the section “Location-dependent synaptic integration effects”). However, at least two passive cable properties can help to level out synaptic responses within a dendritic branch and are therefore worth mentioning. First, synaptic or intrinsic membrane currents injected at any point along a thin dendrite tend to flow inward to the soma or nearest main trunk with relatively little distance-dependent charge attenuation (Rall and Rinzel, 1973; Cuntz et al., 2007; Nevian et al., 2007; see also Chapters 12 and 15). Second, dendritic spines can reduce the location dependence of synaptic voltage response in two different senses:

- 1 High-resistance spine necks can equalize the input resistance at spine heads along the length of a dendrite (Palmer and Stuart, 2009; Gullidge et al., 2012; Harnett et al., 2012); if synaptic conductances at spine heads are also kept constant, spine-head voltage responses and injected synaptic currents will be equal regardless of dendritic location (Gullidge et al., 2012)
- 2 Dendritic input resistance tends to grow from low near the proximal branch point to high near the distal tip; therefore if synaptic conductances are scaled to be larger near the proximal end of the branch and smaller near the distal end, this will tend to equalize *dendritic* voltage responses regardless of location (Katz et al., 2009).

Voltage responses can also be leveled at spine heads and underlying dendritic shafts simultaneously by appropriately manipulating spine neck resistances (higher at distal locations) and synaptic conductances (lower at distal locations).

The third assumption of the 2LM is that different dendrites function independently of each other, that is, they do not interfere with each other's internal synaptic computations. This type of behavior is supported by two biophysical mechanisms, one passive, one active. First, a thin dendrite emanating from a structure with low input resistance, such as the soma, enjoys a significant degree of voltage "privacy" relative to other parts of the cell. This is due to the steep attenuation of voltage signals traveling inward from a distal site (Rall and Rinzel, 1973; Koch et al. 1982; Zador et al., 1995; Nevian et al., 2007), so that large voltage excursions in one dendrite are only seen in attenuated form in other dendrites (Milojkovic et al., 2005; Larkum et al., 2009; Behabadi et al., 2014). In addition to this well known passive attenuation effect, the axo-somatic firing mechanism provides a second mechanism for voltage isolation between dendritic branches, by effectively clamping the somatic potential near the firing threshold (averaged over time). This time-averaged voltage clamping by the axo-somatic spike-generating mechanism was first reported by Holt and Koch (1997) in the course of explaining how "divisive" inhibition at the soma can actually have a subtractive effect on spike rate. Behabadi and Mel (2014) showed that the combination of this active voltage-isolation mechanism and classical passive voltage-compartmentalization effects allows a cell to carry out multiple independent voltage-dependent computations in different dendrites with negligible crosstalk between them (Fig. 16.5).

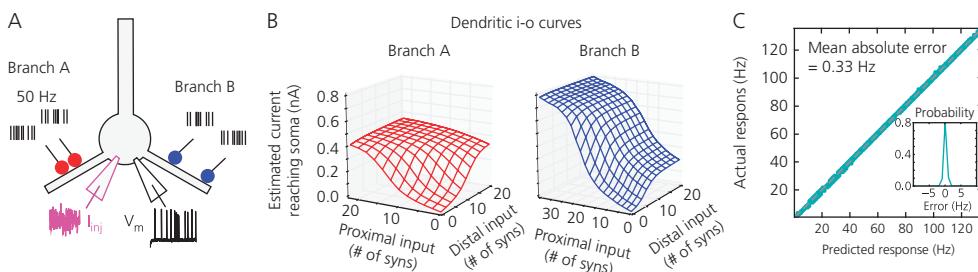


Fig. 16.5 Voltage-dependent synaptic interactions in different dendrites can proceed with nearly perfect independence. **(A)** Two stimulus sites were activated on each dendrite (130 and 150 μm from the soma on branch A and 90 and 190 μm on branch B). **(B)** Estimated time-averaged current reaching the soma in response to the two inputs to branches A and B, respectively, when each branch was stimulated alone. Asymmetric two-dimensional sigmoidal surfaces result from analog nonlinear synaptic location effects discussed in Behabadi et al. (2012), and are a generalization of the one-dimensional sigmoidal input-output (i-o) curves associated with stimulation of a single site (Schiller et al., 2000; Major et al., 2008). To predict the cell's firing rate when the two branches are stimulated together, the currents produced by branches A and B shown in panel (B) are summed and passed through the somatic f - I curve (measured separately by steady current injections; not shown). **(C)** Scatterplot of actual versus predicted firing rates for 384,000 stimulus configurations involving two locations on the two branches. Predictions are close to perfect (mean absolute error = 0.33 Hz), implying that voltage crosstalk is minimal. The inset shows a histogram of spike rate prediction errors (actual-predicted).

Reproduced from Bardia F. Behabadi and Bartlett W. Mel, Mechanisms underlying subunit independence in pyramidal neuron dendrites, *Proceedings of the National Academy of Sciences of the United States of America*, 111(1), pp. 498–503, Figure 3, © 2014, National Academy of Sciences.

Functional significance of the 2LM: linking dendritic computations to circuit-level computations

Beyond its value in predicting neuronal responses to patterns of synaptic excitation, the 2LM provided three benefits. First, the analogy between a single (biological) neuron and a multi-layered artificial neural network allows the large body of theory developed in the field of artificial neural networks to shed light on the computing capabilities of a real neuron, as well as the synaptic learning rules that allow a neuron to access those capabilities.

The 2LM also suggests an intriguing connection between the “arithmetic” of dendritic processing and that of sensory processing. For example, the response properties of one of the principal cell types in the primary visual cortex—the “complex cell” first identified by Hubel and Wiesel—has been described as a two-layer model with a quadratic nonlinearity (sometimes referred to as an “energy model;” Pollen and Ronner, 1982; Ohzawa et al., 1990; Heeger, 1992). To explore this connection, we asked whether this two-layer sensory computation could be carried out entirely within the dendritic tree of a complex cell, rather than requiring a layer of “simple cells” as had generally been assumed by Hubel and Wiesel-inspired models. Using a detailed compartmental model of a pyramidal neuron with active dendrites, we found that when groups of ON- and OFF-center afferents corresponding to simple cell subunits formed excitatory contacts near to each other in the dendrites, the cell as a whole exhibited classical complex cell-like response, including overlapping ON and OFF subfields and position-independent orientation tuning (Mel et al., 1998). In effect, even though there was only a single neuron involved in the simulations, the complex cell behaved as if it were driven by multiple spatially offset simple cells (see also Archie and Mel, 2000). These studies demonstrated that dendritic trees containing NMDA and voltage-dependent Na^+ channels can directly contribute to the formation of nonlinear sensory receptive field properties (Lavzin et al., 2012; Smith et al., 2013).

A third benefit of the 2LM is that compared with single-neuron models with continuous or undefined distance-dependent synaptic interactions, the 2LM, with its “locationless” subunits, greatly facilitates quantitative analysis of a neuron’s learning capacity (Poirazi and Mel, 2001; Wu and Mel, 2009; Legenstein and Maass, 2011).

Problems with the basic 2LM

Notwithstanding the arguments above that a spiny thin dendrite is in some respects well suited to function as a locationless subunit, in the past several years it has become clear that an individual dendrite is not limited to this simple type of integrative behavior. Three complications are worth mentioning. First, it has been appreciated for decades that the non-uniform boundary conditions found in typical dendritic structures produce highly asymmetric voltage attenuation curves, such that voltage signals traveling from a stimulus site inward to the soma are severely attenuated while those travelling outward to the dendritic tips are only mildly attenuated (Rall and Rinzel, 1973; Zador et al., 1995; Nevian et al., 2007). This fact, while promoting subunit independence around a soma as already discussed, makes it unlikely that voltage-dependent interactions between synaptic inputs *within* a dendritic subunit should be invariant to location. Second, as previously mentioned, the input resistance of a thin dendrite typically increases moving outward from the soma to the dendritic tip (Branco and Häusser, 2010), once again due to the different boundary conditions found at the somatic (low input resistance) versus terminal (high input resistance) ends of the branch. A third source of location dependence in a dendritic branch results from the fact that the readout of the dendrite is at the soma, so a proximal voltage signal will produce a larger response than the same signal generated more distally.

Major et al. (2008) showed that these three effects together lead to a systematic progression in the form of the NMDA-dependent sigmoidal input–output curves produced by focal glutamate stimulation at different distances from the soma along a thin dendritic branch. In particular, as the stimulation site moves farther out along the branch, the local spike threshold decreases steadily since the threshold is inversely related to the input resistance (Rhodes, 2006; Gidon and Segev, 2012; Jadi et al., 2012). The peak amplitude of the input–output curve also declines steadily as the stimulus moves further away from the soma, due to conventional distance-dependent voltage attenuation (Milojkovich et al., 2005; Major et al., 2008; Behabadi et al., 2012) (Fig. 16.6B, C).

An interesting side effect of the spatial dependence of a dendrite's sigmoidal input–output curve is that conventional notions of stimulus strength become sorely inadequate. For example, whereas we would naively expect a stimulus of a given intensity delivered to a proximal site to produce a larger effect at the soma than the same stimulus delivered distally, the somatic response to the distal stimulus can in fact be larger if the input intensity is sufficient to trigger a local spike distally but not proximally (compare blue curve with red and green ones in Fig. 16.6C for an input of ~12 synapses). In an even odder twist, the somatic response can be largest for a stimulus delivered mid-dendrite, compared with the same stimulus delivered more proximally or more distally (compare the green with the red and blue curves in Fig. 16.6C, for ~30 synapses).

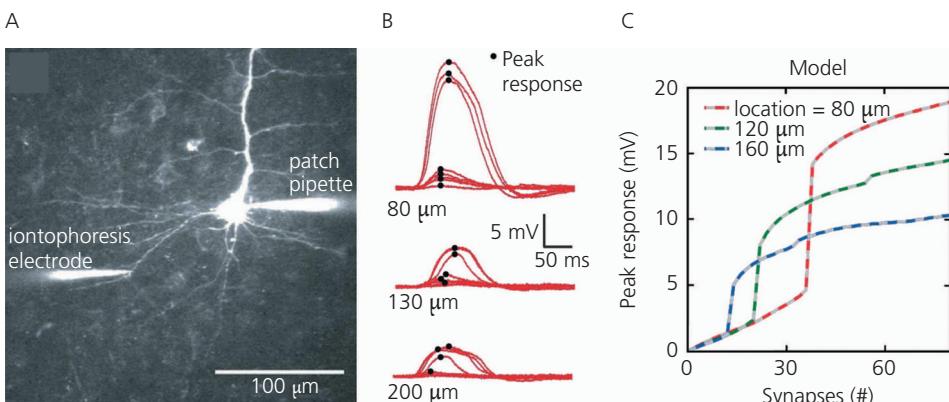


Fig. 16.6 Location dependence of dendritic input–output curves. (A) Experimental setup, from Major et al. (2008): glutamate iontophoresis activates synapses at specific dendritic locations. (B) Voltage traces recorded at the soma for various stimulus sites along the dendritic length. As stimulation intensity is increased, a threshold is crossed and a local “NMDA spike” is generated. (C) A compartmental model shows a similar location dependence of the threshold and peak somatic voltage response. Curves show peak voltage responses recorded at the soma, analogous to the peaks of the traces shown in part B (indicated by black dots). Thus, even when only a single focal excitatory input is involved, a dendrite can generate a spectrum of sigmoidal input–output curves whose thresholds and magnitudes depend on the stimulus location. This fact is irreconcilable with the locationless subunit assumption of the basic 2LM.

Parts A and B Reprinted from Major, Guy, Alon Polsky, Winfried Denk, Jackie Schiller, and David W. Tank. “Spatiotemporally Graded NMDA Spike/Plateau Potentials in Basal Dendrites of Neocortical Pyramidal Neurons.” *Journal of Neurophysiology* 99, no. 5 (May 1, 2008): 2584–2601, with permission from (c) The American Physiological Society. Part C reproduced from Bardia F. Behabadi, Alon Polsky, Monika Jadi, Jackie Schiller, and Bartlett W. Mel, Location-dependent excitatory synaptic interactions in pyramidal neuron dendrites, *PLoS Computational Biology*, 8(7), e1002599, Figure 3b, © 2012, The Authors.

Location-dependent synaptic integration effects

To explore location-dependent synaptic integration effects in thin dendrites, we moved beyond the simplest case of a single focal stimulus, or diffuse stimulation of a dendrite, to the case when synaptic inputs are delivered to two spatially separated sites on the same dendritic branch. Behabadi et al. (2012) addressed the case of two excitatory inputs (E–E), and Jadi et al. (2012) addressed the case of one excitatory and one inhibitory input (E–I). Given the close agreement between the recordings from brain slices and from both detailed and simplified compartmental models (see Fig. 16.7 for a comparison of detailed and simplified circuit models), and the parallel results for E–E and E–I interactions, for simplicity we show in Fig. 16.8 only the results from a simplified (4-compartment)

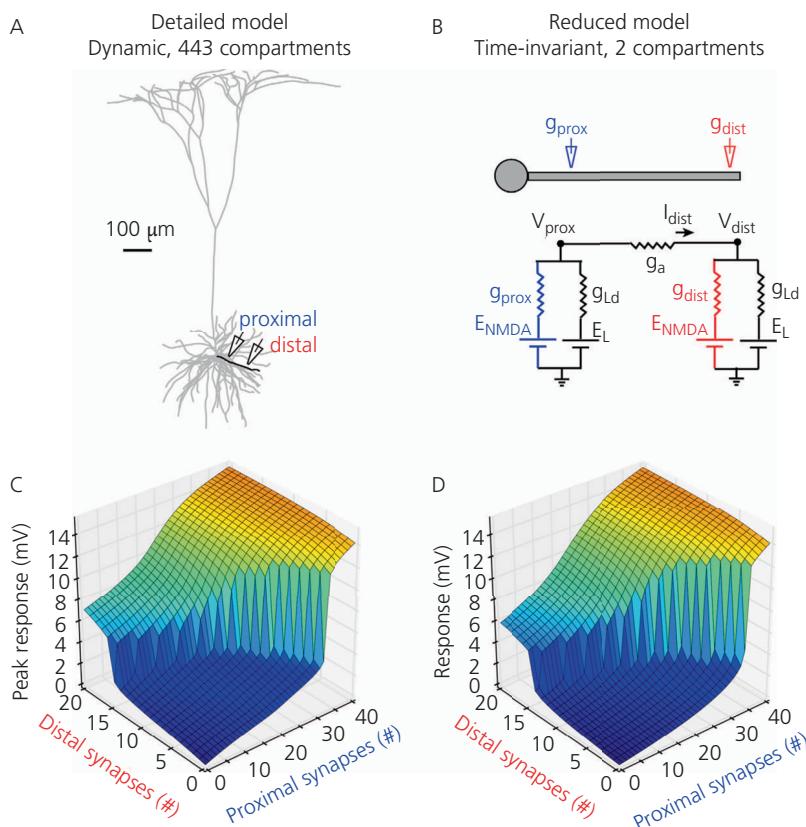


Fig. 16.7 Comparison of excitatory proximal–distal interaction in a detailed compartmental model with fully modeled temporal dynamics, and AMPA- and NMDA-type synapses activated at 90 and 150 μm by 50-Hz double-pulse inputs (A) versus a highly simplified two-compartment model with just five time-invariant conductances (B). Axial, distal leak, and proximal leak conductances were 2.5, 0.25, and 4 arbitrary units (AU), respectively. NMDA peak conductance was 0.5 AU per synapse. (C–D). Location-dependence of peak “somatic” responses is nearly indistinguishable in the two models.

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model, and consolidate the E–E and E–I spatial integration effects on a single combined plot. The main findings from the two studies could be summarized as follows:

- 1 The time-averaged response of a thin dendrite to two spatially separated inputs can be described as a two-dimensional sigmoidal function, in a generalization of the conventional one-dimensional sigmoidal input–output curve associated with a single input site (Fig. 16.6C). For co-localized inputs (Fig. 16.8, two subplots on the main diagonal), the input–output relation is symmetric and effectively reduces to the one-dimensional case.
- 2 For spatially separated inputs (Fig. 16.8, lower right subplot), the sigmoid function is more complex and asymmetric. The function is most easily deconstructed by considering how a proximal input modulates the input–output curve of a distal input, and vice versa. The proximal input, when viewed as the modulator, has a multiplicative effect on the distal input–output curve, lowering its threshold (i.e., left-shifting the input–output curve) and increasing its amplitude. The distal input, when viewed as the modulator, strictly lowers the threshold of the proximal input–output curve without affecting its amplitude. (See Vu and Krasne (1992) for analogous effects in passive dendrites.)
- 3 The modulatory effects of excitation and inhibition are roughly equal and opposite: where an excitatory modulator lowers the threshold, an inhibitory modulator raises it; where an excitatory modulator boosts the magnitude of the input–output curve, an inhibitory modulator reduces it. Furthermore, when excitatory and inhibitory modulators are co-localized, their effects roughly cancel (see Jadi et al., 2014).

Having worked out some basic rules of synaptic integration involving two spatially separated inputs, it is interesting to consider whether or how these rules generalize to scenarios involving more stimuli delivered to more locations on the same dendrite. In particular, if the response to a single focal input to a dendrite is described by a one-dimensional sigmoid function, and the response to two inputs is described by a two-dimensional sigmoid function (whose parameters depend on their relative and absolute locations), then it is natural to ask how many spatial degrees of freedom are needed to accurately capture the overall spatial analog processing capabilities of a single thin dendrite? In other words, how many spatial “pixels” does the dendrite effectively contain as it reads out an arbitrary steady-state pattern of excitation/inhibition? A preliminary investigation of this issue involving excitation only suggests that the sigmoidal input–output function of a thin dendrite has a maximum dimensionality in the range of three to four (Jin et al., 2012).

Excitatory and inhibitory modulation effects combine in rational ways

An important question arises in considering the functional implications of the nonlinear synaptic location effects summarized in Fig. 16.8: do the effects of two or more modulatory inputs at different dendritic locations combine “rationally”? For example, if the effect of a proximal excitatory modulator is to lower the threshold and increase the gain of a driver input’s sigmoidal input–output curve (Fig. 16.8, lower right panel), and the effect of a distal inhibitory modulator is to raise the threshold of the input–output curve without affecting its gain, then if both modulators are activated together, can the net effect of the combined modulation be purely multiplicative, that is, involving an increase in gain with no change in threshold? We simulated this scenario in a reduced model as shown in Fig. 16.9. We found that the two modulatory effects do combine in the expected way (see Fig. 16.9C), and when an axo-somatic spiking mechanism is included in the pipeline (Fig. 16.9D), a nearly pure multiplicative modulation is seen.

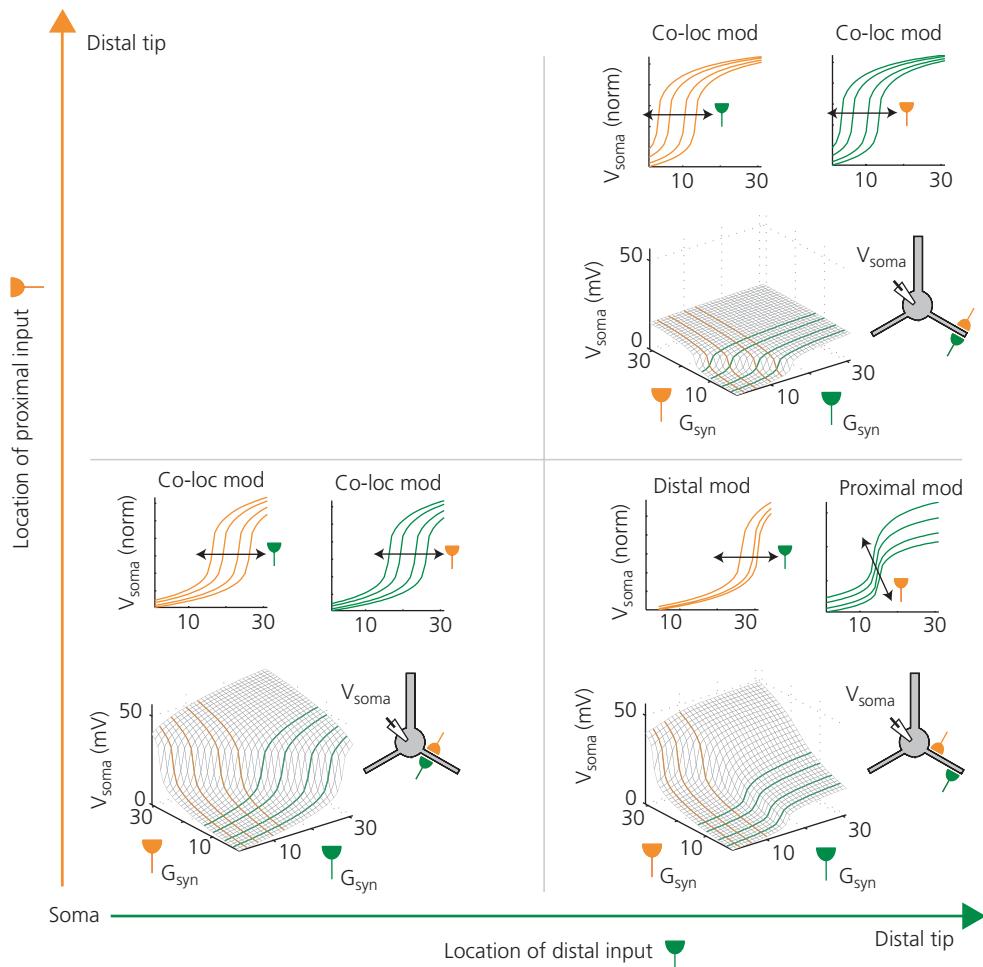


Fig. 16.8 Summary of two-input synaptic location effects. Surface plots show effects of simultaneous stimulation by two inputs (neuron schematic shows locations in each case), delivered to a four-compartment time-invariant circuit model analogous to that shown in Fig. 16.7(C) (with one somatic and three dendritic compartments). Synaptic inputs are in normalized conductance units. Two panels on the main diagonal show cases with colocalized inputs at different distances from the soma. Green and orange line plots in the top row of each panel show color-coded slices from surface plots to highlight the modulatory effect one input has on the input–output curve of the other. In each line plot, the double-ended arrow indicates that excitatory modulation lowers the threshold and/or increases the curve amplitude, whereas inhibitory modulation at the same site acts roughly oppositely, i.e., increases the threshold and/or reduces the amplitude.

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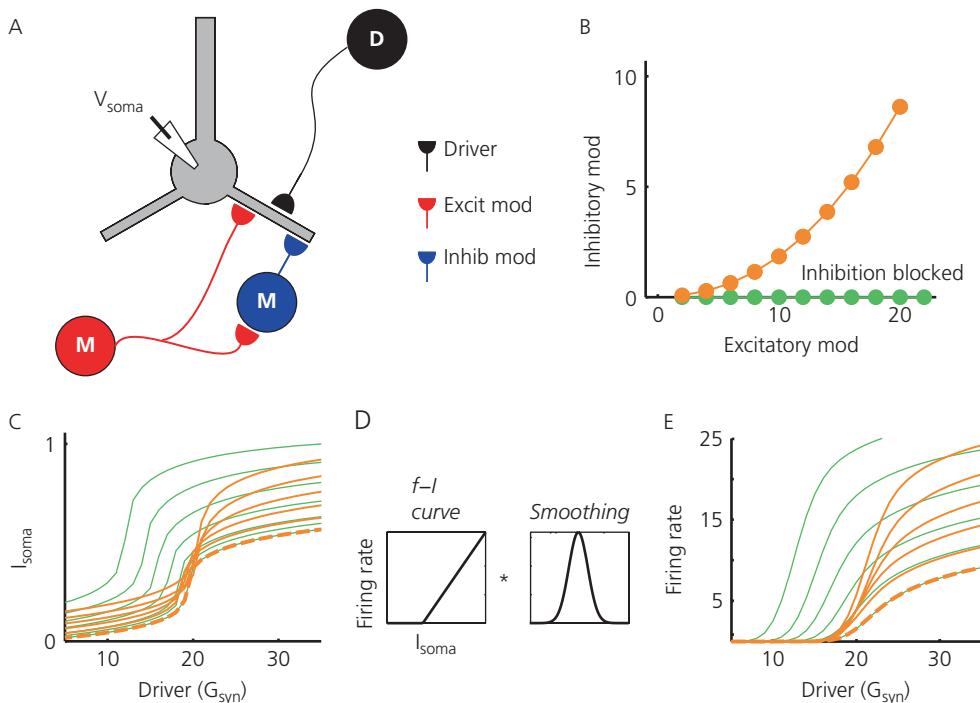


Fig. 16.9 A compound modulation circuit leads to pure multiplicative scaling of a driver input's i-o curve. (A) Schematic of modulatory and driver inputs impinging on a thin dendrite. (B) Orange scatter data show the relation between excitatory modulation strength (red input in A) and inhibitory modulation strength (blue input in A). Green data points indicate the case with pure excitatory modulation, i.e., with inhibition “blocked.” (C) Orange curves show subthreshold voltage calculated using a four-compartment model (the same as was used in Fig. 16.8). The effect of the compound modulator is to increase the amplitude and slightly lower the threshold. For comparison, green curves show modulation with inhibition blocked. (D) Transformation to firing rate is modeled by applying a thresholded $f-I$ curve followed by convolution with a smoothing kernel. (E) Firing rate curves obtained by applying the transformation in D to the data in C. Orange curves show approximate multiplicative scaling. Green curves show case with inhibition blocked for comparison.

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This scenario illustrates how the analog nonlinear spatial processing capabilities of active dendrites could add computational flexibility to the surrounding neural circuit. In particular, a modulatory input (contextual, attentional, cross-modal, etc.) can tailor its effect on a neuron by biasing its direct excitatory contacts closer to or further from the soma to achieve the desired mix of gain-boosting versus threshold-lowering effects, while choosing its connection strength onto soma- versus dendrite-targeting interneurons to achieve the desired mix of gain suppression versus threshold elevation. It is not yet known whether neurons take advantage of this mechanism to customize their circuit-level computations. High-resolution connectome data will be needed to settle the question.

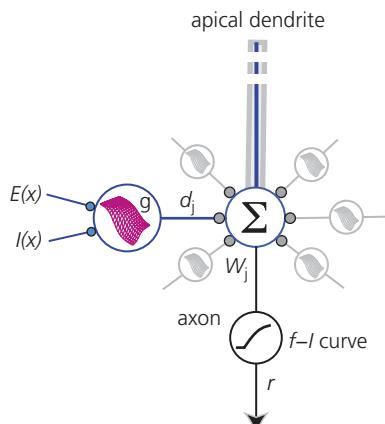


Fig. 16.10 Elaboration of the basic two-layer model of a stellate dendritic tree shown in Fig. 16.4. Dendritic subunits are shown here having multi-dimensional sigmoidal input–output functions that depend on the spatial distributions of excitatory and inhibitory inputs to that dendrite. The thick vertical branch suggests an apical tree.

Conclusions

It remains an important goal of systems neuroscience to develop valid simplified models of individual neurons, without which an understanding of local circuits containing hundreds to thousands of neurons, let alone large-scale neural systems containing millions to billions of neurons, will be nearly impossible to achieve. In this chapter we have discussed a progression of simple models of neurons with active dendrites, culminating in an augmented two-layer model with multi-dimensional sigmoidal subunits, aimed at compactly describing the input–output function of a generic stellate-shaped dendritic tree (Fig. 16.10). This model was developed primarily to describe a single subtree within the dendritic arbor of a hippocampal or neocortical pyramidal neuron, such as the basal subtree, the apical oblique dendrites, or the apical tuft (Mel, et al., 1998; Larkum et al., 2009). It is important to keep this limited scope in mind since interactions *between* dendritic subtrees undoubtedly enhance the computing capabilities of a single neuron (Caulier and Connors, 1994; Golding and Spruston, 1998; Larkum et al., 1999, 2001; Rhodes and Llinas, 2001; Kepcs et al., 2002; Häusser and Mel, 2003; Gasparini et al., 2004; Larkum et al., 2004; Williams, 2004; Jarsky et al., 2005; Harnett et al., 2015). Further work will be needed to fully grasp these more complicated subnetwork interaction effects, and to determine to what extent the same simplified models can be applied to the many different types of neurons in the CNS (e.g., striatal medium spiny neurons, chandelier cells, spinal motor neurons, Purkinje cells, retinal ganglion cells, etc.). Contributions from both experimental and modeling studies will continue to be essential for finding answers to these fascinating questions.

References

- Abbott LF (1999) Lapicque's introduction of the integrate-and-fire model neuron (1907). *Brain Research Bulletin* 50:303–304.
- Agmon-Snir H, Segev I (1993) Signal delay and input synchronization in passive dendritic structures. *Journal of Neurophysiology* 70:2066–2085.

- Anderson CH, Van Essen DC (1987) Shifter circuits: a computational strategy for dynamic aspects of visual processing. *Proceedings of the National Academy of Sciences of the United States of America* **84**:6297–6301.
- Antic SD, Zhou W-L, Moore AR, Short SM, Ikonomu KD (2010) The decade of the dendritic NMDA spike. *Journal of Neuroscience Research* **88**:2991–3001.
- Archie KA, Mel BW (2000) A model for intradendritic computation of binocular disparity. *Nature Neuroscience* **3**:54–63.
- Beaulieu C, Colonnier M (1985) A laminar analysis of the number of round-asymmetrical and flat-symmetrical synapses on spines, dendritic trunks, and cell bodies in area 17 of the cat. *Journal of Comparative Neurology* **231**:180–189.
- Behabadi BF, Mel BW (2014) Mechanisms underlying subunit independence in pyramidal neuron dendrites. *Proceedings of the National Academy of Sciences of the United States of America* **111**:498–503.
- Behabadi BF, Polsky A, Jadi M, Schiller J, Mel BW (2012) Location-dependent excitatory synaptic interactions in pyramidal neuron dendrites. *PLoS Computational Biology* **8**:e1002599.
- Bejan A (2000). *Shape and Structure, From Engineering to Nature*. Cambridge: Cambridge University Press.
- Bernander O, Koch C, Douglas RJ (1994) Amplification and linearization of distal synaptic input to cortical pyramidal cells. *Journal of Neurophysiology* **72**:2743–2753.
- Bi GQ, Poo MM (1998) Synaptic modifications in cultured hippocampal neurons: dependence on spike timing, synaptic strength, and postsynaptic cell type. *Journal of Neuroscience* **18**:10464–10472.
- Borg-Graham LJ, Grzywacz NM (1992) A model of the directional selectivity circuit in retina: transformations by neurons singly and in concert. In: *Single Neuron Computation* (McKenna TM, Davis JL, Zornetzer SF, eds), pp. 347–375. New York: Academic Press.
- Branco T, Häusser M (2010) The single dendritic branch as a fundamental functional unit in the nervous system. *Current Opinion in Neurobiology* **20**:494–502.
- Cash S, Yuste R (1999) Linear summation of excitatory inputs by CA1 pyramidal neurons. *Neuron* **22**:383–394.
- Cauller LJ, Connors BW (1994) Synaptic physiology of horizontal afferents to layer I in slices of rat SI neocortex. *Journal of Neuroscience* **14**:751–762.
- Chklovskii DB, Schikorski T, Stevens CF (2002) Wiring optimization in cortical circuits. *Neuron* **34**:341–347.
- Cook EP, Johnston D (1997) Active dendrites reduce location-dependent variability of synaptic input trains. *Journal of Neurophysiology* **78**:2116–2128.
- Crick F (1984) Function of the thalamic reticular complex: the searchlight hypothesis. *Proceedings of the National Academy of Sciences of the United States of America* **81**:4586–4590.
- Cuntz H, Borst A, Segev I (2007) Optimization principles of dendritic structure. *Theoretical Biology and Medical Modelling* **4**:21.
- Dan Y, Poo M-M (2004) Spike timing-dependent plasticity of neural circuits. *Neuron* **44**: 23–30.
- De Schutter E, Bower JM (1994) Simulated responses of cerebellar Purkinje cells are independent of the dendritic location of granule cell synaptic inputs. *Proceedings of the National Academy of Sciences of the United States of America* **91**:4736–4740.
- Durbin R, Rumelhart DE (1989) Product units: a computationally powerful and biologically plausible extension to backpropagation networks. *Neural Computation* **1**:133–142.
- Elston GN, Rosa MG (1998) Morphological variation of layer III pyramidal neurones in the occipitotemporal pathway of the macaque monkey visual cortex. *Cerebral Cortex* **8**:278–294.
- Fino E, Packer AM, Yuste R (2013) The logic of inhibitory connectivity in the neocortex. *The Neuroscientist* **19**:228–237.
- Fox CA, Barnard JW (1957) A quantitative study of the Purkinje cell dendritic branchlets and their relationship to afferent fibres. *Journal of Anatomy* **91**:299–313.

- Froemke RC, Poo M-M, Dan Y (2005) Spike-timing-dependent synaptic plasticity depends on dendritic location. *Nature* **434**:221–225.
- Gasparini S, Migliore M, Magee JC (2004) On the initiation and propagation of dendritic spikes in CA1 pyramidal neurons. *Journal of Neuroscience* **24**:11046–11056.
- Gidon A, Segev I (2012) Principles governing the operation of synaptic inhibition in dendrites. *Neuron* **75**:330–341.
- Golding NL, Spruston N (1998) Dendritic sodium spikes are variable triggers of axonal action potentials in hippocampal CA1 pyramidal neurons. *Neuron* **21**, no. 5 : 1189–1200.
- Golding NL, Staff NP, Spruston N (2002) Dendritic spikes as a mechanism for cooperative long-term potentiation. *Nature* **418**:326–331.
- Golding NL, Mickus TJ, Katz Y, Kath WL, Spruston N (2005) Factors mediating powerful voltage attenuation along CA1 pyramidal neuron dendrites. *Journal of Physiology* **568**:69–82.
- Gordon U, Polksky A, Schiller J (2006) Plasticity compartments in basal dendrites of neocortical pyramidal neurons. *Journal of Neuroscience* **26**:12717–12726.
- Grossberg S, Mingolla E, Ross WD (1997) Visual brain and visual perception: how does the cortex do perceptual grouping? *Trends in Neurosciences* **20**:106–111.
- Gulledge AT, Carnevale NT, Stuart GJ (2012) Electrical advantages of dendritic spines. *PloS ONE* **7**:e36007.
- Harnett MT, Makara JK, Spruston N, Kath WL, Magee JC (2012) Synaptic amplification by dendritic spines enhances input cooperativity. *Nature* **491**:599–602.
- Harnett MT, Magee JC, Williams SR (2015) Distribution and function of HCN channels in the apical dendritic tuft of neocortical pyramidal neurons. *Journal of Neuroscience* **35**:1024–1037.
- Harris KD, Shepherd GMG (2015) The neocortical circuit: themes and variations. *Nature Neuroscience* **18**:170–181.
- Häusser M, Mel BW (2003) Dendrites: bug or feature? *Current Opinion in Neurobiology* **13**, no. 3 (June 2003): 372–383.
- Heeger DJ (1992) Normalization of cell responses in cat striate cortex. *Visual Neuroscience* **9**:181–197.
- Holt GR, Koch C (1997) Shunting inhibition does not have a divisive effect on firing rates. *Neural Computation* **9**:1001–1013.
- Hopfield JJ (1982) Neural networks and physical systems with emergent collective computational abilities. *Proceedings of the National Academy of Sciences of the United States of America* **79**:2554–2558.
- Hubel DH, Wiesel TN (1962) Receptive fields, binocular interaction and functional architecture in the cat's visual cortex. *Journal of Physiology* **160**:106–154.
- Jadi M, Polksky A, Schiller J, Mel BW (2012) Location-dependent effects of inhibition on local spiking in pyramidal neuron dendrites. *PLoS Computational Biology* **8**:e1002550.
- Jadi MP, Behabadi BF, Poleg-Polsky A, Schiller J, Mel BW (2014) An augmented two-layer model captures nonlinear analog spatial integration effects in pyramidal neuron dendrites. *Proceedings of the IEEE* **102**:782–798.
- Jarosky T, Roxin A, Kath WL, Spruston N (2005) Conditional dendritic spike propagation following distal synaptic activation of hippocampal CA1 pyramidal neurons. *Nature Neuroscience* **8**:1667–1676.
- Jin L, Behabadi BF, Mel BW (2012) Dimensionality of dendritic computation. Presentation at “Neuroscience 2012”.
- Johnston D, Magee JC, Colbert CM, Cristie BR (1996) Active properties of neuronal dendrites. *Annual Review of Neuroscience* **19**:165–186.
- Kandel ER, Spencer WA (1961) Electrophysiological properties of an archicortical neuron. *Annals of the New York Academy of Sciences* **94**:570–603.
- Katona G, Kaszás A, Turi GF, Hájos N, Tamás G, Vizi ES, Rózsa B (2011) Roller coaster scanning reveals spontaneous triggering of dendritic spikes in CA1 interneurons. *Proceedings of the National Academy of Sciences of the United States of America* **108**:2148–2153.

- Katz Y, Menon V, Nicholson DA, Geinisman Y, Kath WL, Spruston N (2009) Synapse distribution suggests a two-stage model of dendritic integration in CA1 pyramidal neurons. *Neuron* **63**:171–177.
- Kepcs A, Wang XJ, Lisman J (2002). Bursting neurons signal input slope. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* **22**, no. 20 (October 15): 9053–9062.
- Koch C, Poggio T, Torre V (1983) Nonlinear interactions in a dendritic tree: localization, timing, and role in information processing. *Proceedings of the National Academy of Sciences of the United States of America* **80**:2799–2802.
- Koch, C, T Poggio, and V Torre (1982) Retinal ganglion cells: a functional interpretation of dendritic morphology. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **298**, no. 1090 (July 27): 227–263.
- Koester HJ, Sakmann B (1998) Calcium dynamics in single spines during coincident pre- and postsynaptic activity depend on relative timing of back-propagating action potentials and subthreshold excitatory postsynaptic potentials. *Proceedings of the National Academy of Sciences of the United States of America* **95**:9596–9601.
- Larkum ME, Zhu JJ, Sakmann B (1999) A new cellular mechanism for coupling inputs arriving at different cortical layers. *Nature* **398**:338–341.
- Larkum ME, Zhu JJ, Sakmann B (2001) Dendritic mechanisms underlying the coupling of the dendritic with the axonal action potential initiation zone of adult rat layer 5 pyramidal neurons. *Journal of Physiology* **533**:447–466.
- Larkum ME, Nevian T, Sandler M, Polsky A, Schiller J (2009) Synaptic integration in tuft dendrites of layer 5 pyramidal neurons: a new unifying principle. *Science* **325**:756–760.
- Larkum ME, Senn W, Lüscher HR (2004) “Top-down dendritic input increases the gain of layer 5 pyramidal neurons.” *Cerebral Cortex* (New York, N.Y.: 1991) **14**, no. 10 (October 2004): 1059–1070. doi:10.1093/cercor/bhh065.
- Legenstein R, Maass W (2011) Branch-specific plasticity enables self-organization of nonlinear computation in single neurons. *Journal of Neuroscience* **31**:10787–10802.
- London M, Häusser M (2005) Dendritic computation. *Annual Review of Neuroscience* **28**:503–532.
- London M, Segev I (2001) Synaptic scaling in vitro and in vivo. *Nature Neuroscience* **4**:853–855.
- Losonczy A, Makara JK, Magee JC (2008) Compartmentalized dendritic plasticity and input feature storage in neurons. *Nature* **452**:436–441.
- McBain CJ, Fisahn A (2001) Interneurons unbound. *Nature Reviews Neuroscience* **2**:11–23.
- Magee JC (1998) Dendritic hyperpolarization-activated currents modify the integrative properties of hippocampal CA1 pyramidal neurons. *Journal of Neuroscience* **18**:7613–7624.
- Magee JC, Cook EP (2000) Somatic EPSP amplitude is independent of synapse location in hippocampal pyramidal neurons. *Nature Neuroscience* **3**:895–903.
- Magee JC, Johnston D (1997) A synaptically controlled, associative signal for Hebbian plasticity in hippocampal neurons. *Science* **275**:209–213.
- Major G, Polsky A, Denk W, Schiller J, Tank DW (2008) Spatiotemporally graded NMDA spike/plateau potentials in basal dendrites of neocortical pyramidal neurons. *Journal of Neurophysiology* **99**, no. 5 (May 1): 2584–2601. doi:10.1152/jn.00011.2008.
- Major G, Larkum ME, Schiller J (2013) Active properties of neocortical pyramidal neuron dendrites. *Annual Review of Neuroscience* **36**:1–24.
- Makara JK, Magee JC (2013) Variable dendritic integration in hippocampal CA3 pyramidal neurons. *Neuron* **80**:1438–1450.
- Marder E, Eisen JS (1984) Electrically coupled pacemaker neurons respond differently to same physiological inputs and neurotransmitters. *Journal of Neurophysiology* **51**:1362–1374.
- Maria L, Rapoport S, Polsky A, Garion L, Schiller J (2012) Nonlinear dendritic processing determines angular tuning of barrel cortex neurons in vivo. *Nature* **490**, no. 7420 (October 18): 397–401. doi:10.1038/nature11451.

- Markram H, Lübke J, Frotscher M, Sakmann B (1997) Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. *Science* **275**:213–215.
- Megías M, Emri Z, Freund TF, Gulyás AI (2001) Total number and distribution of inhibitory and excitatory synapses on hippocampal CA1 pyramidal cells. *Neuroscience* **102**:527–540.
- Mel BW (1986) A connectionist learning model for 3-D mental rotation, zoom, and pan. In: *Proceedings of the Eighth Annual Conference of the Cognitive Science Society*, pp. 562–571. Hillsdale, NJ: Lawrence Erlbaum Associates.
- Mel BW (1987) MURPHY: a robot that learns by doing. In: *Neural Information Processing Systems (NIPS 1987)* (Anderson DZ, ed.), pp. 544–553. New York: American Institute of Physics. Available at: <http://papers.nips.cc/paper/92-murphy-a-pobot-that-learns-by-doing>.
- Mel BW (1992a) NMDA-based pattern discrimination in a modeled cortical neuron. *Neural Computation* **4**:502–517.
- Mel BW (1992b) The clusteron: toward a simple abstraction for a complex neuron. In: *Advances in Neural Information Processing Systems 5* (Moody J, Hanson S, Lippmann R, eds), pp. 35–42. San Mateo, CA: Morgan Kaufmann.
- Mel BW (1993) Synaptic integration in an excitable dendritic tree. *Journal of Neurophysiology* **70**:1086–1101.
- Mel BW (1994) Information processing in dendritic trees. *Neural Computation* **6**: 1031–1085.
- Mel BW, Ruderman DL, Archie KA (1998) Translation-invariant orientation tuning in visual “Complex” cells could derive from intradendritic computations. *Journal of Neuroscience* **18**:4325–4334.
- Milojkovic BA, Radovicic MS, Antic SD (2005) A strict correlation between dendritic and somatic plateau depolarizations in the rat prefrontal cortex pyramidal neurons. *Journal of Neuroscience* **25**:3940–3951.
- Nevian T, Larkum ME, Polsky A, Schiller J. (2007) Properties of basal dendrites of layer 5 pyramidal neurons: a direct patch-clamp recording study. *Nature Neuroscience* **10**:206–214.
- Ohzawa I, DeAngelis GC, Freeman RD (1990) Stereoscopic depth discrimination in the visual cortex: neurons ideally suited as disparity detectors. *Science* **249**:1037–1041.
- Palmer LM, Stuart Greg J (2009) Membrane potential changes in dendritic spines during action potentials and synaptic input. *Journal of Neuroscience* **29**:6897–6903.
- Palmer LM, Shai AS, Reeve JE, Anderson HL, Paulsen O, Larkum ME (2014) NMDA spikes enhance action potential generation during sensory input. *Nature Neuroscience* **17**:383–390.
- Patil MM, Linster C, Lubenov E, Hasselmo ME (1998) Cholinergic agonist carbachol enables associative long-term potentiation in piriform cortex slices. *Journal of Neurophysiology* **80**:2467–2474.
- Petreanu L, Mao T, Sternson SM, Svoboda K (2009) The subcellular organization of neocortical excitatory connections. *Nature* **457**:1142–1145.
- Piskorowski RA, Chevaleyre V (2012) Synaptic integration by different dendritic compartments of hippocampal CA1 and CA2 pyramidal neurons. *Cellular and Molecular Life Sciences* **69**:75–88.
- Poirazi P, Mel BW (2001) Impact of active dendrites and structural plasticity on the memory capacity of neural tissue. *Neuron* **29**:779–796.
- Poirazi P, Brannon T, Mel BW (2003a) Arithmetic of subthreshold synaptic summation in a model CA1 pyramidal cell. *Neuron* **37**:977–987.
- Poirazi P, Brannon T, Mel BW (2003b) Pyramidal neuron as two-layer neural network. *Neuron* **37**:989–999.
- Pollen DA, Ronner SF (1982) Spatial computation performed by simple and complex cells in the visual cortex of the cat. *Vision Research* **22**:101–118.
- Polsky A, Mel BW, Schiller J (2004) Computational subunits in thin dendrites of pyramidal cells. *Nature Neuroscience* **7**:621–627.
- Polsky A, Mel BW, Schiller J (2009) Encoding and decoding bursts by nmda spikes in basal dendrites of layer 5 pyramidal neurons. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* **29**, no. 38 (September 23): 11891–11903. doi:10.1523/JNEUROSCI.5250–08.2009.

- Rall W (1964) Theoretical significance of dendritic trees for neuronal input–output relations. In: *Neural Theory and Modeling* (Reiss RF, ed.), pp. 73–97. Stanford, CA: Stanford University Press.
- Rall W, Rinzel J (1973) Branch input resistance and steady attenuation for input to one branch of a dendritic neuron model. *Biophysical Journal* **13**:648–687.
- Rall W, Segev I (1987) Functional possibilities for synapses on dendrites and on dendritic spines. In: *Synaptic Function* (Edelman GM, Gall WE, Cowan WM, eds), pp. 605–636. New York: Wiley.
- Raphael G, Tsianos GA, Loeb GE (2010) Spinal-like regulator facilitates control of a two-degree-of-freedom wrist. *Journal of Neuroscience* **30**:9431–9444.
- Raymond JL, Lisberger SG, Mauk MD (1996) The cerebellum: a neuronal learning machine? *Science* **272**:1126–1131.
- Rhodes P (2006) The properties and implications of NMDA spikes in neocortical pyramidal cells. *Journal of Neuroscience* **26**:6704–6715.
- Rhodes, Paul A, and Rodolfo R Llinás (2001) Apical tuft input efficacy in layer 5 pyramidal cells from rat visual cortex. *The Journal of Physiology* **536**, no. Pt 1 (October 1): 167–187. doi:10.1111/j.1469-7793.2001.00167.x.
- Rosenblatt F (1962) *Principles of Neurodynamics: Perceptrons and the Theory of Brain Mechanisms*. Washington, DC: Spartan Books.
- Routh BN, Johnston D, Harris K, Chitwood RA (2009) Anatomical and electrophysiological comparison of CA1 pyramidal neurons of the rat and mouse. *Journal of Neurophysiology* **102**:2288–2302.
- Rumelhart DE, Hinton GE, McClelland JL (1986) A general framework for parallel distributed processing. In: *Parallel Distributed Processing: Explorations in the Microstructure of Cognition, Vol. 1* (Rumelhart DE, McClelland JL, eds), pp. 45–76. Cambridge, MA: Bradford.
- Schiller J, Schiller Y, Stuart G, Sakmann B (1997) Calcium action potentials restricted to distal apical dendrites of rat neocortical pyramidal neurons. *Journal of Physiology* **505**:605–616.
- Shepherd G (1998) *The Synaptic Organization of the Brain*. New York: Oxford University Press.
- Shepherd GM, Brayton RK (1987) Logic operations are properties of computer-simulated interactions between excitable dendritic spines. *Neuroscience* **21**:151–165.
- Shepherd GM, Brayton RK, Miller JP, Segev I, Rinzel J, Rall W (April 1985) Signal enhancement in distal cortical dendrites by means of interactions between active dendritic spines. *Proceedings of the National Academy of Sciences of the United States of America* **82**:2192–2195.
- Shipman SL, Herring BE, Suh YH, Roche KW, Nicoll JA (2013) Distance-dependent scaling of AMPARs is cell-autonomous and GluA2 dependent. *Journal of Neuroscience* **33**:13312–13319.
- Silver RA (2010) Neuronal arithmetic. *Nature Reviews Neuroscience* **11**:474–489.
- Smith SL., Smith IT, Branco T, Michael H (2013) Dendritic spikes enhance stimulus selectivity in cortical neurons *in vivo*. *Nature* **503**, no. 7474 (November 7): 115–120. doi:10.1038/nature12600.
- Sivyer B, Williams SR (2013) Direction selectivity is computed by active dendritic integration in retinal ganglion cells. *Nature Neuroscience* **16**:1848–1856.
- Spencer WA, Kandel ER (1961) Electrophysiology of hippocampal neurons: IV. Fast prepotentials. *Journal of Neurophysiology* **24**:272–285.
- Spruston N (2008) Pyramidal neurons: dendritic structure and synaptic integration. *Nature Reviews Neuroscience* **9**:206–221.
- Stuart GJ, Sakmann B (1994) Active propagation of somatic action potentials into neocortical pyramidal cell dendrites. *Nature* **367**:69–72.
- Stuart G, Spruston N (1998) Determinants of voltage attenuation in neocortical pyramidal neuron dendrites. *Journal of Neuroscience* **18**:3501–3510.
- Ulfhake B, Kellerth JO (1981) A quantitative light microscopic study of the dendrites of cat spinal alpha-motoneurons after intracellular staining with horseradish peroxidase. *Journal of Comparative Neurology* **202**:571–583.

- Vu ET, Krasne FB (1992) Evidence for a computational distinction between proximal and distal neuronal inhibition. *Science* **255**:1710–1712.
- Williams SR (2004) Spatial compartmentalization and functional impact of conductance in pyramidal neurons. *Nature Neuroscience* **7**:961–967.
- Williams SR, Stuart GJ (2000) Site independence of EPSP time course is mediated by dendritic I(h) in neocortical pyramidal neurons. *Journal of Neurophysiology* **83**:3177–3182.
- Woolf TB, Shepherd GM, Greer CA (1991) Local information processing in dendritic trees: subsets of spines in granule cells of the mammalian olfactory bulb. *Journal of Neuroscience* **11**:1837–1854.
- Wu XE, Mel BW (2009) Capacity-enhancing synaptic learning rules in a medial temporal lobe online learning model. *Neuron* **62**:31–41.
- Zador AM, Pearlmutter BA (1996) VC dimension of an integrate-and-fire neuron model. In: *Proceedings of the Ninth Annual Conference on Computational Learning Theory*, pp. 10–18. New York: ACM.
- Zador AM, Claiborne BJ, Brown TH (1991) Nonlinear pattern separation in single hippocampal neurons with active dendritic membrane. In: *Advances in Neural Information Processing Systems 4* (Moody J, Hanson S, Lippmann R, eds), pp. 51–58. San Mateo, CA: Morgan Kaufmann.
- Zador AM, Agmon-Snir H, Segev I (1995) The morphoelectrotonic transform: a graphical approach to dendritic function. *Journal of Neuroscience* **15**:1669–1682.

Chapter 17

Modeling dendrite shape

Hermann Cuntz

Summary

The functional role of voltage-gated channels has been extensively studied in computational models of dendrites, but the role of dendritic shape itself has long eluded a rigorous quantitative analysis because of a lack of corresponding models. Several novel modeling techniques have recently emerged that enable the generation of synthetic dendritic trees for most types of neurons. These models are useful for studying how aspects of dendritic computation depend on specific features of dendritic morphology and the impact of pathological changes of dendritic shape. The models also allow one to study general principles and relations of morphological parameters in dendritic trees and to understand circuit formation during development. With detailed models of single neurons now reaching a high degree of biological realism, it will be valuable to implement such models in simulations of entire neural circuits.

Introduction

Imagine a jigsaw puzzle extending in three dimensions with billions of pieces, each piece having thousands of tabs and blanks to connect it to other pieces. What a challenge it would be to design the shape of this puzzle, and what a complex and highly entangled structure would result! Conceptually, this is the engineering problem that the brain has to solve to build the circuits that underlie behavior. Each circuit is highly interconnected, with each neuron receiving signals from and sending out signals to thousands of other neurons. The dendritic structures at which neurons receive inputs from other cells need to make precise contacts with the complementary axonal trees of the input neurons. The result is a dense, space-filling network, as shown in three-dimensional electron microscopic reconstructions of the cortex, leaving essentially no wasted space (Fig. 17.1; Seung, 2012).

In order to solve this organizational challenge, dendrites and axons elaborate sophisticated tree-like structures with fine branches and complex arborizations. While the dendrites of different cell types exhibit a most remarkable variety of shapes and sizes (Ramón y Cajal, 1995; DeFelipe, 2010; see also Chapters 1 and 2), neurons of the same cell type are strikingly similar, hinting that connectivity has an underlying role in determining their shape. The morphological complexity of neural circuits really stands in stark contrast to reoccurring patterns of dendritic as well as axonal trees of individual cell types, for which we are starting to gain a good understanding. For example, it is known that the anatomy of the single dendritic tree of any given cell type shapes synaptic integration (Rall, 1959; Rall et al., 1967; Rall and Rinzel, 1973; Segev and London, 2000; Gullidge et al., 2005; London and Häusser, 2005; Cuntz et al., 2014) and the corresponding neural firing behavior

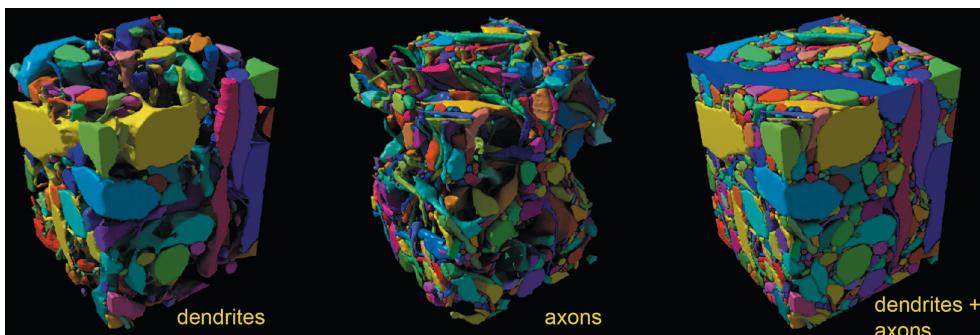


Fig. 17.1 Electron microscopic reconstruction of a volume of cerebral cortex. A $6 \times 6 \times 7.5 \mu\text{m}$ cube of L5 mouse somatosensory cortex from a region of neuropil selected to contain no blood vessels or cell bodies. The slices were obtained with the automated tape-collecting ultramicrotome scanning electron microscopy technique (ATUM-SEM; Hayworth et al., 2014). While the aldehyde fixation in this block emphasizes the dense packing since it removes some of the extracellular space, the tissue *in vivo* is believed to be similarly dense.

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(Mainen and Sejnowski, 1996; Vetter et al., 2001; Krichmar et al., 2002; van Ooyen et al., 2002; van Elburg and van Ooyen, 2010).

While the importance of the precise geometry of dendritic trees in synaptic integration and computation is undisputed, numerical models that capture the functional aspects of single neurons and networks are typically based on either highly simplified morphologies (e.g., represented by a ball for the soma and a stick for the dendrite) or on morphologies obtained directly as reconstructions using light or electron microscopy. In these functional models the morphology *per se* cannot be parameterized and the role of morphological parameters typically cannot be investigated. Furthermore, single morphological reconstructions, which are usually each obtained from different individual animals, cannot be combined in network models in a realistic configuration since they do not match one another. This would be like trying to solve a jigsaw puzzle when each piece comes from a different set, and poses a difficult challenge for large-scale network modeling projects (e.g., Markram, 2006).

Modeling has become an established tool for identifying underlying principles in the complex dynamics of neural circuits (since, e.g., Hodgkin and Huxley, 1952; Rall, 1959). It is generally accepted that the generation of models will be essential if we are to combine our overall knowledge about the brain into a functional holistic concept. Modeling the intricate branched structure in neural circuits is therefore a key step toward achievement of this goal (Ascoli, 2002a; van Ooyen, 2003; Cuntz et al., 2014). The resulting models can reveal the functionality of the underlying circuits with respect to computation and the way in which they connect. Thus far, no unifying theory exists to link the structure of circuits to their particular role in computation and connectivity. However, a number of models have recently emerged that are able to describe the morphology of single cells at an exquisite level of detail (Fig. 17.2). This chapter provides a brief overview of some of the more promising methods as well as some of their individual applications. It concludes with an outlook of how these can combine to eventually solve the jigsaw puzzle of the brain.

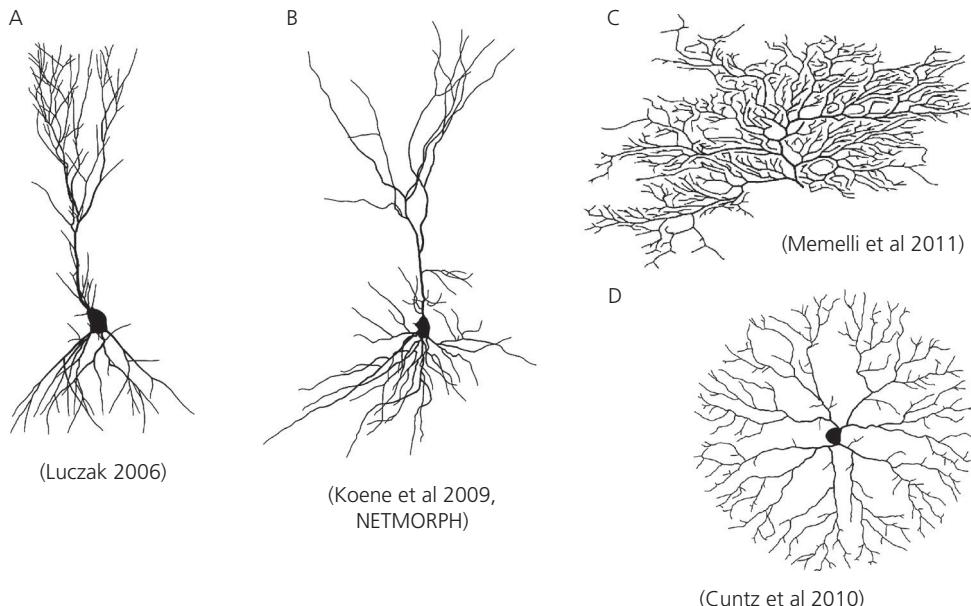


Fig. 17.2 Examples of morphological models of dendrite structure. **(A)** Diffusion-limited aggregate-based synthetic morphology of a CA1 hippocampal pyramidal cell (Luczak, 2006). **(B)** Morphogenesis based on growth cone simulations leading to a synthetic L2/3 cortical pyramidal cell dendrite (Koene et al., 2009). **(C)** Synthetic cerebellar Purkinje cell generated by simulating self-referential forces such as somatotropism, inertia, and self-avoidance (Memelli et al., 2013). **(D)** Synthetic starburst amacrine cell modelled to satisfy optimal wiring constraints (Cuntz et al., 2010). Panels A and B were processed by the TREES toolbox (Cuntz et al., 2010) for improved visualization.

Part A reprinted from *Journal of Neuroscience Methods*, 157(1), Artur Luczak, Spatial embedding of neuronal trees modeled by diffusive growth, pp. 132–141, Copyright 2006, Elsevier. With permission from Elsevier. Part B reproduced from *Neuroinformatics*, 7(3), pp 195–210, NETMORPH: A framework for the stochastic generation of large scale neuronal networks with realistic neuron morphologies, Randal A. Koene, Betty Tijms, Peter van Hees, Frank Postma, Alexander de Ridder, Ger J. A. Ramakers, Jaap van Pelt, and Arjen van Ooyen © 2009, Springer Science and Business Media. With permission from Springer Science and Business Media. Part C reproduced from Heraldo Memelli, Benjamin Torben-Nielsen, and James Kozloski, Self-referential forces are sufficient to explain different dendritic morphologies, *Frontiers in Neuroinformatics*, 7, p.1. © 2013 Memelli, Torben-Nielsen and Kozloski. Part D reproduced from Hermann Cuntz, Friedrich Forstner, Alexander Borst, and Michael Häusser, Location-one rule to grow them all: a general theory of neuronal branching and its practical application, *PLoS Computational Biology*, 6(8), e1000877, © 2010, The Authors.

Dendritic trees as fractal structures

The tree structures of dendrites are fractal in nature. Their irregular, seemingly stochastic, branches are self-similar across various scales, meaning that any smaller part of a dendritic tree could itself be mistaken for a dendritic tree in its own right. A principal feature of fractals, their so-called fractal dimension, measures how the structure behaves throughout various scales. The fractal dimension of dendritic trees can be calculated in a variety of ways (Smith et al., 1996) and stochastic models can reproduce the fractal features of dendrites. One of the simplest ways

to derive synthetic dendritic tree-like structures is based on self-organization of patterns arising from reaction–diffusion systems where different substances diffuse spatially and react with each other (Turing, 1952), or from diffusion-limited aggregates (DLAs) where particles diffuse in space to only aggregate when they encounter a stable substrate (Witten and Sander, 1981, 1983). Synthetic tree structures resulting from DLAs (Fig. 17.3) can be set to match the fractal dimension observed in real dendrites and reproduce a number of other dendritic morphological traits (Caserta et al., 1990; Hentschel and Fine, 1994, 1996, 2003). Other simple tree structures based on reaction–diffusion systems showcase dendritic growth under developmental-like conditions mimicking the interactions between substances (Sugimura et al., 2007) that underlie dendritic space filling, a feature of the single cell that allows it to maximize its number of potential connection partners.

While such models have provided conceptual advances in our understanding of the principles underlying dendritic structure, it is only recently that DLAs have been extended to sophisticated three-dimensional models that accurately reproduce the statistics of real dendritic trees (Luczak, 2006, 2014). By synthesizing realistic hippocampal granule and pyramidal cells, this work showed that additional steps are required in the generational process, such as pruning of unrealistic small branches (Fig. 17.2A). For the generation of such DLAs, particles are confined to a given volume and neighboring synthetic dendrites can be generated simultaneously when they compete for floating particles. Unfortunately a dense tissue, as in Fig. 17.1, cannot be achieved with this method since parts of the DLA are unreachable for floating particles that would rather aggregate on the outside before reaching the inside of the structure. However, the spatial embedding of neurons can be studied in three dimensions by altering the shape of the available space and the position of other neurons in the nervous tissue. Results from such studies have highlighted the importance of the spanning volume for the shape of dendrites. When the spanning volume was drastically changed, dendrite trees grown under otherwise similar

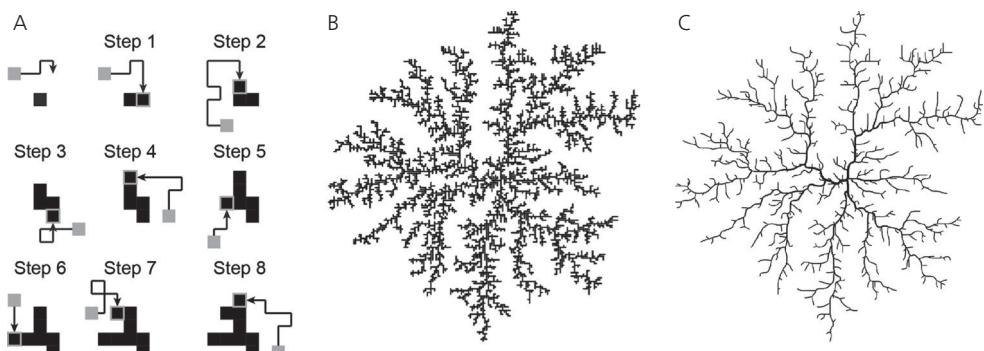


Fig. 17.3 Diffusion-limited aggregates (DLAs). (A) Particles (gray) diffuse freely on a random walk until they encounter the aggregate where they stabilize (black). Nine steps in the construction procedure are illustrated. When a particle reaches the direct neighborhood of the existing aggregate, it stabilizes (black with gray border) and becomes part of the aggregate. (B) When a sufficient number of particles have aggregated, stochastic fractal tree structures emerge. (C) When small branches are pruned and synthetic taper is added, dendrite-like structures can be obtained (here using the TREES toolbox).

conditions resulted in different cell types. A particular advantage of this type of approach for generating synthetic dendrites is the minimal number of parameters required: models are fully constrained by the definition of available volume besides post-processing of trees by pruning of dendrites and adjusting diameters.

A more controlled approach for generating fractal synthetic trees employs variations of so-called Lindenmayer systems (L-systems; Lindenmayer, 1968), which are extensively used to generate synthetic plants and botanical trees (Prusinkiewicz and Lindenmayer, 2004). L-systems apply a geometric interpretation of formal grammars to generate fractal structures iteratively. The basis for this approach is illustrated in Fig. 17.4 with a simple non-stochastic local growth rule. This is a very elegant way to produce complex structures from simple rules. However, in order to simulate dendritic morphologies, the corresponding growth rules need to be stochastic in nature and global constraints, such as a maximum total length, are required. Since this type of model in principle allows for a limitless selection of rules and parameters, it can only be applied to dendrites when combined with sophisticated optimization procedures to match the models with their biological counterparts. In order to find simple Lindenmayer-like systems that describe dendritic morphologies, a search for a set of local rules that depend on a strict minimum of fundamental parameters has been undertaken (Hillman, 1979; Ascoli, 1999). For example, the diameter of a dendrite could taper with a certain rate as a branch grows, and bifurcations as well as branch terminations can occur as a function of local diameter. Do such rules hold true for dendrites?

Relations between some local parameters do indeed exist. For example, branch order, number of terminations, bifurcation asymmetry, and branch radius show correlations in certain cell types (Donohue and Ascoli, 2008). Motoneurons can be modeled by fitting relationships between membrane area, stem diameter, and number of termination points as well as path-length relations (Burke et al., 1992) or by matching the fractal dimension locally and assuming somatofugal tropism (Marks and Burke, 2007a,b). However, in most cases the parameter relationships are not strong enough to fully determine the dendritic morphology and use of these methods has now tended to revert to optimizing global branching statistics to generate faithful synthetic dendritic and axonal trees. What kind of biological mechanism would provide such a global cue is unclear, and it remains possible that local rules will be discovered that describe the growth process exactly. The earlier approaches to this kind of modeling are reviewed

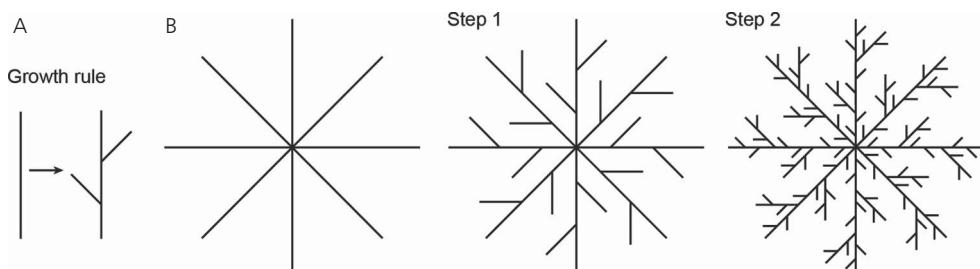


Fig. 17.4 Lindenmayer systems (L-systems). This figure illustrates the principles of a Lindenmayer system using the example of a simple growth rule. **(A)** Illustration of the geometrical growth rule that is applied on each individual segment at each iteration. **(B)** Two iterations are shown after the initial condition on the left.

extensively in a series of papers by Ascoli and colleagues (Ascoli et al., 2001a,b; Ascoli, 2002b). A software package called L-neuron (Ascoli and Krichmar, 2000) combines a number of L-system-like descriptions, including the ones mentioned here (Hillman, 1979; Burke et al., 1992) as well as other similar approaches (Senft and Ascoli, 1999). Further, a number of other newer approaches exist (Eberhard et al., 2006; Lindsay et al., 2007; Torben-Nielsen et al., 2008; Wolf et al., 2013).

Simulation of dendritic development

The growth process expressed in stochastic L-systems that reproduce dendritic branching statistics does not on its own offer any functional interpretation or conclusions about the biological processes that led to the final tree structures. Ideally, mechanisms at the level of growth cones in dendrites should be modeled to understand what happens during development. However, including simple concepts of biological growth in the generational process can already help to provide a better mechanistic understanding, and can reproduce the intermediate morphological steps during biological dendrite growth. Purely stochastic branching processes (Galton–Watson; see, e.g., Kliemann, 1987), where the branching probability in each branch is independent of other branches, have been useful for understanding the emergence of branching patterns and some general branching statistics (Ireland et al., 1985; Kliemann, 1987; Carriquiry et al., 1991; Uemura et al., 1995). This indicates that the chances of growth cone splitting might be independent of other factors in the developing cell. In fact, by combining a stochastic branching process with self-referential forces, realistic morphological models were achieved in a recent study (Memelli et al., 2013; Fig. 17.2C).

Modeling the molecular mechanisms of dendritic growth has led to a number of approaches to simulate dendritic tree structure. Explicit elongation and branching rules were for example used to model the topology of dendritic trees (van Pelt and Verwer, 1983) and growth cones have been modeled in a variety of ways (van Veen and van Pelt, 1992; Li and Qin, 1996; van Pelt and Schierwagen, 2004). Also, the aforementioned reaction–diffusion systems (Sugimura et al., 2007) are useful for validating the molecular mechanisms, for example substances that act as attractors and suppressors, that lead to dendrite development. In recent work (Torben-Nielsen and De Schutter, 2014), realistic synthetic dendritic trees were generated by simulating growth cones that individually sense the environment, which consists of other growth cones and the substrates in which they grow, such as molecular gradients or tissue boundaries. Finally, neurite outgrowth in developing neuronal networks can be studied in terms of its dependence on neural activity using adequate models (van Ooyen, 1994; van Ooyen and van Pelt, 1994).

As was the case for the L-systems-like approaches, a large number of models that simulate the developmental process of dendrite growth at various levels of complexity have tested the physical and biological constraints of dendritic morphology (van Pelt et al., 2001; van Pelt and Uylings, 2002; Zubler and Douglas, 2009), but some recent models have reached a high degree of realism for generating synthetic dendrites and axons (Koene et al., 2009; Fig. 17.2B). With this method, the developmental stages of single neurons are also well reproduced (Fig. 17.5), opening up the possibility of precisely matching the morphological models using time-lapse reconstructions from dendrites growing during development (Lee et al., 2013). This work on modeling dendritic development has been summarized by Arjen van Ooyen (van Ooyen, 2003, 2011; Graham and van Ooyen, 2006).



Fig. 17.5 Growth cone simulations. Simulation of the growth process of a sample L2/3 cortical pyramidal cell by reproducing developmental elongation and bifurcation.

Reproduced from *Neuroinformatics*, 7(3), pp 195–210, NETMORPH: a framework for the stochastic generation of large scale neuronal networks with realistic neuron morphologies, Randal A. Koene, Betty Tijms, Peter van Hees, Frank Postma, Alexander de Ridder, Ger J. A. Ramakers, Jaap van Pelt, and Arjen van Ooyen © 2009, Springer Science and Business Media. With permission from Springer Science and Business Media.

Morphological models based on functional constraints

In order to gain a more fundamental understanding of why dendritic trees look the way they do, it is useful to study generational processes that are based on actual functional constraints. The one requirement common to all dendrites is that they need to connect with often distant input sources. More than a century ago, Ramón y Cajal observed that dendrites and axons connect in an efficient manner that does not waste space, cellular material, or conduction time in the neural circuitry (Ramón y Cajal 1995, pp. 115–125). The branching statistics of dendritic and axonal morphologies (Cherniak, 1992; Chklovskii, 2004; Shepherd et al., 2005), as well as of entire neural circuits (Klyachko and Stevens, 2003; Chklovskii and Koulakov, 2004; Wen and Chklovskii, 2008; Wen et al., 2009; Bullmore and Sporns, 2012), reflect the selection pressure to reduce wiring costs. Are dendrites and axons then ideally matched to anatomically optimize their connections with each other in terms of Cajal's rules?

In order to study the relation between dendrite shape and connectivity, dendritic and axonal trees have been compared to minimum spanning trees (MSTs; Mitchison, 1991), as well as combinations of MSTs and preservation of certain branching statistics (Tamori, 1993). MST algorithms from graph theory build tree structures that minimize the total amount of wiring, thereby conserving cellular material, when applied on a set of initial target locations distributed in space. One way to implement the MST algorithm is to connect at each iteration the closest unconnected target to the existing tree (Prim, 1957). In such a greedy notation, in which at each iteration the local optimum is selected, the distance or cost function can be extended to penalize long path lengths

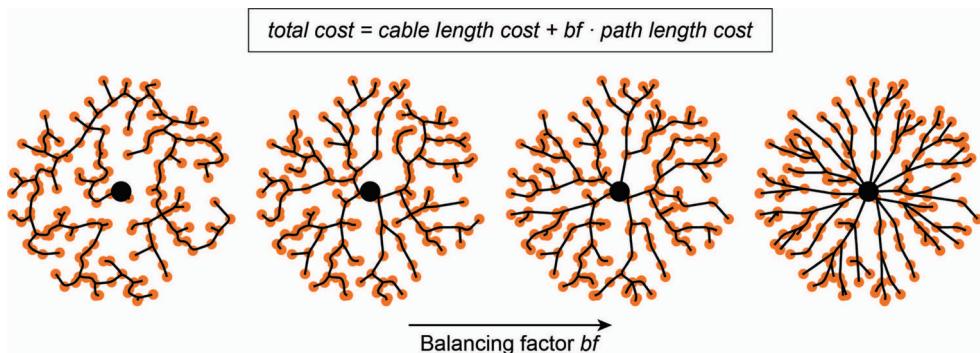


Fig. 17.6 Minimum spanning trees. From left to right, the same set of target points (orange circles) were connected to a starting node (large black dot) according to the minimum spanning tree algorithm with increasing balancing factor, bf , between the two wiring costs: total cable length and sum of all paths.

Reproduced (slightly modified) from Cuntz H (2012) The dendritic density field of a cortical pyramidal cell. *Frontiers in Neuroanatomy* 6:2.

between any point on the tree and the root, thereby also conserving conduction times, the second of the three optimal wiring laws of Cajal (Fig. 17.6).

Using this strategy, highly realistic synthetic dendritic trees were generated with a fairly simple and quick algorithmic procedure applicable to a wide variety of cell types (Cuntz et al., 2007, 2010), for example fly interneurons (Cuntz et al., 2007, 2008), dentate gyrus granule cells (Schneider et al., 2014), retinal starburst amacrine cells, cortical pyramidal cells from different layers, and at different developmental stages (Cuntz et al., 2010), as well as neocortical axons (Budd et al., 2010). A balancing factor bf , which weighs the two costs against each other in the cost function, represents the one and only parameter of the model once the targets are defined. Interestingly, different cell types possess very different bf values, indicating that for pyramidal or granule cells with high bf values, short conduction times are important enough to expend on extra cable length, whereas this is not the case for Purkinje cells in the cerebellum.

This approach allows the most common branching features of dendrites to be combined in one precise equation that describes how the total dendritic length L scales with the number of branch points bp , the number of targets n , and the volume V that the dendritic tree spans (Cuntz et al., 2012):

$$L = \sqrt[3]{3/4\pi} \cdot \sqrt[3]{V} n^{2/3} = \sqrt[3]{3/4\pi} \cdot \sqrt[3]{V} (\alpha \cdot bp)^{2/3} \quad (17.1)$$

The parameter α determines the ratio between bp and n and only depends on the value of bf . This is a good example of the usefulness of such models, and the scaling law was shown to be true experimentally between n (where n is the number of synaptic puncta) and L for periglomerular neurons in the olfactory bulb and between bp and L for the entirety of the database at NeuroMorpho.org (Ascoli, 2006) including morphological reconstructions for a wide variety of cell types and species (Cuntz et al., 2012; Fig. 17.7). Similar scaling principles hold between electrotonic length and compartmentalization of dendrites and the number of synapses (Cuntz et al., 2012), suggesting that there may be profound implications for function and computation linked to this morphological principle. All these results speak in favor of the wiring constraints originally proposed by Ramón y Cajal (1995).

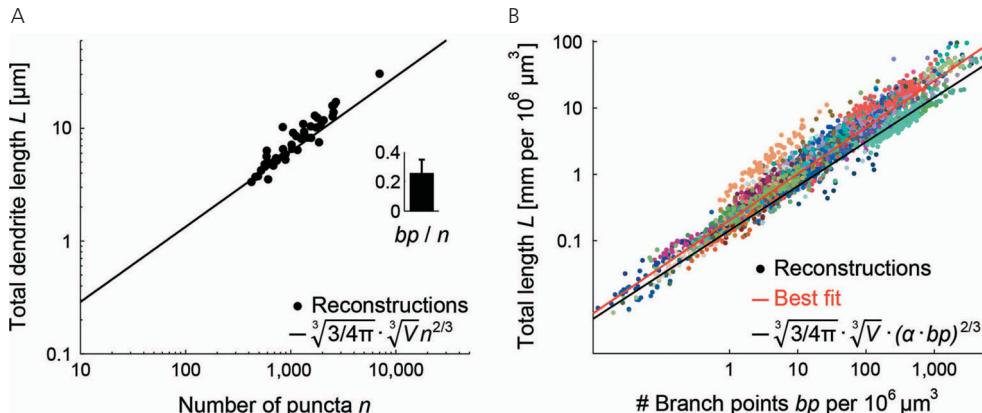


Fig. 17.7 Dendritic scaling law from optimal wiring constraints. (A) Total dendrite length L versus number of synaptic puncta n in experimental data from adult newborn periglomerular neurons in the olfactory bulb (black dots; Mizrahi, 2007; Livneh et al., 2009). Data follow the same trend found in minimum spanning trees expressed by $\sqrt[3]{3/4\pi} \cdot \sqrt[3]{V} n^{2/3}$. The inset shows the ratio between the number of branch points, bp , and number of synaptic puncta n . (B) Relation between total dendrite length L and number of branch points, bp , for all reconstructions from the morphological database NeuroMorpho.org (Ascoli, 2006). All data strictly follow the equation predicted with the minimum spanning tree morphological model. L and bp were normalized by the volume $V = 1,000,000 \mu\text{m}^3$ that the dendrite covers. The lower bound power law (black line) is shown for $\alpha = 0.29$ corresponding to $bf = 0.5$ in the model. Each datapoint is one neuron, with colors corresponding to different datasets (usually neuron types). The best fit (red line) has a power of 0.70.

Part A adapted from Hermann Cuntz, Alexandre Mathy, and Michael Häusser, A scaling law derived from optimal dendritic wiring, *Proceedings of the National Academy of Sciences of the United States of America*, 109(27), pp.11014–11018, Figure 3d, Copyright © 2012, The National Academy of Sciences. Part B adapted from Hermann Cuntz, Alexandre Mathy, and Michael Häusser, A scaling law derived from optimal dendritic wiring, *Proceedings of the National Academy of Sciences of the United States of America*, 109(27), pp.11014–11018, Figure 2a, Copyright © 2012, The National Academy of Sciences.

Such wiring criteria do not, however, constrain dendritic diameters *per se*. Several studies have mapped dendritic diameters post hoc onto the wiring diagrams obtained using minimum spanning trees (Cuntz et al., 2007, 2008, 2010; Schneider et al., 2014). They obtained a faithful match for the characteristic diameter taper in real dendrites by assuming that the diameter optimizes dendritic democracy, meaning that it renders the somatic impact of the synaptic inputs across the dendrite as near equal as possible (Magee and Cook, 2000; Häusser, 2001). Numerical simulations have shown that a quadratic diameter taper satisfies these conditions, which turns out to be a good match for realistic diameter tapers (Fig. 17.8; Cuntz et al., 2007).

An ambitious endeavor has attempted to further relate dendritic morphology to specific computations that a dendrite needs to perform by numerical optimization of L-system-like models (Stiefel and Sejnowski, 2007; Torben-Nielsen and Stiefel, 2009, 2010a,b; Stiefel and Torben-Nielsen, 2014). Interestingly, for the example of fly interneurons (Torben-Nielsen and Stiefel, 2010b), these methods showed that it was possible to generate dendritic morphologies that were better at performing the computation, in this case wide-field visual motion integration, than the real counterparts. This indicates that either the full extent of this dendrite's computation was not considered,

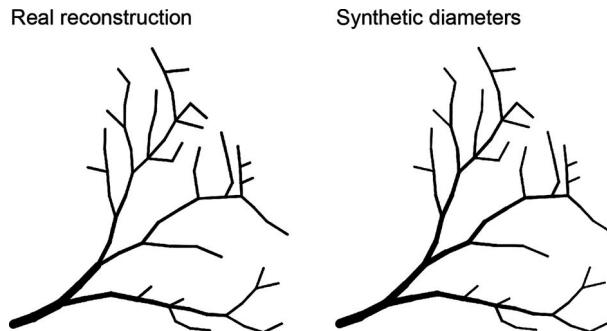


Fig. 17.8 Quadratic diameter taper to optimize synaptic democracy. A subtree of a fly horizontal system tangential cell of the lobula plate is shown as a reconstruction from the microscope (left) and with the diameters entirely replaced by a quadratic diameter taper (right) that optimizes the current transfer from the tip of the dendrites to the root to ensure synaptic democracy (Häusser, 2001; Cuntz et al., 2007).

or that the computation does not play a prime role in shaping the structure of these neurons. Incidentally, optimizing for a cost function that just considered wiring criteria led to quantitatively more realistic results (Torben-Nielsen and Stiefel, 2010b).

Conclusions and outlook on complete morphological network models

This chapter has discussed various strategies for generating synthetic dendritic branching structures in morphological models. In the quest for the best model, it is necessary to measure how well a model performs. Branching statistics of single neurons have been used to fit the parameters of morphological models, or to compare the resulting synthetic dendritic trees with their real counterparts. A number of statistics have been proposed, using both metric and topological measures (Uylings and van Pelt, 2002), and the further development of these statistics is still a subject of active research (see, e.g., Ascoli et al., 2008; Torben-Nielsen and Cuntz, 2014). Branching statistics for given dendritic trees can be calculated using freely available software tools (Scorcioni et al., 2008; Cuntz et al., 2010, 2011; Torben-Nielsen, 2014). However, these statistics are strongly dependent on the context in which a dendrite grows, and so may scale with animal size or depend on tissue constraints and the cellular neighborhood.

For this reason, branching statistics for individual cells of a given cell type are not a homogeneous group, and different types of individual cells might grow with the same branching rule but differ in the particular context in which they grow. As such, horizontal system (HS) and vertical system (VS) neurons in the fly and their precise branching statistics can be modeled with the same exact branch rule (Cuntz et al., 2008) but one obtains easily distinguishable morphologies because of the different area that their dendrites span. Additionally, competition for input space between neighboring neurons can serve as a contextual cue (Samsonovich and Ascoli, 2003). One consequence under certain conditions, both at the level of the network and the single-cell morphology, is dendritic tiling (Cuntz et al., 2010).

An interesting example of the complication caused by context is provided by apical and basal dendrites of pyramidal cells. On the one hand, statistical models indicate a larger difference

between apical and basal dendrites of a single cell than between dendrites of different cell types (Donohue and Ascoli, 2008). On the other hand, apical and basal dendrites can be constructed using the exact same growth rule (Cuntz, 2012), with the only difference being that the cell's axonal output is located in the layer where the basal dendrite is located. In this example, the context, i.e. the spanning field of the dendrite, fully determines the differences in shape and the distribution of all branching parameters.

As we have seen, obviously different morphologies can sometimes be modeled with the same underlying growth principle. Consequently, they could be considered to belong to the same cell type morphologically. This makes branching statistics a questionable measure for fitting and testing a morphological model. Along the same lines, reconstructions without the context of other cells and tissue constraints are not very informative, and indeed there are solutions available that take context into account (Rein et al., 2002; Chiang et al., 2011; Oberlaender et al., 2012). Only a few morphological models exist to circumvent the use of branching statistics, yet some of them achieve such a high level of detail that they pass the "Turing test" (Cuntz et al., 2010), in which experts in the field are no longer able to distinguish the synthetic trees from the real ones. Validation of morphological models can also be achieved by measuring how "easy" it is to produce a certain dendritic morphology with a given stochastic model and its adjusted parameter set (Luczak, 2010). However, this is only meaningful in the case of morphological models that integrate the context into the construction process (Luczak, 2006; Cuntz et al., 2008; Torben-Nielsen and De Schutter, 2014). Thus, novel morphological models are required. Data from cellular connectomics approaches will be invaluable in this quest since complete morphologies will become available that are embedded in their surrounding circuit and therefore their particular context (Denk and Horstmann, 2004; Briggman et al., 2011; Helmstaedter et al., 2013).

The methods described here can all be scaled to generate large numbers of individualized dendritic morphologies (e.g., Schneider et al., 2014; Fig. 17.9). Combining them into complete circuits also requires consideration of the space-packing issues mentioned at the beginning of this chapter, as well as their connectivity, and therefore the axons in the circuit must be taken into account. Because axons are thinner and can extend much further, axonal reconstructions are rare, but a few morphological models of axons based on the same principles as those for dendrites do exist (Koene et al., 2009; Budd et al., 2010). If one assumes that connectivity between cells is stochastic

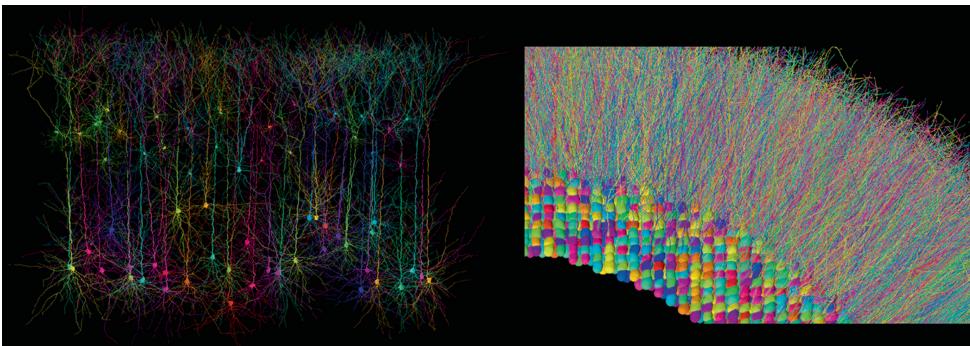


Fig. 17.9 Studying circuits using morphological models. Synthetic cortical pyramidal cells from L2/3, L4, and L5 (left; Cuntz et al., 2010) and a model with synthetic dendrites for all approximately 1.2 million granule cells in the rat dentate gyrus (right; Schneider et al., 2014).

and depends on opportunistic anatomical proximity, the morphological models of dendrites and axons are useful for estimating circuit connectivity (McAssey et al., 2014; van Ooyen et al., 2014). Conversely, the shape of dendrites can be seen as optimizing a given axonal organization (Wen and Chklovskii, 2008; Wen et al., 2009; Cuntz, 2012).

It will also be useful to combine the existing models and to find out how biology implements the optimal wiring constraints that define dendrite structure. In the meantime, existing morphological models can be used in their present form in a number of applications, for example to populate network models (Schneider et al., 2014), to “repair” experimental reconstructions, for example by re-creating branches damaged during the experimental procedure (Anwar et al., 2009; van Pelt et al., 2014), to study dendritic integration (Cuntz et al., 2012, 2013), and to study the changes associated with pathology. Ultimately, morphological models should be able to reproduce the dense circuits in the brain, predicting from the connectivity of these circuits the shapes and locations of all intertwined dendrites, axons, and even glia.

Acknowledgments

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References

- Anwar H, Riachi I, Schürmann F, Markram H (2009) An approach to capturing neuron morphological diversity. In: *Computational Modeling Methods for Neuroscientists* (De Schutter E, ed.), pp. 211–231. Cambridge, MA: MIT Press.
- Ascoli GA (1999) Progress and perspectives in computational neuroanatomy. *Anatomical Record* 257:195–207.
- Ascoli GA (2002a) *Computational Neuroanatomy—Principles and Methods*. Totowa, NJ: Humana Press.
- Ascoli GA (2002b) Neuroanatomical algorithms for dendritic modelling. *Network* 13:247–260.
- Ascoli GA (2006) Mobilizing the base of neuroscience data: the case of neuronal morphologies. *Nature Reviews Neuroscience* 7:318–324.
- Ascoli GA, Krichmar JL (2000) L-neuron: a modeling tool for the efficient generation and parsimonious description of dendritic morphology. *Neurocomputing* 32–33:1003–1011.
- Ascoli GA, Krichmar JL, Nasuto SJ, Senft SL (2001a) Generation, description and storage of dendritic morphology data. *Philosophical Transactions of the Royal Society B: Biological Sciences* 356:1131–1145.
- Ascoli GA, Krichmar JL, Scorcioni R, Nasuto SJ, Senft SL (2001b) Computer generation and quantitative morphometric analysis of virtual neurons. *Anatomy and Embryology* 204:283–301.
- Ascoli GA, et al. (2008) Petilla terminology: nomenclature of features of GABAergic interneurons of the cerebral cortex. *Nature Reviews Neuroscience* 9:557–568.
- Briggman KL, Helmstaedter M, Denk W (2011) Wiring specificity in the direction-selectivity circuit of the retina. *Nature* 471:183–188.
- Budd JML, Kovács K, Ferecskó AS, Buzás P, Eysel UT, Kisvárday ZF (2010) Neocortical axon arbors trade-off material and conduction delay conservation. *PLoS Computational Biology* 6:e1000711.
- Bullmore ET, Sporns O (2012) The economy of brain network organization. *Nature Reviews Neuroscience* 13:336–349.
- Burke RE, Marks WB, Ulvhake B (1992) A parsimonious description of motoneuron dendritic morphology using computer simulation. *Journal of Neuroscience* 12:2403–2416.
- Carriquiry AL, Ireland WP, Kliemann W, Uemura E (1991) Statistical evaluation of dendritic growth models. *Bulletin of Mathematical Biology* 53:579–589.

- Caserta F, Stanley HE, Eldred WD, Daccord G, Hausman RE, Nittmann J (1990) Physical mechanisms underlying neurite outgrowth: a quantitative analysis of neuronal shape. *Physical Review Letters* **64**:95–98.
- Cherniak C (1992) Local optimization of neuron arbors. *Biological Cybernetics* **66**:503–510.
- Chiang A-S, et al. (2011) Three-dimensional reconstruction of brain-wide wiring networks in *Drosophila* at single-cell resolution. *Current Biology* **21**:1–11.
- Chklovskii DB (2004) Synaptic connectivity and neuronal morphology: two sides of the same coin. *Neuron* **43**:609–617.
- Chklovskii DB, Koulakov AA (2004) Maps in the brain: what can we learn from them? *Annual Review of Neuroscience* **27**:369–392.
- Cuntz H (2012) The dendritic density field of a cortical pyramidal cell. *Frontiers in Neuroanatomy* **6**:2.
- Cuntz H, Borst A, Segev I (2007) Optimization principles of dendritic structure. *Theoretical Biology and Medical Modelling* **4**:21.
- Cuntz H, Forstner F, Haag J, Borst A (2008) The morphological identity of insect dendrites. *PLoS Computational Biology* **4**:e1000251.
- Cuntz H, Forstner F, Borst A, Häusser M (2010) One rule to grow them all: a general theory of neuronal branching and its practical application. *PLoS Computational Biology* **6**:e1000877.
- Cuntz H, Forstner F, Borst A, Häusser M (2011) The TREES toolbox—probing the basis of axonal and dendritic branching. *Neuroinformatics* **9**:91–96.
- Cuntz H, Mathy A, Häusser M (2012) A scaling law derived from optimal dendritic wiring. *Proceedings of the National Academy of Sciences of the United States of America* **109**:11014–11018.
- Cuntz H, Forstner F, Schnell B, Ammer G, Raghu SV, Borst A (2013) Preserving neural function under extreme scaling. *PLoS ONE* **8**:e71540.
- Cuntz H, Remme MWH, Torben-Nielsen B (2014) *The Computing Dendrite—From Structure to Function*. New York: Springer.
- DeFelipe J (2010) *Cajal's Butterflies of the Soul: Science and Art*. Oxford University Press.
- Denk W, Horstmann H (2004) Serial block-face scanning electron microscopy to reconstruct three-dimensional tissue nanostructure. *PLoS Biology* **2**:e329.
- Donohue DE, Ascoli GA (2008) A comparative computer simulation of dendritic morphology. *PLoS Computational Biology* **4**:e1000089.
- Eberhard JP, Wanner AA, Wittum G (2006) NeuGen: a tool for the generation of realistic morphology of cortical neurons and neural networks in 3D. *Neurocomputing* **70**:327–342.
- van Elburg RAJ, van Ooyen A (2010) Impact of dendrite size and dendrite topology on burst firing in pyramidal cells. *PLoS Computational Biology* **6**:e1000781.
- Graham BP, van Ooyen A (2006) Mathematical modelling and numerical simulation of the morphological development of neurons. *BMC Neuroscience* **7**(Suppl. 1):S9.
- Gulledge AT, Kampa BM, Stuart GJ (2005) Synaptic integration in dendritic trees. *Journal of Neurobiology* **64**:75–90.
- Hayworth KJ, Morgan JL, Schalek R, Berger DR, Hildebrand DGC, Lichtman JW (2014) Imaging ATUM ultrathin section libraries with WaferMapper: a multi-scale approach to EM reconstruction of neural circuits. *Frontiers in Neural Circuits* **8**:68.
- Helmstaedter M, Briggman KL, Turaga SC, Jain V, Seung HS, Denk W (2013) Connectomic reconstruction of the inner plexiform layer in the mouse retina. *Nature* **500**:168–174.
- Hentschel HGE, Fine A (1994) Instabilities in cellular dendritic morphogenesis. *Physical Review Letters* **73**:3592–3595.
- Hentschel HGE, Fine A (1996) Diffusion-regulated control of cellular dendritic morphogenesis. *Proceedings of the Royal Society B: Biological Sciences* **263**:1–8.

- Hentschel HGE, Fine A (2003) Early dendritic and axonal morphogenesis. In: *Modeling Neural Development* (van Ooyen A, ed.), pp. 49–74. Cambridge, MA: MIT Press.
- Hillman DE (1979) Neuronal shape parameters and substructures as a basis of neuronal form. In: *The Neurosciences, Fourth Study Program* (Schmitt FO, Worden FG, eds), pp. 477–498. Cambridge, MA: MIT Press.
- Hodgkin AL, Huxley AF (1952) A quantitative description of membrane current and its application to conduction and excitation in nerve. *Journal of Physiology* **117**:500–544.
- Häusser M (2001) Synaptic function: dendritic democracy. *Current Biology* **11**:R10–R12.
- Ireland W, Heidel J, Uemura E (1985) A mathematical model for the growth of dendritic trees. *Neuroscience Letters* **54**:243–249.
- Kliemann W (1987) A stochastic dynamical model for the characterization of the geometrical structure of dendritic processes. *Bulletin of Mathematical Biology* **49**:135–152.
- Klyachko VA, Stevens CF (2003) Connectivity optimization and the positioning of cortical areas. *Proceedings of the National Academy of Sciences of the United States of America* **100**:7937–7941.
- Koene RA, Tijms B, van Hees P, Postma F, de Ridder A, Ramakers GJA, van Pelt J, van Ooyen A (2009) NETMORPH: a framework for the stochastic generation of large scale neuronal networks with realistic neuron morphologies. *Neuroinformatics* **7**:195–210.
- Krichmar JL, Nasuto SJ, Scorcioni R, Washington SD, Ascoli GA (2002) Effects of dendritic morphology on CA3 pyramidal cell electrophysiology: a simulation study. *Brain Research* **941**:11–28.
- Lee P-C, He H-Y, Lin C, Ching Y, Cline HT (2013) Computer aided alignment and quantitative 4D structural plasticity analysis of neurons. *Neuroinformatics* **11**:249–257.
- Li GH, Qin C De (1996) A model for neurite growth and neuronal morphogenesis. *Mathematical Biosciences* **132**:97–110.
- Lindenmayer A (1968) Mathematical models for cellular interactions in development. II. Simple and branching filaments with two-sided inputs. *Journal of Theoretical Biology* **18**:300–315.
- Lindsay KA, Maxwell DJ, Rosenberg JR, Tucker G (2007) A new approach to reconstruction models of dendritic branching patterns. *Mathematical Biosciences* **205**:271–296.
- Livneh Y, Feinstein N, Klein M, Mizrahi A (2009) Sensory input enhances synaptogenesis of adult-born neurons. *Journal of Neuroscience* **29**:86–97.
- London M, Häusser M (2005) Dendritic computation. *Annual Review of Neuroscience* **28**:503–532.
- Luczak A (2006) Spatial embedding of neuronal trees modeled by diffusive growth. *Journal of Neuroscience Methods* **157**:132–141.
- Luczak A (2010) Measuring neuronal branching patterns using model-based approach. *Frontiers of Computational Neuroscience* **4**:135.
- Luczak A (2014) Shaping of neurons by environmental interaction. In: *The Computing Dendrite—From Structure to Function* (Cuntz H, Remme MWH, Torben-Nielsen B, eds), pp. 79–90. New York: Springer.
- McAssey MP, Bijma F, Tarigan B, van Pelt J, van Ooyen A, de Gunst M (2014) A morpho-density approach to estimating neural connectivity. *PLoS ONE* **9**:e86526.
- Magee JC, Cook EP (2000) Somatic EPSP amplitude is independent of synapse location in hippocampal pyramidal neurons. *Nature Neuroscience* **3**:895–903.
- Mainen ZF, Sejnowski TJ (1996) Influence of dendritic structure on firing pattern in model neocortical neurons. *Nature* **382**:363–366.
- Markram H (2006) The blue brain project. *Nature Reviews Neuroscience* **7**:153–160.
- Marks WB, Burke RE (2007a) Simulation of motoneuron morphology in three dimensions. I. Building individual dendritic trees. *Journal of Comparative Neurology* **503**:685–700.
- Marks WB, Burke RE (2007b) Simulation of motoneuron morphology in three dimensions. II. Building complete neurons. *Journal of Comparative Neurology* **503**:701–716.

- Memelli H, Torben-Nielsen B, Kozloski J (2013) Self-referential forces are sufficient to explain different dendritic morphologies. *Frontiers of Neuroinformatics* 7:1.
- Mitchison G (1991) Neuronal branching patterns and the economy of cortical wiring. *Proceedings of the Royal Society B: Biological Sciences* 245:151–158.
- Mizrahi A (2007) Dendritic development and plasticity of adult-born neurons in the mouse olfactory bulb. *Nature Neuroscience* 10:444–452.
- Oberlaender M, de Kock CPJ, Bruno RM, Ramirez A, Meyer HS, Dercksen VJ, Helmstaedter M, Sakmann B (2012) Cell type-specific three-dimensional structure of thalamocortical circuits in a column of rat vibrissal cortex. *Cerebral Cortex* 22:2375–2391.
- van Ooyen A (1994) Activity-dependent neural network development. *Network* 5:401–423.
- van Ooyen A (2003) *Modeling Neural Development*. Cambridge, MA: MIT Press.
- van Ooyen A (2011) Using theoretical models to analyse neural development. *Nature Reviews Neuroscience* 12:311–326.
- van Ooyen A, van Pelt J (1994) Activity-dependent outgrowth of neurons and overshoot phenomena in developing neural networks. *Journal of Theoretical Biology* 167:27–43.
- van Ooyen A, Duijnhouwer J, Remme MWH, van Pelt J (2002) The effect of dendritic topology on firing patterns in model neurons. *Network* 13:311–325.
- van Ooyen A, Carnell A, de Ridder S, Tarigan B, Mansvelder HD, Bijma F, de Gunst M, van Pelt J (2014) Independently outgrowing neurons and geometry-based synapse formation produce networks with realistic synaptic connectivity. *PLoS ONE* 9:e85858.
- van Pelt J, Schierwagen AK (2004) Morphological analysis and modeling of neuronal dendrites. *Mathematical Biosciences* 188:147–155.
- van Pelt J, Uylings HBM (2002) Branching rates and growth functions in the outgrowth of dendritic branching patterns. *Network* 13:261–281.
- van Pelt J, Verwer RWH (1983) The exact probabilities of branching patterns under terminal and segmental growth hypotheses. *Bulletin of Mathematical Biology* 45:269–285.
- van Pelt J, van Ooyen A, Uylings HBM (2001) The need for integrating neuronal morphology databases and computational environments in exploring neuronal structure and function. *Anatomy and Embryology* 204:255–265.
- van Pelt J, van Ooyen A, Uylings HBM (2014) Axonal and dendritic density field estimation from incomplete single-slice neuronal reconstructions. *Frontiers of Neuroanatomy* 8:54.
- Prim RC (1957) Shortest connection networks and some generalizations. *Bell System Technical Journal* 36:1389–1401.
- Prusinkiewicz P, Lindenmayer A (2004) *The Algorithmic Beauty of Plants*. <http://algorithmicbotany.org/papers/#abop>
- Rall W (1959) Branching dendritic trees and motoneuron membrane resistivity. *Experimental Neurology* 52:491–527.
- Rall W, Rinzel J (1973) Branch input resistance and steady attenuation for input to one branch of a dendritic neuron model. *Biophysical Journal* 13:648–687.
- Rall W, Burke RE, Smith TG, Nelson PG, Frank K (1967) Dendritic location of synapses and possible mechanisms for the monosynaptic EPSP in motoneurons. *Journal of Neurophysiology* 30:1169–1193.
- Ramón y Cajal S (1995) *Histology of the Nervous System of Man and Vertebrates* (Swanson LW, transl.). Oxford: Oxford University Press.
- Rein K, Zöckler M, Mader MT, Grübel C, Heisenberg M (2002) The Drosophila standard brain. *Current Biology* 12:227–231.
- Samsonovich A V, Ascoli GA (2003) Statistical morphological analysis of hippocampal principal neurons indicates cell-specific repulsion of dendrites from their own cell. *Journal of Neuroscience Research* 71:173–187.

- Schneider CJ, Cuntz H, Soltesz I (2014) Linking macroscopic with microscopic neuroanatomy using synthetic neuronal populations. *PLoS Computational Biology* **10**:e1003921.
- Scorcioni R, Polavaram S, Ascoli GA (2008) L-Measure: a web-accessible tool for the analysis, comparison and search of digital reconstructions of neuronal morphologies. *Nature Protocols* **3**:866–876.
- Segev I, London M (2000) Untangling dendrites with quantitative models. *Science* **290**:744–750.
- Senft SL, Ascoli GA (1999) Reconstruction of brain networks by algorithmic amplification of morphometry data. *Lectures Notes in Computer Science* **1606**:25–33.
- Seung HS (2012) *Connectome: How the Brain's Wiring Makes Us Who We Are*. Boston, MA: Houghton Mifflin Harcourt.
- Shepherd GMG, Stepanyants A, Bureau I, Chklovskii DB, Svoboda K (2005) Geometric and functional organization of cortical circuits. *Nature Neuroscience* **8**:782–790.
- Smith TG, Lange GD, Marks WB (1996) Fractal methods and results in cellular morphology—dimensions, lacunarity and multifractals. *Journal of Neuroscience Methods* **69**:123–136.
- Stiefel KM, Sejnowski TJ (2007) Mapping function onto neuronal morphology. *Journal of Neurophysiology* **98**:513–526.
- Stiefel KM, Torben-Nielsen B (2014) Optimized dendritic morphologies for noisy inputs. In: *The Computing Dendrite—From Structure to Function* (Cuntz H, Remme MWH, Torben-Nielsen B, eds), pp. 147–158. New York: Springer.
- Sugimura K, Shimono K, Uemura T, Mochizuki A (2007) Self-organizing mechanism for development of space-filling neuronal dendrites. *PLoS Computational Biology* **3**:e212.
- Tamori Y (1993) Theory of dendritic morphology. *Physical Review E* **48**:3124–3129.
- Torben-Nielsen B (2014) An efficient and extendable Python library to analyze neuronal morphologies. *Neuroinformatics* **12**:619–622.
- Torben-Nielsen B, Cuntz H (2014) Introduction to dendritic morphology. In: *The Computing Dendrite—From Structure to Function* (Cuntz H, Remme MWH, Torben-Nielsen B, eds), pp. 3–22. New York: Springer.
- Torben-Nielsen B, De Schutter E (2014) Context-aware modeling of neuronal morphologies. *Frontiers of Neuroanatomy* **8**:92.
- Torben-Nielsen B, Stiefel KM (2009) Systematic mapping between dendritic function and structure. *Network* **20**:69–105.
- Torben-Nielsen B, Stiefel KM (2010a) An inverse approach for elucidating dendritic function. *Frontiers of Computational Neuroscience* **4**:128.
- Torben-Nielsen B, Stiefel KM (2010b) Wide-field motion integration in fly vs cells: insights from an inverse approach. *PLoS Computational Biology* **6**:e1000932.
- Torben-Nielsen B, Vanderlooy S, Postma EO (2008) Non-parametric algorithmic generation of neuronal morphologies. *Neuroinformatics* **6**:257–277.
- Turing AM (1952) The chemical basis of morphogenesis. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* **237**:37–72.
- Uemura E, Carriquiry A, Kliemann W, Goodwin J (1995) Mathematical modeling of dendritic growth in vitro. *Brain Research* **671**:187–194.
- Uylings HBM, van Pelt J (2002) Measures for quantifying dendritic arborizations. *Network* **13**:397–414.
- van Veen MP, van Pelt J (1992) A model for outgrowth of branching neurites. *Journal of Theoretical Biology* **159**:1–23.
- Vetter P, Roth A, Häusser M (2001) Propagation of action potentials in dendrites depends on dendritic morphology. *Journal of Neurophysiology* **85**:926–937.
- Wen Q, Chklovskii DB (2008) A cost-benefit analysis of neuronal morphology. *Journal of Neurophysiology* **99**:2320–2328.

- Wen Q, Stepanyants A, Elston GN, Grosberg AY, Chklovskii DB (2009) Maximization of the connectivity repertoire as a statistical principle governing the shapes of dendritic arbors. *Proceedings of the National Academy of Sciences of the United States of America* **106**:12536–12541.
- Witten TA, Sander LM (1981) Diffusion-limited aggregation, a kinetic critical phenomenon. *Physical Review Letters* **47**:1400–1403.
- Witten TA, Sander LM (1983) Diffusion-limited aggregation. *Physical Review B* **27**:5686–5697.
- Wolf S, Grein S, Queisser G (2013) Employing NeuGen 2.0 to automatically generate realistic morphologies of hippocampal neurons and neural networks in 3D. *Neuroinformatics* **11**:137–148.
- Zubler F, Douglas RJ (2009) A framework for modeling the growth and development of neurons and networks. *Frontiers of Computational Neuroscience* **3**:25.

Chapter 18

Functional plasticity at dendritic synapses

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Summary

In this chapter we aim to summarize the experiments and theory that are relevant to the role of dendrites in plasticity. Since the vast majority of synapses form connections onto the dendritic arbor of a neuron, synaptic plasticity is fundamentally a dendritic phenomenon. Dendrites integrate incoming information to elicit local dendritic spikes that help trigger plasticity mechanisms. However, synapses far from the soma may be relatively more isolated from axonally initiated action potentials which are important—although not always required—for long-term synaptic plasticity. As the excitability of dendritic arbors is itself plastic, synaptic plasticity must be coordinated with dendritic plasticity for control of neuronal computations and neural circuit functions.

Introduction

Neurons in the brain are connected via synapses, which convey information and carry out computations. Synapses are dynamically modified by changes in patterns of activity, on both short and long time scales ranging from milliseconds to days and weeks. Such activity-dependent synaptic plasticity is thought to underlie many important phenomena, including circuit refinement during development (Katz and Shatz, 1996; Cline, 1998; Crair, 1999; Zhang and Poo, 2001; Knudsen, 2002; Feldman and Brecht, 2005; Hensch, 2005), learning and memory (Bliss and Collingridge, 1993; Stevens, 1998; Malenka and Nicoll, 1999; Abbott and Nelson, 2000; Maren, 2001, 2005; Sjöström and Nelson, 2002; Lynch, 2004; Kim and Linden, 2007), and perhaps other cognitive functions related to adapting neural systems to learned associations, environmental statistics, and reward contingencies.

Importantly, most excitatory synaptic inputs are formed upon the dendrites rather than the soma or the axon of a neuron. This means synaptic plasticity is intimately connected with dendritic properties, such as their ion channel densities and their morphologies (London and Häusser, 2005; Sjöström et al., 2008). As first described by Ramón y Cajal (1894), dendritic morphology defines neuronal type and is often used to classify cells (Harvey and Svoboda, 2007; Parekh and Ascoli, 2013; Blackman et al., 2014; Ferreira et al., 2014).

Synaptic plasticity is determined by pre- and postsynaptic activity patterns. As the properties of the dendritic arbor govern postsynaptic activity, it follows that dendrites must shape synaptic plasticity. In other words, the type of dendritic excitability associated with a given synapse must partially determine plasticity at that synapse. Likewise, the location of an input in the dendritic arbor will influence plasticity, because electrical and chemical signals are transformed by the dendrite.

The properties of dendrites can be modified by changes in state or activity. Dendritic excitability can be rapidly adjusted via neuromodulation. For example, β -adrenergic, cholinergic, and dopaminergic receptors can modulate the propagation of action potentials (APs) in dendrites (see the section “Modulation of backpropagating action potentials”) (Hoffman and Johnston, 1999). In addition, forms of dendritic plasticity can be induced by changes in activity patterns. This activity-dependent regulation of dendritic excitability means that synaptic plasticity shapes dendritic excitability, just as dendritic excitability affects synaptic plasticity. Synaptic and dendritic computations are thus intimately linked to each other. An activity-dependent loop of reciprocal interactions between synaptic and dendritic forms of plasticity is thus created via a complex set of interdependences (Sjöström et al., 2008).

In this chapter, we begin with a brief historical perspective, after which we review the basic properties and key mechanisms of synaptic plasticity. We then describe how dendrites shape and control synaptic plasticity, and how plasticity in turn can alter the properties of dendrites. We thus aim to synthesize experimental and theoretical results that are relevant to the role of dendrites in plasticity.

Plasticity: a historical background

Hebb's theory of learning and memory

In his well-known 1949 book, *The Organization of Behavior*, Canadian neuropsychologist Donald Hebb argued that learning and memory in the brain could be achieved by strengthening of already existing connections in assemblies of interconnected cells when these neurons are repeatedly and persistently activated simultaneously. Specifically, for a pair of neurons A and B that are connected, he postulated that (Hebb, 1949):

When an axon of cell A is near enough to excite B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased.

This postulated mechanism could help explain how neurons could be wired together in an assembly as a result of past experience, thereby coding associations in the connective strengths amongst repeatedly coactivated neurons. Hebb argued that such assemblies could represent percepts and could form a substrate for cognition. This view thus requires closed loops and re-entrant paths in neuronal circuits that can sustain reverberating activity over a period of time, where these activity patterns represent the stimulus that triggered it in the first place. If the stimulus is noisy or only partially complete, the assembly could fill in and reliably recall the full percept by ensuring, via reciprocal connectivity, that the full assembly of cells is activated. The assembly can also be intrinsically excited in the brain in the absence of the sensory stimulus that originally brought about its existence by way of Hebbian plasticity, which would represent thought instead of perception.

The Hebbian postulate is succinct and carefully formulated, which is probably one reason why it is so widely cited. It is also directly testable by experiment. Even so, it is important to appreciate that Hebb was not the first to argue that a mechanism of this kind could underpin learning in the brain (for a review see Markram et al., 2011).

Several key features of synaptic plasticity are implied by Hebb's postulate. First of all, the pre-synaptic cell A is active before the postsynaptic cell B, since cell A is in part responsible for spike generation by cell B. Second, cell A does not act alone in evoking the firing of cell B. Rather, it takes part in firing it, which indicates the need for cooperation among inputs. Third, potentiation is specific to active synapses only, so inactive synapses should be unaffected, a concept that is known as

input specificity. Fourth, Hebbian plasticity is intrinsically unstable, because synchronous activity results in synaptic growth, which only results in even tighter synchrony, thus resulting in a positive feedback loop. Finally, Hebb's postulate makes no distinction between synapses at different locations in the dendritic arbor, but treats them all equivalently. Today, we know that the location of a synapse in the dendritic tree has important implications for plasticity, because cable filtering and active conductances in the dendritic arbor shape electrical signals as they propagate to and from the synapse (Rall and Shepherd, 1968; Rall and Segev, 1987) (see Chapters 14 and 15). As we detail in this chapter, dendrites thus considerably increase the computational repertoire of neurons.

The discovery of long-term potentiation and depression

Approximately two decades after Hebb published his postulate, the first experimental discoveries were found to support his conjecture that synapses should be strengthened if they are repeatedly involved in driving a cell. Using high-frequency electrical stimulation in the dentate gyrus of the rabbit hippocampus, Bliss and Lømo found that evoked responses would grow and remain at elevated levels (Lømo, 1966; Bliss and Lømo, 1970, 1973), thereby outlasting other previously described phenomena such as short-term facilitation or post-tetanic potentiation. Here, consistency with Hebb's postulate was assumed because high-frequency stimulation should drive postsynaptic cells, thus explaining why it would lead to persistent growth of the response amplitude. A few years later Douglas and Goddard (1975) named this phenomenon long-term potentiation (LTP), and subsequently revealed that LTP exhibits cooperativity as anticipated by Hebb (see Fig. 18.1 and McNaughton et al., 1978).

Long-term depression (LTD) is also required for information storage in neuronal networks since this process can act together with LTP to achieve stability of the system (Sejnowski, 1977; Willshaw and Dayan, 1990; Malenka, 1993). The first studies reported heterosynaptic depression, i.e., synaptic weakening occurred at inactive inputs when synapses activated at high frequency underwent LTP (Lynch et al., 1977; Levy and Steward, 1979). The most commonly used protocol for homosynaptic LTD was first discovered in the hippocampus (Dudek and Bear, 1992; Mulkey and Malenka, 1992). This LTD paradigm requires 1-Hz stimulation for 15 minutes, which some have argued is not necessarily biologically plausible (Perrett et al., 2001). Nevertheless, the concept of frequency-dependent plasticity emerged from these studies: high-frequency stimulation elicited LTP, whereas low-frequency stimulation promoted LTD, and this was true for brain regions as different as the neocortex and the hippocampus (Kirkwood et al., 1993).

A brief history of timing in synaptic plasticity

One could argue that associative learning requires proper timing of pre- and postsynaptic activity, because association is closely related to coincidence in time. Even so, most early experimental studies did not investigate timing in plasticity. Levy and Steward (1983) carried out one of the first studies that directly varied relative timing in plasticity experiments. They elicited a train of strong stimuli in one pathway before or after a train of weak stimuli in another pathway, and found a marked temporal asymmetry: strong-before-weak stimulation resulted in LTD in the weak input, whereas weak-before-strong activation evoked LTP in the weak input. Although these authors only explored differently timed trains of activity and not individual spikes, this represented an important conceptual step toward exploring and understanding the role of spike timing in plasticity.

Simultaneous somatic and dendritic recordings (Stuart and Sakmann, 1994) have shown that somatically evoked APs backpropagate into the dendrites of neocortical layer 5 (L5) pyramidal cells, which could also be visualized using calcium imaging (Markram and Sakmann, 1994). This

backpropagating action potential (bAP) is thus perfectly suited to act as an associative signal for plasticity at active synapses, triggering either LTD (Debanne et al., 1994) or LTP (Magee and Johnston, 1997) depending on factors such as timing and rate (Sjöström et al., 2001).

Markram et al. (1997) later showed that plasticity was sensitive to the precise timing of individual APs in connected pairs of neocortical L5 pyramidal neurons, by demonstrating that LTP was triggered by the presynaptic cell spiking 10 ms before the postsynaptic cell, whereas the opposite ordering yielded LTD. Only bidirectionally connected pairs were used to demonstrate this key result, so what was pre-before-post for the synapse onto one cell was post-before-pre for the reciprocal synapse onto the other cell, thus elegantly showing that relative timing alone determined the sign of plasticity.

Around the same time, work in cultured hippocampal neurons (Bi and Poo, 1998) and *Xenopus laevis* tadpole optic tectum (Zhang et al., 1998) showed that the timing sensitivity of synaptic plasticity was acute, with a difference of a few milliseconds resulting in either LTP or LTD. These studies also mapped out in higher resolution the relation between spike timing and synaptic plasticity, showing that a timing difference of more than 50–100 ms between pre- and postsynaptic activity resulted in no plasticity, with a hallmark temporal asymmetry (see Fig. 18.2). Later, in a theoretical study by Song et al. (2000), this type of phenomenology was named spike-timing-dependent plasticity (STDP). Importantly, both rate-dependent and timing-dependent forms of long-term synaptic plasticity can be observed at the same synapses (Sjöström et al., 2001; Froemke et al., 2006), presumably because they rely on similar underlying mechanisms.

Key properties of classical LTP

The discovery by Bliss and Lømo (1973) of LTP in the hippocampal dentate gyrus of the rabbit led to a large number of studies exploring this phenomenon in the hippocampus as well as in other brain regions (Malenka and Nicoll, 1999). Because the vast majority of these studies were done on Schaffer collateral and commissural inputs from CA3 onto CA1 neurons in the hippocampus, we call this form of plasticity classical LTP and we describe its key features here.

Synapse specificity and the NMDA receptor

To a first approximation, LTP is synapse specific, which means that only the activated synapses are potentiated (Brown et al., 1990; Bliss and Collingridge, 1993; Malenka and Nicoll, 1999). This property is important for achieving optimal information storage capacity in a neuron (Malenka and Nicoll, 1999), and is in addition implied by the Hebbian postulate. Mechanistically, the input specificity of LTP can be explained by its dependence on NMDA-receptor-mediated Ca^{2+} influx (see the section “Key triggers of synaptic plasticity”) (Malenka et al., 1988; Lisman, 1989; Bliss and Collingridge, 1993). The NMDA receptor dependence may additionally help explain the need for high-frequency stimulation, which is more likely to occur in high-frequency activity paradigms (Sjöström and Nelson, 2002).

Early studies would typically use a control pathway to ensure stability of recordings, which would as a side effect demonstrate input specificity (e.g., Bliss and Lømo, 1973; Lynch et al., 1977; Andersen et al., 1977; McNaughton et al., 1978). However, even input specificity shown with such relatively crude tools may break down under certain circumstances (e.g., Hoffman et al., 2002). With more refined tools, it has become clear that synapse specificity breaks down over short distances. In hippocampal organotypic slice cultures, potentiation is smeared out across inputs adjoining the activated input, at least for synapses on the same dendritic branch separated by a distance of less

than 70 μm (Engert and Bonhoeffer, 1997). But such spreading of LTP can also occur across neighboring hippocampal neurons separated by 100 μm or less by way of the diffusible messenger nitric oxide (Schuman and Madison, 1994), presumably via presynaptically expressed plasticity. A lack of synapse specificity is thus not exclusively due to dendritic mechanisms. Similar results have been found in the developing *Xenopus* optic tectum, although here the degree of specificity depends on the age of the animal: as dendritic Ca^{2+} signals become compartmentalized with maturation, synapse specificity may improve (Tao et al., 2001).

Synapse specificity can also break down during the induction of plasticity. Using two-photon uncaging in hippocampal slices, it was shown that induction of plasticity at a given spine would lower the threshold for subsequent plasticity at nearby synapses located approximately 10 μm distant (Harvey and Svoboda, 2007). Such crosstalk between synapses can be mediated by the spread of Ca^{2+} -activated Ras-ERK signaling to neighboring spines (Harvey et al., 2008). Another mechanism is synaptic tagging, by which synaptically triggered protein translation results in LTP of tagged synapses (Schuman, 1997; Frey and Morris, 1998; Govindarajan et al., 2011). The crosstalk could thus arise by weakly stimulated synapses hijacking the tag of a strongly stimulated neighboring synapse to enable their own potentiation under circumstances that would otherwise not permit LTP.

LTP is cooperative and associative

Classically, LTP has cooperative and associative properties (McNaughton et al., 1978; Levy and Steward, 1979; Barionuevo and Brown, 1983; Zalutsky and Nicoll, 1992; Kirkwood and Bear, 1994; Debanne et al., 1996). Cooperativity was first described by McNaughton et al. (1978), and denotes the observation that individual inputs on their own are too weak to reach the threshold for LTP induction, even with high-frequency stimulation, but a sufficient number of inputs that cooperate will potentiate (Fig. 18.1). This need for cooperativity is implied in Hebb's postulate, since the connection from cell A to cell B should be strengthened if cell A "takes part in firing" cell B (Hebb, 1949), which is important for the creation of Hebbian assemblies by wiring cells together in sets that code for the same percept. In agreement, Shatz (1992) coined the phrase "cells that fire together wire together" to illustrate how presynaptic neurons cooperate as visual circuits learn about percepts.

Associativity is often used in the classical literature to denote the fact that with two electrodes—one set to strong stimulation to recruit many input fibers so as to surpass the threshold for LTP, and the other set to weak levels below the plasticity threshold—the weak input will potentiate if stimulated simultaneously with the strong input (Bliss and Collingridge, 1993; Malenka, 2003). Both associativity and cooperativity thus denote the same underlying fundamental phenomenology and theoretical concept, which tends to make their usage confusing and inconsistent. In fact, it has been argued that any difference between associativity and cooperativity is semantic (McNaughton, 2003). Nevertheless, these two important concepts are thought to enable learning in the brain in a way that is reminiscent of behavioral associative learning (Rescorla, 1988).

Mechanistically, it has been argued that cooperativity and associativity can be explained by the need for Ca^{2+} influx through NMDA receptors to trigger LTP (e.g., Bliss and Collingridge, 1993; Malenka, 2003). Only when NMDA receptors are sufficiently depolarized by a large enough number of activated inputs will they open to elicit the strong Ca^{2+} signals that are needed to trigger LTP. In this elegant view, the properties of the NMDA receptor alone set the threshold for LTP, which can explain several key concepts in synaptic plasticity, including synapse specificity, frequency dependence, and cooperativity/associativity.

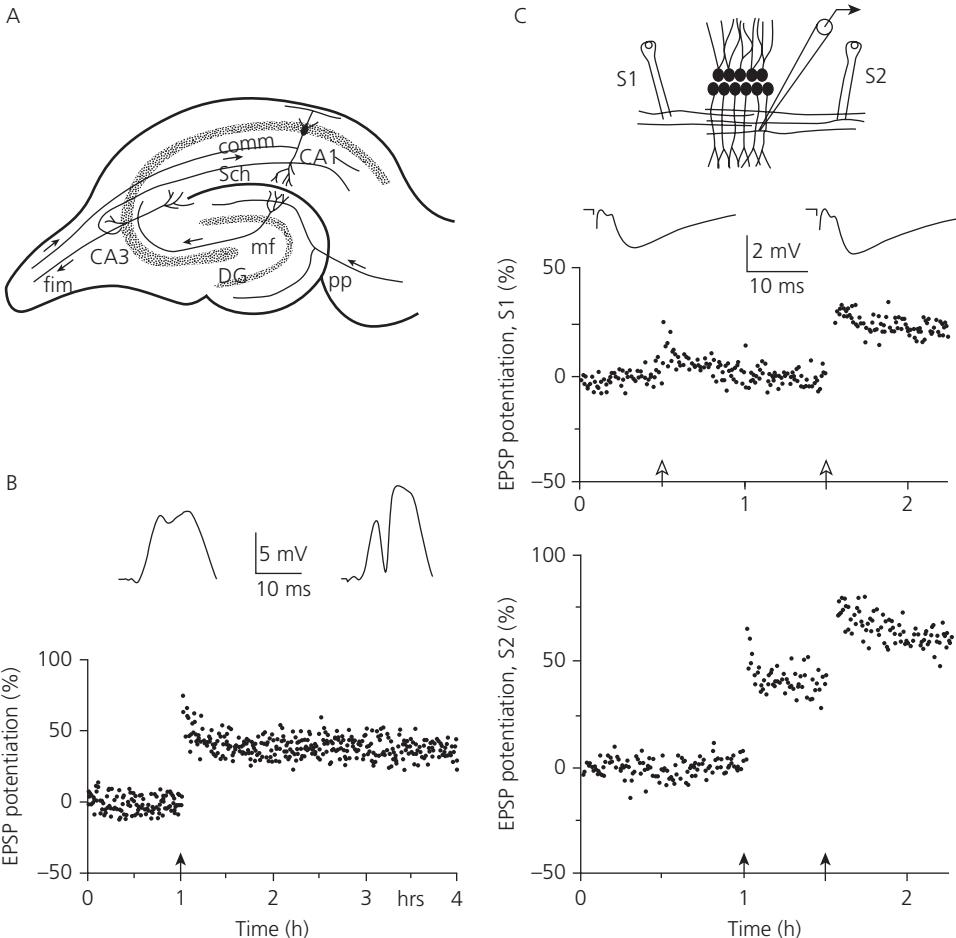


Fig. 18.1 Classical LTP exhibits cooperativity, associativity, input specificity, and E-S potentiation. **(A)** Schematic of the hippocampus showing the cornu ammonis regions 1 and 3 (CA1, CA3) and the dentate gyrus (DG) as well as the perforant path (pp), the mossy fibers (mf), the fimbria (fim), the Schaeffer collaterals (Sch), and the commissural fibers (comm). **(B)** Dentate gyrus field responses before (top left) and after (top right) induction of LTP by 200-ms-long 250-Hz tetanization of the perforant path *in vivo*. The increased slope of the upward stroke indicates the growth of the population synaptic response, whereas the increased downward population spike response reflects a combination of LTP and an increase in postsynaptic excitability, termed E-S potentiation (Zhang and Linden, 2003). The population EPSP time course (bottom) shows a stable baseline before LTP induction (arrow) followed by a rapid synaptic strengthening after the tetanus. **(C)** This experiment involves a weak (S1) and a strong pathway (S2) stimulated in parallel on alternating trials (schematic inset top and time course plots below). When the weak S1 pathway is tetanized (left open arrow), it does not potentiate because it is not strong enough to elicit LTP. However, when the strong S2 pathway is tetanized (left closed arrow), its responses grow because a sufficient number of afferent fibers were recruited by the stronger stimulation strength, thus demonstrating the cooperativity requirement of LTP. At the same time, note that the weak S1 pathway does not potentiate, showing the input specificity of LTP. When both the weak S1 and the strong S2 pathways are tetanized simultaneously (open and closed arrows to the right), the responses of both pathways increase, which illustrates the associative properties of classical LTP.

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Cooperativity can, however, arise in many different ways, notably via dendritic mechanisms (see Sjöström and Nelson, 2002; Sjöström et al., 2008). Perhaps counterintuitively, even in the presence of suprathreshold postsynaptic spiking LTP requires sufficiently strong subthreshold depolarization (Sjöström et al., 2001). The need for cooperativity could also arise from a requirement for local dendritic spikes in plasticity. A well-established example of local dendritic spikes is based on the NMDA receptor, which when bound to glutamate in large numbers temporarily becomes a voltage-gated ion channel (Schiller et al., 1998, 2000; Schiller and Schiller, 2001). Alternatively, local spikes can be based on a combination of NMDA receptors and voltage-gated Ca^{2+} channels (Wei et al., 2001). However, these local dendritic spikes may in some circumstances trigger LTD rather than LTP (Holthoff et al., 2004)—although recent *in vivo* evidence favors LTP as the outcome of local dendritic plateau potentials (Gambino et al., 2014). These candidate dendritic mechanisms for cooperativity will be discussed in greater detail later (see the section “Are back-propagating action potentials needed for plasticity?” and subsequent text).

Cooperativity can also rely on downstream signaling mechanisms. In mouse hippocampal CA1 fast-spiking interneurons, sufficient activation of Ca^{2+} -permeable alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors triggers calcium-induced release of calcium from internal stores, which in turns elicits LTD (Camire and Topolnik, 2014). Intriguingly, this form of cooperativity requires not only a sufficient number of inputs but also needs them to be located sufficiently close to each other along the dendrite.

Forms of cooperativity such these (Schiller et al., 2000; Gambino et al., 2014) that rely on local dendritic properties are particularly interesting because they promote dendritic clustering of synapses that are closely related in terms of information content (Larkum and Nevian, 2008). This view on plasticity is a departure from the more standard Hebbian and connectionist take, and provides neurons with tremendously increased computational power, since individual dendritic branches can be seen as distinct computational elements (Poirazi and Mel, 2001).

LTP is unstable

LTP is nominally a positive feedback process that can destabilize neural circuits. This is because correlated activity in connected cells causes these connections to grow stronger, which in turn promotes correlated activity, and so on. Other mechanisms are therefore required to control the potential runaway excitation due to this positive feedback (Watt and Desai, 2010). There are several such stabilizing mechanisms that have been discovered. One of the most important was discovered by Gina Turrigiano and colleagues—this is a negative-feedback mechanism that homeostatically scales synaptic strengths up or down to keep overall activity levels within bounds (Turrigiano, 1999, 2008; Turrigiano and Nelson, 2000, 2004). There are several other stability-promoting mechanisms that we will mention later, including heterosynaptic LTD (Lynch et al., 1977), metaplasticity (Bienenstock et al., 1982; Bear et al., 1987), short-term depression (Abbott et al., 1997), and plasticity of inhibitory synapses (Lamsa et al., 2010).

The longevity of LTP

By definition, LTP is long lasting, which intuitively makes good sense since the purpose of LTP is to store information for long periods of time. It should be noted, however, that certain theoretical treatments of memory mechanisms require synaptic weights to continuously adjust as new information is stored (Abraham and Robins, 2005), which is in keeping with some experimental findings that plasticity tends to reverse and be constantly adjusted by on-going activity (Zhou and Poo, 2004). Nevertheless, the persistence of synaptic weights is an integral part of several memory

theories, notably that of Hebb (1949). So with the disclaimer that LTP may under some circumstances not last for long periods (Abraham, 2003; Zhou and Poo, 2004), it is widely believed LTP does and should persist for up to days, weeks, and maybe even months (Staubli and Lynch, 1987; Abraham et al., 2002). In fact, Bliss and colleagues monitored LTP for 10 hours in anesthetized animals and for 3 days in awake animals (Bliss et al., 1973; Bliss and Lømo, 1973).

LTP exhibits two or possibly more phases, known as early and late LTP (Bliss and Collingridge, 1993; Kandel, 2001; Abraham, 2003; Reymann and Frey, 2007). Typically, the early phase lasts between 1 and 3 hours and does not require protein synthesis. The late phase of LTP, however, persists for at least a day and requires both translation and transcription (Kandel, 2001; Reymann and Frey, 2007). As was discussed by Vlatkovic and Schuman in Chapter 5, there is evidence showing that LTP-related translation may occur in dendrites (Sutton and Schuman, 2006), close to or even in the spine (Ostroff et al., 2002; Tanaka et al., 2008). Here, dendrites may serve an important role in compartmentalizing the molecular machinery that controls plasticity (see the section “Biochemical compartmentalization”).

LTP is accompanied by changes in excitability

Plasticity is a property of many systems and biological structures beyond synapses. Regulation of information processing in the brain can also be achieved by persistent regulation of ionic conductances in different specialized neuronal compartment such as dendrites, axon, and soma. Neuronal intrinsic excitability is defined by the properties and the subcellular distribution of ion channels. The degree of excitability will shape the capacity of a neuron to generate an output signal, namely the AP, from a specified input signal (usually the postsynaptic potential). As voltage-gated ion channels are scattered in spines, dendrites, and axon (Nimchinsky et al., 2002), their modulation is likely to affect the shape of the amplitude of the excitatory postsynaptic potential (EPSP) and its propagation (Reyes, 2001), and consequently change the input-output function of the neuron. Lasting activity-dependent changes in overall intrinsic excitability have been documented in several areas of the brain (Aizenman and Linden, 2000; Daoudal et al., 2002; Cudmore and Turrigiano, 2004). For example, in deep cerebellar nuclei and granule cells it was found that somatic current injection-induced spiking was persistently increased after brief application of 100-Hz tetanization trains to the nearby white matter (Aizenman and Linden, 2000; Armano et al., 2000). This form of plasticity was dependent on NMDA receptor activation, relied on postsynaptic Ca^{2+} elevation, and could be induced with a brief postsynaptic stimulation without any synaptic stimulation (Aizenman and Linden, 2000; Armano et al., 2000). Other mechanisms dependent on Ca^{2+} influx and protein kinase A activation are also responsible for persistent changes in synaptic plasticity in L5 pyramidal cells (Cudmore and Turrigiano, 2004). On the other hand, hyperpolarization or recruitment of inhibitory synaptic stimulation can also lead to a persistent increase in excitability in some cells (Nelson et al., 2003). This opposite regulation is probably needed for differential regulation of neuronal networks.

While most of these studies have tried to infer local changes in dendritic branches from single electrode dendritic or somatic recordings, there is some direct evidence for local dendritic changes in excitability (Aizenman et al., 2003; Smith and Otis, 2003; Fan et al., 2005; Sutton et al., 2006). Kim et al. (2007) showed that LTP in CA1 neurons induced endocytosis of the A-type K^+ channel (I_A) subunit $\text{K}_v4.2$ through a rapid clathrin-mediated mechanism. Interestingly, I_A was exclusively removed from spines that had experienced synaptic stimulation during LTP induction, making the internalization of $\text{K}_v4.2$ subunits seemingly synapse specific (Kim et al., 2007). This study demonstrates that very restricted regulation of dendritic compartment excitability can occur.

Although the NMDA receptor seems to be preferentially linked to synaptic plasticity, these receptors can also participate in long-lasting plasticity of neuronal excitability (Wang et al., 2003). Bliss and Lømo (1973) found that in parallel with LTP of field EPSPs, high-frequency stimulation also induced an increase in discharge probability to a given synaptic input. Since this increases the probability that an EPSP will trigger a spike, it has been termed EPSP-spike potentiation (E-S potentiation). Interestingly, changes in E-S coupling can be bidirectional, and a reduction may occur upon the induction of LTD (Daoudal et al., 2002). E-S plasticity has important implications for information processing since it can rapidly modulate the input–output relationship of a neuron, and may result from changes in dendritic excitability (see the section “Plasticity of dendritic excitability”) (Frick et al., 2004).

Spike-timing-dependent plasticity

Critically for studies of dendritic integration and input–output coupling, LTP and LTD can be induced by repetitively pairing pre- and postsynaptic APs, where the precise spike timing determines the sign and magnitude of synaptic modification. This paradigm or protocol for inducing LTP or LTD is known as STDP. When experimentally pairing an EPSP with an AP, one can typically observe LTP if the presynaptic cell fires an AP a few milliseconds before the postsynaptic cell, whereas the opposite temporal order results in LTD (Fig. 18.2) (Abbott and Nelson, 2000; Caporale and Dan, 2008; Buchanan and Mellor, 2010; Feldman, 2012). At most synapses these timing requirements are roughly within 10–100 ms. It is worth noting that pre- before postsynaptic LTP is consistent with the Hebbian postulate (Hebb, 1949) whereas timing-dependent LTD is an extension to Hebb’s theory. The rapid, and from a biological view surprisingly sharp, 1-ms transition between LTP and LTD (Fig. 18.2; Zhang et al., 1998)—which has been observed in neocortex with great temporal acuity (Celikel et al., 2004)—can be considered a hallmark feature of STDP. This sharp temporal transition in STDP can, under some circumstances, be smeared out by burst spiking (Kampa et al., 2006; Nevian and Sakmann, 2006) and by temporal noise in the form of spike jitter (Pawlak et al., 2013), giving the impression of a graded switch between LTP and LTD that lasts for many milliseconds. Because EPSPs cooperate via dendritic integration to generate postsynaptic spikes, and because bAPs provide an associational signal for synaptic plasticity, there is a close relationship between STDP and studies of dendritic properties, including their biophysics, structure, and electrogenesis.

STDP has been observed in its above-mentioned classical form in a variety of brain regions, including L2/3 and L5 of the neocortex and the hippocampus, striatum, and amygdala among others (Abbott and Nelson, 2000; Sjöström et al., 2008; Feldman, 2012). Different synapses may have different timing and mechanistic requirements for induction of STDP. In the electric lobe of the mormyrid weakly electric fish, for example, the timing requirements of STDP are inverted, with pre- leading postsynaptic firing resulting in LTD and the opposite ordering evoking LTP (Bell et al., 1997; Han et al., 2000). Similar inverted forms of STDP—sometimes called anti-STDP or anti-Hebbian STDP—have also been found at inhibitory connections onto neocortical L2/3 pyramidal neurons (Holmgren and Zilberman, 2001), at excitatory corticostriatal synapses (Fino et al., 2005), and in the cerebellum (Wang et al., 2000). Similarly, anticorrelated activity produces strengthening of excitatory synapses onto specific subsets of hippocampal interneurons (Lamsa et al., 2007; Camire and Topolnik, 2014), although it is still unclear how this form of plasticity relates to STDP since the precise timing requirements have not yet been fully explored. At neocortical L4 spiny stellate cell synapses, timing-dependent LTD is found within a narrow 40-ms wide window regardless of temporal ordering (Egger et al., 1999), which may help reduce cortical feed-forward amplification of thalamocortical inputs.

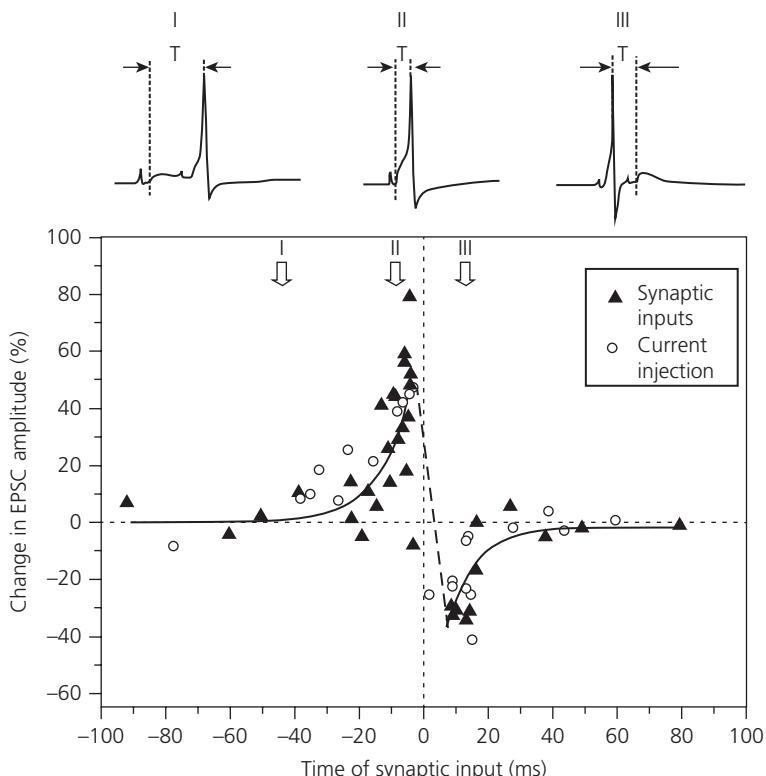


Fig. 18.2 In STDP, synaptic potentiation and depression of a weak subthreshold input depends critically on its millisecond timing relative to a strong suprathreshold input. This illustrates the associative properties of STDP. The plasticity of retinotectal inputs to tectal cells was studied *in vivo* in *Xenopus laevis* tadpoles, developmental stage 40–41. In each experiment, one suprathreshold and one subthreshold input were stimulated repetitively at 1 Hz for 100 s, while the relative timing between the two inputs was varied across experiments (see x-axis and inset top). The y-axis shows the relative percentage change in the strength of the weak input only. Open circles denote experiments in which postsynaptic spiking of the tectal cell was produced by current injection instead of a strong retinotectal input.

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STDP typically shares several key features with classical LTP (see the section “Key properties of classical LTP”): STDP is dependent upon NMDA receptor activation (Markram et al., 1997; Bi and Poo, 1998; Feldman, 2000), it is frequency dependent (Sjöström et al., 2001; Froemke et al., 2006), and it exhibits cooperativity (Sjöström et al., 2001) as well as associativity (Zhang et al., 1998). Whether or not STDP is intrinsically stable depends on its properties as well as on overall firing rates and will be discussed in more detail in the section “STDP may help stabilize synaptic weights in the dendritic tree”. Although it has not been formally established that STDP persists for days and weeks, several studies indirectly support longevity on the order of hours (e.g., Schuett et al., 2001;

Wolters et al., 2005; Young et al., 2007). Much like classical LTP, STDP can be reversed by ongoing activity (Zhou et al., 2003). However, as discussed below, STDP does have some interesting features that are quite distinct from those of classical LTP.

Associative STDP may enable dendritic synaptic competition

In the STDP paradigm, it has been argued that a sufficient condition for associativity is provided by the postsynaptic AP (Debanne et al., 1994, 1996; Zhang et al., 1998), rather than solely via activation of the NMDA receptor, as in the case of classical LTP (Bliss and Collingridge, 1993; Malenka, 2003). For example, in the *Xenopus* tadpole tectum, subthreshold weak retinotectal inputs will potentiate or depress depending on their millisecond timing relative to an input that is suprathreshold with respect to the postsynaptic AP (Zhang et al., 1998). These are, in other words, cases of associative LTP and LTD, respectively. Interestingly, this form of plasticity also enables timing-dependent competition among inputs, so that inputs activated early will win out compared with those activated later, in the sense that early predictive inputs will tend to potentiate and the late “postdictive” inputs will tend to weaken. Computer simulations have shown that such timing-dependent competition among inputs will tend to shorten response latencies and shorten their durations, because early inputs will eventually take over and predominantly drive the postsynaptic cell (Song et al., 2000).

More important, however, is probably the fact that competition among afferents is essential for ocular dominance plasticity (Wiesel and Hubel, 1965; Gandhi et al., 2008; Espinosa and Stryker, 2012). With standard Hebbian correlation-based learning, synaptic competition is not intrinsic but has to be added by way of other mechanisms such as heterosynaptic LTD, synaptic scaling, or the Bienenstock, Cooper, and Munro (BCM) rule (Bienenstock et al., 1982; Miller and MacKay, 1994; Miller, 1996; Turrigiano, 2008). What distinguishes STDP from a classical rate-based LTP in this regard is thus the existence of the timing-dependent LTD window, which enables the synaptic competition that is inherent to STDP (Miller and MacKay, 1994; Song et al., 2000). Paradoxically, this temporal window for spike-timing-dependent LTD does not actually require postsynaptic spiking in neocortical L5 pyramidal cells—repeated strong subthreshold postsynaptic depolarization tens of milliseconds before activation of an input is sufficient for induction of LTD (Sjöström et al., 2004). This raises the interesting possibility that timing-dependent plasticity could be achieved locally in the dendrites of neurons completely in the absence of postsynaptic spiking (Goldberg et al., 2002). Such dendritic STDP has in fact been found in neocortical L5 pyramidal cells (Gordon et al., 2006). Therefore axonal spiking output is not necessary for timing-dependent plasticity (see the section “The role of local dendritic spikes in plasticity”) (Lisman and Spruston, 2005, 2010), but cellular information storage can be achieved solely via associative interactions mediated by local dendritic spikes (Kim et al., 2015).

Cooperativity in STDP depends on dendrite excitability

Given that, in STDP, inputs that are activated a few milliseconds before the postsynaptic spike will potentiate (Fig. 18.2), it may be surprising that timing-dependent LTP still exhibits a cooperativity requirement that remains even in the presence of postsynaptic spiking (Sjöström et al., 2001). This cooperativity requirement can be explained by the need for boosting bAPs that would otherwise fail as they travel decrementally into dendritic arbors, where the boosting can be provided via sufficient dendritic subthreshold depolarization by the synaptic inputs themselves (Sjöström and Häusser, 2006). In the absence of boosting, failing bAPs may not provide sufficient depolarization to unblock glutamate-bound NMDA receptors to trigger LTP (Sjöström et al., 2008).

Mechanistically, boosting of bAPs by depolarization may result from more efficient recruitment of fast sodium channels by a preceding subthreshold depolarization (Stuart and Häusser, 2001), or by an equivalent inactivation of I_A that would otherwise contribute to failure of the bAP (Hoffman et al., 1997; Migliore et al., 1999). We will discuss boosting of bAPs in greater detail in the section “Modulation of backpropagating action potentials”.

STDP may help stabilize synaptic weights in the dendritic tree

Although classical LTP is inherently unstable, STDP is on the contrary inherently stable, at least under some circumstances. This is because the temporal window for LTD tends to be larger than that of LTP (Feldman, 2000; Sjöström et al., 2001), which means that with linearly summing spike pair interactions, high-frequency firing naturally tends to promote LTD at the expense of LTP (Abbott and Nelson, 2000). Conversely, low-frequency firing would tend to favor LTP instead of LTD, since inputs that drive postsynaptic spiking would be in the causal temporal ordering that results in potentiation (Abbott and Nelson, 2000). Generally, linearly summing STDP thus has a built-in tendency to keep activity levels within reasonable bounds. In addition, high levels of postsynaptic activity can also dynamically broaden the timing window of LTD, which thus further increases the likelihood of synaptic weakening during periods of high levels of spiking (Debanne et al., 1994; Sjöström et al., 2003).

Unfortunately, spike pairings in STDP do not always summate linearly, which means STDP does have prominent frequency dependence, with LTP dominating at high frequencies and a relatively larger propensity for LTD at low rates (Sjöström et al., 2001; Froemke et al., 2006). This frequency dependence may ruin the intrinsic stability of STDP if activity levels are driven beyond a critical frequency (Tsodyks, 2002).

Nevertheless, because STDP is critically determined by the timing of the postsynaptic spike as it backpropagates into the dendrite, the intrinsic stability of STDP suggests that dendritic levels of activation may help keep a neuron stable. As a corollary, the reliability of AP backpropagation in an individual cell may thus be important for ensuring stability (Sjöström et al., 2008). In hippocampal CA1 neurons, synaptic weights are on average scaled with distance so that distal ones tend to be stronger than proximal ones, with the net result that the typical efficacy at the soma is similar for distal and proximal inputs (Magee and Cook, 2000) (although see Williams and Stuart, 2002). Such dendritic democracy of synaptic strength (Häusser, 2001) could in fact arise due to specific inverted forms of STDP (Rumsey and Abbott, 2004, 2006), highlighting the importance of the bAP in balancing the weights of a neuron. Indeed, it has been pointed out that LTP caused by local dendritic spikes—in the absence of spiking output and of bAPs—may pose a particularly difficult balancing problem for a neuron with prominent dendritic arborization, as it would tend to destabilize the weights in the dendritic tree (Goldberg et al., 2002). Additional research is required before we understand how dendritic-spike-dependent LTP can be kept in check to ensure balancing of synaptic weights in the arborizations. Presumably, specific local negative-feedback mechanisms are required (Goldberg et al., 2002).

STDP depends on rate and on dendritic location

STDP has some very interesting higher-order complexities when APs are occurring at frequencies high enough to permit spike triplet or quadruplet interactions, which can lead to LTD or LTP for conditions that could perhaps at a first blush seem less than immediately obvious (Froemke and Dan, 2002; Pfister and Gerstner, 2006; Clopath et al., 2010). For example, in short triplet bursts

where postsynaptic spiking leads presynaptic spiking, LTD is induced, even if the overall set of spike timings would be expected to produce LTP (Froemke and Dan, 2002). This suggests that synaptic plasticity may be more sensitive to the specifics of firing onset rather than what happens later in a high-frequency train of spikes, especially at short-term depressing synapses. However, this onset specificity of plasticity breaks down at high firing frequencies where LTP is favored irrespective of timing (Sjöström et al., 2001; Froemke et al., 2006), although it is still unclear when such high firing rates occur in the neocortex *in vivo* (Lee et al., 2006; O'Connor et al., 2010; Wolfe et al., 2010; Barth and Poulet, 2012).

Finally, STDP depends critically on the location of a synapse in the dendritic tree, which can even lead to a complete inversion in the sign of plasticity in some circumstances (Froemke et al., 2005, 2010; Letzkus et al., 2006; Sjöström and Häusser, 2006; Kampa et al., 2007) (see the section “Synaptic plasticity depends on dendritic location”).

Stability-promoting mechanisms

As mentioned earlier, classical LTP is intrinsically unstable, because correlated firing promotes strengthening of synaptic connections, leading to increased correlated firing. This positive-feedback loop can thus result in runaway excitation, so learning in the brain must be counterbalanced by other means to avoid pathological activity states. Fortunately, there are several stability-promoting mechanisms that may overcome this intrinsic instability.

Homeostatic plasticity

Homeostatic plasticity relies on a negative-feedback loop, so that too much or too little activity changes the synaptic gain globally in a neuron to maintain neuronal activity within an optimal range (Turrigiano and Nelson, 2004; Turrigiano, 2007; Watt and Desai, 2010). Although homeostatic plasticity typically operates on a slow 24-hour time scale, these mechanisms can in some circumstances be induced an order of magnitude faster (van Welie et al., 2004, 2006; Frank et al., 2006; Sutton et al., 2006; Ibata et al., 2008). Still, homeostasis must not impede learning mechanisms, which rely on more rapid fluctuations in neuronal activity that carry relevant information (Turrigiano and Nelson, 2004).

Synaptic scaling is an important stability-promoting mechanism that scales synaptic strengths up or down to counteract excessive changes in activity. This homeostatic mechanism has been shown in the mammalian central nervous system and in neocortical cultures (Turrigiano et al., 1998) and was later verified *in vivo* (Desai et al., 2002; Keck et al., 2013). In the visual cortex, synaptic scaling is developmentally regulated as it can only occur in specific layers at specific times, typically during the critical period (Desai et al., 2002; Maffei et al., 2004; Maffei et al., 2006; Maffei and Turrigiano, 2008). Typically, regulation of excitatory synaptic inputs can be mediated by translocation of AMPA (Turrigiano et al., 1998) or NMDA receptors (Watt et al., 2000) in and out of the postsynaptic density. Presynaptic mechanisms leading to the regulation of the release machinery also exist (Bacci et al., 2001; Paradis et al., 2001; Burrone et al., 2002), although presynaptic changes are expected to also alter the dynamics of the scaled synapses, which has knock-on effects for synaptic information transfer (Abbott et al., 1997; Tsodyks and Markram, 1997). Synaptic scaling mechanisms also occur for inhibitory synapses. Adjustments in the relative weight of excitation and inhibition provide a powerful means of stabilizing neural networks (Rutherford et al., 1998). Activity deprivation in the cortex leads to a reduction in the number of GABA receptors clustering at the synapses and to actual reduction in the number of functional inhibitory synapses (Kilman

et al., 2002). Thus, opposing activity-dependent modulation of synaptic scaling of excitatory and inhibitory synapses is crucial for network stability (Rutherford et al., 1997, 1998; Kilman et al., 2002; Liu, 2004; Swanwick et al., 2005; Karmarkar and Buonomano, 2006). The balancing of excitation and inhibition within neuronal networks has also been demonstrated *in vivo* after monocular deprivation (Maffei et al., 2004). As different types of inhibitory inputs may selectively target different dendritic compartments (Markram et al., 2004; Pouille and Scanziani, 2004; Wang et al., 2004; Silberberg and Markram, 2007), it is possible that subregions of the dendritic tree are selectively balanced in such a manner (Goldberg et al., 2002). It was recently shown *in vivo* that synaptic scaling can later be followed by a reduction in inhibition, demonstrating that multiple types of homeostatic mechanism can work together to counterbalance instability (Keck et al., 2013).

The intrinsic excitability of neurons may also change homeostatically to account for activity patterns that are out of reasonable bounds (Desai, 2003; Zhang and Linden, 2003). Theoretical work has demonstrated that activity-dependent changes in intrinsic membrane properties could maintain stability, despite perturbations such as channel turnover and cell growth (LeMasson et al., 1993). Indeed, behavior predicted by this modeling work has been revealed experimentally in cultured lobster stomatogastric ganglion neurons (Turrigiano et al., 1994) as well as in cortical culture (Desai et al., 1999). Interestingly, both synaptic scaling and homeostatic changes in excitability can be localized to specific dendritic regions (see the section “Homeostatic changes in dendritic excitability”) (Pozo and Goda, 2010; Turrigiano, 2011).

Heterosynaptic plasticity

Heterosynaptic plasticity is an additional or alternative mechanism that may act similarly to homeostatic plasticity to control circuit excitability. Heterosynaptic modifications refer to changes that occur at unstimulated inputs after induction of synaptic plasticity at a different set of stimulated inputs that undergo homosynaptic plasticity.

The classical form of heterosynaptic plasticity is heterosynaptic LTD, first observed in hippocampal commissural inputs after induction of LTP with high-frequency stimulation to the Schaffer collaterals (Lynch et al., 1977). The reduction in commissural field potentials occurred simultaneously with potentiation of the Schaffer collateral response, demonstrating that heterosynaptic LTD can occur almost simultaneously with homosynaptic LTP. Royer and Paré (2003) later discovered an intriguing form of heterosynaptic LTP in amygdala, dependent on release of postsynaptic Ca^{2+} from internal stores after induction of homosynaptic LTD. Heterosynaptic changes in intercalated neurons of the amygdala occurred specifically to putative neighboring inputs along the dendrites of these cells, leaving other inputs unaffected. Thus interactions among nearby synapses and perhaps the local dendritic geometry might play important roles in regulating heterosynaptic plasticity.

These changes can effectively reorganize cortical receptive fields and tuning curves *in vivo*. In the adult rat auditory cortex, pairing nucleus basalis stimulation with a tone of a given frequency shifts the peak of the tuning curve toward the paired stimulus (Bakin and Weinberger, 1996; Kilgard and Merzenich, 1998; Froemke et al., 2007). This seems to be due to LTP of excitatory and inhibitory inputs together at the paired frequency, in combination with heterosynaptic LTD induced at the original best frequency within 10 minutes (Froemke et al., 2013). Other inputs were unchanged, leaving the general shapes of tuning curves intact while changing the peak preferences of many neurons within the cortical network.

However, it is important to note that spike-timing-dependent LTD can also lead to similar adjustments of cortical tuning curves. As the LTD time window is generally longer than the LTP window, inputs that are spontaneously active and uncorrelated with postsynaptic spiking will tend

to depress (Song et al., 2000). This would then help to shift tuning curves or compensate for the increases in synaptic strength at inputs correlated with postsynaptic spiking (Schuett et al., 2001; Allen et al., 2003; Celikel et al., 2004; Meliza and Dan, 2006; Jacob et al., 2007).

Metaplasticity

Synaptic plasticity itself is also plastic, so that high activity levels may reduce the overall propensity for LTP in excitatory cells, whereas low activity levels do the opposite (Bienenstock et al., 1982; Bear et al., 1987; Abraham and Bear, 1996). One of the best-known paradigms describing this form of plasticity is the BCM sliding threshold model (Bienenstock et al., 1982). In this scheme, the sign and amplitude of plasticity are determined by the postsynaptic spike rate. Correlated low-frequency activity will result in LTD, whereas LTP occurs at spike frequencies above a threshold level. A critical element of the BCM model is that this threshold is not fixed but is instead determined by the recent history of activity (Bear et al., 1987). When activity levels are high, the threshold for LTP is increased; conversely, when activity is low, the threshold is decreased. The fact that the threshold is sliding is what prevents the system from reaching saturation, and it furthermore enables competition among inputs. This competition feature is critical for explaining the ocular dominance plasticity found by Hubel and Wiesel (Wiesel and Hubel, 1965; reviewed in Espinosa and Stryker, 2012), which cannot be achieved with classical rate-dependent plasticity without a sliding threshold (Miller and MacKay, 1994). By contrast, STDP intrinsically provides competition (Song et al., 2000) (see the section “STDP may help stabilize synaptic weights in the dendritic tree”).

Several studies have found electrophysiological evidence in support of the BCM learning rule, for example in the visual cortex (Kirkwood et al., 1996; Philpot et al., 2003; Sawtell et al., 2003; Philpot et al., 2007) and the hippocampus (Abraham et al., 2001; Chevaleyre and Castillo, 2004; O’Connor et al., 2005). For example, light deprivation induces activity-dependent modifications of synaptic responses in the visual cortex, shifting the threshold toward a global state favorable for LTP induction (Kirkwood et al., 1996). This reduced threshold for LTP induction may be due to a shift in NMDA receptor subunit composition, i.e., a lower NR2A/NR2B ratio (Yashiro and Philpot, 2008).

In its original formulation, the BCM rule is global in the sense that the sliding threshold is regulated by the overall activity of the cell, which in turn affects all synapses equally in the same cell. However, the BCM rule could in principle be local to the dendritic tree (compare, e.g., Graupner and Brunel, 2012). It is also possible to relate the BCM rule to the STDP paradigm (Karmarkar and Buonomano, 2002; Izhikevich and Desai, 2003). Because STDP depends critically on the bAP, which in turn is governed by dendritic properties (see the section “Are backpropagating action potentials needed for plasticity?”) (Sjöström et al., 2008), a formulation of the BCM rule that is dendritically determined can exist. The Ca^{2+} level during induction of plasticity is one possible mediator of such a dendritic BCM rule (see the section “Key triggers of synaptic plasticity”) (Sjöström and Nelson, 2002).

Short-term plasticity as a circuit stabilizer

Short-term plasticity may also help neuronal circuits to self-stabilize. In particular, short-term depression of excitation causes synaptic drive to die off rapidly during high-frequency activation (Abbott et al., 1997). In addition, excitatory inputs may undergo short-term depression faster than some inhibitory synapses do, which during high-frequency activity shifts the balance toward inhibition (Varela et al., 1999; Galarreta and Hestrin, 1998; Blackman et al., 2013). Excitatory inputs onto some inhibitory cell types such as cortical Martinotti cells (Silberberg and Markram, 2007) and hippocampal late-persistent interneurons (Pouille and Scanziani, 2004) also dramatically increases via short-term facilitation when activity levels are high (reviewed in Blackman et al., 2013). Because these interneurons

specifically target the dendrites of excitatory cells, both spiking output and plasticity of pyramidal cells is very efficiently shut down by recruitment of Martinotti cells (Bar-Ilan et al., 2012; Gidon and Segev, 2012). Interestingly, recent results indicate that homeostatic synaptic plasticity in the neocortex may be mediated by genetic programs that specifically promote inhibition onto excitatory neurons while inducing excitation onto somatostatin-positive inhibitory neurons (Spiegel et al., 2014). Since cortical Martinotti cells are well demarcated by somatostatin expression (Wang et al., 2004; Toledo-Rodriguez et al., 2005), this suggests an intriguing link between Martinotti-cell-mediated dendritic inhibition (Silberberg and Markram, 2007) and homeostatic synaptic scaling (Turriagano, 2008).

Inhibitory plasticity

Inhibitory synapses are also plastic, and can be modified by changes in experience or activity (Lamsa et al., 2010; Vogels et al., 2013). This provides another synapse-specific manner by which excitability can be controlled. However, perhaps due to the diversity of interneuron subtypes (Fishell and Rudy, 2011; DeFelipe et al., 2013), it has been difficult thus far to discover basic learning rules for long-term inhibitory plasticity, at least in cortical circuits. *In vivo*, it seems clear that forms of excitatory and inhibitory plasticity are coordinated to balance inhibition with excitation over development and in the adult cortex (Hensch, 2005; Dorrrn et al., 2010; Froemke et al., 2013).

Recent studies in cortical brain slices indicate that there may be complex interactions between excitatory and inhibitory forms of plasticity within the dendrites of the same postsynaptic neuron (Wang and Maffei, 2014). In L5 neurons of mouse auditory cortex, pre- and postsynaptic spike pairing induces LTP of inhibitory inputs regardless of temporal order. This potentiation seems to normalize the ratio between excitation and inhibition, as the magnitude of inhibitory LTP is greater when excitatory events are initially much greater in amplitude than co-evoked inhibitory events (D'Amour and Froemke, 2015). This form of inhibitory plasticity requires NMDA receptors, perhaps as a mechanism to sense the relative strength of excitatory input, for coordination of excitatory–inhibitory balance by correlated spike pairing.

In contrast, Wang and Maffei (2014) found that in L4 star pyramids, the amounts of inhibitory and excitatory plasticity were inversely correlated. In other words, when inhibitory inputs were strongly potentiated by pairing high-frequency bursts of pre- and postsynaptic APs, an excitatory input onto the same postsynaptic cell tended to be depressed. Further work will be required to understand how excitatory and inhibitory plasticity are regulated together to ensure that inhibition is properly calibrated and balanced with excitation.

Key triggers of synaptic plasticity

At many synapses, there seem to be two fundamental stages for modifying synaptic strength: the *induction* or initiation of synaptic modifications, and the *expression* mechanisms required to maintain these changes over a range of time scales from seconds to days or longer. Here we will focus mainly on the induction of LTP and LTD, in terms of dendritic components of synaptic transmission and integration that contribute to long-term synaptic plasticity.

The induction of LTP and LTD at most synapses requires NMDA receptor activation by coincident pre- and postsynaptic activity. This leads to an influx of Ca^{2+} through the NMDA receptor channel and activation of downstream Ca^{2+} -dependent second-messenger systems (Malenka and Nicoll, 1999; Feldman, 2009). Conventionally, these NMDA receptors are thought to be postsynaptic (although see below and Duguid and Sjöström, 2006). Postsynaptic NMDA receptors act as molecular coincidence detectors due to their nonlinear current–voltage properties. Unlike most AMPA receptors (which have linear current–voltage relationships), the NMDA receptor channel

is partially blocked by extracellular Mg^{2+} (Fig. 18.3A). At hyperpolarized potentials, NMDA receptor-mediated currents are small relative to AMPA receptor-mediated responses. However, as local depolarization increases, this Mg^{2+} block is relieved and NMDA receptors are able to pass more current when activated by presynaptic glutamate release (Mayer et al., 1984; Nowak et al., 1984). These channels are also highly permeable to Ca^{2+} (MacDermott et al., 1986; Mayer and Westbrook, 1987), and NMDA receptor-dependent Ca^{2+} currents are thought to underlie the induction of LTP and LTD (Collingridge et al., 1983; Malenka and Nicoll, 1999).

Dendritic calcium imaging has provided compelling evidence that NMDA receptors function in this manner. Yuste and Denk (1995) imaged spines in CA1 pyramidal cell dendrites. They found that bAPs and synaptic stimulation both led to increases in Ca^{2+} in single spines, but pairing spikes with synaptic activation produced supralinear increases in spine Ca^{2+} greater than the predicted sum of the combined signals (Fig. 18.3B). Backpropagating APs activate voltage-gated Ca^{2+} channels, leading to influx of Ca^{2+} largely through L-type channels as well as other subtypes depending on dendritic location (Markram and Sakmann, 1994; Magee et al., 1998; Yasuda et al., 2003; Hoogland and Saggau, 2004). Synaptic stimulation produces influx of Ca^{2+} from several different sources, predominantly via NMDA receptors, but also from Ca^{2+} channels and Ca^{2+} -permeable AMPA receptors in some cases (Yuste et al., 1999). The supralinear increase in Ca^{2+} after pairing pre- and postsynaptic activation is probably a result of the sensitivity of NMDA receptors to depolarized potentials.

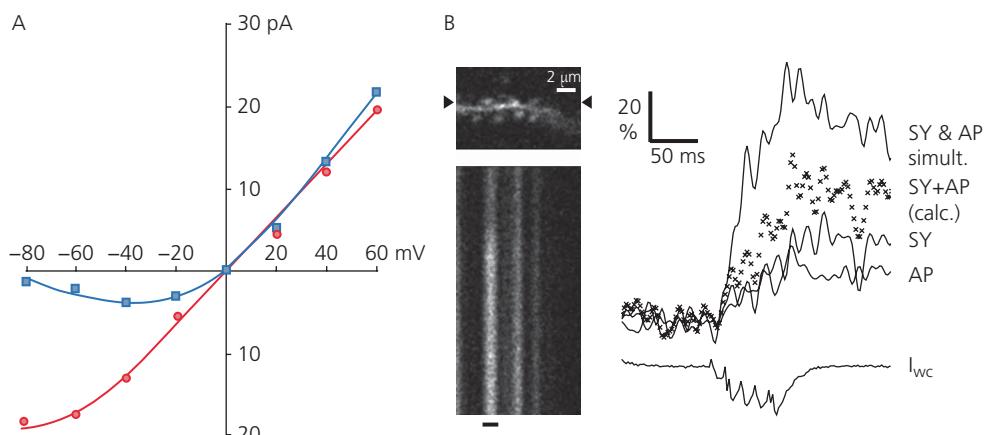


Fig. 18.3 The nonlinear response profile of NMDA receptors results in supralinear postsynaptic calcium signaling. (A) NMDA receptor currents show outward rectification in normal artificial cerebrospinal fluid (blue line) but not in nominally Mg^{2+} -free artificial cerebrospinal fluid (red line), because the voltage dependence of the NMDA receptor is mediated by Mg^{2+} ions at hyperpolarized voltages. (B) Two-photon calcium imaging in hippocampal CA1 pyramidal cell spines reveals the supralinear Ca^{2+} signals (Yuste and Denk, 1995) that trigger LTP (Sjöström and Nelson, 2002; Sjöström et al., 2008). Synaptic activation (SY) and APs (AP) alone arithmetically sum to a lower Ca^{2+} signal (SY + AP calc.) than when both are elicited simultaneously (SY and AP simult.).

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The time course and amplitude of postsynaptic increases in Ca^{2+} are believed to control the sign and magnitude of long-term synaptic plasticity, a notion known as the calcium hypothesis (Lisman, 1989; Sjöström and Nelson, 2002). Specifically, transient and high-amplitude increases in postsynaptic Ca^{2+} of the order of 1 s and $>10 \mu\text{M}$ induced LTP, while prolonged and lower-amplitude increases in Ca^{2+} for about 1 minute and $<1 \mu\text{M}$ induced LTD (Fig. 18.4). These levels were determined by calcium imaging in hippocampal slices combined with photolysis of caged Ca^{2+} compounds in order to release Ca^{2+} in a temporally specific and calibrated manner (Yang

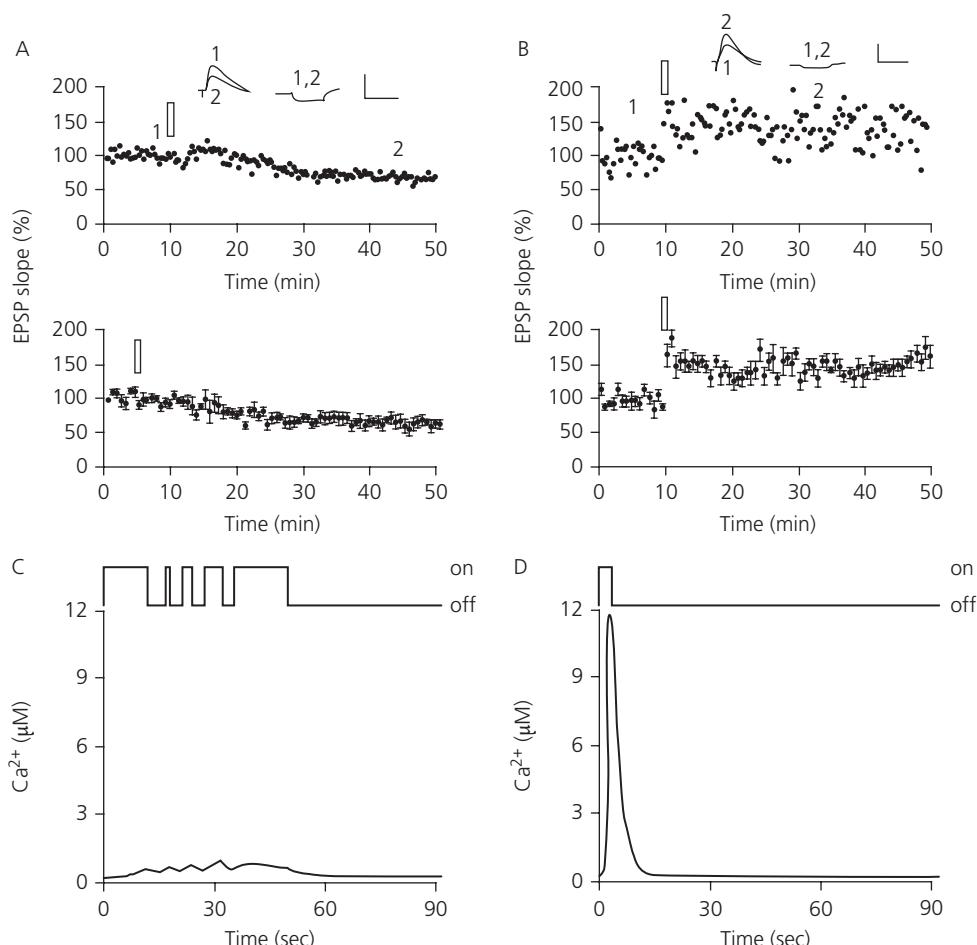


Fig. 18.4 Induction of long-term synaptic modification is triggered by postsynaptic elevations of Ca^{2+} . LTD is induced (A) by modest, prolonged elevations of postsynaptic Ca^{2+} (B). LTP, on the other hand, is elicited (C) by high, brief elevations of postsynaptic Ca^{2+} (D). Ca^{2+} signals were measured with fura-2 in hippocampal CA1 neurons, and Ca^{2+} transients were evoked by flash photolysis of caged Ca^{2+} .

Adapted from Shao-Nian Yang, Yun-Gui Tang, and Robert S. Zucker, Selective induction of LTP and LTD by post-synaptic $[\text{Ca}^{2+}]_{\text{i}}$ Elevation, *Journal of Neurophysiology*, 81(2), pp. 781–787, Figure 2, Copyright © 1999, The American Physiological Society.

et al., 1999). Thus postsynaptic increases in intracellular Ca^{2+} are sufficient for induction of LTP (Malenka et al., 1988).

How do these types of Ca^{2+} signals relate to patterns of activity used to induce LTP and LTD? Hansel et al. (1997) found in rat visual cortical slices that tetanization protocols for induction of LTD or LTP led to modest or large increases in intracellular Ca^{2+} , respectively. This is consistent with the rate-based view of synaptic plasticity in which moderate increases in presynaptic activity or postsynaptic depolarization generally induce LTD while stronger increases in activity or depolarization induce LTP (Lisman, 1989; Feldman, 2009).

Pairing pre- and postsynaptic activity can also increase intracellular Ca^{2+} . In hippocampal CA1 pyramidal neurons, bAPs paired with synaptic stimulation produce large increases in Ca^{2+} and induce LTP (Magee and Johnston, 1997). This LTP is blocked by APV and MK-801 (NMDA receptor antagonists) as well as nimodipine (an L-type Ca^{2+} blocker), indicating that both synaptic and spike-triggered increase in Ca^{2+} is required for LTP induction (Fig. 18.5). In L5 cortical pyramidal neurons, Koester and Sakmann (1998) found that the relative timing of bAPs was important for controlling the summation between spike and synaptic Ca^{2+} . In particular, summation was supralinear when the postsynaptic spike came after synaptic stimulation, but the increase was sublinear if the spike was elicited before synaptic stimulation. This bidirectional regulation of Ca^{2+} signaling is consonant with induction of STDP by pre- and postsynaptic spike pairing in the hippocampus (Nishiyama et al., 2000; but see Buchanan and Mellor, 2010) and cortex (Froemke et al., 2005), in which pre- before postsynaptic spiking induces LTP and post- before presynaptic spiking induces LTD. Whereas pre- before postsynaptic spiking probably induces LTP because of supralinear Ca^{2+} influxes, it has remained less clear why the inverse temporal order induces LTD. Postsynaptic spiking prior to synaptic stimulation might simply be mistimed relative to maximal NMDA receptor activation, and/or bAPs might lead to Ca^{2+} -dependent desensitization of NMDA receptors (Froemke et al., 2005). In either case, the Ca^{2+} hypothesis would predict that a lower Ca^{2+} influx should lead to depression of excitatory synapses (Lisman, 1989; Sjöström and Nelson, 2002). The Ca^{2+} hypothesis, however, is not universally accepted. For example, Nevian and Sakmann (2006) found that, at connections between neocortical L2/3 pyramidal cells, spine Ca^{2+} levels governed the magnitude of plasticity, whereas the sign of plasticity was determined by metabotropic glutamate receptors. In the hippocampus, the activity requirements of STDP are furthermore not generally agreed on (Buchanan and Mellor, 2010), which implies that the Ca^{2+} hypothesis alone may not predict the outcome of plasticity at hippocampal synapses either.

To further complicate the otherwise so simple and elegant Ca^{2+} hypothesis, presynaptic NMDA receptors may also be involved in the induction of long-term potentiation (Humeau et al., 2003; Park et al., 2014) and depression (Sjöström et al., 2003; Rodriguez-Moreno et al., 2013), often in mechanisms associated with metabotropic glutamate receptors (Bender et al., 2006) and endocannabinoid receptors (Sjöström et al., 2003; Min and Nevian, 2012). These presynaptic NMDA receptors may only be functionally expressed in developing circuits (Corlew et al., 2007), probably closely linked to the critical period in visual cortex (Larsen et al., 2014). Presynaptic NMDA receptor signaling may work together with release of retrograde messengers triggered by postsynaptic Ca^{2+} signals to induce STDP (Sjöström et al., 2003). Presynaptic coincidence detection provides a high degree of control over the patterns of activity required to induce LTP or LTD at functionally different synapses by ensuring a degree of coordination between pre- and postsynaptic compartments during the expression of plasticity (Duguid and Sjöström, 2006). This also implies that dendritic events can have consequences for neighboring presynaptic neurons as well as postsynaptic cells.

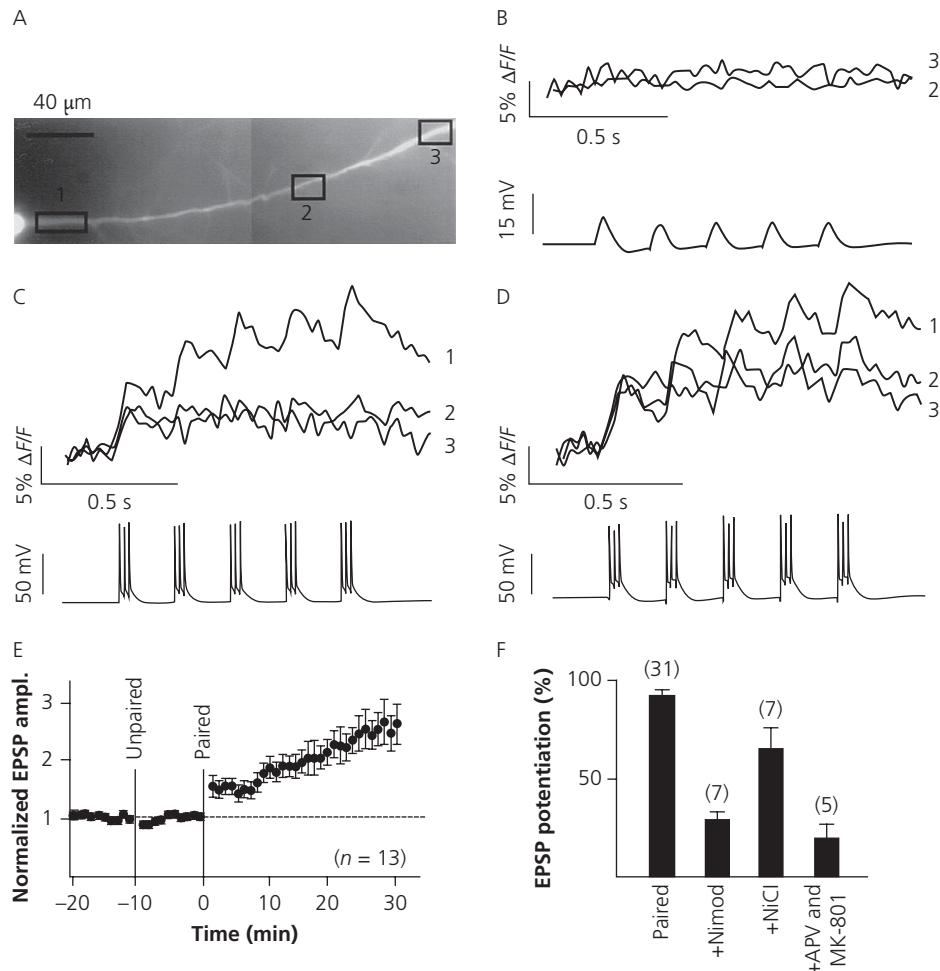


Fig. 18.5 Backpropagating APs provide an associative signal for LTP. **(A)** calcium imaging with fura-2 from three locations in a CA1 pyramidal cell dendrite. **(B)** Synaptic stimulation elicited only modest dendritic Ca^{2+} elevations. **(C)** Postsynaptic spikes alone elicited larger and more widespread Ca^{2+} signals. The bottom trace shows somatic voltage. **(D)** Postsynaptic spikes combined with synaptic stimulation resulted in even larger Ca^{2+} signals in dendritic regions 2 and 3. **(E)** LTP is triggered by postsynaptic spikes paired with EPSPs, but not by unpaired stimulation. EPSPs or bAPs alone are thus not sufficient for LTP. **(F)** LTP induced by pre- and postsynaptic spike pairing was blocked by the L-type Ca^{2+} channel blocker nimodipine or by the NMDA receptor blockers APV/MK-801.

Reprinted from Jeffrey C. Magee and Daniel Johnston, A synaptically controlled, associative signal for Hebbian plasticity in hippocampal neurons, *Science*, 275(5297) pp. 125–127 © 1997, The American Association for the Advancement of Science. Reprinted with permission from AAAS.

Synaptic plasticity depends on dendritic location

It is now clear that different synapses within the same neuron are subject to distinct patterns of pre- and postsynaptic activity, leading to considerable variation in membrane potential and intracellular signaling dynamics from synapse to synapse. Much of this diversity arises from the specific location of individual synapses within the dendritic arbor, due to electrotonic filtering of bAPs, initiation of dendritic spikes, and heterogeneity of dendritic ion channel distributions. Consequentially, NMDA receptor signaling (among other things) should vary as a function of dendritic location due to the nonlinearity of NMDA receptor Ca^{2+} influx (Fig. 18.3). Therefore, long-term synaptic plasticity might also be expected to depend on dendritic location.

Several studies in neocortical pyramidal neurons have identified distinct learning rules for STDP within the same cell type, depending on where individual inputs are located within the apical dendrites (Froemke et al., 2010). In general, in L2/3 and L5 pyramidal neurons, proximal inputs closer to the cell body and site of somatic AP generation display conventional STDP time windows. That is, repetitive short pre-before-post pairings when the spike follows the EPSP within about 10 ms induce LTP, while post-before-pre pairings when spikes precede EPSPs within about 50 ms induce LTD. However, inputs further from the soma show differences in excitatory STDP in a cell-type specific manner.

In L5 pyramidal neurons there is a spatial gradient of plasticity along apical and basal dendrites. As the somatic rise time of EPSPs is correlated with the dendritic distance of the site of synaptic activation (Jack and Redman, 1971; Sjöström and Häusser, 2006), rise time can be used as a proxy for dendritic location. Two studies of L5 pyramidal neurons found that pre-before-post pairing induced LTD instead of LTP when the rise times were relatively long (Letzkus et al., 2006; Sjöström and Häusser, 2006). Sjöström and Häusser (2006) examined this at monosynaptic connections between L2/3 or L5 neurons and L5 pyramidal cells, confirming that rise time accurately reflected the dendritic location of putative synaptic contact sites up to 500–700 μm from the soma. Pairing pre- and postsynaptic spike bursts induced long-term changes in synaptic strength where the sign and magnitude of synaptic modification varied as a smooth function of rise time (Fig. 18.6A). Similar results were obtained by Letzkus et al. (2006), although the pairing procedure and dependence on the exact rise time were somewhat different. Interestingly, post-before-pre pairing also showed a conversion to the opposite form of plasticity for events with slower rise times; i.e., post-before-pre induced LTP at more distally located events (Fig. 18.6B).

In contrast, at distal sites in L2/3 apical dendrites more than 100 μm from the soma, pre-before-post pairing induced a smaller magnitude of LTP than that observed proximally, but the time window for LTD induced by post-before-pre pairing was considerably longer (Fig. 18.6C). This broadening of the temporal requirements for LTD could be mimicked by blocking I_A , which leads to widening of bAPs in the dendrites. Consequentially, simulations indicated that only inputs that were temporally correlated and transient should remain at distal locations after STDP-based synaptic refinement (Froemke et al., 2005).

There are some mechanistic differences between STDP at L5 and L2/3 synapses. Sjöström and Häusser (2006) found that, similar to post-before-pre LTD, pre-before-post LTD at distal dendritic locations required endocannabinoid signaling. At L2/3 synapses during post-before-pre spike pairing, the postsynaptic AP suppresses NMDA receptor activation (Froemke et al., 2005). Influx of Ca^{2+} triggered by postsynaptic spiking produced a rapid calcineurin-dependent decrease in NMDA receptor EPSPs. The time window for NMDA receptor suppression at both proximal and distal sites on L2/3 apical dendrites matched the time window for induction of LTD, indicating that NMDA receptor suppression set the timing requirements for LTD.

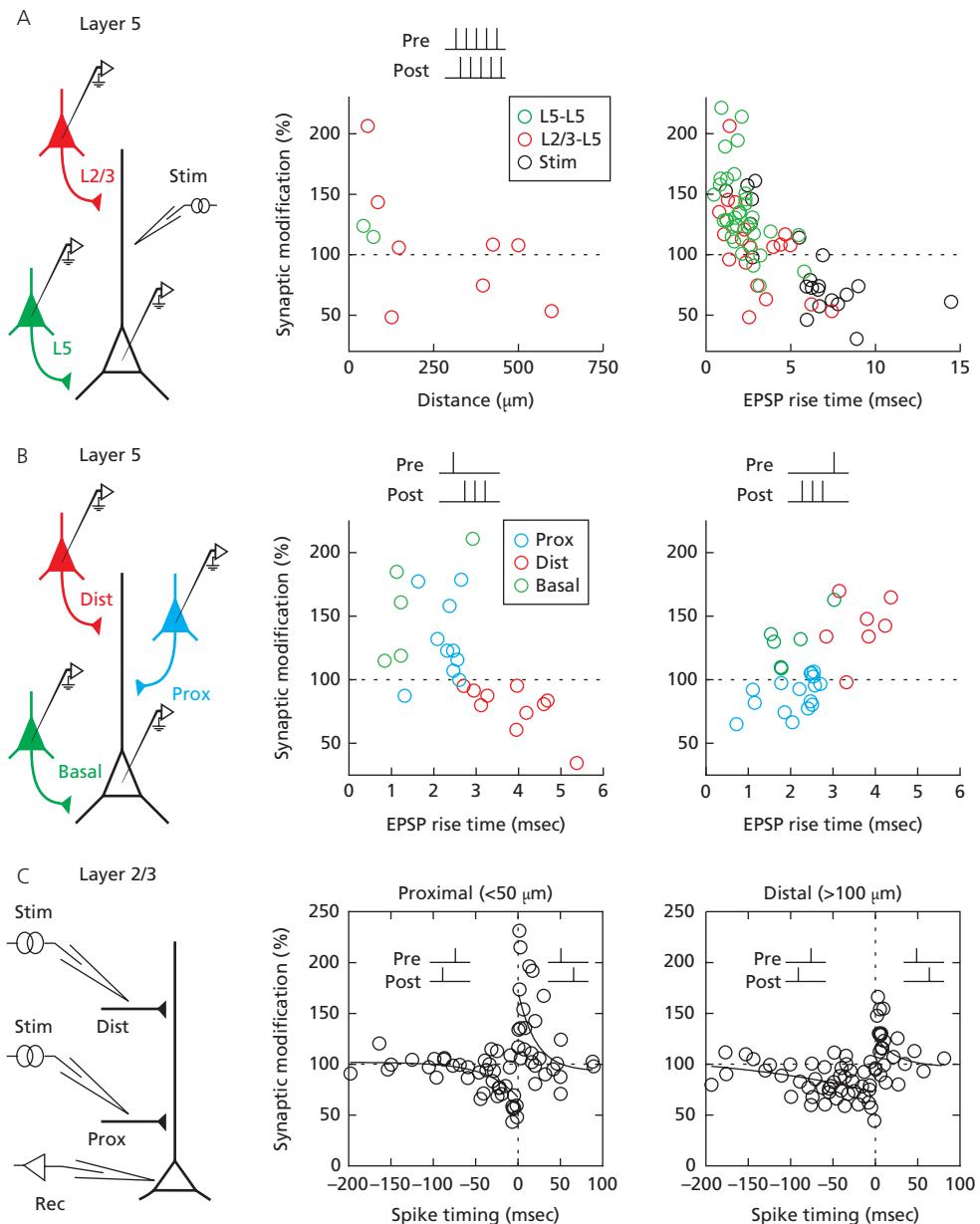


Fig. 18.6 STDP timing rules depend on dendritic synapse location. **(A)** STDP of L2/3 (red) and L5 (green) EPSPs in L5 pyramidal neurons. STDP was induced by repetitively pairing of pre- and postsynaptic APs at +10 ms. Center: the sign of synaptic modification was negatively correlated with distance of putative synaptic contact. Right: the sign of synaptic modification was negatively correlated with EPSP rise time. Dendritic depolarization can switch the sign of distal plasticity from LTD to LTP (see Fig. 18.7), which is a form of cooperativity in STDP (modified from Sjöström and Häusser, 2006). **(B)** STDP of proximal (blue) and distal (red) apical L2/3 inputs, and basal (green) L5 inputs, to L5 pyramidal neurons. Somatic EPSP rise time was used as a proxy for synaptic location, with fast EPSPs

Importantly, however, there may be common principles of dendritic integration that may underlie the changes in STDP windows in L2/3 and L5 dendrites. NMDA receptor activation is necessary in all cases, and Ca^{2+} channels also play important roles in shaping the dendritic and synaptic responses to paired pre- and postsynaptic activity. APs and paired pre- and postsynaptic activity were broader in distal dendrites, as measured with electrophysiological recordings (Froemke et al., 2005; Letzkus et al., 2006; Sjöström and Häusser, 2006) and calcium imaging (Froemke et al., 2005; Sjöström and Häusser, 2006). Biophysical modeling based on NMDA receptor gating and relief of Mg^{2+} blockade (Kampa et al., 2004) was used to examine the role of dendritic spikes in STDP at various apical locations (Letzkus et al., 2006). The model predicted a continuous shift from an asymmetric, Hebbian STDP window at proximal locations to a window with a larger post-before-pre LTD region more distally and a window with post-before-pre LTP at further distal locations in the tuft. Therefore, although the effector mechanisms and molecules for expression of synaptic modifications may be distinct, different synapses may each require dendritic spikes to gate NMDA receptors for induction of long-term synaptic plasticity (Froemke et al., 2010).

Modulation of backpropagating action potentials

Neuromodulators such as dopamine and acetylcholine are inherently involved in associative learning such as reward-mediated learning. It is well established that neuromodulation can affect STDP in many different brain regions and cell types (Bissiere et al., 2003; Lin et al., 2003; Couey et al., 2007; Seol et al., 2007; Pawlak and Kerr, 2008; Shen et al., 2008; Zhang et al., 2009). Because neuromodulators can affect the properties of dendritic ion channels by phosphorylation and dephosphorylation (Levitan, 1994; Tokuda and Hatase, 1998; Cantrell and Catterall, 2001; Davis et al., 2001), they are likely to affect intrinsic dendritic excitability, AP backpropagation, and consequently induction of long-term synaptic modification.

The functional role of changes in dendritic excitability via ion channel modulation has been most extensively studied in CA1 pyramidal cells with regard to the backpropagation of somatic APs into the dendrites (Johnston et al., 1999; Tsubokawa, 2000). Acetylcholine can increase the amplitude of dendritic bAPs by either changing Na^+ channel inactivation (Colbert and Johnston, 1998) or reducing K^+ channel opening (Shen et al., 2005, 2007). Norepinephrine can also enhance backpropagation by its action on I_A (Hoffman and Johnston, 1998), and could participate in coincidence detection. A relatively weak synaptic stimulus paired with norepinephrinergic input,

Fig. 18.6 (Continued)

(<2.7 ms) indicating proximal EPSPs and slow rise times (>2.7 ms) indicating distal EPSPs. EPSP rise time was negatively correlated with the sign and magnitude of the change in synaptic strength for pre- before postsynaptic pairings (middle), but positively correlated with the sign and magnitude of changes in synaptic strength for post- before presynaptic pairings (right), leading to a switch to anti-Hebbian STDP for more distal inputs (modified from Letzkus et al., 2006). (C) STDP of proximal (<50 μm) and distal (>100 μm) inputs onto apical dendrites of L2/3 visual cortical pyramidal neurons. Post-before presynaptic pairing at -50 to -100 ms induced LTD distally (right) but not proximally (middle) (modified from Froemke et al., 2005).

Reproduced from Robert C. Froemke, Johannes J. Letzkus, Björn M. Kampa, Giao B. Hang, and Greg J. Stuart, Dendritic synapse location and neocortical spike-timing-dependent plasticity, *Frontiers in Synaptic Neuroscience*, 2:29. © 2010, The Authors.

which increases the amplitude of bAPs, could promote removal of the Mg^{2+} blockade of NMDA receptors and thus trigger long-lasting synaptic plasticity (Watabe et al., 2000). Acetylcholine decreases I_A to enhance bAPs (Hoffman and Johnston, 1999). Similarly, activation of β_2 adrenergic receptors increases entry of Ca^{2+} to spines following bAPs, by potentiating T-type channels (Hoogland and Saggau, 2004). Serotonin, on the other hand, leads to dendritic hyperpolarization, thus limiting the extent of backpropagation and reducing Ca^{2+} influx (Sandler and Ross, 1999). Dopaminergic receptor activation enhances I_h and reduces dendritic excitability in L5 pyramidal neurons in the entorhinal cortex (Rosenkranz and Johnston, 2006), but has no apparent effect on backpropagation in prefrontal cortical pyramidal cells, although it changes input resistance and excitability (Gulledge and Stuart, 2003). However, in striatal spiny projection neurons expressing D_2 receptors, rapidly applied and spatially restricted dopamine modulation prevents the backpropagation of APs by inhibiting specific voltage-gated Ca^{2+} channels (Day et al., 2008).

Are backpropagating action potentials needed for plasticity?

STDP depends on the precise relative timing of pre- and postsynaptic spikes, which suggests that bAPs are essential for synapses to detect postsynaptic activity. However, bAPs are not strictly necessary for induction of LTP. For example, when paired with low-frequency presynaptic stimulation, depolarization of the postsynaptic membrane by current injection is sufficient to induce LTP (Malinow and Tsien, 1990). Conversely, LTP in neocortical L5 pyramidal cells due to pairing of high-frequency pre- and postsynaptic spike trains is abolished by postsynaptic hyperpolarization, even though postsynaptic somatic APs are seemingly unaffected (Sjöström et al., 2001; Sjöström and Häusser, 2006), suggesting that spikes are not sufficient for LTP. These observations, however, do not necessarily indicate that bAPs are not necessary for LTP, but might instead reveal another level of complexity in the regulatory mechanisms of plasticity. How bAPs invade the dendritic arbor to provide one of the two components needed to trigger the NMDA-receptor-based spine coincidence detector has a profound impact on the outcome of plasticity. Interestingly, the reliability of AP backpropagation in dendrites varies tremendously among neuronal types (Stuart et al., 1997; Häusser et al., 2000; Vetter et al., 2001) and physiological conditions. Therefore, in some circumstances, bAPs will fail to invade the dendritic arbor and may thus not be sufficient to depolarize distal NMDA receptors (Figs 18.5C and 18.7). In this situation, high-frequency correlated pre- and postsynaptic firing fails to induce LTP even though the Hebbian rule seems to be satisfied (Figs 18.6 and 18.7) (Sjöström et al., 2001; Sjöström and Häusser, 2006). Not only does the bAP provide the synapse with information about whether the cell fired or not, it also conveys contextual information about the state of excitability of the dendrite, which is determined by factors such as activity and neuromodulation. The impact of such factors on the dendrite varies with cell type. In hippocampal CA1 pyramidal cells the spike typically fails to invade distal dendrites chiefly because there is a steep somato-dendritic gradient of I_A that is detrimental to AP backpropagation (Hoffman et al., 1997). Without this K^+ channel gradient, APs would backpropagate reliably, as dendritic Na^+ channel densities are in fact comparable with those at the soma (Stuart and Sakmann, 1994; Magee and Johnston, 1995, 1997). Because depolarization inactivates I_A after approximately 10 ms, sufficiently large EPSPs can temporarily inactivate dendritic I_A channels, and thereby momentarily increase the reliability of AP backpropagation into apical and radial oblique dendrites (Hoffman et al., 1997; Migliore et al., 1999; Gasparini et al., 2007). In neocortical L5 pyramidal cells, a combination of insufficiently high densities of dendritic voltage-activated Na^+ and Ca^{2+} conductances and the dendritic geometry of these cells (Vetter et al., 2001) is probably responsible for the relatively poor reliability of AP backpropagation in distal dendrites. In these cells, appropriately timed

subthreshold depolarization can rescue distal bAPs (Larkum et al., 2001) by recruiting additional voltage-gated Na^+ channels (Larkum et al., 2001; Stuart and Häusser, 2001).

At excitatory inputs onto neocortical L5 neurons, plasticity gradually changes from LTP to LTD with distance of the synapse from the soma (Fig. 18.6A, B), which seems to reflect how bAPs fail as they backpropagate into distal dendritic compartments (Sjöström and Häusser, 2006). However, cooperation among synapses can depolarize dendritic compartments and boost failing bAPs, switching plasticity at distal synapses from LTD to LTP (Stuart and Häusser, 2001; Sjöström and Häusser, 2006).

It is thus clear that the information carried by bAPs does not generally distribute equally among synapses. In fact, boosting of bAPs can be branch specific: when an individual branch of the dendritic tree is depolarized by current injection, bAPs preferentially backpropagate into that arm of the arbor (Magee et al., 1998). Although branch-specific boosting of bAPs has not been well studied, one might imagine that this mechanism could promote potentiation of neighboring inputs that possibly carry related information. The clustering of relevant synapses theoretically shows computational advantages for the storage of information (Mel, 1992; Poirazi and Mel, 2001), and recent evidence supports the notion of such synaptic clustering *in vivo* (Makino and Malinow, 2011; Fu et al., 2012; Takahashi et al., 2012) (although see Jia et al., 2010). Synaptic clustering, however, can also be achieved with local dendritic spikes in the absence of bAPs, so this is not evidence *per se* for the relevance of bAPs in plasticity *in vivo*.

To show the relevance of bAPs in the intact brain, an important first step is to reveal their existence *in vivo*. Two-photon calcium imaging provided some of the first evidence that L2/3 pyramidal cells support AP backpropagation *in vivo*, although to a smaller degree than *in vitro* (Svoboda et al., 1999). Robust backpropagation in L2/3 and L5 pyramidal cells was later shown using both electrophysiology and optical methods (Helmchen et al., 1999; Waters et al., 2003). But the existence of bAPs in the intact brain does not in and of itself indicate their involvement in synaptic plasticity: the key question is what impact, if any, bAPs have on synaptic strength. By pairing subthreshold visual stimulation with APs elicited by brief somatic current injection, plasticity that is indistinguishable from STDP has been shown in the intact brain (Meliza and Dan, 2006; Pawlak et al., 2013). Importantly, Pawlak et al. (2013) showed that spike pairing not only altered synaptic strength in a timing-dependent manner, but also the spiking output of the neurons. This adds an important operative dimension to STDP since neuronal receptive fields are typically defined by the stimulus-specific tuning of neuronal spiking, and not by subthreshold responses. With STDP, it is thus possible to retrain neurons to alter their functional specificity. These studies show that bAPs could be important for plasticity, but one key criticism remains: these bAPs were artificially elicited by current injection (Lisman and Spruston, 2005, 2010). Whether natural stimuli evoke bAPs that also trigger synaptic plasticity remains to be established. Alternatively, natural stimuli that lead to disinhibition of cortical neurons *in vivo* (Froemke et al., 2007) or evoke NMDA spikes in the intact brain (Gambino et al., 2014) might more effectively gate long-term synaptic modifications and adjustments of cortical receptive fields.

The role of local dendritic spikes in plasticity

Other types of dendritic spikes beyond bAPs might be important for long-term synaptic plasticity (Lisman and Spruston, 2005, 2010). Local dendritic spikes are triggered when sufficient depolarization is reached, resulting in a nonlinear input–output relationship (Figs 18.7 and 18.8). Depending on the cell type, these spikes are supported by distinctive conductances. For example, in Purkinje cells, whose dendrites lack voltage-gated Na^+ channels (Stuart and Häusser,

1994), voltage-gated Ca^{2+} channels generate dendritic spikes (Llinás and Sugimori, 1980). In hippocampal pyramidal cells, dendritic spikes are produced by concerted activation of Na^+ (Golding and Spruston, 1998) and Ca^{2+} conductances (Golding et al., 1999). In neocortical pyramidal cells, the relevant conductances change in a location-dependent manner. Proximal dendrites are more prone to Na^+ channel-mediated dendritic spikes (Stuart et al., 1997; Larkum et al., 2007; Nevian et al., 2007), while voltage-gated Ca^{2+} channels contribute more to distal dendritic spikes (Schiller et al., 1997; Larkum et al., 1999a,b). These regenerative events remain confined within the dendritic region where they initiated, with this spatial restriction largely due to the passive membrane properties of the dendritic tree (Vetter et al., 2001), and in some cases by voltage-gated potassium conductances (Etzion and Grossman, 1998; Golding et al., 1999; Gasparini et al., 2004; Khavandgar et al., 2005).

Interestingly, NMDA receptors can also produce local dendritic spikes, known as NMDA spikes (Schiller et al., 2000; Nevian et al., 2007; Lavzin et al., 2012). Because of their outward rectification (Fig. 18.3), glutamate-bound NMDA receptors are basically voltage-gated ion channels, and are thus ideally suited for supporting local regenerative events (Fig. 18.7C). This form of local dendritic spike requires relatively large amounts of glutamate release, but is also limited to the region where the glutamate is released.

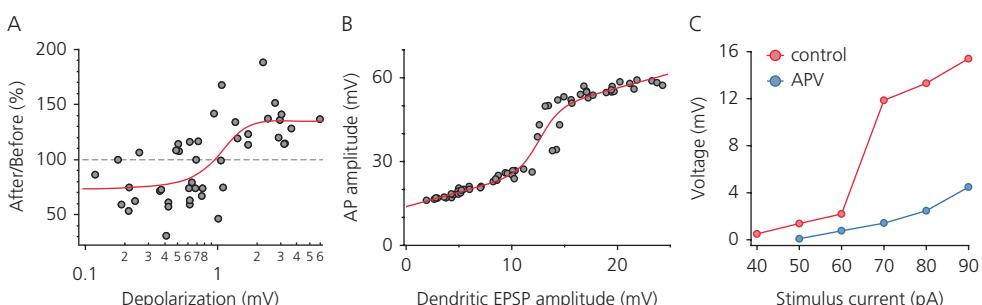


Fig. 18.7 LTP of distal synapses onto L5 pyramidal cells, amplification of bAPs, and local dendritic spikes all exhibit nonlinearities reminiscent of cooperativity. **(A)** LTP at distal L5 pyramidal cell inputs requires that the somatic EPSP amplitude is greater than 1.0 mV (Sjöström and Häusser, 2006). Strong synaptic stimulation may sufficiently depolarize to enable potentiation at low frequency (Debanne et al., 1998; Feldman, 2000; Bi and Poo, 2001). High-frequency firing may also potentiate weak synapses close to the soma (Markram et al., 1997; Sjöström et al., 2001). **(B)** APs fail as they backpropagate into distal apical dendrites of L5 pyramidal cells, but sufficient depolarization can rescue bAPs by boosting the fast Na^+ current (Stuart and Häusser, 2001). The 13-mV threshold for bAP amplification is equivalent to 3.6 mV in the soma. **(C)** NMDA spikes induce a supralinear input-output relationship in L4 spiny stellate neurons with minimal extracellular stimulation of a single distal dendrite (Lavzin et al., 2012). Supralinearity is lost when NMDA receptors are blocked with APV (AP5).

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Irrespective of the underlying ion channels, dendritic spikes can satisfy the depolarization requirement of the spine coincidence detector, and there is solid evidence that dendritic spikes can indeed trigger plasticity (Golding et al., 2002; Holthoff et al., 2004; Kampa et al., 2006; Remy and Spruston, 2007; Hardie and Spruston, 2009; Gambino et al., 2014; Kim et al., 2015). In hippocampal CA1 neurons, LTP of distal stratum lacunosum moleculare inputs can be evoked by sufficiently strong synaptic activation in the absence of somatic spiking (Fig. 18.8; Golding et al., 2002). To obtain LTP, synaptic inputs need to reach the threshold for distal dendritic spikes. This means that this dendritic mechanism also results in a form of cooperativity, which may facilitate associative learning.

Although individual dendritic spikes can lead to LTP in hippocampal CA1 neurons (Holthoff et al., 2004; Remy and Spruston, 2007), single-shot LTD is elicited in neocortical L5 pyramidal cells (Holthoff et al., 2004). Experiments in the intact brain are thus needed to ascertain what the outcome is under more realistic circumstances. However, clarifying the role of dendritic spikes

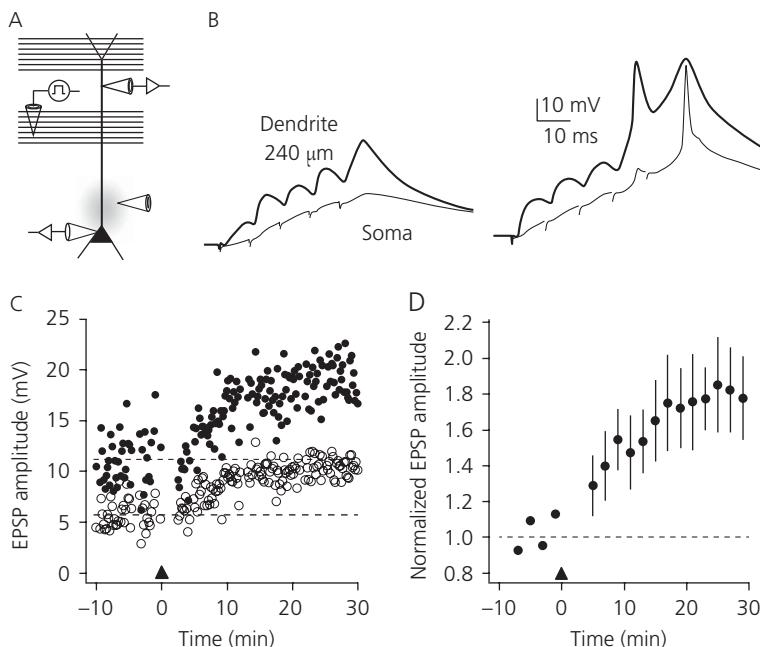


Fig. 18.8 LTP at stratum radiatum (SR) synapses onto hippocampal CA1 neurons can be induced by dendritic spikes in the absence of bAPs. **(A)** Responses to SR stimulation were recorded in the soma and dendrites. During theta-burst stimulation, tetrodotoxin (TTX) was puffed locally to abolish bAPs. **(B)** SR theta-burst stimulation evoked dendritic spikes that were attenuated at the soma. **(C)** SR-synapse LTP could occur independently of bAPs. EPSPs measured both dendritically (filled circles) and somatically (open circles) in the presence of somatic TTX exhibited prominent LTP. **(D)** Ensemble data ($n = 4$) of dendritic EPSP amplitudes in LTP experiments where bAPs were blocked indicated that this form of LTP induction was robust.

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in plasticity *in vivo* has been experimentally challenging. Recently, Gambino and colleagues provided compelling *in vivo* evidence for the role of dendritic spikes in LTP (Gambino et al., 2014). By rhythmically stimulating the whiskers at 8 Hz and recording compound postsynaptic potentials from L2/3 pyramidal cells, they showed that LTP could be induced after a 1-minute-long stimulation. These whisker-deflection-evoked plateau potentials were reminiscent of local dendritic NMDA spikes and required the cooperative activation of two different inputs: the lemniscal and paralemniscal pathways (Gambino et al., 2014).

It is thus not clear if dendritic spikes or bAPs are the key determinants of synaptic plasticity, but they need not be mutually exclusive. The defining regenerative event may simply be different depending on factors such as cell type, developmental stage, and network state (Schulz, 2010). In fact, both mechanisms might be working hand in hand: for example, bAPs may alter the threshold of local dendritic spikes or the degree to which they propagate (Larkum et al., 1999b). In the end, depolarization sufficient to unblock NMDA receptors is likely to induce plasticity regardless of its source (McNaughton et al., 1978; Gustafsson and Wigström, 1986; Kelso et al., 1986; Gustafsson et al., 1987; Clopath et al., 2010). The question remains, however, whether somatic APs typically determine plasticity under *in vivo* conditions, or if local dendritic spikes are the dominating triggering factor.

Dendrites control synaptic plasticity

How do dendrites influence synaptic plasticity? The distinct morphology and biophysics of the dendritic tree can compartmentalize chemical and electrical signals, thus determining the signals that are available at the synapse. As we will discuss in detail in this section, such spatial restriction of signaling impacts both the synapse specificity and cooperativity of synaptic learning rules. Compartmentalization can occur at two different levels—at dendritic branches or in individual spines (Branco and Häusser, 2010; Kastellakis et al., 2015). Since their discovery by Santiago Ramón y Cajal, the function of spines has been subject to much debate. Spines are morphological compartments that cover many dendritic trees. They exhibit a wide range of morphologies, typically consisting of an approximately micron-wide spine head and a narrower spine neck of variable length (Harris, 1999). Spines may in part serve a structural role (see Chapter 19): to enhance the number of possible connections and, therefore, the potential for plasticity (Stepanyants et al., 2002; Chklovskii et al., 2004). Nevertheless, since synapses can form directly onto dendritic shafts, and since not all neurons possess spines, the function of spines must extend beyond merely connecting neurons to each other (Koch and Zador, 1993). It has been suggested that spines serve a combination of three different functions: to connect, to electrically compartmentalize, and to biochemically compartmentalize (Yuste, 2011). Here, we review their role in dendritic compartmentalization and how this may impact synaptic plasticity.

Electrical compartmentalization

At the very beginning of the twentieth century the Ramón y Cajal (1904) suggested that spines may serve as electrical compartments, an idea that was later elaborated by several other investigators (Chang, 1952; Shepherd et al., 1985; Rall and Segev, 1987; Segev and Rall, 1988). The advent of advanced imaging techniques has allowed researchers to directly test this idea (Yuste, 2013). Spines contain voltage-gated Ca^{2+} channels and other active conductances (Yuste and Denk, 1995). Minimal synaptic stimulation can elicit strong entry of Ca^{2+} through NMDA receptors in spines even though Mg^{2+} should block the NMDA receptor channel at resting membrane potential. This suggests that EPSPs in spine heads are sufficiently large to relieve NMDA

receptors of Mg^{2+} blockade. More recently, Araya and colleagues found that glutamate uncaging combined with minimal stimulation evoked EPSPs that were inversely correlated with spine neck length (Araya et al., 2007, 2014). High synaptic conductance means low spine head resistance and consequently greater attenuation of voltage across the spine neck resistance. In other words, only if the synaptic conductance is sufficiently high will the spine neck resistance appreciably reduce the synaptic driving force and consequently the amplitude of the somatic EPSP (Koch and Zador, 1993). This means that the range of physiologically relevant synaptic conductances is critically important for the potential contribution of spine neck resistance to synaptic plasticity. Measurement of spine resistance has yielded inconsistent results. Previous studies based on electron microscopy (Harris and Stevens, 1989), diffusion measurements (Svoboda et al., 1996), or voltage-sensitive dye imaging (Palmer and Stuart, 2009) reported values ranging from 1 to 500 M Ω for spine neck resistance. Using calcium imaging, Harnett et al. (2012) found high spine neck resistances, consistently on the order of 500 M Ω , across different spines. Interestingly, Bloodgood and Sabatini (2005) found evidence for occasional high spine neck resistance in organotypic culture of CA1 neurons, sometimes approaching 1 G Ω . Recent evidence obtained using super-resolution stimulated emission depletion (STED) microscopy favors a long-tailed distribution of spine resistances (Tønnesen et al., 2014), with the typical spine neck resistance being approximately 60 M Ω , with only 5% larger than 500 M Ω .

Is spine neck resistance relevant for plasticity? Although this is still a matter of debate, recent results suggest that it could be. Using multicompartamental computer modeling, Gull edge et al. (2012) showed that a spine neck resistance of as little as 50 M Ω —which is approximately the same as was recently observed with STED microscopy (Tønnesen et al., 2014)—could standardize EPSP amplitudes across the dendritic tree, reducing the location-dependent variability of synaptic responses. However, Gull edge et al. (2012) showed that spine neck resistance would have to be larger than 500 M Ω to alter somatic EPSP amplitude by more than 20%, suggesting that regulation of spine resistance is not an efficient way of adjusting synaptic weights.

Still, earlier studies showed that spines do undergo morphological changes after induction of synaptic plasticity, with LTP going hand-in-hand with spine growth (Matsuzaki et al., 2004), while LTD is associated with spine shrinkage (Zhou et al., 2004). Induction of LTP in addition leads to a shortening of the spine neck length (Araya et al., 2014). Also, synaptic weight is generally correlated with synaptic volume and related measures of spine morphology (Schikorski and Stevens, 1997; Nusser et al., 1998; Takumi et al., 1999; Matsuzaki et al., 2001; Murthy et al., 2001; Kasai et al., 2003) (but see Cingolani and Goda, 2008). These findings hint that spine size may help determine synapse strength, although not necessarily directly via electrical compartmentalization.

STED microscopy has also indicated that LTP leads to changes in spine neck resistance (Tønnesen et al., 2014) and not just spine size (Matsuzaki et al., 2004). Glutamate uncaging in a Mg^{2+} -free environment was used to evoke LTP, and this caused an enlargement of spine neck width and a shortening of spine neck length that led to a 50% drop in spine neck resistance (Tønnesen et al., 2014). This was predicted to bring about only a slight increase in dendritic EPSP amplitude, even though the spine EPSP amplitude would typically drop by 20–40%. Again, spine electrical compartmentalization seems an unlikely means of directly regulating synaptic efficacy, thus implying little or no direct impact on LTP expression. However, the drop in spine neck resistance after LTP is likely to alter subsequent activation of NMDA receptors, since spine voltage will not reach as close to the reversal potential (Tønnesen et al., 2014). This is in general agreement with earlier studies showing that spine neck resistance probably affects the induction of LTP, because head depolarization is strongest in spines that are well isolated from the dendrite, leading to stronger Ca $^{2+}$ signals and presumably increased propensity for LTP (Araya et al., 2006; Grunditz et al., 2008; Bloodgood

et al., 2009; Holbro et al., 2010). Conversely, spine neck shortening in LTP could form a substrate for metaplasticity mechanisms, serving to downregulate the likelihood of LTP in already strongly potentiated spines. Such a mechanism could explain why strong synaptic connections potentiate less well than weak ones (Liao et al., 1992; Bi and Poo, 1998; Montgomery et al., 2001; Sjöström et al., 2001).

Taken together, these studies make it seem unlikely that electrical compartmentalization generally underlies the expression of plasticity. They do, however, make a very compelling case for the involvement of spine neck resistance in regulating the induction of plasticity. We are thus faced with reciprocal interactions between plasticity and spine biophysics: spine neck morphology governs the induction of plasticity, and in return plasticity modulates morphology. This process could increase the computing power and stability of spiny cells.

Biochemical compartmentalization

As outlined earlier, the postsynaptic spine contains the coincidence detection machinery, i.e., NMDA receptors as well as other Ca^{2+} sources that trigger synaptic plasticity. This chemical compartmentalization provides an ideal way for the spine to restrict Ca^{2+} to individual synapses, to ensure synapse specificity as well as to render the molecular machinery of plasticity more efficacious by localizing related enzymes close to each other (Malenka and Nicoll, 1999; for review, see Colgan and Yasuda, 2014).

Confinement of synaptic Ca^{2+} transients in spines has been shown with imaging techniques (Muller and Connor, 1991; Yuste and Denk, 1995). That spines are generally chemically isolated was first demonstrated by direct measurements of diffusion using fluorescence recovery after photo-bleaching (FRAP) (Svoboda et al., 1996), which have since been reproduced several times (e.g., Bloodgood and Sabatini, 2005; Araya et al., 2006; Grunditz et al., 2008; Holbro et al., 2010). A spine may therefore constitute a protected environment for diffusible messengers such as Ca^{2+} , cAMP, and other second messengers, thereby preventing them from reaching other synapses. However, it is important to note that the spine does not restrict all signaling molecules. A gradient of Ras activation, for example, occurs both in the activated spine and in the parent dendrite, but to a lesser degree in the dendrite (Yasuda et al., 2006; Harvey et al., 2008). Other molecules relevant to plasticity may also be restricted to spines. One good example is the kinase CaMKII, which has been hypothesized to maintain information stored at synapses (Lisman, 1989; Lisman et al., 2002). This view, however, has more recently been debated (Lisman et al., 2012), as it has become clear that CaMKII remains activated for only a few minutes after induction (Lee et al., 2009), suggesting that it may be necessary for the induction but not the maintenance of plasticity. What is not subject to debate, however, is that this kinase is actively transported into spines and is activated after induction of LTP (Shen and Meyer, 1999; Otmakhov et al., 2004; Lee et al., 2009). In addition, the targeting of CaMKII mRNA into the parent dendrite as well as its translation is essential for synaptic plasticity and for behavioral learning (Miller et al., 2002). There is also evidence that polyribosomes redistribute from parent dendrites into spines during LTP (Ostroff et al., 2002) (see Chapter 5). This physical compartmentalization provided by spines thus endows neurons with a privileged environment that is important for controlling a key subset of biochemical processes specific to synaptic plasticity.

Interestingly, this biochemical compartmentalization could be modified by plasticity. As mentioned earlier, spines are continuously motile and they undergo rapid and essentially incessant morphological changes (Matus, 2005; Oertner and Matus, 2005). Changes in spine morphology alter diffusion (Majewska et al., 2000), so elongation and shortening of the spine neck renders the spine diffusionaly more or less isolated from the parent dendrite (Korkotian et al., 2004). Using a

low-affinity Ca^{2+} indicator in combination with two-photon imaging, Noguchi et al. (2005) also showed that spines with small necks concentrate NMDA-receptor-mediated Ca^{2+} signals better than do those with large necks. Similarly, Bloodgood and Sabatini (2005) discovered that synaptic plasticity alters diffusion between spines and parent dendrite. It is important to note, however, that the diffusion properties of Ca^{2+} probably differ from those of Ca^{2+} -sensitive dyes. For example, dye-bound Ca^{2+} diffuses more freely and is not physiologically buffered, resulting in an overestimation of Ca^{2+} diffusion (Higley and Sabatini, 2008). Nevertheless, much like with the electrical compartmentalization discussed earlier, these observations suggest reciprocal interactions between plasticity and biochemical compartmentalization. On the one hand, the spatial confinement provided by the spine may increase peak Ca^{2+} levels, thus reducing the threshold for LTP (Korkotian et al., 2004; Noguchi et al., 2005). On the other hand, synaptic plasticity alters spine morphology (Matsuzaki et al., 2004; Zhou et al., 2004) and the diffusive properties of spines (Bloodgood and Sabatini, 2005), thereby resulting in a closed loop. Disentangling these interactions will require much more work.

Plasticity of dendritic excitability

Intrinsic electrical properties of neurons are largely determined by their expression of voltage- and Ca^{2+} -gated ion channels (Johnston and Narayanan, 2008). Mechanistically, the modulation of intrinsic excitability is complex due to the significant variety and functional heterogeneity of the ion channels involved as well as the incomplete portrait of their expression within neurons (Child and Benarroch, 2014). Regulation of the functionality of ion channels includes a variety of mechanisms such as phosphorylation from kinases or translocation to and from the plasma membrane (Johnston and Narayanan, 2008). Activity-dependent changes in intrinsic excitability can either provide positive cell-wide feedback much like Hebbian plasticity does for individual synapses (e.g., Aizenman and Linden, 2000; Cudmore and Turrigiano, 2004), or they can result in negative-feedback plasticity that is homeostatic (e.g., Desai et al., 1999; van Welie et al., 2004, 2006; Frank et al., 2006; Sutton et al., 2006). Changes in intrinsic excitability can be mediated by alterations of the axon initial segment, for example by its reversible movement away from the soma to reduce excitability after periods with high levels of activity (Grubb and Burrone, 2010). Here, however, we next focus on plasticity of intrinsic excitability that is specifically dendritically localized (Zhang and Linden, 2003; Campanac and Debanne, 2007; Sjöström et al., 2008; Debanne and Poo, 2010).

Hebbian changes in dendritic excitability

Changes in dendritic excitability can work in synergy with Hebbian synaptic plasticity, presumably to assist in information storage (Sjöström et al., 2008). It has been demonstrated that upon theta-burst induction of LTP in hippocampal CA1 pyramidal cells, there is an NMDA receptor-dependent increase in dendritic excitability that remains localized to a region no larger than a few hundred microns (Frick et al., 2004). In those dendritic compartments, excitability is increased by a hyperpolarizing shift of the inactivation curve of I_A . As a consequence, APs backpropagate more reliably, which means subsequent induction of LTP at nearby synapses is likely facilitated. Although not yet studied in detail, this might therefore result in heterosynaptic metaplasticity. A subsequent study revealed that I_A -based chemically induced plasticity could be spatially restricted to individual spines (Kim et al., 2007), although here expression was based on ion channel trafficking. Regardless, I_A -based plasticity of intrinsic dendritic excitability could help explain E-S potentiation in classical LTP (Frick et al., 2004). Earlier studies, on the other hand, suggested that changes

in inhibition underlie E-S potentiation (Abraham et al., 1987; Chavez-Noriega et al., 1989), but it was later shown that E-S potentiation can be induced in the absence of inhibition (Hess and Gustafsson, 1990; Asztely and Gustafsson, 1994; Jester et al., 1995; although see Staff and Spruston, 2003). Besides, simultaneous LTP of excitation and inhibition may actually help preserve the balance between excitation and inhibition (Lamsa et al., 2005). Indeed, it has been argued for some time that local modifications of dendritic excitability may be the principal cause of E-S potentiation (Wathey et al., 1992).

Several other studies have reported changes in dendritic intrinsic excitability after induction of LTP, although these have found an involvement of the hyperpolarization-activated “sag” conductance I_h (Wang et al., 2003; Campanac et al., 2008) or of Na^+ channels (Xu et al., 2005), instead of I_A (Frick et al., 2004). Although synaptic and intrinsic forms of plasticity typically seem to act synergistically, this is not always the case: maximal synaptic depression may result in increased intrinsic excitability (Brager and Johnston, 2007).

Interestingly, changes in intrinsic dendritic excitability are not restricted to classical LTP, but can be found in the more recent STDP paradigm as well: in the hippocampus, such dendritic plasticity is bidirectional and acutely temporally sensitive in a manner very similar to STDP (Campanac and Debanne, 2008). Timing-dependent plasticity of dendritic excitability furthermore remains quite dendritically localized, and thus provides a surprising degree of synapse specificity (Campanac and Debanne, 2008). Localized plasticity of intrinsic dendritic excitability may, in other words, work in concert with temporally sensitive synaptic plasticity learning rules such as STDP (Campanac and Debanne, 2007; Debanne and Poo, 2010). Timing-based LTP and LTD can also give rise to the corresponding up- or downregulation of excitability in the presynaptic cell (Ganguly et al., 2000; Li et al., 2004). Here, it would be interesting to know if the altered excitability is due to changes in the dendrites of the presynaptic neuron.

In the hippocampus, changes in dendritic excitability have knock-on effects on subsequent co-incidence detection in dendrites (Xu et al., 2006). This in turn may of course affect the plasticity of both synapses and dendrites. We are thus faced with a complicated and intriguing interplay between intrinsic and synaptic forms of plasticity (see “Conclusions”).

Interestingly, it has been found that the coupling between local dendritic spikes and the soma of hippocampal CA1 pyramidal cells can be modified in an arbor-specific manner, thus resulting in a form of branch-strength potentiation (Losonczy et al., 2008). Triggering of this mechanism requires NMDA receptor activation, and further relies on regulation of dendritic $K_{v4.2}$ potassium channels for expression (Losonczy et al., 2008). As this mechanism is somewhat input-specific, individual dendritic compartments could help store information. Theoretical work has demonstrated that this form of plasticity permits individual neurons to bind input features in a self-organized manner (Legenstein and Maass, 2011). This fine-grained compartmentalization of dendritic plasticity suggests that neurons could in principle function as individual mini-networks, to bind features at the single-cell level (Poirazi and Mel, 2001; Häusser and Mel, 2003; Poirazi et al., 2003; Costa and Sjöström, 2011).

Homeostatic changes in dendritic excitability

Dendrites can also mediate local negative-feedback mechanisms that ensure homeostasis (Yu and Goda, 2009). For example, in dissociated hippocampal neurons, global suppression of activity leads to rapid local dendritic upregulation of AMPA receptors specifically in a region where NMDA receptors are antagonized (Sutton et al., 2006). Regulation can also be presynaptic, yet specific to local dendritic activity: in hippocampal cultures, the local level of dendritic depolarization sets the probability of release (Branco et al., 2008).

Theta-burst firing in CA1 pyramidal cells leads to an increase in the sag current I_h in the apical dendrite, which requires bAPs and NMDA receptor activation (Fan et al., 2005). This results in a global decrease in neuronal excitability displayed by lower somatic input resistance and a rightward shift of the f - I curve. Because this form of intrinsic dendritic plasticity helps downregulate cell excitability after potentiation, it should be homeostatic. An earlier study, however, reported the opposite outcome (see above and Wang et al., 2003). What could explain this discrepancy? Campanac et al. (2008) provided a solution to this conundrum. They found that near-maximal LTP induced by theta-burst pairing produced a homeostatic upregulation of I_h in CA1 pyramidal cell dendrites (Campanac et al., 2008). However, more moderate levels of LTP elicited E-S potentiation via downregulation of I_h (Campanac et al., 2008). These findings suggest that two opposing forms of dendritic plasticity are active simultaneously. If stimulation strength is high, negative-feedback homeostasis is activated, but if levels of activation are low, positive-feedback Hebbian learning is elicited. Dendritic excitability may thus be differentially regulated depending on levels of activity to ensure stability as well as efficient information storage (Sjöström et al., 2008).

Conclusions

In this chapter, we have taken a dendritic perspective on functional synaptic plasticity. We have argued that synaptic plasticity is fundamentally a dendritic phenomenon, since the vast majority of inputs are made onto the dendrites. Dendrites serve to integrate the information delivered by synapses, and based on this incoming activity they may trigger different forms of synaptic plasticity. But the excitability of dendritic arbors is also regulated in an activity-dependent manner. As a result, there is a close interrelationship between the plasticity of synapses and dendrites that provides rich dynamics for adjusting and refining computations at the synaptic, dendritic, cellular, and network levels (Sjöström et al., 2008). To ensure optimal performance of neuronal microcircuits, and to avoid pathological states such as epilepsy, synaptic strength and dendritic excitability must therefore be regulated together.

Although we have made leaps in our understanding of dendritic function with the advent of technical advances such as two-photon imaging, multicompartmental computer modeling, dendritic recordings, and optogenetics, there is considerable work left to do. One important problem is to understand how the strength of synapses is adjusted to correct for or to take advantage of dendritic geometry. For example, distal synapses have less influence than more proximal synapses on axonally generated APs, which puts them at a disadvantage in the temporal competition to become potentiated by STDP. But STDP rules themselves are location dependent (Froemke et al., 2010). This location dependence is partly due to decremental AP backpropagation, which provides the associative signal for STDP. In this view, distal synapses would be prone to LTD, whereas proximal inputs would tend to potentiate (Letzkus et al., 2006; Sjöström and Häusser, 2006; Hardingham et al., 2007), which could lead to an imbalance of synaptic weights in the dendritic tree. The corresponding problem also exists for plasticity mediated by local dendritic spikes (Goldberg et al., 2002). However, CA1 pyramidal neurons and neocortical L5 pyramidal cells both seem to have relatively well-balanced dendritic arbors (Magee and Cook, 2000; Williams and Stuart, 2002). Perhaps homeostatic mechanisms normalize weights spatially in dendrites to ensure that arbors remain well balanced (Turrigiano, 2011; Pozo and Goda, 2010). Much additional research is needed to address these problems.

In addition, there are mechanisms to alter local dendritic excitability (Frick et al., 2004; Lošonczi et al., 2008). This could affect the reliability of AP backpropagation locally, or it could increase the propensity for subsequent local dendritic spikes, both of which might promote

additional potentiation of synaptic strength as well as of local dendritic excitability. The region of increased dendritic excitability may thus expand and grow, potentially leading to pathological states such as epilepsy. Perhaps negative-feedback mechanisms kick in to ensure that such runaway growth of dendritic excitability does not occur (Fan et al., 2005; Campanac et al., 2008). Perhaps the presynaptic side is additionally regulated to reduce synaptic strength locally (Branco et al., 2008). However, it remains unclear how all these mechanisms are precisely coordinated to maintain neuronal branch-specific excitability that is both balanced and within reasonable bounds.

What is clear, however, is that regarding the point neuron as *the* computational unit is a serious oversimplification, because neurons have dendrites, which actively contribute to single-neuron computations, to synaptic plasticity, and to intrinsic plasticity. Instead, we must consider individual dendritic neurons as powerful mini-networks in and of themselves (Poirazi and Mel, 2001; Häusser and Mel, 2003; Poirazi et al., 2003; Larkum et al., 2009). Here is one case where Donald Hebb was uncharacteristically wrong: he argued that dendrites are exclusively for connecting the presynaptic to the postsynaptic neuron (Hebb, 1972). However, to fully appreciate the functional plasticity at dendritic synapses, we must depart from Hebbian dogma and instead think outside the point-neuron box. Given our rapidly expanding experimental toolkit, this will no doubt be an exciting departure.

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References

- Abbott LF, Nelson SB (2000) Synaptic plasticity: taming the beast. *Nature Neuroscience* **3**:1178–1183.
- Abbott LF, Varela JA, Sen K, Nelson SB (1997) Synaptic depression and cortical gain control. *Science* **275**:220–224.
- Abraham WC (2003) How long will long-term potentiation last? *Philosophical Transactions of the Royal Society B: Biological Sciences* **358**:735–744.
- Abraham WC, Bear MF (1996) Metaplasticity: the plasticity of synaptic plasticity. *Trends in Neurosciences* **19**:126–130.
- Abraham WC, Robins A (2005) Memory retention—the synaptic stability versus plasticity dilemma. *Trends in Neurosciences* **28**:73–78.
- Abraham WC, Gustafsson B, Wigström H (1987) Long-term potentiation involves enhanced synaptic excitation relative to synaptic inhibition in guinea-pig hippocampus. *Journal of Physiology* **394**:367–380.
- Abraham WC, Mason-Parker SE, Bear MF, Webb S, Tate WP (2001) Heterosynaptic metaplasticity in the hippocampus *in vivo*: a BCM-like modifiable threshold for LTP. *Proceedings of the National Academy of Sciences of the United States of America* **98**:10924–10929.
- Abraham WC, Logan B, Greenwood JM, Dragunow M (2002) Induction and experience-dependent consolidation of stable long-term potentiation lasting months in the hippocampus. *Journal of Neuroscience* **22**:9626–9634.
- Aizenman CD, Linden DJ (2000) Rapid, synaptically driven increases in the intrinsic excitability of cerebellar deep nuclear neurons. *Nature Neuroscience* **3**:109–111.
- Aizenman CD, Akerman CJ, Jensen KR, Cline HT (2003) Visually driven regulation of intrinsic neuronal excitability improves stimulus detection *in vivo*. *Neuron* **39**:831–842.

- Allen CB, Celikel T, Feldman DE (2003) Long-term depression induced by sensory deprivation during cortical map plasticity in vivo. *Nature Neuroscience* **6**:291–299.
- Andersen P, Sundberg SH, Sveen O, Wigstrom H (1977) Specific long-lasting potentiation of synaptic transmission in hippocampal slices. *Nature* **266**:736–737.
- Araya R, Jiang J, Eisenthal KB, Yuste R (2006) The spine neck filters membrane potentials. *Proceedings of the National Academy of Sciences of the United States of America* **103**:17961–17966.
- Araya R, Nikolenko V, Eisenthal KB, Yuste R (2007) Sodium channels amplify spine potentials. *Proceedings of the National Academy of Sciences of the United States of America* **104**:12347–12352.
- Araya R, Vogels TP, Yuste R (2014) Activity-dependent dendritic spine neck changes are correlated with synaptic strength. *Proceedings of the National Academy of Sciences of the United States of America* **111**:E2895–E2904.
- Armano S, Rossi P, Taglietti V, D’Angelo E (2000) Long-term potentiation of intrinsic excitability at the mossy fiber-granule cell synapse of rat cerebellum. *Journal of Neuroscience* **20**:5208–5216.
- Asztely F, Gustafsson B (1994) Dissociation between long-term potentiation and associated changes in field EPSP waveform in the hippocampal CA1 region: an *in vitro* study in guinea pig brain slices. *Hippocampus* **4**:148–156.
- Bacci A, Coco S, Pravettoni E, Schenk U, Armano S, Frassoni C, Verderio C, De Camilli P, Matteoli M (2001) Chronic blockade of glutamate receptors enhances presynaptic release and downregulates the interaction between synaptophysin-synaptobrevin-vesicle-associated membrane protein 2. *Journal of Neuroscience* **21**:6588–6596.
- Bakin JS, Weinberger NM (1996) Induction of a physiological memory in the cerebral cortex by stimulation of the nucleus basalis. *Proceedings of the National Academy of Sciences of the United States of America* **93**:11219–11224.
- Bar-Ilan L, Gidon A, Segev I (2012) The role of dendritic inhibition in shaping the plasticity of excitatory synapses. *Frontiers in Neural Circuits* **6**:118.
- Barrio Nuevo G, Brown TH (1983) Associative long-term potentiation in hippocampal slices. *Proceedings of the National Academy of Sciences of the United States of America* **80**:7347–7351.
- Barth AL, Poulet JF (2012) Experimental evidence for sparse firing in the neocortex. *Trends in Neurosciences* **35**:345–355.
- Bear MF, Cooper LN, Ebner FF (1987) A physiological basis for a theory of synapse modification. *Science* **237**:42–48.
- Bell CC, Han VZ, Sugawara Y, Grant K (1997) Synaptic plasticity in a cerebellum-like structure depends on temporal order. *Nature* **387**:278–281.
- Bender VA, Bender KJ, Brasier DJ, Feldman DE (2006) Two coincidence detectors for spike timing-dependent plasticity in somatosensory cortex. *Journal of Neuroscience* **26**:4166–4177.
- Bi GQ, Poo MM (1998) Synaptic modifications in cultured hippocampal neurons: dependence on spike timing, synaptic strength, and postsynaptic cell type. *Journal of Neuroscience* **18**:10464–10472.
- Bi GQ, Poo MM (2001) Synaptic modification by correlated activity: Hebb’s postulate revisited. *Annual Review of Neuroscience* **24**:139–166.
- Bienenstock EL, Cooper LN, Munro PW (1982) Theory for the development of neuron selectivity: orientation specificity and binocular interaction in visual cortex. *Journal of Neuroscience* **2**:32–48.
- Bissiere S, Humeau Y, Luthi A (2003) Dopamine gates LTP induction in lateral amygdala by suppressing feedforward inhibition. *Nature Neuroscience* **6**:587–592.
- Blackman AV, Abrahamsson T, Costa RP, Lalanne T, Sjöström PJ (2013) Target cell-specific short-term plasticity in local circuits. *Frontiers in Synaptic Neuroscience* **5**:11.
- Blackman AV, Grabschchnig S, Legenstein R, Sjöström PJ (2014) A comparison of manual neuronal reconstruction from biocytin histology or 2-photon imaging: morphometry and computer modeling. *Frontiers in Neuroanatomy* **8**:65.

- Bliss TV, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* **361**:31–39.
- Bliss TV, Lømo T (1970) Plasticity in a monosynaptic cortical pathway. *Journal of Physiology* **207**:61P.
- Bliss TV, Lømo T (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *Journal of Physiology* **232**:331–356.
- Bliss TVP, Lømo T, Gardner-Medwin AR (1973) Synaptic plasticity in the hippocampal formation. In: *Macromolecules and Behaviour* (Ansell G, Bradley PB, eds), pp. 193–203. London: Macmillan.
- Bloodgood BL, Sabatini BL (2005) Neuronal activity regulates diffusion across the neck of dendritic spines. *Science* **310**:866–869.
- Bloodgood BL, Giessel AJ, Sabatini BL (2009) Biphasic synaptic Ca influx arising from compartmentalized electrical signals in dendritic spines. *PLoS Biology* **7**:e1000190.
- Brager DH, Johnston D (2007) Plasticity of intrinsic excitability during long-term depression is mediated through mGluR-dependent changes in I(h) in hippocampal CA1 pyramidal neurons. *Journal of Neuroscience* **27**:13926–13937.
- Branco T, Häusser M (2010) The single dendritic branch as a fundamental functional unit in the nervous system. *Current Opinion in Neurobiology* **20**:494–502.
- Branco T, Staras K, Darcy KJ, Goda Y (2008) Local dendritic activity sets release probability at hippocampal synapses. *Neuron* **59**:475–485.
- Brown TH, Kairiss EW, Keenan CL (1990) Hebbian synapses: biophysical mechanisms and algorithms. *Annual Review of Neuroscience* **13**:475–511.
- Buchanan KA, Mellor JR (2010) The activity requirements for spike timing-dependent plasticity in the hippocampus. *Frontiers in Synaptic Neuroscience* **2**:11.
- Burrone J, O'Byrne M, Murthy VN (2002) Multiple forms of synaptic plasticity triggered by selective suppression of activity in individual neurons. *Nature* **420**:414–418.
- Camire O, Topolnik L (2014) Dendritic calcium nonlinearities switch the direction of synaptic plasticity in fast-spiking interneurons. *Journal of Neuroscience* **34**:3864–3877.
- Campanac E, Debanne D (2007) Plasticity of neuronal excitability: Hebbian rules beyond the synapse. *Archives Italiennes de Biologie* **145**:277–287.
- Campanac E, Debanne D (2008) Spike timing-dependent plasticity: a learning rule for dendritic integration in rat CA1 pyramidal neurons. *Journal of Physiology* **586**:779–793.
- Campanac E, Daoudal G, Ankri N, Debanne D (2008) Downregulation of dendritic I(h) in CA1 pyramidal neurons after LTP. *Journal of Neuroscience* **28**:8635–8643.
- Cantrell AR, Catterall WA (2001) Neuromodulation of Na⁺ channels: an unexpected form of cellular plasticity. *Nature Reviews Neuroscience* **2**:397–407.
- Caporale N, Dan Y (2008) Spike timing-dependent plasticity: a Hebbian learning rule. *Annual Review of Neuroscience* **31**:25–46.
- Celikel T, Szostak VA, Feldman DE (2004) Modulation of spike timing by sensory deprivation during induction of cortical map plasticity. *Nature Neuroscience* **7**:534–541.
- Chang HT (1952) Cortical neurons with particular reference to the apical dendrites. *Cold Spring Harbor Symposia on Quantitative Biology* **17**:189–202.
- Chavez-Noriega LE, Bliss TV, Halliwell JV (1989) The EPSP-spike (E-S) component of long-term potentiation in the rat hippocampal slice is modulated by GABAergic but not cholinergic mechanisms. *Neuroscience Letters* **104**:58–64.
- Chevaleyre V, Castillo PE (2004) Endocannabinoid-mediated metaplasticity in the hippocampus. *Neuron* **43**:871–881.
- Child ND, Benarroch EE (2014) Differential distribution of voltage-gated ion channels in cortical neurons: implications for epilepsy. *Neurology* **82**:989–999.
- Chklovskii DB, Mel BW, Svoboda K (2004) Cortical rewiring and information storage. *Nature* **431**:782–788.

- Cingolani LA, Goda Y (2008) Actin in action: the interplay between the actin cytoskeleton and synaptic efficacy. *Nature Reviews Neuroscience* **9**:344–356.
- Cline HT (1998) Topographic maps: developing roles of synaptic plasticity. *Current Biology*, **8**, R836–839.
- Clopath C, Busing L, Vasilaki E, Gerstner W (2010) Connectivity reflects coding: a model of voltage-based STDP with homeostasis. *Nature Neuroscience* **13**:344–352.
- Colbert CM, Johnston D (1998) Protein kinase C activation decreases activity-dependent attenuation of dendritic Na^+ current in hippocampal CA1 pyramidal neurons. *Journal of Neurophysiology* **79**:491–495.
- Colgan LA, Yasuda R (2014) Plasticity of dendritic spines: subcompartmentalization of signaling. *Annual Review of Physiology* **76**:365–385.
- Collingridge GL, Kehl SJ, McLennan H (1983) Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. *Journal of Physiology* **334**:33–46.
- Corlew R, Wang Y, Ghermazien H, Erisir A, Philpot BD (2007) Developmental switch in the contribution of presynaptic and postsynaptic NMDA receptors to long-term depression. *Journal of Neuroscience* **27**:9835–9845.
- Costa RP, Sjöström PJ (2011) One cell to rule them all, and in the dendrites bind them. *Frontiers in Synaptic Neuroscience* **3**:1–2.
- Couey JJ, Meredith RM, Spijker S, Poorthuis RB, Smit AB, Brussaard AB, Mansvelder HD (2007) Distributed network actions by nicotine increase the threshold for spike-timing-dependent plasticity in prefrontal cortex. *Neuron* **54**:73–87.
- Crair MC (1999) Neuronal activity during development: permissive or instructive? *Current Opinion in Neurobiology* **9**:88–93.
- Cudmore RH, Turrigiano GG (2004) Long-term potentiation of intrinsic excitability in LV visual cortical neurons. *Journal of Neurophysiology* **92**:341–348.
- D'Amour JA, Froemke RC (2015) Inhibitory and excitatory spike-timing-dependent plasticity in the auditory cortex. *Neuron* **86**:514–528.
- Daoudal G, Hanada Y, Debanne D (2002) Bidirectional plasticity of excitatory postsynaptic potential (EPSP)-spike coupling in CA1 hippocampal pyramidal neurons. *Proceedings of the National Academy of Sciences of the United States of America* **99**:14512–14517.
- Davis MJ, Wu X, Nurkiewicz TR, Kawasaki J, Gui P, Hill MA, Wilson E (2001) Regulation of ion channels by protein tyrosine phosphorylation. *American Journal of Physiology Heart and Circulatory Physiology* **281**:H1835–H1862.
- Day M, Wokosin D, Plotkin JL, Tian X, Surmeier DJ (2008) Differential excitability and modulation of striatal medium spiny neuron dendrites. *Journal of Neuroscience* **28**:11603–11614.
- Debanne D, Poo MM (2010) Spike-timing dependent plasticity beyond synapse—pre- and post-synaptic plasticity of intrinsic neuronal excitability. *Frontiers in Synaptic Neuroscience* **2**:21.
- Debanne D, Gähwiler BH, Thompson SM (1994) Asynchronous pre- and postsynaptic activity induces associative long-term depression in area CA1 of the rat hippocampus in vitro. *Proceedings of the National Academy of Sciences of the United States of America* **91**:1148–1152.
- Debanne D, Gähwiler BH, Thompson SM (1996) Cooperative interactions in the induction of long-term potentiation and depression of synaptic excitation between hippocampal CA3–CA1 cell pairs in vitro. *Proceedings of the National Academy of Sciences of the United States of America* **93**:11225–11230.
- Debanne D, Gähwiler BH, Thompson SM (1998) Long-term synaptic plasticity between pairs of individual CA3 pyramidal cells in rat hippocampal slice cultures. *Journal of Physiology* **507**:237–247.
- Defelipe J, et al. (2013) New insights into the classification and nomenclature of cortical GABAergic interneurons. *Nature Reviews Neuroscience* **14**:202–216.
- Desai NS (2003) Homeostatic plasticity in the CNS: synaptic and intrinsic forms. *Journal of Physiology Paris* **97**:391–402.
- Desai NS, Rutherford LC, Turrigiano GG (1999) Plasticity in the intrinsic excitability of cortical pyramidal neurons. *Nature Neuroscience* **2**:515–520.

- Desai NS, Cudmore RH, Nelson SB, Turrigiano GG (2002) Critical periods for experience-dependent synaptic scaling in visual cortex. *Nature Neuroscience* 5:783–789.
- Dorrn AL, Yuan K, Barker AJ, Schreiner CE, Froemke RC (2010) Developmental sensory experience balances cortical excitation and inhibition. *Nature* 465:932–936.
- Douglas RM, Goddard GV (1975) Long-term potentiation of the perforant path-granule cell synapse in the rat hippocampus. *Brain Research* 86:205–215.
- Dudek SM, Bear MF (1992) Homosynaptic long-term depression in area CA1 of hippocampus and effects of N-methyl-D-aspartate receptor blockade. *Proceedings of the National Academy of Sciences of the United States of America* 89:4363–4367.
- Duguid I, Sjöström PJ (2006) Novel presynaptic mechanisms for coincidence detection in synaptic plasticity. *Current Opinion in Neurobiology* 16:312–322.
- Egger V, Feldmeyer D, Sakmann B (1999) Coincidence detection and changes of synaptic efficacy in spiny stellate neurons in rat barrel cortex. *Nature Neuroscience* 2:1098–1105.
- Engert F, Bonhoeffer T (1997) Synapse specificity of long-term potentiation breaks down at short distances. *Nature* 388:279–284.
- Espinosa JS, Stryker MP (2012) Development and plasticity of the primary visual cortex. *Neuron* 75:230–249.
- Etzion Y, Grossman Y (1998) Potassium currents modulation of calcium spike firing in dendrites of cerebellar Purkinje cells. *Experimental Brain Research* 122:283–294.
- Fan Y, Fricker D, Brager DH, Chen X, Lu HC, Chitwood RA, Johnston D (2005) Activity-dependent decrease of excitability in rat hippocampal neurons through increases in I(h). *Nature Neuroscience* 8:1542–1551.
- Feldman DE (2000) Timing-based LTP and LTD at vertical inputs to layer II/III pyramidal cells in rat barrel cortex. *Neuron* 27:45–56.
- Feldman DE (2009) Synaptic mechanisms for plasticity in neocortex. *Annual Review of Neuroscience* 32:33–55.
- Feldman DE (2012) The spike-timing dependence of plasticity. *Neuron* 75:556–571.
- Feldman DE, Brecht M (2005) Map plasticity in somatosensory cortex. *Science* 310:810–815.
- Ferreira TA, Blackman AV, Oyrer J, Jayabal S, Chung AJ, Watt AJ, Sjöström PJ, Van Meyel DJ (2014) Neuronal morphometry directly from bitmap images. *Nature Methods* 11:982–984.
- Fino E, Glowinski J, Venance L (2005) Bidirectional activity-dependent plasticity at corticostriatal synapses. *Journal of Neuroscience* 25:11279–11287.
- Fishell G, Rudy B (2011) Mechanisms of inhibition within the telencephalon: “where the wild things are.” *Annual Review of Neuroscience* 34:535–567.
- Frank CA, Kennedy MJ, Goold CP, Marek KW, Davis GW (2006) Mechanisms underlying the rapid induction and sustained expression of synaptic homeostasis. *Neuron* 52:663–677.
- Frey U, Morris RG (1998) Synaptic tagging: implications for late maintenance of hippocampal long-term potentiation. *Trends in Neurosciences* 21:181–188.
- Frick A, Magee J, Johnston D (2004) LTP is accompanied by an enhanced local excitability of pyramidal neuron dendrites. *Nature Neuroscience* 7:126–135.
- Froemke RC, Dan Y (2002) Spike-timing-dependent synaptic modification induced by natural spike trains. *Nature* 416:433–438.
- Froemke RC, Poo MM, Dan Y (2005) Spike-timing-dependent synaptic plasticity depends on dendritic location. *Nature* 434:221–225.
- Froemke RC, Tsay IA, Raad M, Long JD, Dan Y (2006) Contribution of individual spikes in burst-induced long-term synaptic modification. *Journal of Neurophysiology* 95:1620–1629.
- Froemke RC, Merzenich MM, Schreiner CE (2007) A synaptic memory trace for cortical receptive field plasticity. *Nature* 450:425–429.

- Froemke RC, Letzkus JJ, Kampa BM, Hang GB, Stuart GJ (2010) Dendritic synapse location and neocortical spike-timing-dependent plasticity. *Frontiers in Synaptic Neuroscience* **2**:29.
- Froemke RC, Carcea I, Barker AJ, Yuan K, Seybold BA, Martins AR, Zaika N, Bernstein H, Wachs M, Levis PA, Polley DB, Merzenich MM, Schreiner CE (2013) Long-term modification of cortical synapses improves sensory perception. *Nature Neuroscience* **16**:79–88.
- Fu M, Yu X, Lu J, Zuo Y (2012) Repetitive motor learning induces coordinated formation of clustered dendritic spines in vivo. *Nature* **483**:92–95.
- Galarreta M, Hestrin S (1998) Frequency-dependent synaptic depression and the balance of excitation and inhibition in the neocortex. *Nature Neuroscience* **1**:587–594.
- Gambino F, Pages S, Kehayas V, Baptista D, Tatti R, Carleton A, Holtmaat A (2014) Sensory-evoked LTP driven by dendritic plateau potentials in vivo. *Nature* **515**:116–119.
- Gandhi SP, Yanagawa Y, Stryker MP (2008) Delayed plasticity of inhibitory neurons in developing visual cortex. *Proceedings of the National Academy of Sciences of the United States of America* **105**:16797–16802.
- Ganguly K, Kiss L, Poo M (2000) Enhancement of presynaptic neuronal excitability by correlated presynaptic and postsynaptic spiking. *Nature Neuroscience* **3**:1018–1026.
- Gasparini S, Migliore M, Magee JC (2004) On the initiation and propagation of dendritic spikes in CA1 pyramidal neurons. *Journal of Neuroscience* **24**:11046–11056.
- Gasparini S, Losonczy A, Chen X, Johnston D, Magee JC (2007) Associative pairing enhances action potential back-propagation in radial oblique branches of CA1 pyramidal neurons. *Journal of Physiology* **580**:787–800.
- Gidon A, Segev I (2012) Principles governing the operation of synaptic inhibition in dendrites. *Neuron* **75**:330–341.
- Goldberg J, Holthoff K, Yuste R (2002) A problem with Hebb and local spikes. *Trends in Neurosciences* **25**:433–435.
- Golding NL, Spruston N (1998) Dendritic sodium spikes are variable triggers of axonal action potentials in hippocampal CA1 pyramidal neurons. *Neuron* **21**:1189–1200.
- Golding NL, Jung HY, Mickus T, Spruston N (1999) Dendritic calcium spike initiation and repolarization are controlled by distinct potassium channel subtypes in CA1 pyramidal neurons. *Journal of Neuroscience* **19**:8789–8798.
- Golding NL, Staff NP, Spruston N (2002) Dendritic spikes as a mechanism for cooperative long-term potentiation. *Nature* **418**:326–331.
- Gordon U, Polksy A, Schiller J (2006) Plasticity compartments in basal dendrites of neocortical pyramidal neurons. *Journal of Neuroscience* **26**:12717–12726.
- Govindarajan A, Israely I, Huang SY, Tonegawa S (2011) The dendritic branch is the preferred integrative unit for protein synthesis-dependent LTP. *Neuron* **69**:132–146.
- Graupner M, Brunel N (2012) Calcium-based plasticity model explains sensitivity of synaptic changes to spike pattern, rate, and dendritic location. *Proceedings of the National Academy of Sciences of the United States of America* **109**:3991–3996.
- Grubbs MS, Burrone J (2010) Activity-dependent relocation of the axon initial segment fine-tunes neuronal excitability. *Nature* **465**:1070–1074.
- Grunditz A, Holbro N, Tian L, Zuo Y, Oertner TG (2008) Spine neck plasticity controls postsynaptic calcium signals through electrical compartmentalization. *Journal of Neuroscience* **28**:13457–13466.
- Gulledge AT, Stuart GJ (2003) Action potential initiation and propagation in layer 5 pyramidal neurons of the rat prefrontal cortex: absence of dopamine modulation. *Journal of Neuroscience* **23**:11363–11372.
- Gulledge AT, Carnevale NT, Stuart GJ (2012) Electrical advantages of dendritic spines. *PLoS ONE* **7**:e36007.
- Gustafsson B, Wigström H (1986) Hippocampal long-lasting potentiation produced by pairing single volleys and brief conditioning tetani evoked in separate afferents. *Journal of Neuroscience* **6**:1575–1582.

- Gustafsson B, Wigström H, Abraham WC, Huang YY (1987) Long-term potentiation in the hippocampus using depolarizing current pulses as the conditioning stimulus to single volley synaptic potentials. *Journal of Neuroscience* 7:774–780.
- Han VZ, Grant K, Bell CC (2000) Reversible associative depression and nonassociative potentiation at a parallel fiber synapse. *Neuron* 27:611–622.
- Hansel C, Artola A, Singer W (1997) Relation between dendritic Ca^{2+} levels and the polarity of synaptic long-term modifications in rat visual cortex neurons. *European Journal of Neuroscience* 9:2309–2322.
- Hardie J, Spruston N (2009) Synaptic depolarization is more effective than back-propagating action potentials during induction of associative long-term potentiation in hippocampal pyramidal neurons. *Journal of Neuroscience* 29:3233–3241.
- Hardingham NR, Hardingham GE, Fox KD, Jack JJ (2007) Pre-synaptic efficacy directs normalization of synaptic strength in layer 2/3 rat neocortex following paired activity. *Journal of Neurophysiology* 97:2965–2975.
- Harnett MT, Makara JK, Spruston N, Kath WL, Magee JC (2012) Synaptic amplification by dendritic spines enhances input cooperativity. *Nature* 491:599–602.
- Harris KM (1999) Structure, development, and plasticity of dendritic spines. *Current Opinion in Neurobiology* 9:343–348.
- Harris KM, Stevens JK (1989) Dendritic spines of CA 1 pyramidal cells in the rat hippocampus: serial electron microscopy with reference to their biophysical characteristics. *Journal of Neuroscience* 9:2982–2997.
- Harvey CD, Svoboda K (2007) Locally dynamic synaptic learning rules in pyramidal neuron dendrites. *Nature* 450:1195–1200.
- Harvey CD, Yasuda R, Zhong H, Svoboda K (2008) The spread of Ras activity triggered by activation of a single dendritic spine. *Science* 321:136–140.
- Häusser M (2001) Synaptic function: dendritic democracy. *Current Biology* 11:R10–R12.
- Häusser M, Mel B (2003) Dendrites: bug or feature? *Current Opinion in Neurobiology* 13:372–383.
- Häusser M, Spruston N, Stuart GJ (2000) Diversity and dynamics of dendritic signaling. *Science* 290:739–744.
- Hebb DO (1949) *The Organization of Behavior*. New York: Wiley.
- Hebb DO (1972) *A Textbook of Psychology*. Montreal: W. B. Saunders.
- Helmbchen F, Svoboda K, Denk W, Tank DW (1999) In vivo dendritic calcium dynamics in deep-layer cortical pyramidal neurons. *Nature Neuroscience* 2:989–996.
- Hensch TK (2005) Critical period plasticity in local cortical circuits. *Nature Reviews Neuroscience* 6:877–888.
- Hess G, Gustafsson B (1990) Changes in field excitatory postsynaptic potential shape induced by tetanization in the CA1 region of the guinea-pig hippocampal slice. *Neuroscience* 37:61–69.
- Higley MJ, Sabatini BL (2008) Calcium signaling in dendrites and spines: practical and functional considerations. *Neuron* 59:902–913.
- Hoffman DA, Johnston D (1998) Downregulation of transient K^+ channels in dendrites of hippocampal CA1 pyramidal neurons by activation of PKA and PKC. *Journal of Neuroscience* 18:3521–3528.
- Hoffman DA, Johnston D (1999) Neuromodulation of dendritic action potentials. *Journal of Neurophysiology* 81:408–411.
- Hoffman DA, Magee JC, Colbert CM, Johnston D (1997) K^+ channel regulation of signal propagation in dendrites of hippocampal pyramidal neurons. *Nature* 387:869–875.
- Hoffman DA, Sprengel R, Sakmann B (2002) Molecular dissection of hippocampal theta-burst pairing potentiation. *Proceedings of the National Academy of Sciences of the United States of America* 99:7740–7745.
- Holbro N, Grunditz A, Wiegert JS, Oertner TG (2010) AMPA receptors gate spine $\text{Ca}(2+)$ transients and spike-timing-dependent potentiation. *Proceedings of the National Academy of Sciences of the United States of America* 107:15975–15980.

- Holmgren CD, Zilberman Y (2001) Coincident spiking activity induces long-term changes in inhibition of neocortical pyramidal cells. *Journal of Neuroscience* **21**:8270–8277.
- Holthoff K, Kovalchuk Y, Yuste R, Konnerth A (2004) Single-shock LTD by local dendritic spikes in pyramidal neurons of mouse visual cortex. *Journal of Physiology* **560**:27–36.
- Hoogland TM, Saggau P (2004) Facilitation of L-type Ca^{2+} channels in dendritic spines by activation of β_2 adrenergic receptors. *Journal of Neuroscience* **24**:8416–8427.
- Humeau Y, Shaban H, Bissière S, Lüthi A (2003) Presynaptic induction of heterosynaptic associative plasticity in the mammalian brain. *Nature* **426**:841–845.
- Ibata K, Sun Q, Turrigiano GG (2008) Rapid synaptic scaling induced by changes in postsynaptic firing. *Neuron* **57**:819–826.
- Izhikevich EM, Desai NS (2003) Relating STDP to BCM. *Neural Computation* **15**:1511–1523.
- Jack JJB, Redman SJ (1971) The propagation of transient potentials in some linear cable structures. *Journal of Physiology* **215**:283–320.
- Jacob V, Brasier DJ, Erchova I, Feldman D, Shulz DE (2007) Spike timing-dependent synaptic depression in the in vivo barrel cortex of the rat. *Journal of Neuroscience* **27**:1271–1284.
- Jester JM, Campbell LW, Sejnowski TJ (1995) Associative EPSP-spike potentiation induced by pairing orthodromic and antidromic stimulation in rat hippocampal slices. *Journal of Physiology* **484**:689–705.
- Jia H, Rochefort NL, Chen X, Konnerth A (2010) Dendritic organization of sensory input to cortical neurons in vivo. *Nature* **464**:1307–1312.
- Johnston D, Narayanan R (2008) Active dendrites: colorful wings of the mysterious butterflies. *Trends in Neurosciences* **31**:309–316.
- Johnston D, Hoffman DA, Colbert CM, Magee JC (1999) Regulation of back-propagating action potentials in hippocampal neurons. *Current Opinion in Neurobiology* **9**:288–292.
- Kampa BM, Clements J, Jonas P, Stuart GJ (2004) Kinetics of Mg^{2+} unblock of NMDA receptors: implications for spike-timing dependent synaptic plasticity. *Journal of Physiology* **556**:337–345.
- Kampa BM, Letzkus JJ, Stuart GJ (2006) Requirement of dendritic calcium spikes for induction of spike-timing-dependent synaptic plasticity. *Journal of Physiology* **574**:283–290.
- Kampa BM, Letzkus JJ, Stuart GJ (2007) Dendritic mechanisms controlling spike-timing-dependent synaptic plasticity. *Trends in Neurosciences* **30**:456–463.
- Kandel ER (2001) The molecular biology of memory storage: a dialogue between genes and synapses. *Science* **294**:1030–1038.
- Karmarkar UR, Buonomano DV (2002) A model of spike-timing dependent plasticity: one or two coincidence detectors? *Journal of Neurophysiology* **88**:507–513.
- Karmarkar UR, Buonomano DV (2006) Different forms of homeostatic plasticity are engaged with distinct temporal profiles. *European Journal of Neuroscience* **23**:1575–1584.
- Kasai H, Matsuzaki M, Noguchi J, Yasumatsu N, Nakahara H (2003) Structure–stability–function relationships of dendritic spines. *Trends in Neurosciences* **26**:360–368.
- Kastellakis G, Cai DJ, Mednick SC, Silva AJ, Poirazi P (2015) Synaptic clustering within dendrites: An emerging theory of memory formation. *Progress in Neurobiology* **126**:19–35.
- Katz LC, Shatz CJ (1996) Synaptic activity and the construction of cortical circuits. *Science* **274**:1133–1138.
- Keck T, Keller GB, Jacobsen RI, Eysel UT, Bonhoeffer T, Hubener M (2013) Synaptic scaling and homeostatic plasticity in the mouse visual cortex in vivo. *Neuron* **80**:327–334.
- Kelso SR, Ganong AH, Brown TH (1986) Hebbian synapses in hippocampus. *Proceedings of the National Academy of Sciences of the United States of America* **83**:5326–5330.
- Khavandgar S, Walter JT, Sageser K, Khodakhah K (2005) Kv1 channels selectively prevent dendritic hyperexcitability in rat Purkinje cells. *Journal of Physiology* **569**:545–557.
- Kilgard MP, Merzenich MM (1998) Cortical map reorganization enabled by nucleus basalis activity. *Science* **279**:1714–1718.

- Kilman V, Van Rossum MC, Turrigiano GG (2002) Activity deprivation reduces miniature IPSC amplitude by decreasing the number of postsynaptic GABA(A) receptors clustered at neocortical synapses. *Journal of Neuroscience* **22**:1328–1337.
- Kim SJ, Linden DJ (2007) Ubiquitous plasticity and memory storage. *Neuron* **56**:582–592.
- Kim Y, Hsu C-L, Cembrowski MS, Mensh BD, Spruston N (2015) Dendritic sodium spikes are required for long-term potentiation at distal synapses on hippocampal pyramidal neurons. *eLife* **4**:e06414. <http://elifesciences.org/content/early/2015/08/06/eLife.06414>
- Kim J, Jung SC, Clemens AM, Petralia RS, Hoffman DA (2007) Regulation of dendritic excitability by activity-dependent trafficking of the A-type K⁺ channel subunit Kv4.2 in hippocampal neurons. *Neuron* **54**:933–947.
- Kirkwood A, Bear MF (1994) Hebbian synapses in visual cortex. *Journal of Neuroscience* **14**:1634–1645.
- Kirkwood A, Dudek SM, Gold JT, Aizenman CD, Bear MF (1993) Common forms of synaptic plasticity in the hippocampus and neocortex in vitro. *Science* **260**:1518–1521.
- Kirkwood A, Rioult MC, Bear MF (1996) Experience-dependent modification of synaptic plasticity in visual cortex. *Nature* **381**:526–528.
- Knudsen EI (2002) Instructed learning in the auditory localization pathway of the barn owl. *Nature* **417**:322–328.
- Koch C, Zador A (1993) The function of dendritic spines: devices subserving biochemical rather than electrical compartmentalization. *Journal of Neuroscience* **13**:413–422.
- Koester HJ, Sakmann B (1998) Calcium dynamics in single spines during coincident pre- and postsynaptic activity depend on relative timing of back-propagating action potentials and subthreshold excitatory postsynaptic potentials. *Proceedings of the National Academy of Sciences of the United States of America* **95**:9596–9601.
- Korkotian E, Holcman D, Segal M (2004) Dynamic regulation of spine-dendrite coupling in cultured hippocampal neurons. *European Journal of Neuroscience* **20**:2649–2663.
- Lamsa K, Heeroma JH, Kullmann DM (2005) Hebbian LTP in feed-forward inhibitory interneurons and the temporal fidelity of input discrimination. *Nature Neuroscience* **8**:916–924.
- Lamsa KP, Heeroma JH, Somogyi P, Rusakov DA, Kullmann DM (2007) Anti-Hebbian long-term potentiation in the hippocampal feedback inhibitory circuit. *Science* **315**:1262–1266.
- Lamsa KP, Kullmann DM, Woodin MA (2010) Spike-timing dependent plasticity in inhibitory circuits. *Frontiers in Synaptic Neuroscience*, 2:8.
- Larkum ME, Nevian T (2008) Synaptic clustering by dendritic signalling mechanisms. *Current Opinion in Neurobiology* **18**:321–331.
- Larkum ME, Kaiser KMM, Sakmann B (1999a) Calcium electrogenesis in distal apical dendrites of layer 5 pyramidal cells at a critical frequency of back-propagating action potentials. *Proceedings of the National Academy of Sciences of the United States of America* **96**:14600–14604.
- Larkum ME, Zhu JJ, Sakmann B (1999b) A new cellular mechanism for coupling inputs arriving at different cortical layers. *Nature* **398**:338–341.
- Larkum ME, Zhu JJ, Sakmann B (2001) Dendritic mechanisms underlying the coupling of the dendritic with the axonal action potential initiation zone of adult rat layer 5 pyramidal neurons. *Journal of Physiology* **533**:447–466.
- Larkum ME, Waters J, Sakmann B, Helmchen F (2007) Dendritic spikes in apical dendrites of neocortical layer 2/3 pyramidal neurons. *Journal of Neuroscience* **27**:8999–9008.
- Larkum ME, Nevian T, Sandler M, Polsky A, Schiller J (2009) Synaptic integration in tuft dendrites of layer 5 pyramidal neurons: a new unifying principle. *Science* **325**:756–760.
- Larsen RS, Smith IT, Miriyala J, Han JE, Corlew RJ, Smith SL, Philpot BD (2014) Synapse-specific control of experience-dependent plasticity by presynaptic NMDA receptors. *Neuron* **83**:879–893.
- Lavzin M, Rapoport S, Polsky A, Garion L, Schiller J (2012) Nonlinear dendritic processing determines angular tuning of barrel cortex neurons in vivo. *Nature* **490**:397–401.

- Lee AK, Manns ID, Sakmann B, Brecht M (2006) Whole-cell recordings in freely moving rats. *Neuron* **51**:399–407.
- Lee SJ, Escobedo-Lozoya Y, Szatmari EM, Yasuda R (2009) Activation of CaMKII in single dendritic spines during long-term potentiation. *Nature* **458**:299–304.
- Legenstein R, Maass W (2011) Branch-specific plasticity enables self-organization of nonlinear computation in single neurons. *Journal of Neuroscience* **31**:10787–10802.
- Lemasson G, Marder E, Abbott LF (1993) Activity-dependent regulation of conductances in model neurons. *Science* **259**:1915–1917.
- Letzkus JJ, Kampa BM, Stuart GJ (2006) Learning rules for spike timing-dependent plasticity depend on dendritic synapse location. *Journal of Neuroscience* **26**:10420–10429.
- Levitin IB (1994) Modulation of ion channels by protein phosphorylation and dephosphorylation. *Annual Review of Physiology* **56**:193–212.
- Levy WB, Steward O (1979) Synapses as associative memory elements in the hippocampal formation. *Brain Research* **175**:233–245.
- Levy WB, Steward O (1983) Temporal contiguity requirements for long-term associative potentiation/depression in the hippocampus. *Neuroscience* **8**:791–797.
- Li C, Lu J, Wu C, Duan S, Poo M (2004) Bidirectional modification of presynaptic neuronal excitability accompanying spike timing-dependent synaptic plasticity. *Neuron* **41**:257–268.
- Liao D, Jones A, Malinow R (1992) Direct measurement of quantal changes underlying long-term potentiation in CA1 hippocampus. *Neuron* **9**:1089–1097.
- Lin YW, Min MY, Chiu TH, Yang HW (2003) Enhancement of associative long-term potentiation by activation of beta-adrenergic receptors at CA1 synapses in rat hippocampal slices. *Journal of Neuroscience* **23**:4173–4181.
- Lisman J (1989) A mechanism for the Hebb and the anti-Hebb processes underlying learning and memory. *Proceedings of the National Academy of Sciences of the United States of America* **86**:9574–9578.
- Lisman J, Spruston N (2005) Postsynaptic depolarization requirements for LTP and LTD: a critique of spike timing-dependent plasticity. *Nature Neuroscience* **8**:839–841.
- Lisman J, Spruston N (2010) Questions about STDP as a general model of synaptic plasticity. *Frontiers in Synaptic Neuroscience* **3**:5.
- Lisman J, Schulman H, Cline H (2002) The molecular basis of CaMKII function in synaptic and behavioural memory. *Nature Reviews Neuroscience* **3**:175–190.
- Lisman J, Yasuda R, Raghavachari S (2012) Mechanisms of CaMKII action in long-term potentiation. *Nature Reviews Neuroscience* **13**:169–182.
- Liu G (2004) Local structural balance and functional interaction of excitatory and inhibitory synapses in hippocampal dendrites. *Nature Neuroscience* **7**:373–379.
- Llinás R, Sugimori M (1980) Electrophysiological properties of *in vitro* Purkinje cell dendrites in mammalian cerebellar slices. *Journal of Physiology* **305**:197–213.
- Lømo T (1966) Frequency potentiation of excitatory synaptic activity in the dentate area of the hippocampal formation. *Acta Physiologica Scandinavica* **68**(Suppl. 277):128.
- London M, Häusser M (2005) Dendritic computation. *Annual Review of Neuroscience* **28**:503–532.
- Losonczy A, Makara JK, Magee JC (2008) Compartmentalized dendritic plasticity and input feature storage in neurons. *Nature* **452**:436–441.
- Lynch MA (2004) Long-term potentiation and memory. *Physiological Review* **84**:87–136.
- Lynch GS, Dunwiddie T, Gribkoff V (1977) Heterosynaptic depression: a postsynaptic correlate of long-term potentiation. *Nature* **266**:737–739.
- Macdermott AB, Mayer ML, Westbrook GL, Smith SJ, Barker JL (1986) NMDA-receptor activation increases cytoplasmic calcium concentration in cultured spinal cord neurones. *Nature* **321**:519–522.
- McNaughton BL (2003) Long-term potentiation, cooperativity and Hebb's cell assemblies: a personal history. *Philosophical Transactions of the Royal Society B: Biological Sciences* **358**:629–634.

- McNaughton BL, Douglas RM, Goddard GV (1978) Synaptic enhancement in fascia dentata: cooperativity among coactive afferents. *Brain Research* **157**:277–293.
- Maffei A, Turrigiano G (2008) The age of plasticity: developmental regulation of synaptic plasticity in neocortical microcircuits. *Progress in Brain Research* **169**:211–223.
- Maffei A, Nelson SB, Turrigiano GG (2004) Selective reconfiguration of layer 4 visual cortical circuitry by visual deprivation. *Nature Neuroscience* **7**:1353–1359.
- Maffei A, Nataraj K, Nelson SB, Turrigiano GG (2006) Potentiation of cortical inhibition by visual deprivation. *Nature* **443**:81–84.
- Magee JC, Cook EP (2000) Somatic EPSP amplitude is independent of synapse location in hippocampal pyramidal neurons. *Nature Neuroscience* **3**:895–903.
- Magee JC, Johnston D (1995) Characterization of single voltage-gated Na^+ and Ca^{2+} channels in apical dendrites of rat CA1 pyramidal neurons. *Journal of Physiology* **487**:67–90.
- Magee JC, Johnston D (1997) A synaptically controlled, associative signal for Hebbian plasticity in hippocampal neurons. *Science* **275**:209–213.
- Magee J, Hoffman D, Colbert C, Johnston D (1998) Electrical and calcium signaling in dendrites of hippocampal pyramidal neurons. *Annual Review of Physiology* **60**:327–346.
- Majewska A, Tashiro A, Yuste R (2000) Regulation of spine calcium dynamics by rapid spine motility. *Journal of Neuroscience* **20**:8262–8268.
- Makino H, Malinow R (2011) Compartmentalized versus global synaptic plasticity on dendrites controlled by experience. *Neuron* **72**:1001–1011.
- Malenka RC (1993) Long-term depression: not so depressing after all. *Proceedings of the National Academy of Sciences of the United States of America* **90**:3121–3123.
- Malenka RC (2003) The long-term potential of LTP. *Nature Reviews Neuroscience* **4**:923–926.
- Malenka RC, Nicoll RA (1999) Long-term potentiation—a decade of progress? *Science* **285**:1870–1874.
- Malenka RC, Kauer JA, Zucker RS, Nicoll RA (1988) Postsynaptic calcium is sufficient for potentiation of hippocampal synaptic transmission. *Science* **242**:81–84.
- Malinow R, Tsien RW (1990) Presynaptic enhancement shown by whole-cell recordings of long-term potentiation in hippocampal slices. *Nature* **346**:177–180.
- Maren S (2001) Neurobiology of Pavlovian fear conditioning. *Annual Review of Neuroscience* **24**:897–931.
- Maren S (2005) Synaptic mechanisms of associative memory in the amygdala. *Neuron* **47**:783–786.
- Markram H, Sakmann B (1994) Calcium transients in dendrites of neocortical neurons evoked by single subthreshold excitatory postsynaptic potentials via low-voltage-activated calcium channels. *Proceedings of the National Academy of Sciences of the United States of America* **91**:5207–5211.
- Markram H, Lübke J, Frotscher M, Sakmann B (1997) Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. *Science* **275**:213–215.
- Markram H, Toledo-Rodriguez M, Wang Y, Gupta A, Silberberg G, Wu C (2004) Interneurons of the neocortical inhibitory system. *Nature Reviews Neuroscience* **5**:793–807.
- Markram H, Gerstner W, Sjöström PJ (2011) A history of spike-timing-dependent plasticity. *Frontiers in Synaptic Neuroscience* **3**:4.
- Matsuzaki M, Ellis-Davies GC, Nemoto T, Miyashita Y, Iino M, Kasai H (2001) Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons. *Nature Neuroscience* **4**:1086–1092.
- Matsuzaki M, Honkura N, Ellis-Davies GC, Kasai H (2004) Structural basis of long-term potentiation in single dendritic spines. *Nature* **429**:761–766.
- Matus A (2005) Growth of dendritic spines: a continuing story. *Current Opinion in Neurobiology* **15**:67–72.
- Mayer ML, Westbrook GL (1987) Permeation and block of N-methyl-D-aspartic acid receptor channels by divalent cations in mouse cultured central neurones. *Journal of Physiology* **394**:501–527.

- Mayer ML, Westbrook GL, Guthrie PB (1984) Voltage-dependent block by Mg²⁺ of NMDA responses in spinal cord neurones. *Nature* **309**:261–263.
- Mel BW (1992) The clusteron: toward a simple abstraction for a complex neuron. In: *Advances in Neural Information Processing Systems* (Moody J, Hanson S, Lippmann R, eds), pp. 35–42. San Mateo, CA: Morgan Kaufmann.
- Meliza CD, Dan Y (2006) Receptive-field modification in rat visual cortex induced by paired visual stimulation and single-cell spiking. *Neuron* **49**:183–189.
- Migliore M, Hoffman DA, Magee JC, Johnston D (1999) Role of an A-type K⁺ conductance in the back-propagation of action potentials in the dendrites of hippocampal pyramidal neurons. *Journal of Computational Neuroscience* **7**:5–15.
- Miller KD (1996) Synaptic economics: competition and cooperation in synaptic plasticity. *Neuron* **17**:371–374.
- Miller KD, Mackay DJC (1994) The role of constraints in Hebbian learning. *Neural Computation* **6**:100–126.
- Miller S, Yasuda M, Coats JK, Jones Y, Martone ME, Mayford M (2002) Disruption of dendritic translation of CaMKIIα impairs stabilization of synaptic plasticity and memory consolidation. *Neuron* **36**:507–519.
- Min R, Nevian T (2012) Astrocyte signaling controls spike timing-dependent depression at neocortical synapses. *Nature Neuroscience* **15**:746–753.
- Montgomery JM, Pavlidis P, Madison DV (2001) Pair recordings reveal all-silent synaptic connections and the postsynaptic expression of long-term potentiation. *Neuron* **29**:691–701.
- Mulkey RM, Malenka RC (1992) Mechanisms underlying induction of homosynaptic long-term depression in area CA1 of the hippocampus. *Neuron* **9**:967–975.
- Muller W, Connor JA (1991) Dendritic spines as individual neuronal compartments for synaptic Ca²⁺ responses. *Nature* **354**:73–76.
- Murthy VN, Schikorski T, Stevens CF & Zhu Y (2001) Inactivity produces increases in neurotransmitter release and synapse size. *Neuron*, **32**, 673–682.
- Nelson AB, Krispel CM, Sekirnjak C, Du Lac S (2003) Long-lasting increases in intrinsic excitability triggered by inhibition. *Neuron* **40**:609–620.
- Nevian T, Sakmann B (2006) Spine Ca²⁺ signaling in spike-timing-dependent plasticity. *Journal of Neuroscience* **26**:11001–11013.
- Nevian T, Larkum ME, Polsky A, Schiller J (2007) Properties of basal dendrites of layer 5 pyramidal neurons: a direct patch-clamp recording study. *Nature Neuroscience* **10**:206–214.
- Nimchinsky EA, Sabatini BL, Svoboda K (2002) Structure and function of dendritic spines. *Annual Review of Physiology* **64**:313–353.
- Nishiyama M, Hong K, Mikoshiba K, Poo MM, Kato K (2000) Calcium stores regulate the polarity and input specificity of synaptic modification. *Nature* **408**:584–588.
- Noguchi J, Matsuzaki M, Ellis-Davies GC, Kasai H (2005) Spine-neck geometry determines NMDA receptor-dependent Ca²⁺ signaling in dendrites. *Neuron* **46**:609–622.
- Nowak L, Bregestovski P, Ascher P, Herbet A, Prochiantz A (1984) Magnesium gates glutamate-activated channels in mouse central neurones. *Nature* **307**:462–465.
- Nusser Z, Lujan R, Laube G, Roberts JD, Molnar E, Somogyi P (1998) Cell type and pathway dependence of synaptic AMPA receptor number and variability in the hippocampus. *Neuron* **21**:545–559.
- O'Connor DH, Wittenberg GM, Wang SS (2005) Dissection of bidirectional synaptic plasticity into saturable unidirectional processes. *Journal of Neurophysiology* **94**:1565–1573.
- O'Connor DH, Peron SP, Huber D, Svoboda K (2010) Neural activity in barrel cortex underlying vibrissa-based object localization in mice. *Neuron* **67**:1048–1061.
- Oertner TG, Matus A (2005) Calcium regulation of actin dynamics in dendritic spines. *Cell Calcium* **37**:477–482.
- Ostroff LE, Fiala JC, Allwardt B, Harris KM (2002) Polyribosomes redistribute from dendritic shafts into spines with enlarged synapses during LTP in developing rat hippocampal slices. *Neuron* **35**:535–545.

- Otmakhov N, Tao-Cheng JH, Carpenter S, Asrican B, Dosemeci A, Reese TS, Lisman J (2004) Persistent accumulation of calcium/calmodulin-dependent protein kinase II in dendritic spines after induction of NMDA receptor-dependent chemical long-term potentiation. *Journal of Neuroscience* **24**:9324–9331.
- Palmer LM, Stuart GJ (2009) Membrane potential changes in dendritic spines during action potentials and synaptic input. *Journal of Neuroscience* **29**:6897–6903.
- Paradis S, Sweeney ST, Davis GW (2001) Homeostatic control of presynaptic release is triggered by postsynaptic membrane depolarization. *Neuron* **30**:737–749.
- Parekh R, Ascoli GA (2013) Neuronal morphology goes digital: a research hub for cellular and system neuroscience. *Neuron* **77**:1017–1038.
- Park H, Popescu A, Poo MM (2014) Essential role of presynaptic NMDA receptors in activity-dependent BDNF secretion and corticostriatal LTP. *Neuron* **84**:1009–1022.
- Pawlak V, Kerr JN (2008) Dopamine receptor activation is required for corticostriatal spike-timing-dependent plasticity. *Journal of Neuroscience* **28**:2435–2446.
- Pawlak V, Greenberg DS, Sprekeler H, Gerstner W, Kerr JN (2013) Changing the responses of cortical neurons from sub- to suprathreshold using single spikes *in vivo*. *eLife* **2**:e00012.
- Perrett SP, Dudek SM, Eagleman D, Montague PR, Friedlander MJ (2001) LTD induction in adult visual cortex: role of stimulus timing and inhibition. *Journal of Neuroscience* **21**:2308–2319.
- Pfister JP, Gerstner W (2006) Triplets of spikes in a model of spike timing-dependent plasticity. *Journal of Neuroscience* **26**:9673–9682.
- Philpot BD, Espinosa JS, Bear MF (2003) Evidence for altered NMDA receptor function as a basis for metaplasticity in visual cortex. *Journal of Neuroscience* **23**:5583–5588.
- Philpot BD, Cho KK, Bear MF (2007) Obligatory role of NR2A for metaplasticity in visual cortex. *Neuron* **53**:495–502.
- Poirazi P, Mel BW (2001) Impact of active dendrites and structural plasticity on the memory capacity of neural tissue. *Neuron* **29**:779–796.
- Poirazi P, Brannon T, Mel BW (2003) Pyramidal neuron as two-layer neural network. *Neuron* **37**:989–999.
- Pouille F, Scanziani M (2004) Routing of spike series by dynamic circuits in the hippocampus. *Nature* **429**:717–723.
- Pozo K, Goda Y (2010) Unraveling mechanisms of homeostatic synaptic plasticity. *Neuron* **66**:337–351.
- Rall W, Segev I (1987) Functional possibilities for synapses on dendrites and dendritic spines. In: *Synaptic Function* (Edelman GM, Gall WE, Cowan WM, eds), pp. 605–636. New York: Wiley.
- Rall W, Shepherd GM (1968) Theoretical reconstruction of field potentials and dendrodendritic synaptic interactions in olfactory bulb. *Journal of Neurophysiology* **31**:884–915.
- Ramón Y Cajal S (1894) The Croonian Lecture: la fine structure des centres nerveux. *Proceedings of the Royal Society of London* **55**:444–468.
- Ramón Y Cajal S (1904) *Textura del Sistema Nervioso del Hombre y de los Vertebrados*. Madrid: Moya.
- Remy S, Spruston N (2007) Dendritic spikes induce single-burst long-term potentiation. *Proceedings of the National Academy of Sciences of the United States of America* **104**:17192–17197.
- Rescorla RA (1988) Behavioral studies of Pavlovian conditioning. *Annual Review of Neuroscience* **11**:329–352.
- Reyes A (2001) Influence of dendritic conductances on the input-output properties of neurons. *Annual Review of Neuroscience* **24**:653–675.
- Reymann KG, Frey JU (2007) The late maintenance of hippocampal LTP: requirements, phases, “synaptic tagging,” “late-associativity” and implications. *Neuropharmacology* **52**:24–40.
- Rodriguez-Moreno A, Gonzalez-Rueda A, Banerjee A, Upton AL, Craig MT, Paulsen O (2013) Presynaptic self-depression at developing neocortical synapses. *Neuron* **77**:35–42.
- Rosenkranz JA, Johnston D (2006) Dopaminergic regulation of neuronal excitability through modulation of I_h in layer V entorhinal cortex. *Journal of Neuroscience* **26**:3229–3244.

- Royer S, Paré D (2003) Conservation of total synaptic weight through balanced synaptic depression and potentiation. *Nature* **422**:518–522.
- Rumsey CC, Abbott LF (2004) Equalization of synaptic efficacy by activity- and timing-dependent synaptic plasticity. *Journal of Neurophysiology* **91**:2273–2280.
- Rumsey CC, Abbott LF (2006) Synaptic democracy in active dendrites. *Journal of Neurophysiology* **96**:2307–2318.
- Rutherford LC, Dewan A, Lauer HM, Turrigiano GG (1997) Brain-derived neurotrophic factor mediates the activity-dependent regulation of inhibition in neocortical cultures. *Journal of Neuroscience* **17**:4527–4535.
- Rutherford LC, Nelson SB, Turrigiano GG (1998) BDNF has opposite effects on the quantal amplitude of pyramidal neuron and interneuron excitatory synapses. *Neuron* **21**:521–530.
- Sandler VM, Ross WN (1999) Serotonin modulates spike backpropagation and associated $[Ca^{2+}]_i$ changes in the apical dendrites of hippocampal CA1 pyramidal neurons. *Journal of Neurophysiology* **81**:216–224.
- Sawtell NB, Frenkel MY, Philpot BD, Nakazawa K, Tonegawa S, Bear MF (2003) NMDA receptor-dependent ocular dominance plasticity in adult visual cortex. *Neuron* **38**:977–985.
- Schikorski T, Stevens CF (1997) Quantitative ultrastructural analysis of hippocampal excitatory synapses. *Journal of Neuroscience* **17**:5858–5867.
- Schiller J, Schiller Y (2001) NMDA receptor-mediated dendritic spikes and coincident signal amplification. *Current Opinion in Neurobiology* **11**:343–348.
- Schiller J, Schiller Y, Stuart G, Sakmann B (1997) Calcium action potentials restricted to distal apical dendrites of rat neocortical pyramidal neurons. *Journal of Physiology* **505**:605–616.
- Schiller J, Schiller Y, Clapham DE (1998) NMDA receptors amplify calcium influx into dendritic spines during associative pre- and postsynaptic activation. *Nature Neuroscience* **1**:114–118.
- Schiller J, Major G, Koester HJ, Schiller Y (2000) NMDA spikes in basal dendrites of cortical pyramidal neurons. *Nature* **404**:285–289.
- Schuett S, Bonhoeffer T, Hubener M (2001) Pairing-induced changes of orientation maps in cat visual cortex. *Neuron* **32**:325–337.
- Schulz JM (2010) Synaptic plasticity in vivo: more than just spike-timing? *Frontiers in Synaptic Neuroscience* **2**:140.
- Schuman EM (1997) Synapse specificity and long-term information storage. *Neuron* **18**:339–342.
- Schuman EM, Madison DV (1994) Locally distributed synaptic potentiation in the hippocampus. *Science* **263**:532–536.
- Segev I, Rall W (1988) Computational study of an excitable dendritic spine. *Journal of Neurophysiology* **60**:499–523.
- Sejnowski TJ (1977) Storing covariance with nonlinearly interacting neurons. *Journal of Mathematical Biology* **4**:303–321.
- Seol GH, Ziburkus J, Huang S, Song L, Kim IT, Takamiya K, Huganir RL, Lee HK, Kirkwood A (2007) Neuromodulators control the polarity of spike-timing-dependent synaptic plasticity. *Neuron* **55**:919–929.
- Shatz CJ (1992) The developing brain. *Scientific American* **267**:60–67.
- Shen K, Meyer T (1999) Dynamic control of CaMKII translocation and localization in hippocampal neurons by NMDA receptor stimulation. *Science* **284**:162–166.
- Shen W, Hamilton SE, Nathanson NM, Surmeier DJ (2005) Cholinergic suppression of KCNQ channel currents enhances excitability of striatal medium spiny neurons. *Journal of Neuroscience* **25**:7449–7458.
- Shen W, Tian X, Day M, Ulrich S, Tkatch T, Nathanson NM, Surmeier DJ (2007) Cholinergic modulation of Kir2 channels selectively elevates dendritic excitability in striatopallidal neurons. *Nature Neuroscience* **10**:1458–1466.
- Shen W, Flajolet M, Greengard P, Surmeier DJ (2008) Dichotomous dopaminergic control of striatal synaptic plasticity. *Science* **321**:848–851.

- Shepherd GM, Brayton RK, Miller JP, Segev I, Rinzel J, Rall W (1985) Signal enhancement in distal cortical dendrites by means of interactions between active dendritic spines. *Proceedings of the National Academy of Sciences of the United States of America* **82**:2192–2195.
- Silberberg G, Markram H (2007) Disynaptic inhibition between neocortical pyramidal cells mediated by Martinotti cells. *Neuron* **53**:735–746.
- Sjöström PJ, Häusser M (2006) A cooperative switch determines the sign of synaptic plasticity in distal dendrites of neocortical pyramidal neurons. *Neuron* **51**:227–238.
- Sjöström PJ, Nelson SB (2002) Spike timing, calcium signals and synaptic plasticity. *Current Opinion in Neurobiology* **12**:305–314.
- Sjöström PJ, Turrigiano GG, Nelson SB (2001) Rate, timing, and cooperativity jointly determine cortical synaptic plasticity. *Neuron* **32**:1149–1164.
- Sjöström PJ, Turrigiano GG, Nelson SB (2003) Neocortical LTD via coincident activation of presynaptic NMDA and cannabinoid receptors. *Neuron* **39**:641–654.
- Sjöström PJ, Turrigiano GG, Nelson SB (2004) Endocannabinoid-dependent neocortical layer-5 LTD in the absence of postsynaptic spiking. *Journal of Neurophysiology* **92**:3338–3343.
- Sjöström PJ, Ranz EA, Roth A, Häusser M (2008) Dendritic excitability and synaptic plasticity. *Physiological Reviews* **88**:769–840.
- Smith SL, Otis TS (2003) Persistent changes in spontaneous firing of Purkinje neurons triggered by the nitric oxide signaling cascade. *Journal of Neuroscience* **23**:367–372.
- Song S, Miller KD, Abbott LF (2000) Competitive Hebbian learning through spike-timing-dependent synaptic plasticity. *Nature Neuroscience* **3**:919–926.
- Spiegel I, Mardinly AR, Gabel HW, Bazinet JE, Couch CH, Tzeng CP, Harmin DA, Greenberg ME (2014) Npas4 regulates excitatory-inhibitory balance within neural circuits through cell-type-specific gene programs. *Cell* **157**:1216–1229.
- Staff NP, Spruston N (2003) Intracellular correlate of EPSP-spike potentiation in CA1 pyramidal neurons is controlled by GABAergic modulation. *Hippocampus* **13**:801–805.
- Staubli U, Lynch G (1987) Stable hippocampal long-term potentiation elicited by “theta” pattern stimulation. *Brain Research* **435**:227–234.
- Stepanyants A, Hof PR, Chklovskii DB (2002) Geometry and structural plasticity of synaptic connectivity. *Neuron* **34**:275–288.
- Stevens CF (1998) A million dollar question: does LTP = memory? *Neuron* **20**:1–2.
- Stuart G, Häusser M (1994) Initiation and spread of sodium action potentials in cerebellar Purkinje cells. *Neuron* **13**:703–712.
- Stuart GJ, Häusser M (2001) Dendritic coincidence detection of EPSPs and action potentials. *Nature Neuroscience* **4**:63–71.
- Stuart GJ, Sakmann B (1994) Active propagation of somatic action potentials into neocortical pyramidal cell dendrites. *Nature* **367**:69–72.
- Stuart G, Spruston N, Sakmann B, Häusser M (1997) Action potential initiation and backpropagation in neurons of the mammalian CNS. *Trends in Neurosciences* **20**:125–131.
- Sutton MA, Schuman EM (2006) Dendritic protein synthesis, synaptic plasticity, and memory. *Cell* **127**:49–58.
- Sutton MA, Ito HT, Cressy P, Kempf C, Woo JC, Schuman EM (2006) Miniature neurotransmission stabilizes synaptic function via tonic suppression of local dendritic protein synthesis. *Cell* **125**:785–799.
- Svoboda K, Tank DW, Denk W (1996) Direct measurement of coupling between dendritic spines and shafts. *Science* **272**:716–719.
- Svoboda K, Helmchen F, Denk W, Tank DW (1999) Spread of dendritic excitation in layer 2/3 pyramidal neurons in rat barrel cortex in vivo. *Nature Neuroscience* **2**:65–73.
- Swanwick CC, Murthy NR, Kapur J (2005) Activity-dependent scaling of GABAergic synapse strength is regulated by brain-derived neurotrophic factor. *Molecular and Cellular Neurosciences* **31**:481–492.

- Takahashi N, Kitamura K, Matsuo N, Mayford M, Kano M, Matsuki N, Ikegaya Y (2012) Locally synchronized synaptic inputs. *Science* **335**:353–356.
- Takumi Y, Ramirez-Leon V, Laake P, Rinvik E, Ottersen OP (1999) Different modes of expression of AMPA and NMDA receptors in hippocampal synapses. *Nature Neuroscience* **2**:618–624.
- Tanaka J, Horiike Y, Matsuzaki M, Miyazaki T, Ellis-Davies GC, Kasai H (2008) Protein synthesis and neurotrophin-dependent structural plasticity of single dendritic spines. *Science* **319**:1683–1687.
- Tao HW, Zhang LI, Engert F, Poo M (2001) Emergence of input specificity of LTP during development of retinotectal connections in vivo. *Neuron* **31**:569–580.
- Tokuda M, Hatase O (1998) Regulation of neuronal plasticity in the central nervous system by phosphorylation and dephosphorylation. *Molecular Neurobiology* **17**:137–156.
- Toledo-Rodriguez M, Goodman P, Illic M, Wu C, Markram H (2005) Neuropeptide and calcium-binding protein gene expression profiles predict neuronal anatomical type in the juvenile rat. *Journal of Physiology* **567**:401–413.
- Tønnesen J, Katona G, Rózsa B, Nägerl UV (2014) Spine neck plasticity regulates compartmentalization of synapses. *Nature Neuroscience* **17**:678–685.
- Tsodyks M (2002) Spike-timing-dependent synaptic plasticity—the long road towards understanding neuronal mechanisms of learning and memory. *Trends in Neurosciences* **25**:599–600.
- Tsodyks MV, Markram H (1997) The neural code between neocortical pyramidal neurons depends on neurotransmitter release probability. *Proceedings of the National Academy of Sciences of the United States of America* **94**:719–723.
- Tsubokawa H (2000) Control of Na^+ spike backpropagation by intracellular signaling in the pyramidal neuron dendrites. *Molecular Neurobiology* **22**:129–141.
- Turrigiano GG (1999) Homeostatic plasticity in neuronal networks: the more things change, the more they stay the same. *Trends in Neurosciences* **22**:221–227.
- Turrigiano G (2007) Homeostatic signaling: the positive side of negative feedback. *Current Opinion in Neurobiology* **17**:318–324.
- Turrigiano GG (2008) The self-tuning neuron: synaptic scaling of excitatory synapses. *Cell* **135**:422–435.
- Turrigiano G (2011) Too many cooks? Intrinsic and synaptic homeostatic mechanisms in cortical circuit refinement. *Annual Review of Neuroscience* **34**:89–103.
- Turrigiano GG, Nelson SB (2000) Hebb and homeostasis in neuronal plasticity. *Current Opinion in Neurobiology* **10**:358–364.
- Turrigiano GG, Nelson SB (2004) Homeostatic plasticity in the developing nervous system. *Nature Reviews Neuroscience* **5**:97–107.
- Turrigiano G, Abbott LF, Marder E (1994) Activity-dependent changes in the intrinsic properties of cultured neurons. *Science* **264**:974–977.
- Turrigiano GG, Leslie KR, Desai NS, Rutherford LC, Nelson SB (1998) Activity-dependent scaling of quantal amplitude in neocortical neurons. *Nature* **391**:892–896.
- Varela JA, Song S, Turrigiano GG, Nelson SB (1999) Differential depression at excitatory and inhibitory synapses in visual cortex. *Journal of Neuroscience* **19**(11):4293–4304.
- Vetter P, Roth A, Häusser M (2001) Propagation of action potentials in dendrites depends on dendritic morphology. *Journal of Neurophysiology* **85**:926–937.
- Vogels TP, Froemke RC, Doyon N, Gilson M, Haas JS, Liu R, Maffei A, Miller P, Wierenga CJ, Woodin MA, Zenke F, Sprekeler H (2013) Inhibitory synaptic plasticity: spike timing-dependence and putative network function. *Frontiers in Neural Circuits* **7**:119.
- Wang L, Maffei A (2014) Inhibitory plasticity dictates the sign of plasticity at excitatory synapses. *Journal of Neuroscience* **34**:1083–1093.
- Wang SS, Denk W, Häusser M (2000) Coincidence detection in single dendritic spines mediated by calcium release. *Nature Neuroscience* **3**:1266–1273.

- Wang Z, Xu NL, Wu CP, Duan S, Poo MM (2003) Bidirectional changes in spatial dendritic integration accompanying long-term synaptic modifications. *Neuron* **37**:463–472.
- Wang Y, Toledo-Rodriguez M, Gupta A, Wu C, Silberberg G, Luo J, Markram H (2004) Anatomical, physiological and molecular properties of Martinotti cells in the somatosensory cortex of the juvenile rat. *Journal of Physiology* **561**:65–90.
- Watabe AM, Zaki PA, O'Dell TJ (2000) Coactivation of beta-adrenergic and cholinergic receptors enhances the induction of long-term potentiation and synergistically activates mitogen-activated protein kinase in the hippocampal CA1 region. *Journal of Neuroscience* **20**:5924–5931.
- Waters J, Larkum M, Sakmann B, Helmchen F (2003) Supralinear Ca^{2+} influx into dendritic tufts of layer 2/3 neocortical pyramidal neurons in vitro and in vivo. *Journal of Neuroscience* **23**:8558–8567.
- Wathey JC, Lytton WW, Jester JM, Sejnowski TJ (1992) Computer simulations of EPSP-spike (E-S) potentiation in hippocampal CA1 pyramidal cells. *Journal of Neuroscience* **12**:607–618.
- Watt AJ, Desai NS (2010) Homeostatic plasticity and STDP: keeping a neuron's cool in a fluctuating world. *Frontiers in Synaptic Neuroscience* **2**:5.
- Watt AJ, Van Rossum MC, Macleod KM, Nelson SB, Turrigiano GG (2000) Activity coregulates quantal AMPA and NMDA currents at neocortical synapses. *Neuron* **26**:659–670.
- Wei DS, Mei YA, Bagal A, Kao JP, Thompson SM, Tang CM (2001) Compartmentalized and binary behavior of terminal dendrites in hippocampal pyramidal neurons. *Science* **293**:2272–2275.
- van Welie I, van Hooft JA, Wadman WJ (2004) Homeostatic scaling of neuronal excitability by synaptic modulation of somatic hyperpolarization-activated I_h channels. *Proceedings of the National Academy of Sciences of the United States of America* **101**:5123–5128.
- van Welie I, van Hooft JA, Wadman WJ (2006) Background activity regulates excitability of rat hippocampal CA1 pyramidal neurons by adaptation of a K^+ conductance. *Journal of Neurophysiology* **95**:2007–2012.
- Wiesel TN, Hubel DH (1965) Comparison of the effects of unilateral and bilateral eye closure on cortical unit responses in kittens. *Journal of Neurophysiology* **28**:1029–1040.
- Williams SR, Stuart GJ (2002) Dependence of EPSP efficacy on synapse location in neocortical pyramidal neurons. *Science* **295**:1907–1910.
- Willshaw D, Dayan P (1990) Optimal plasticity from matrix memories: what goes up must come down. *Neural Computation* **2**:85–93.
- Wolfe J, Houweling AR, Brecht M (2010) Sparse and powerful cortical spikes. *Current Opinion in Neurobiology* **20**:306–312.
- Wolters A, Schmidt A, Schramm A, Zeller D, Naumann M, Kunesch E, Benecke R, Reiners K, Classen J (2005) Timing-dependent plasticity in human primary somatosensory cortex. *Journal of Physiology* **565**:1039–1052.
- Xu J, Kang N, Jiang L, Nedergaard M, Kang J (2005) Activity-dependent long-term potentiation of intrinsic excitability in hippocampal CA1 pyramidal neurons. *Journal of Neuroscience* **25**:1750–1760.
- Xu NL, Ye CQ, Poo MM, Zhang XH (2006) Coincidence detection of synaptic inputs is facilitated at the distal dendrites after long-term potentiation induction. *Journal of Neuroscience* **26**:3002–3009.
- Yang SN, Tang YG, Zucker RS (1999) Selective induction of LTP and LTD by postsynaptic $[\text{Ca}^{2+}]_i$ elevation. *Journal of Neurophysiology* **81**:781–787.
- Yashiro K, Philpot BD (2008) Regulation of NMDA receptor subunit expression and its implications for LTD, LTP, and metaplasticity. *Neuropharmacology* **55**:1081–1094.
- Yasuda R, Sabatini BL, Svoboda K (2003) Plasticity of calcium channels in dendritic spines. *Nature Neuroscience* **6**:948–955.
- Yasuda R, Harvey CD, Zhong H, Sobczyk A, Van Aelst L, Svoboda K (2006) Supersensitive Ras activation in dendrites and spines revealed by two-photon fluorescence lifetime imaging. *Nature Neuroscience* **9**:283–291.

- Young JM, Waleszczyk WJ, Wang C, Calford MB, Dreher B, Obermayer K (2007) Cortical reorganization consistent with spike timing-but not correlation-dependent plasticity. *Nature Neuroscience* **10**:887–895.
- Yu LM, Goda Y (2009) Dendritic signalling and homeostatic adaptation. *Current Opinion in Neurobiology* **19**:327–335.
- Yuste R (2011) Dendritic spines and distributed circuits. *Neuron* **71**:772–781.
- Yuste R (2013) Electrical compartmentalization in dendritic spines. *Annual Review of Neuroscience* **36**:429–449.
- Yuste R, Denk W (1995) Dendritic spines as basic functional units of neuronal integration. *Nature* **375**:682–684.
- Yuste R, Majewska A, Cash SS, Denk W (1999) Mechanisms of calcium influx into hippocampal spines: heterogeneity among spines, coincidence detection by NMDA receptors, and optical quantal analysis. *Journal of Neuroscience* **19**:1976–1987.
- Zalutsky RA, Nicoll JA (1992) Mossy fiber long-term potentiation shows specificity but no apparent cooperativity. *Neuroscience Letters* **138**:193–197.
- Zhang W, Linden DJ (2003) The other side of the engram: experience-driven changes in neuronal intrinsic excitability. *Nature Reviews Neuroscience* **4**:885–900.
- Zhang LI, Poo MM (2001) Electrical activity and development of neural circuits. *Nature Neuroscience* **4**(Suppl.):1207–1214.
- Zhang LI, Tao HW, Holt CE, Harris WA, Poo M (1998) A critical window for cooperation and competition among developing retinotectal synapses. *Nature* **395**:37–44.
- Zhang JC, Lau PM, Bi GQ (2009) Gain in sensitivity and loss in temporal contrast of STDP by dopaminergic modulation at hippocampal synapses. *Proceedings of the National Academy of Sciences of the United States of America* **106**:13028–13033.
- Zhou Q, Poo MM (2004) Reversal and consolidation of activity-induced synaptic modifications. *Trends in Neurosciences* **27**:378–383.
- Zhou Q, Tao HW, Poo MM (2003) Reversal and stabilization of synaptic modifications in a developing visual system. *Science* **300**:1953–1957.
- Zhou Q, Homma KJ, Poo MM (2004) Shrinkage of dendritic spines associated with long-term depression of hippocampal synapses. *Neuron* **44**:749–757.

Chapter 19

Structural plasticity in dendrites and spines

Tobias Bonhoeffer and Pico Caroni

Summary

This chapter examines some of the most important studies on the topic of structural plasticity in dendrites and spines, placing them into the context of information storage in the brain. We start with an assessment of the classical literature, which mostly relied on conventional light and electron microscopy. However, most emphasis is on studies using state-of-the-art imaging approaches, which allow microscopic structural changes to be followed over time, including in awake animals performing complex behavioral tasks. We end by considering five particularly important open questions, providing an outlook into the future, and consider where research on structural plasticity in relationship to synaptic plasticity and memory could stand in a few years' time.

Introduction

The idea that structural changes in neurons are important for the ability of the nervous system to adapt to challenges in the outside world has captivated generations of neuroscientists. James, Cajal, and Hebb (James, 1890; Ramón y Cajal, 1911; Hebb, 1949) were important proponents of this idea, which suggests that such changes may also be of crucial importance for the storage of information in the nervous system. While changes in the axonal tree of neurons have also been observed and studied, the changes that occur on the postsynaptic side, i.e., on dendrites and in particular on their tiny protrusions, dendritic spines, have received much more attention. This is partly for practical reasons—spines are much more easily detected than their presynaptic counterparts, axonal boutons. Beyond that, however, it also seems that the very existence of a highly specialized “organelle,” approximately 1–5 µm in size, ideally suited for connecting up nearby neuronal processes, strongly suggests that spines may have a pivotal role in shaping and modifying neuronal circuits. This implies that structural changes in dendrites and spines are not serendipitous events but occur for important reasons. Today we have—we think—good ideas about what these reasons may be, but final proof is still lacking.

In this chapter we revisit the role that structural plasticity, in particular that of dendrites and spines, plays in the nervous system. We start by reviewing earlier literature, where due to technical limitations inferences had to be made on the basis of observations in fixed nervous tissue. The advent of new imaging techniques—and in particular two-photon microscopy—has dramatically changed this picture, and we now know much more about the role that these forms of plasticity may play for the functioning of the brain, in particular for learning and memory. But still, many unanswered questions remain.

The literature on dendritic plasticity is vast. It reaches from structurally motivated electron microscopic studies, through biophysical and computational analyses, to questions of learning and memory, and even clinical syndromes like fragile X. We cannot possibly cover everything in this chapter and have therefore selected a couple of topics which we think fit particularly well into the current framework. As always, that is not to say that other things would not be interesting or that there has not been substantial progress elsewhere. It is simply a subjective selection of topics that had to be made in order to limit the chapter to a reasonable size.

Early studies: relating large-scale structural alterations to experience

The idea that synapses, and hence dendritic structures, might be structurally plastic and assemble and disassemble in the adult brain to encode new memories can be traced back to Ramón y Cajal (1893). Because of the overwhelming technical challenges associated with detecting a few changing synapses induced upon learning within the vast adult neuropil, conclusive tests of these ideas had to await the development of methods for longitudinal imaging of individual synaptic structures *in vivo*. Nevertheless, already early on, the notion of a structurally plastic brain had too much appeal for its exploration to be postponed to some distant future. Accordingly, scientists used post-mortem Golgi impregnation, confocal microscopy, and electron microscopy methods to search for evidence that the structure of brain circuits might be modified by experience. These studies led to the development of experimental paradigms that are either still central to current efforts or now deserve to be taken up again with more powerful longitudinal *in vivo* methods. While some details and the exact magnitude of some of the phenomena may have to be reassessed, many of the early observations have by and large been confirmed with more modern approaches. Leaving aside pathology (for that see Chapter 24), the findings demonstrate large-scale alterations in the complexity of dendritic trees in response to major changes in life circumstances such as chronic stress or sexual maturation, and changes in synapse densities upon skill learning.

As a case in point, chronic immobilization stress is particularly effective in inducing alterations in dendritic arborization patterns. These effects were first noticed in hippocampal pyramidal neurons, where reductions of up to 30% in dendritic length and branch numbers have been reported within 10 days of chronic restraint (Watanabe et al., 1992). Similar dendritic shrinkage has been associated with elevated levels of cortisone, suggesting that stress hormones might act in the hippocampus, possibly through glutamatergic signaling, to reduce dendritic complexity (McEwen, 1999). Interestingly, the effects of stress in the hippocampus were more pronounced on apical than on basal dendrites (Magariños and McEwen, 1995). Notably, stress did not universally produce dendritic shrinkage in all brain areas. The same protocols were reported to induce dendritic expansion in some subpopulations of neurons in basolateral amygdala (Vyas et al., 2002). Furthermore, different subpopulations of amygdala neurons were affected to different degrees by chronic immobilization or by chronic unpredictable stress (Mitra et al., 2005). Taken together, these studies suggest that different forms of chronic stress might have potent consequences for the structure of some dendritic trees in limbic structures such as the hippocampus and amygdala. The functional consequences of—or reasons for—these alterations and the circumstances under which they can be reversed have remained unclear. In principle, however, understanding how environmental factors such as stress influence the structure of dendrites and synapses in young animals and in adults might provide a productive entry point for how understanding gene–environment relationships could impact neuronal structural plasticity on one hand and mental health on the other.

In female rodents, experimental enhancement of levels of the sex hormone estradiol *in situ* induced major alterations in dendritic structure in hypothalamic nuclei and in the hippocampus (Cooke and Woolley, 2005). In addition, spine and synapse densities have been reported to fluctuate by up to 30% in hippocampal CA1 pyramidal neurons during the estrus cycle, a process thought to involve the actions of estradiol and progesterone (Woolley and McEwen, 1992). The highest spine densities were reported when estradiol levels were highest. In the ventromedial nucleus of the hypothalamus (VMN) estradiol increased spine densities and dendritic branching, but reduced the length of a specific type of long primary dendrite that extends out of the VMN (Calizo and Flanagan-Cato, 2000). Comparable structural circuit alterations were reportedly induced by estradiol in the arcuate nucleus of the hypothalamus (Christensen et al., 2011). The mechanism through which estradiol induces the assembly of new spines appears to involve activation of the cofilin kinase LIMK1 and actin polymerization, which might synergize with functional validation of those synapses to stabilize new spines in response to specific signals. Recent studies could indeed relate these mechanistic insights to behavior and show that the new spines induced in specific neuronal subpopulations by estradiol are essential for sexual receptivity (Christensen et al., 2011). The estradiol/sexual receptivity scenario provides a nice example of how initial reports of specific large-scale structural plasticity in the adult could be confirmed and expanded, providing a paradigm for investigating how defined circuits remodel in response to hormones to mediate specific behavioral functions. As illustrated by studies in a number of additional systems, hormones provide an excellent entry point for investigating how specific structural alterations in defined sets of synapses and dendrites influence biologically relevant behaviors.

Not surprisingly, early attempts to relate skill learning to the establishment of new synapses have faced more challenges than those focusing on large-scale alterations in response to major changes in the outer or inner environment of the brain. Investigation of the structural changes following skill learning has mainly been based on synapse counts using electron or confocal microscopy, focusing on candidate brain regions such as the hippocampus or motor cortex, with and without learning. In many, but not all, studies synapse densities were reported to be enhanced in hippocampal CA1 stratum radiatum upon trace eyeblink conditioning or spatial training (Andersen and Trommald, 1995). A careful study involving spatial training in rats reported an 11% increase in average spine densities along basal dendrites of pyramidal neurons in CA1 (Moser et al., 1994). Furthermore, the acquisition of complex motor skills was shown to increase synapse densities in rat motor and cerebellar cortex (Black et al., 1990) and to increase the frequency of multispine boutons, a putative hallmark of recent synaptogenesis (Kleim et al., 1996; Jones et al., 1999). Enhanced synapse densities were also consistently reported upon exposure of rats to environmental enrichment. Together, these studies support the notion that long-lasting skill learning involves alterations in the arrangement and densities of synapses, but technical limitations only allowed a comparatively crude level of analysis incompatible with firm conclusions.

In summary, studies of structural plasticity preceding the advent of methods for repeated imaging of synaptic structures *in vivo* have produced early evidence for structural plasticity of dendrites and spines. Well-defined and biologically relevant experimental paradigms such as the effects of specific hormones on defined circuits and behaviors are likely to enrich future studies of structural plasticity. In addition, careful re-examination of the effects of environmental factors such as chronic stress on dendritic structure may lead to valuable entry points for investigating pathophysiological mechanisms in mental health.

Using live imaging to study structural plasticity

The search for appearing, disappearing, or changing synapses and dendritic spines entered a whole new phase when two-photon microscopy became available and made it possible to study the appearance and disappearance of spines deep in living tissue. Two-photon microscopy allows deep optical penetration of the tissue with excellent resolution in space and time, but most importantly it prevents damage of the living tissue (Denk and Svoboda, 1997). There were early attempts to study the generation of spines in living tissue in a long-term potentiation (LTP) paradigm (Hosokawa et al., 1995) using conventional confocal microscopy; however, confocal microscopy with the necessary space and time resolution produced phototoxic side effects.

Two-photon microscopy was first used to study structural plasticity late in the last millennium resulting in two papers published more or less simultaneously showing for the first time in living tissue that new dendritic protrusions (filopodia or spines) occurred when synapses were enhanced by LTP-like paradigms (Engert and Bonhoeffer, 1999; Maletic-Savatic et al., 1999; Fig. 19.1). In one study (Maletic-Savatic et al., 1999) acute hippocampal slices and local electrode stimulation were used to induce the generation of new spines or filopodia close to the stimulation electrode. There was, however, no simultaneous recording of the synaptic enhancement as it unfolded. The other study (Engert and Bonhoeffer, 1999) used a local perfusion approach to localize the relevant synapses and applied simultaneous pre- and postsynaptic stimulation, demonstrating that, in hippocampal slice cultures, stimuli that generated synaptic strengthening also resulted in the generation of new spines. These two studies together, complemented by a third study using conventional electron microscopy (Toni et al., 1999), firmly established that long-term changes in synaptic strength are paralleled by structural changes occurring at the level of dendritic spines. This then—exactly 50 years after Hebb had first proposed it in his book *The Organization of Behavior*—was an important first step to support the idea that synapses and their morphological counterparts, spines, may be used to store information in the brain. However, it took another decade to provide reasonable evidence that these or similar mechanisms may indeed be used to store information in the intact animal (see later).

These seminal studies were later followed up by additional experiments showing that spines are not only generated when synapses are strengthened, but that the inverse also holds true: spines disappear when synapses are weakened (Nägerl et al., 2004). Furthermore, the study of Nägerl et al. (2004) and others (Zito et al., 2009) showed that despite the fact that new spines can be generated within 20 minutes or so, it takes a substantial amount of time until the spines really possess

Fig. 19.1 Synaptic enhancement is paralleled by the addition of new filopodia/spines in the hippocampus. Tetanic stimulation in a hippocampal slice results in the outgrowth of new filopodia-like structures. Open arrowheads denote dendritic protrusions which disappear; closed arrowheads show new protrusions. Numbers in the panels indicate the number of minutes before/after tetanic stimulation. Four examples of spine growth (**A–D**) after paired pre- and postsynaptic stimulation in hippocampal slice cultures. In each case synaptic potentiation was monitored in parallel with the two-photon imaging revealing the structural changes (blue data points below the single panels). Data were recorded every 30 minutes, with the last panel imaged at the time indicated in the picture. Synaptic transmission was prevented everywhere outside the green superfusion zone. Therefore synaptic potentiation and associated structural changes are only expected to occur there (red dots, addition of spines; blue dots, removal of spines). Gray icons below the electrophysiological records denote when the new spines occurred. Scale bars 3 μm.

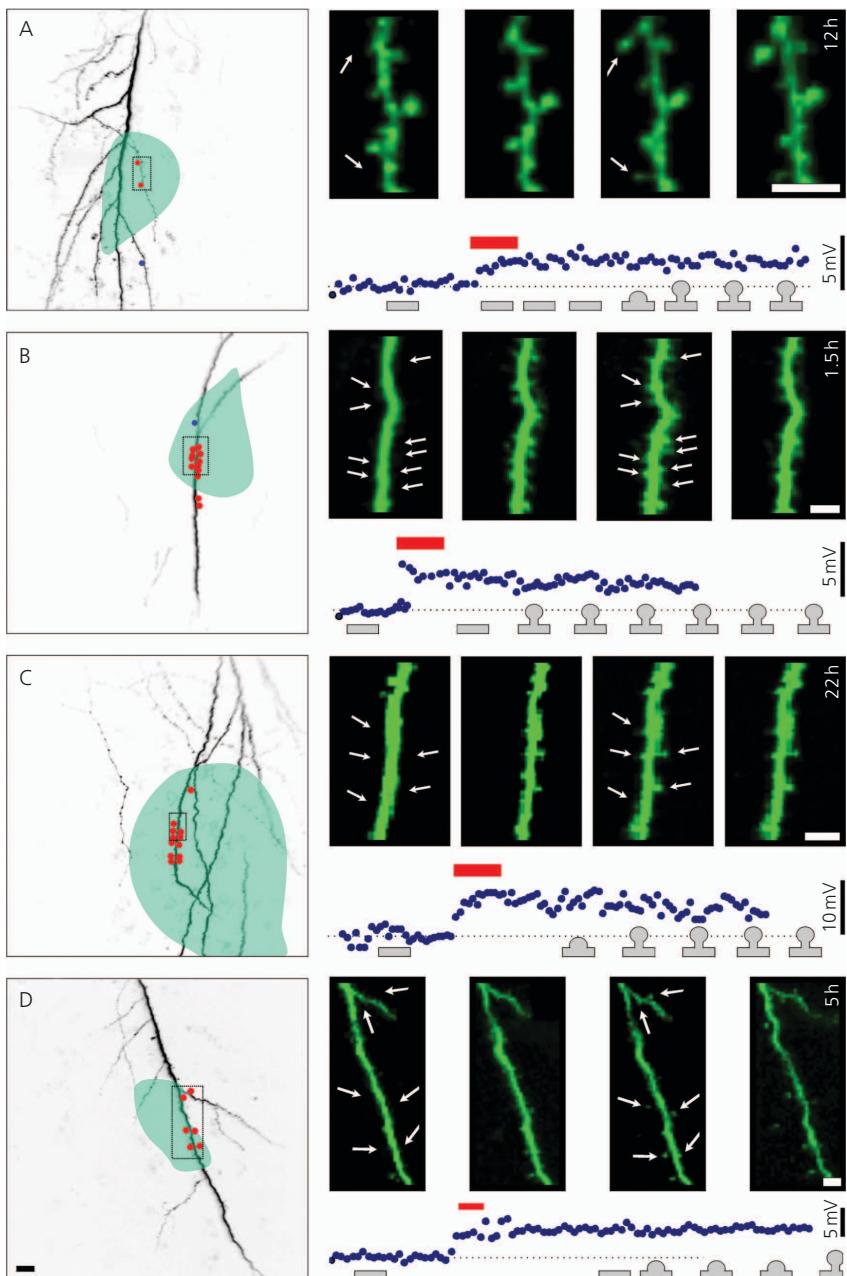


Fig. 19.1 (Continued)

Part A reprinted from M. Maletic-Savatic and R. Malinow, K. Svoboda, Rapid dendritic morphogenesis in CA1 hippocampal dendrites induced by synaptic activity, *Science*, 283(5409) pp. 1923–1927, © 1999, The American Association for the Advancement of Science. Reprinted with permission from AAAS. Part B reprinted by permission from Macmillan Publishers Ltd: *Nature*, 399(6731), Florian Engert and Tobias Bonhoeffer, Dendritic spine changes associated with hippocampal long-term synaptic plasticity, pp.66–70, © 1999 Nature Publishing Group.

functional synapses. The exact time varied in the two studies between 2 and 24 hours, possibly due to the fact that one study concentrated on spontaneous spinogenesis (Zito et al., 2009) whereas the other looked at spines that were generated in response to LTP stimuli (Nägerl et al., 2004), but in general it seems that the structural changes substantially precede the establishment of a functional synapse.

Another seminal series of studies by Kasai and co-workers (Matsuzaki et al., 2004) added a new dimension to morphological plasticity occurring concomitantly with functional changes. These investigators used glutamate uncaging to mimic physiological plasticity paradigms and found that photolysis of glutamate results in massive changes in spine size, which occurred after just a few minutes and lasted at least 100 minutes (Fig. 19.2). Since it is well established that the size of a spine correlates closely with the strength of its synapse (Nusser et al., 1998; Schikorski and Stevens, 1999) spine enlargement was a logical morphological correlate of synaptic enhancement. Many follow-up studies have used this or similar paradigms and have firmly corroborated these results, suggesting that postsynaptic activation alone is sufficient to trigger structural changes and that concomitant presynaptic activity is not required. It needs to be kept in mind, however, that glutamate uncaging is fundamentally different from synaptic release. Therefore it remains to be shown whether similar rules apply and similar changes also occur under more natural synaptic conditions.

One important consideration in the context of newly generated spines has to do with the new contacts they make with presynaptic axons. As already mentioned, it was shown early on that at least after a few hours these new spines do make functional contacts. Later *in vivo* experiments were able to show that new spines can soon mediate synaptic transmission (Kwon

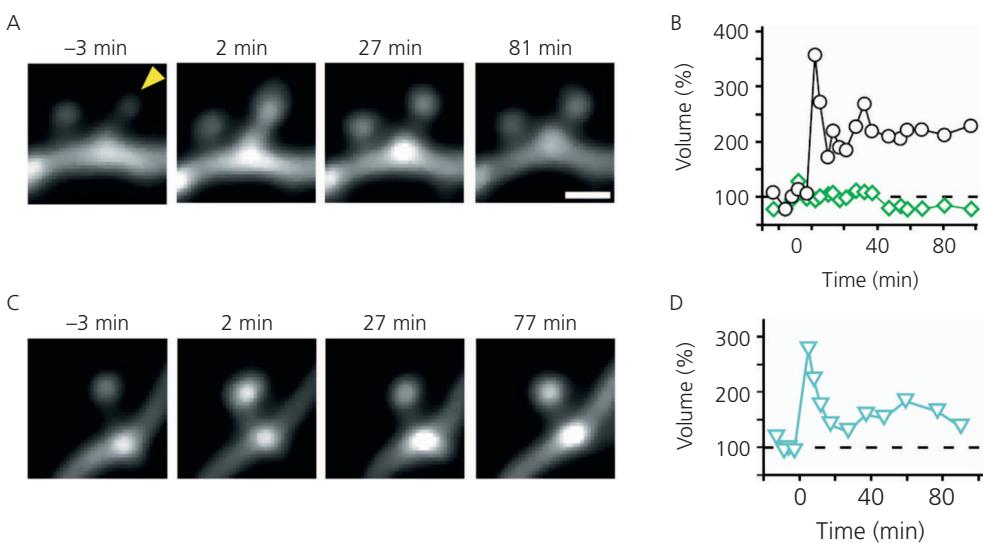


Fig. 19.2 Synaptic enhancement is also paralleled by spine enlargement. (A) Spine enlargement is caused by repetitive glutamate uncaging at the position indicated with the arrowhead. Scale bar 1 μ m. (B) Time course of the spine enlargement compared with a control spine (green). (C–D) Example similar to (A) and (B) only the enlargement was caused by electrical stimulation to the afferent fibers.

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and Sabatini, 2011). However, what remained open in these and all other previous experiments is the question of the identity of the presynaptic fibers that these new spines contact. Are connections made to those fibers that were stimulated during stimulation paradigm, i.e., do the new spines truly contribute to stronger synaptic connections? Or do the new spines connect to arbitrary presynaptic inputs in a much less specific process? Such questions have recently begun to be addressed and preliminary evidence shows that new spines might connect specifically—albeit not exclusively—to previously active presynaptic fibers (Coneva, Bonhoeffer, and Rose, *in preparation*).

To summarize, over the last 15 years it has been firmly established that structural changes at spines are important mediators of synaptic plasticity. Spines can be generated and deleted in plasticity paradigms and they have been shown to contribute to the endurance of synaptic changes in *in vitro* paradigms. Obviously a key question is whether these forms of structural plasticity occur in learning paradigms *in vivo* and whether they contribute to the storage of information in the brain. We will discuss these issues later in this chapter.

Plasticity of dendritic branches in the adult

Over the past decade, several studies have used repeated two-photon imaging of dendrites *in vivo* to address the question of whether entire dendritic branches might remodel in adult cortical or hippocampal principal neurons. These studies found no evidence for changes in the number or length of dendritic branches of pyramidal neurons, concluding that, unlike spines, dendritic trees are stable in the adult (Mizrahi and Katz, 2003; Koleske, 2013). Whether those conclusions will apply generally for excitatory neurons remains to be determined. In addition, the studies did not address dendritic structures during skill learning or upon hormonal changes. However, for the time being, the conclusion is that in the absence of pathology the dendrites of pyramidal neurons might be quite stable in the adult.

In contrast to cortical principal neurons, the dendrites of GABAergic interneurons have been shown to exhibit some structural plasticity in the adult. Already early on it was demonstrated that motor skill learning increased dendritic arborization of stellate interneurons in the cerebellum (Kleim et al., 1997). Later, in the visual cortex, Nedivi and colleagues (Lee et al., 2006) reported that a fraction (one out of 35–45 dendritic tips per cell per week; eight out of 35–45 tips per cell over several months) of dendritic tips exhibit short (up to 10 μm; about 3% per week) alterations in length. This remodeling exhibited striking layer selectivity, and was restricted to upper regions of cortical layers 2/3 (L2/3), where many regulatory inputs to GABAergic neurons are known to terminate (Lee et al., 2006). By contrast, the plasticity did not appear to exhibit obvious specificity for a particular subtype of interneuron within that cortical layer. The plasticity was initially reported in primary visual cortex (Lee et al., 2006) but a subsequent study noted comparable plasticity in somatosensory cortex, and higher plasticity in secondary visual cortex (Chen et al., 2011a). Monocular or binocular deprivation enhanced the dendritic plasticity about three-fold, suggesting that it does respond to strong alterations in circuit activity. Further supporting the notion that the plasticity is influenced by synaptic activity patterns, the dynamic dendritic tips did not cross functional boundaries in the cortex (monocular/binocular), whereas non-dynamic tips did. About 80% of the synapses onto dynamic dendritic tips were excitatory, and the total synapse density as determined by electron microscopy was about one synapse per micron, suggesting that the remodeling might affect on average about ten synapses per tip. The functional significance of this plasticity has not yet been addressed. When considered from a purely numeric point of view, the functional impact of these rearrangements might be modest. However, given the major importance of upper layer

disinhibition for learning and microcircuit function (Letzkus et al., 2011), it is possible that the tips are targeted by subpopulations of excitatory inputs with important gating roles in network function. It will therefore be important to identify presynaptic partners of the dynamic dendritic tips, and to design experiments to address the significance of this particular structural plasticity in modulating attention in learning.

In summary, the issue of whether dendrites of excitatory neurons remodel in the adult remains unresolved. Imaging studies *in vivo* suggest that there might be no or very little remodeling, whereas earlier studies on estrogen and on stress produced evidence that some principal neurons substantially rearrange their dendritic branches in the adult. Resolving these issues clearly calls for further studies using longitudinal *in vivo* imaging methods, possibly coupled with ways to sample relatively large fractions of dendritic trees in a quantitative manner. By contrast, there is evidence that a relatively small fraction of neocortical GABAergic interneuron tips can grow or shrink by about 10 μm within the upper half of L2/3. However, the functional role of this plasticity remains to be determined.

Structure–function relationships of spines

Accepting the strong evidence described above that new spines form and existing spines change their structure, it is important to ask what these kinds of changes may mean from a cellular point of view. Newly generated spines enable the neuron to either enhance existing connections or to form entirely new connections with different presynaptic partners. Both possibilities seem to be the case, even if there might be a preference for the strengthening of existing connections (Coneva, Bonhoeffer, Rose, *in preparation*).

Entirely new connections certainly represent an important role for new spines. It has been proposed that one major function of dendritic spines is that they enable connections to nearby but not immediately adjacent presynaptic partners (e.g., Chklovskii, 2004). In fact it is one of the fundamental geometrical–functional differences between spineless and spiny cells that spiny cells can much more easily establish new connections to presynaptic partners.

The fact that spines change their shape and size means that the net effect of a presynaptic input onto the postsynaptic neuron will be altered by changing the resistance of the spine neck. But importantly, changes in spine neck size and head size also strongly affect compartmentalization of many molecules in the spine head, other than the charge-carrying ions. It was suggested early on, initially theoretically (Rall, 1978; Crick, 1982), that one important aspect of spine function may be compartmentalization, not only in terms of the electrotonic properties of the spine but also for many small molecules (Koch and Zador, 1993). This has been impressively demonstrated by experiments using fluorescence recovery after photobleaching (FRAP) in hippocampal cells (Svoboda et al., 1996), which measured the functional consequences of spines in terms of compartmentalization. Exactly which molecules are relevant for compartmentalization remains an open issue, Ca^{2+} (Grunditz et al., 2008) and CaMKII (Lee et al., 2009) being only two of many candidates.

As pointed out in a recent study (Tønnesen et al., 2014), a problem with these early experiments was that they used two-photon microscopical approaches which do not have the power to truly resolve the width of spine necks, one crucial parameter in these experiments. New super-resolution methods (Klar et al., 2000; Nägerl et al., 2008) now allow this to be probed in living tissue, and the first study along these lines suggests that indeed the width of dendritic spine necks is not only an important determinant for compartmentalization but that it is also affected by synaptic plasticity paradigms (Tønnesen et al., 2014). These experiments nicely extend and relate early electron-microscopical studies (lacking the ability to observe living tissue) and two-photon microscopy experiments (lacking the required spatial resolution), and show how dendritic head and spine size

influence synaptic compartmentalization and how they are regulated by paradigms that influence synaptic transmission.

Interestingly, the Tønnesen et al. (2014) study also shows that the classical categorization of spines into thin, stubby, mushroom-like, and filopodia-like ones may not hold. Instead, the authors report a continuous distribution between these four different categories. Specifically they show that the classical “stubby” spines may be spines with large spine heads and a very thin spine neck, which is not resolved by conventional two-photon microscopy, and which may therefore be misinterpreted as stubby spines. This fits well with other work using electron-microscopic techniques (Arellano et al., 2007), which also concludes that the traditional categorization of spines might not be upheld upon closer scrutiny. Still, there is no question that dendritic protrusions come in many varieties. They can be thorny excrescences, they appear as filopodia, they may take a “stubby,” “mushroom,” or “thin” form (see Chapter 1). Apart from which particular subclassification is most useful, it is reasonable to assume that the exact form of the dendritic spines or protrusions will have a profound influence on their function. Therefore, the precise observation of their structure—ideally, due to their small size, with super-resolution techniques (Klar et al., 2000; Nägerl et al., 2008)—seems of great importance for understanding the precise impact that changes in the nanostructure of spines, i.e., of the spine or protrusion itself, have on neuronal function (see Chapter 1).

Taken together, the structural changes that are observed during synaptic plasticity can have fundamental influences on synaptic transmission as well as the compartmentalization of molecules involved in other ways in the regulation of synaptic transmission. Modern methods now allow us to measure these changes rather precisely, and to relate them to biophysical models. One has to bear in mind, however, that all these experiments so far have had to be conducted in *in vitro* systems, and we do not really know whether what is observed *in vitro* also holds *in vivo*. This is comparable to the first experiments in which it was shown that new spines can be generated by LTP-like protocols *in vitro*, but it took more than 10 years to perform experiments showing that it is plausible that those structural changes really matter for the storage of information in a systems context. We may need to have to wait another 10 years to show that the minute changes on the level of spine necks and spine head size really matter functionally in the intact nervous system.

Neighborhood factors in spine plasticity

Excitability, signaling, and translation in dendrites are all strongly influenced by local distributions of signaling components and synapses. For example, *N*-methyl-D-aspartic acid (NMDA) receptor activation through depolarization-mediated extrusion of Mg²⁺ ions from the ion channel is most effective within a small distance (10–20 μm) of activated synapses (Losonczy et al., 2008). Likewise, while many signaling molecules (e.g., CDC42) are confined to individual spines, some of them, like Rho and Ras, do diffuse locally from the spines where they were activated, and this diffusion can be the basis for enhanced plasticity at neighboring synapses within a distance of about 10 μm (Harvey and Svoboda, 2007; Harvey et al., 2008). Furthermore, local translational events, important for synaptic plasticity, occur in the vicinity of activated synapses, leading to local regulation and accumulation of plasticity-mediating proteins such as Arc, CaMKII, and actin-regulating proteins (Bramham, 2008; Govindarajan et al., 2011). These local signaling events are in turn thought to influence plasticity crosstalk between neighboring synapses, and tagging capture at activated synapses. From the perspective of structural synaptic plasticity, this local regulation of signaling can potentially provide a rich set of mechanisms for local growth, stabilization and destabilization of synapses. As outlined below, there is indeed strong experimental evidence that structural plasticity of synapses is clustered along dendrites.

Synapses located within a distance of 10–20 μm along a dendritic branch can influence each other strongly during synaptic plasticity (Engert and Bonhoeffer, 1997). Thus, studies involving induction of LTP in slice cultures have provided evidence for the preferential establishment and stabilization of clusters of new spines upon LTP, whereas spine losses were much more distributed (De Roo et al., 2008). For example, plasticity-related spine induction involves NO-mediated signaling upon LTP, which promotes clustered spine growth, possibly through local diffusion between synapses (Nikonenko et al., 2013). A recent *in vivo* study monitored the insertion of GluR1 AMPA receptor subunits at individual synapses potentiated through glutamate uncaging, as well as at their close or distant neighbors (Makino and Malinow, 2011). The study reported that GluR1, the insertion of which strengthens synapses in LTP, had a high likelihood of also inserting at postsynapses in the close vicinity of potentiated synapses, whereas GluR2, involved in homeostatic plasticity, did not. Notably, local insertion of GluR1 depended on local signal transduction processes, consistent with the notion that neighboring synapses can take advantage of processes initiated at potentiated synapses through local diffusion of key signaling intermediates.

Does clustered plasticity influence local connectivity patterns? A recent study used calcium imaging to show that clusters of adjacent synapses exhibit a higher tendency to be active at the same time compared with randomly distributed synapses on the same neuron (Kleindienst et al., 2011). That study further provided evidence that many of the coactive postsynaptic neighbors were not innervated by the same presynaptic axon, suggesting that the clustered plasticity might facilitate local convergence of coactive presynaptic inputs onto the same dendritic domain, facilitating their binding through local plasticity mechanisms (Kleindienst et al., 2011). One candidate mechanism might involve presynaptic release of glutamate onto primed postsynaptic dendritic domains (Kwon and Sabatini, 2011). A subsequent *in vivo* study (Takahashi et al., 2012) confirmed these observations for cortical and hippocampal synapses, and provided evidence that establishment of these locally synchronized synaptic inputs involved NMDA receptor-dependent plasticity. Interestingly, and reflecting close local co-regulation of excitation and inhibition in brain microcircuits, clustered structural plasticity of inhibitory synapses was spatially correlated to that of excitatory spine synapses (Chen et al., 2011b).

The first study to report clustered spine plasticity *in vivo* involved developmental learning in barn owls (McBride et al., 2008). In that study, owls established an alternative auditory-visual map due to their vision being distorted (by wearing prisms) during a sensitive period of postnatal development. A detailed anatomical analysis revealed that the extra adaptive synapses were clustered along individual dendritic branches. Furthermore, synapses in non-shifted owls (no prisms worn during the sensitive period) were also clustered within their normal target region. One of the key studies studying structural plasticity in the mammalian neocortex in the context of learning (Fu et al., 2012) also addressed the question of whether dendritic spines induced upon skill learning may be spatially clustered. The study involved learning of new forepaw-dependent motor skill tasks in mice, and used live imaging in the primary motor cortex to longitudinally monitor individual dendritic stretches and their associated spines. The striking finding was that when a new spine appeared upon learning, subsequent spines appearing during later phases of the training procedure frequently emerged in the vicinity of the new spine. The study reported that about a third of the new spines are clustered, and that clustered spines were on average more likely to persist to the end of the experiment than non-clustered new spines. Consistent with the notion that this involved local synaptic plasticity processes, appearance of the new spine was frequently accompanied by enlargement of the preceding spine. Notably, learning of a different motor skill induced the appearance of clustered spines at different positions along dendrites, consistent with

the notion that the clustered plasticity produces local domains of dendrites dedicated to the same representation.

As discussed in detail elsewhere in this book (see Chapter 1), some neurons exhibit complex spines with a handful of presynaptic inputs (e.g., branched spines on Purkinje cells in cerebellar cortex; parallel fiber input), whereas other types of complex spines exhibit up to 30–40 presynaptic inputs (e.g., thorny excrescences in pyramidal neurons in hippocampal CA3; mossy fiber input from dentate gyrus granule cells). While structural plasticity at these complex spines has not yet been investigated, they might provide particularly favorable substrates for clustered plasticity due to local sharing of translational machinery and geometrical arrangements favoring restricted diffusion of signaling molecules within the complex spines. In an electron microscope study looking at cerebellar cortex during acrobatic motor skill training, parallel fibers formed multispine boutons upon learning, which were contacted by pairs of spines onto the same postsynaptic Purkinje cell dendrite (Lee et al., 2013). Notably, contacted spines had simple unbranched morphologies, suggesting formation by new spine outgrowth. They further consistently differed in size, suggesting that they might not have formed at the same time. Moreover, neighboring spines along the same dendrite exhibited postsynaptic densities that were smaller (by about 15%) than average, whereas neighboring spines on different dendrites were not affected. These coordinated local changes might be initiated by either LTP or LTD, and might lead to a specific local focusing by one group of functional inputs onto specific postsynaptic dendrites upon learning. It is not clear whether functionally related inputs eventually converge onto complex spines.

In summary, local environmental factors at dendritic subdomains can have a major impact on both functional and structural synaptic plasticity. Local regulation of plasticity favors the assembly and retention of clusters of adjacent new synapses upon learning. This, in turn, provides powerful potential mechanisms for co-regulation of synapses involved in the same function, and for consolidation and further elaboration of learned skills. This suggests that investigating the relationships between the spatial distribution of synapses at a local scale and the neuronal ensembles recruited upon different aspects of experience may provide exciting new insights into how structure relates to function at dendrites.

Functional consequences of spine plasticity

An important question, of course, is whether the structural plasticity that has been observed in spines in *in vitro* experimental paradigms (Engert and Bonhoeffer, 1999; Maletic-Savatic et al., 1999; Matsuzaki et al., 2004) also occurs in the brain of a behaving animal, and most importantly what purpose it serves under these natural conditions.

Two studies, both published in 2002 (Grutzendler et al., 2002; Trachtenberg et al., 2002), were seminal in this respect because they were the first to look at spine plasticity in the intact rodent brain (Fig. 19.3). Grutzendler et al. (2002) studied the visual cortex (without experimental intervention enhancing plasticity) whereas Trachtenberg et al. (2002) used altered plucking of whiskers from the snout of an animal to study plasticity in the somatosensory cortex. In both studies neurons in the respective primary sensory areas had been labeled genetically and were then followed *in vivo* over days, weeks, and in some cases even many months. They came to somewhat different conclusions, but clearly demonstrated for the first time that it is possible to study spino-genesis and its role in plasticity in the intact brain. These studies were later followed by many others studying deprivation paradigms in the somatosensory cortex (e.g., Holtmaat et al., 2006; Wilbrecht et al., 2010) or in the visual cortex by lesioning part of the retina or the retina in its entirety

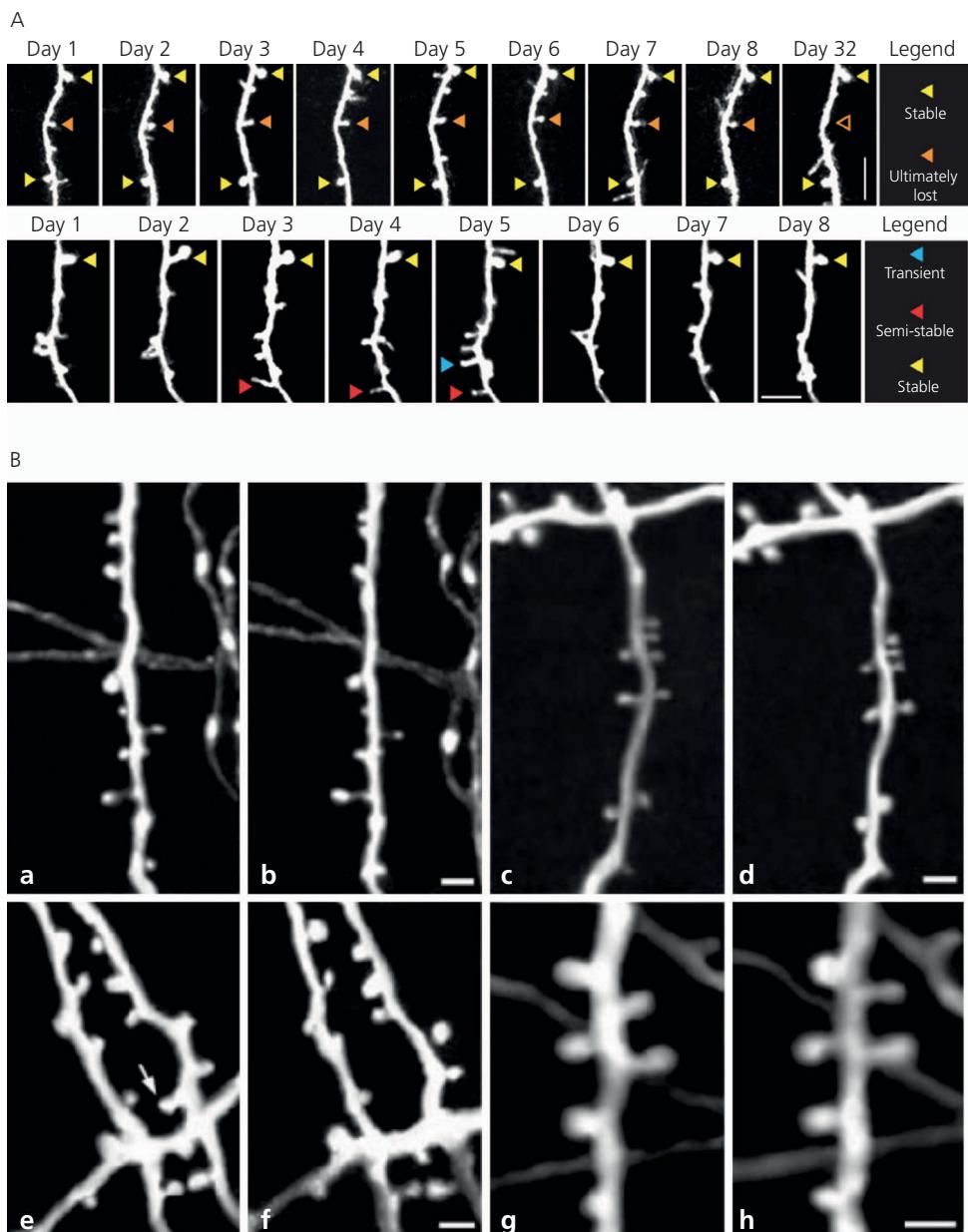


Fig. 19.3 Long-term spine stability and plasticity *in vivo*. **(A)** Neurons genetically labeled with green fluorescent protein were imaged over many days in the somatosensory cortex. Many stable spines (yellow arrowheads) can be seen, but some spines are also transient (blue arrowheads) or semi-stable (red arrowheads). Altering sensory experience changes spine turnover rates (data not shown in this figure). **(B)** Four examples (a/b, c/d, e/f, g/h) of spine stability in the visual cortex of adult mice in which a subset of neurons was labeled with yellow fluorescent protein. Images a/b, c/d, e/f, g/h show examples 3 days apart, demonstrating the stability of spines in the adult visual cortex. The arrow in e shows a spine that is absent at the next time point (f). During the critical period there is substantially more turnover (data not shown in this figure). Scale bars 1 μ m.

(Keck et al., 2008, 2011). In general what has been observed is that periods of high functional plasticity are paralleled by massive structural plasticity, manifesting in much higher spine turnover (sometimes two- to three-fold) than under normal baseline conditions (Holtmaat et al., 2006; Keck et al., 2008; Wilbrecht et al., 2010). When the experimental conditions are such that these changes are thought to be lasting, new persistent spines often result, which are thought to carry the newly acquired information. Interestingly the number of additional spines is often not due to more spines being produced but to those spines that are being generated being substantially more persistent (Holtmaat et al., 2006; Wilbrecht et al., 2010). So, in other words, the rate of generation of spines is not increased but extended spine persistence makes for a longer duration of the respective changes.

These experiments, which mainly investigated structural changes on the basis of changes in the sensory environment, were paralleled by a number of studies investigating whether the changes in dendritic spines, be it their number or size, are likely to be related to the storage of “memories” in the brain. In one of the first studies, the paradigm of monocular deprivation was used to show that the generation of new spines and their persistence is the most likely reason why information that has been acquired once is much more easily relearned a second time (Hofer et al., 2006, 2009; Fig. 19.4A). This effect—called “savings”—was demonstrated in mice that were monocularly deprived for a couple of days early in life then subjected to normal vision again; the same procedure of monocular deprivation was performed again many weeks later when—without prior deprivation—changes such as this would have much smaller effects if any. What was observed is that if an animal had experienced a similar (learning) situation early in life, learning occurs much more reliably and faster. This could be related to the appearance of new spines that emerged during the first plasticity episode and persisted despite full functional recovery. The fact that the growth of additional spines during the second plasticity period could not be seen, yet the functional adaptation occurred much faster and more reliably, suggests that the persistent spines facilitate the second adaptation. Therefore these spines serve to ‘remember’ the previous sensory experiences an animal has had.

Two subsequent studies (Yang et al., 2009; Fu et al., 2012—already discussed in “Neighborhood factors in spine plasticity”) went on to demonstrate that the generation of new spines in the motor cortex correlates with the acquisition of different sorts of motor tasks (Fig. 19.4B). Interestingly, Fu et al. (2012) also found that relearning a task occurred faster and did not involve the generation of new spines, again arguing for persistent spines ‘memorizing’ specific motor tasks. These studies together then provided convincing evidence that the generation of new spines in primary sensory or motor areas is closely tied to the acquisition or ‘learning’ of particular tasks or sensory abilities. The idea that the generation of new spines indeed correlates with learning was further bolstered by experiments which used fear conditioning to show that in this paradigm as well learning goes along with structural changes: fear extinction and fear conditioning are marked by the generation or removal of spines in frontal association cortex (Lai et al., 2012) and auditory cortex (Moczulska et al., 2013). One particularly interesting finding in this context is that fear extinction

Fig. 19.3 (Continued)

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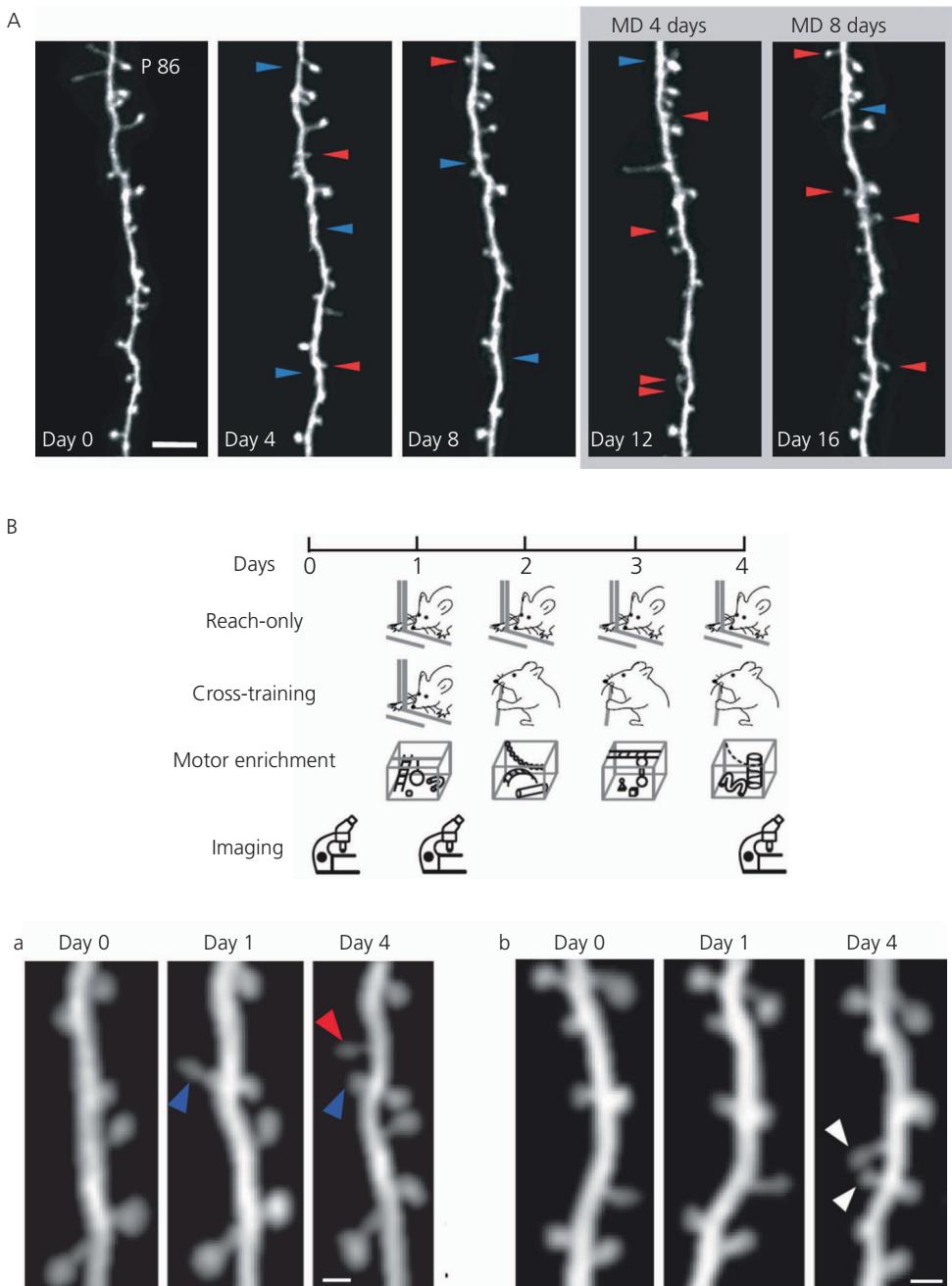


Fig. 19.4 Spine changes occur during monocular deprivation or learning paradigms. **(A)** Spines were imaged every 4 days during a monocular deprivation (MD) paradigm in mouse visual cortex. The number of newly generated spines (red arrowheads) roughly doubles during monocular deprivation, whereas the number of disappearing spines (blue arrowheads) remains roughly constant. Interestingly a second MD in the same animal does not show the increase in spinogenesis anymore, suggesting that spines generated during the first MD may be reutilized in repeated MD (data not shown in the figure). **(B)** Timeline of the experimental paradigm. The timeline shows four days of imaging (imaging icons), followed by four days of each learning paradigm (learning icons). The learning paradigms are: Reach-only, Cross-training, and Motor enrichment.

induces the appearance of spines that were eliminated upon the original fear conditioning to the same particular stimulus, but not to a distinct conditioned stimulus, suggesting that the spines are specifically associated with extinction of one particular association (Lai et al., 2012). Finally, and in a completely different animal model, namely song learning in zebra finches, it was shown that new spines are generated in the nucleus HVC when an animal learns a new song from a tutor (Roberts et al., 2010).

Taken together, there is now considerable evidence from different species as well as different learning paradigms that there is a close correlation between spine plasticity and the ability of an animal to learn. In some experiments it can even be shown that the disappearance and reappearance of spines is specific to learned information. True causality (as opposed to mere correlation) is difficult to prove, but it seems very plausible to assume that structural changes at the level of spines play a key role in many forms of learning.

Structural plasticity of GABAergic synapses

Inhibitory GABAergic neurons can be subdivided into classical inhibitory interneurons that modulate the activity of excitatory networks by inhibiting other interneurons, and those GABAergic neurons such as cerebellar Purkinje cells or striatal medium spiny neurons that have information-processing roles comparable to those of principal glutamatergic neurons. The modulatory interneuron subtypes are generated during embryonic development in ganglionic eminences and migrate to their final destinations throughout the brain. While their roles in network activity have been studied in detail, their roles in learning only came into focus more recently, and their plasticity has not been studied extensively yet.

Like excitatory synapses, inhibitory synapses consist of a majority of stable synapses, and of 5–10% dynamic synapses. Consistent with the notion that many GABAergic neurons respond to the total extent of excitatory network activity, numbers of transient inhibitory presynaptic boutons adjust to the extent of network activity. A majority of GABAergic synapses target dendritic shafts and the somatic compartment of neurons, but about a third target dendritic spines. The latter exhibit much more pronounced structural plasticity, which appears to be coordinated to that of the excitatory synapses onto the same or adjacent spines (Bourne and Harris, 2011). This is reminiscent of clustered plasticity by inhibitory synapses, which is also correlated with that of excitatory synapses in their vicinity, consistent with the notion that excitatory and inhibitory transmission are closely matched locally.

◀
Fig. 19.4 (Continued)

figure). Scale bar 5 µm. (B) Upper panel: timeline of the different learning/environmental enrichment paradigms used in the study by Fu et al. (2012). Learning of a reaching task specifically causes the formation of new spines (a, red and blue arrowheads) in the motor cortex. Interestingly the new spines often occur in clusters (b, white arrowheads). Scale bars 1 µm. Further details can be found in Fu et al. (2012).

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Where this has been investigated, excitatory or inhibitory synapses onto GABAergic neurons exhibited robust rearrangements in response to experience. This structural plasticity has been investigated in some detail in parvalbumin (PV)-positive basket cells, where it has been shown to result in large structural alterations in some forms of learning coupled to behavioral changes such as skill learning or Pavlovian fear conditioning (Ruediger et al., 2011; Donato et al., 2013). The alterations in excitatory or inhibitory synapse densities appear to mediate sustained changes in the state of differentiation of PV-positive neurons and the configuration of local PV-positive neuron networks, which in turn modulate learning, possibly through memory consolidation processes. Enhanced excitatory connectivity onto PV-positive basket cells was induced, together with enhanced fractions of high-PV and high-GAD67 expressing neurons, upon fear conditioning or at the end of maze learning or rotarod training protocols (Donato et al., 2013). The high-PV configuration interfered with incidental memories and might support consolidation of learned skill memories. In contrast, enhanced densities and activity of VIP-positive GABAergic terminals onto PV-positive basket cells were induced together with enhanced fractions of low-PV and low-GAD67 expressing neurons during skill learning. The low-PV configuration enhanced incidental memories and might support consolidation of new information during learning. While synapse rearrangements onto the other types of local GABAergic interneurons have not yet been investigated in detail, it seems reasonable to speculate that they may also affect how these neurons modulate learning and memory.

In conclusion, structural plasticity of inhibitory synapses onto principal neurons seems to resemble that of excitatory synapses. Moreover, where this has been investigated, inhibitory and excitatory synapses were regulated in a coordinated manner. However, the underlying mechanisms remain to be determined. Synapses onto GABAergic interneurons in response to plasticity-inducing stimuli exhibit distinct structural changes, at least in hippocampal spiny interneurons (Scheuss and Bonhoeffer, 2014) and in PV-positive basket cells (Ruediger et al., 2011; Donato et al., 2013). In the latter case it has been shown to be intimately connected to behavioral learning paradigms. It will be interesting to determine whether, how, and in what roles synapses targeting the other subtypes of GABAergic interneurons exhibit structural plasticity upon learning.

Mechanisms that control synapse numbers

A major feature of learning-related structural plasticity is that when synapses are monitored over periods of several days the overall numbers of excitatory synapses onto principal neurons stays roughly constant. This was demonstrated in a particularly striking way by an electron microscopy study of hippocampal slices with or without theta-burst stimulation of Schaffer collateral axons (Bourne and Harris, 2011). The distribution of thin and large spines onto pyramidal neuron dendrites in CA1 did change following this LTP-inducing protocol, but the total area of postsynaptic density per dendrite length remained constant. Likewise, several early longitudinal imaging studies of dendritic spines *in vivo* concluded that spine gains were closely matched by spine losses when they were compared over periods of several days. Against the background of this balanced structural plasticity there are, however, notable circumstances when spine numbers do persistently change in the adult. One of them involves maintaining animals for at least a week under environmentally enriched conditions, a procedure that enhances learning and produces up to a doubling of dendritic spine densities in hippocampus and cortex (Bednarek and Caroni, 2011). Other examples include hormonal changes, as discussed at the beginning of this chapter. Finally, spine densities increase

in juvenile brains and are reduced through synapse elimination toward late adolescence. In this case, recent studies have provided evidence that synapse gains are more predominant during wakefulness whereas synapse losses are more predominant during sleep (Maret et al., 2011). By contrast, in adult animals wakefulness and sleep did not favor global synapse gains or losses, suggesting a specific association between wake–sleep-related bias and conditions under which synapse densities undergo net changes. Sleep was, however, associated with the stabilization of new synapses along dendritic branches upon motor skill learning (Yang et al., 2014).

When considering the mechanisms that might account for balanced gains and losses of spine synapses in individual principal neurons, one key question is whether these operate at the level of entire neurons or locally at the level of dendritic branches or dendritic subdomains. Homeostatic plasticity mechanisms have been mainly considered in the context of balancing global levels of excitation or inhibition onto individual neurons, to ensure signaling within sensitive dynamic ranges and to prevent runaway excitation or persistent silencing (Turrigiano, 2012). For structural plasticity, which has recently also been shown to occur *in vivo* (Keck et al., 2013), the underlying molecular mechanisms might include competition for limited synaptic resources within entire neurons, a type of mechanism for which there is strong experimental evidence in several circuit settings (Lee et al., 2013). In addition, however, local plasticity rules as described in “Neighborhood factors in spine plasticity” section of this chapter can also provide powerful mechanisms to control synapse numbers within local dendritic domains. Again, local competition for synaptic resources can lead to local establishment and/or strengthening of some synapses, balanced by local shrinkage and/or elimination of weaker synapses.

In conclusion, in the absence of specific signals promoting net alterations in synapse numbers, structural plasticity involves balanced strengthening/addition versus weakening/elimination of synapses over periods of 1–2 days. When synapse rearrangements are induced upon learning, gains and losses are likely to be balanced locally, contributing to the assembly of local clusters of functionally related synapses through local homeostatic mechanisms involving competition for limiting synaptic resources.

Conclusion

Much progress has been made in understanding structural plasticity of dendrites and spines and their role in information storage as well as other mechanisms critical for information processing. In particular, the advent of live imaging techniques has led to a much better understanding of these events. We are beginning to see the first solid evidence that structural plasticity of spines may indeed play a pivotal role in learning and memory and we are starting to grasp some of the mechanistic and molecular underpinnings of the associated structural changes. The focus of this work has thus far been primarily on pyramidal neurons, and much less is known about the structural plasticity of GABAergic neurons. But progress has also been made in this area, much facilitated by the modern imaging and genetic labeling techniques that have been developed over the last 10–20 years. While all of these advances are starting to give a coherent picture of the structural plasticity of dendrites and spines and how this plasticity may impact the functioning of the intact nervous system, a number of open questions remain (see Box 19.1). It seems, however, that progress in this area is, if anything, continuously accelerating. It therefore seems likely that already for the next edition of this book we will be able to say much more on the exact role of structural changes of dendrites and spines in a number of ethologically relevant behaviors.

Box 19.1 Open questions

In recent years there have been tremendous advances in understanding the structural plasticity of dendrites and spines. However, a number of important questions remain, the answers to which would considerably enhance our understanding of what spines are good for and how they contribute to information storage and information processing in the mammalian brain.

Are dendritic plasticity and spine changes used for information storage?

Many data appear to suggest that either generation and removal or structural changes in spines are the way the nervous system stores information (Hofer et al., 2009; Yang et al., 2009; Fu et al., 2012). Nonetheless, final proof for this assumption is still outstanding. If it could be shown that during synaptic plasticity new spines are generated, and if then those spines could be ablated in a second part of the experiment and the newly acquired information were abolished, this would be a very strong, perhaps definitive, indication that indeed spines are the crucial organelles involved in the storage of information in the brain. Such experiments, although difficult, are becoming feasible and they will provide exceedingly important information on the role of spines for learning and memory.

Is dendritic structure modulated by behavioral state?

Early experiments have shown massive changes in dendritic structure during behavioral and learning-related events (Black et al., 1990; Magariños and McEwen, 1995; Vyas et al., 2002; Cooke and Woolley, 2005). Most of these experiments were carried out using fixed tissue comparing one experimental cohort with another. More modern experiments, in particular those using chronic *in vivo* imaging approaches, have not reported such dramatic dendritic changes, even though strong changes on the level of spines have been observed. It is important to apply *in vivo* two-photon microscopy to search for the dendritic changes that were reported earlier in order to establish whether they occur in the intact network under physiological conditions.

What is the time course of key molecular components during spinogenesis and spine changes under natural learning conditions?

Many molecules, some of them related to the cytoskeleton (Ras, Rac, CDC42, etc.) and others to pre- or postsynaptic components (Shank, Homer1, etc.) have been reported to be important for the generation of new spines *in vitro* (Bramham, 2008; Harvey et al., 2008; Lee et al., 2009; Caroni et al., 2014). It is important to determine whether these molecules play similar roles *in vivo* and whether and how they change, for example during learning paradigms. In principle it is eminently feasible to label many of these molecular components with genetically encoded indicators and image the orchestration of spine changes in molecular terms *in vivo* during learning paradigms. As always, such experiments come with the problems of overexpression or functional alteration of the labeled molecules, but carefully conducted experiments of that sort would be a big step forward to better understand the molecular events that underlie behaviorally relevant spinogenesis in the intact brain.

Open questions (continued)

What are the functional changes of single spines during the different plasticity paradigms?

A number of experiments have shown convincingly that new spines are generated during plasticity events or that the structure of spines changes substantially under such circumstances (Engert and Bonhoeffer, 1999; Maletic-Savatic et al., 1999; Matsuzaki et al., 2004). However, hardly any studies have so far addressed the question of what these structural changes mean functionally. Therefore it would be very worthwhile – and this is again entirely feasible with current methods – to observe functional (calcium) signals in spines of nervous tissue that has been subjected to behaviorally relevant plasticity events. In such cases one may want to resort to functional plasticity paradigms as they have been established in primary sensory areas, because the structural and functional changes that occur can more easily be related to the well-understood structure of the respective sensory areas. Similar functional data would, however, also be very illuminating in paradigms that have more direct behavioral relevance, such as fear conditioning (Lai et al., 2012) or bird song (Roberts et al., 2010).

Is memory consolidation reflected on the structural level?

Long-term consolidation of memory is thought to occur within the first 24 hours after acquisition. In principle, therefore, if structural plasticity of spines, including the establishment of new spines, does indeed provide long-time traces of what has been learned, the new synapses might have to undergo key stabilization processes during the first 24 hours after learning. Understanding the precise nature of the stabilization process would provide a well-defined temporal framework and an excellent inroad for better relating spine plasticity to memory in learning.

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References

- Andersen P, Trommald M (1995) Possible strategies for finding the substrate for learning-induced changes in the hippocampal cortex. *Journal of Neurobiology* 26:396–402.
- Arellano JI, Benavides-Piccione R, Defelipe J, Yuste R (2007) Ultrastructure of dendritic spines: correlation between synaptic and spine morphologies. *Frontiers in Neuroscience* 1:131–143.
- Bednarek E, Caroni P (2011) β -Adducin is required for stable assembly of new synapses and improved memory upon environmental enrichment. *Neuron* 69:1132–1146.
- Black, J.E., Isaacs, K.R., Anderson, B.J., Alcantara, A.A., and Greenough, W.T. (1990). Learning causes synaptogenesis, whereas motor activity causes angiogenesis, in cerebellar cortex of adult rats. *Proceedings of the National Academy of Sciences of the United States of America* 87:5568–5572.
- Bourne JN, Harris KM (2011) Coordination of size and number of excitatory and inhibitory synapses results in a balanced structural plasticity along mature hippocampal CA1 dendrites during LTP. *Hippocampus* 21:354–373.
- Bramham CR (2008) Local protein synthesis, actin dynamics, and LTP consolidation. *Current Opinion in Neurobiology* 18:524–531.

- Calizo LH, Flanagan-Cato LM (2000) Estrogen selectively regulates spine density within the dendritic arbor of rat ventromedial hypothalamic neurons. *Journal of Neuroscience* **20**:1589–1596.
- Caroni P, Chowdhury A, Lahr M (2014) Synapse rearrangements upon learning: from divergent-sparse connectivity to dedicated sub-circuits. *Trends in Neurosciences* **37**:604–614.
- Chen JL, Flanders GH, Lee W-CA, Lin WC, Nedivi E (2011a) Inhibitory dendrite dynamics as a general feature of the adult cortical microcircuit. *Journal of Neuroscience* **31**:12437–12443.
- Chen X, Leischner U, Rochefort NL, Nelken I, Konnerth A (2011b) Functional mapping of single spines in cortical neurons *in vivo*. *Nature* **475**:501–505.
- Chklovskii DB (2004) Synaptic connectivity and neuronal morphology: two sides of the same coin. *Neuron* **43**:609–617.
- Christensen A, Dewing P, Micevych P (2011) Membrane-initiated estradiol signaling induces spinogenesis required for female sexual receptivity. *Journal of Neuroscience* **31**:17583–17589.
- Cooke BM, Woolley CS (2005) Gonadal hormone modulation of dendrites in the mammalian CNS. *Journal of Neurobiology* **64**:34–46.
- Crick F (1982). Do dendritic spines twitch? *Trends in Neurosciences* **5**:44–46.
- Denk W, Svoboda K (1997) Photon upmanship: why multiphoton imaging is more than a gimmick. *Neuron* **18**:351–357.
- De Roo M, Klauser P, Muller D (2008) LTP promotes a selective long-term stabilization and clustering of dendritic spines. *PLoS Biology* **6**:e219.
- Donato F, Rompani SB, Caroni P (2013) Parvalbumin-expressing basket-cell network plasticity induced by experience regulates adult learning. *Nature* **504**:272–276.
- Engert F, Bonhoeffer T (1997) Synapse specificity of long-term potentiation breaks down at short distances. *Nature* **388**:279–284.
- Engert F, Bonhoeffer T (1999) Dendritic spine changes associated with hippocampal long-term synaptic plasticity. *Nature* **399**:66–70.
- Fu M, Yu X, Lu J, Zuo Y (2012) Repetitive motor learning induces coordinated formation of clustered dendritic spines *in vivo*. *Nature* **483**:92–95.
- Govindarajan A, Israely I, Huang S-Y, Tonegawa S (2011) The dendritic branch is the preferred integrative unit for protein synthesis-dependent LTP. *Neuron* **69**:132–146.
- Grunditz A, Holbro N, Tian L, Zuo Y, Oertner TG (2008) Spine neck plasticity controls postsynaptic calcium signals through electrical compartmentalization. *Journal of Neuroscience* **28**:13457–13466.
- Grutzendler J, Kasthuri N, Gan W (2002) Long-term dendritic spine stability in the adult cortex. *Nature* **420**:812–816.
- Harvey CD, Svoboda K (2007) Locally dynamic synaptic learning rules in pyramidal neuron dendrites. *Nature* **450**:1195–1200.
- Harvey CD, Yasuda R, Zhong H, Svoboda K (2008) The spread of Ras activity triggered by activation of a single dendritic spine. *Science* **321**:136–140.
- Hebb DO (1949) *The Organization of Behavior*. New York: Wiley.
- Hofer SB, Mrsic-Flogel TD, Bonhoeffer T, Hübener M (2006) Prior experience enhances plasticity in adult visual cortex. *Nature Neuroscience* **9**:127–132.
- Hofer SB, Mrsic-Flogel TD, Bonhoeffer T, Hübener M (2009) Experience leaves a lasting structural trace in cortical circuits. *Nature* **457**:313–317.
- Holtmaat A, Wilbrecht L, Knott GW, Welker E, Svoboda K (2006) Experience-dependent and cell-type-specific spine growth in the neocortex. *Nature* **441**:979–983.
- Hosokawa T, Rusakov DA, Bliss TV, Fine A (1995) Repeated confocal imaging of individual dendritic spines in the living hippocampal slice: evidence for changes in length and orientation associated with chemically induced LTP. *Journal of Neuroscience* **15**:5560–5573.
- James W (1890) *The Principles of Psychology, Vols 1 and 2*. New York, Henry Holt and Company.

- Jones TA, Chu CJ, Grande LA, Gregory AD (1999) Motor skills training enhances lesion-induced structural plasticity in the motor cortex of adult rats. *Journal of Neuroscience* **19**:10153–10163.
- Keck T, Mrsic-Flogel TD, Vaz Afonso M, Eysel UT, Bonhoeffer T, Hübener M (2008) Massive restructuring of neuronal circuits during functional reorganization of adult visual cortex. *Nature Neuroscience* **11**:1162–1167.
- Keck T, Scheuss V, Jacobsen RI, Wierenga CJ, Eysel UT, Bonhoeffer T, Hübener M (2011) Loss of sensory input causes rapid structural changes of inhibitory neurons in adult mouse visual cortex. *Neuron* **71**:869–882.
- Keck T, Keller GB, Jacobsen RI, Eysel UT, Bonhoeffer T, Hübener M (2013) Synaptic scaling and homeostatic plasticity in the mouse visual cortex *in vivo*. *Neuron* **80**:327–334.
- Klar TA, Jakobs S, Dyba M, Egner A, Hell SW (2000) Fluorescence microscopy with diffraction resolution barrier broken by stimulated emission. *Proceedings of the National Academy of Sciences of the United States of America* **97**:8206–8210.
- Kleim JA, Lussnig E, Schwarz ER, Comery, T.A., and Greenough, W.T. (1996). Synaptogenesis and Fos expression in the motor cortex of the adult rat after motor skill learning. *Journal of Neuroscience* **16**:4529–4535.
- Kleim JA, Vij K, Ballard DH, Greenough WT (1997) Learning-dependent synaptic modifications in the cerebellar cortex of the adult rat persist for at least four weeks. *Journal of Neuroscience* **17**:717–721.
- Kleindienst T, Winnubst J, Roth-Alpermann C, Bonhoeffer T, Lohmann C (2011) Activity-dependent clustering of functional synaptic inputs on developing hippocampal dendrites. *Neuron* **72**:1012–1024.
- Koch C, Zador A (1993) The function of dendritic spines: devices subserving biochemical rather than electrical compartmentalization. *Journal of Neuroscience* **13**:413–422.
- Koleske AJ (2013) Molecular mechanisms of dendrite stability. *Nature Reviews Neuroscience* **14**:536–550.
- Kwon H-B, Sabatini BL (2011) Glutamate induces de novo growth of functional spines in developing cortex. *Nature* **474**:100–104.
- Lai CSW, Franke TF, Gan W-B (2012) Opposite effects of fear conditioning and extinction on dendritic spine remodelling. *Nature* **483**:87–91.
- Lee W-CA, Huang H, Feng G, Sanes JR, Brown EN, So PT, Nedivi E (2006) Dynamic remodeling of dendritic arbors in GABAergic interneurons of adult visual cortex. *PLoS Biology* **4**:e29.
- Lee S-JR, Escobedo-Lozoya Y, Szatmari EM, Yasuda R (2009) Activation of CaMKII in single dendritic spines during long-term potentiation. *Nature* **458**:299–304.
- Lee KJ, Park IS, Kim H, Greenough WT, Pak DTS, Rhyu IJ (2013) Motor skill training induces coordinated strengthening and weakening between neighboring synapses. *Journal of Neuroscience* **33**:9794–9799.
- Letzkus JJ, Wolff SBE, Meyer EMM, Toyote P, Courtin J, Herry C, Lüthi A (2011) A disinhibitory microcircuit for associative fear learning in the auditory cortex. *Nature* **480**:331–335.
- Losonczy A, Makara JK, Magee JC (2008) Compartmentalized dendritic plasticity and input feature storage in neurons. *Nature* **452**:436–441.
- McBride TJ, Rodriguez-Contreras A, Trinh A, Bailey R, Debello WM (2008) Learning drives differential clustering of axodendritic contacts in the barn owl auditory system. *Journal of Neuroscience* **28**:6960–6973.
- McEwen BS (1999) Stress and hippocampal plasticity. *Annual Review of Neuroscience* **22**:105–122.
- Magariños AM, McEwen BS (1995) Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: involvement of glucocorticoid secretion and excitatory amino acid receptors. *Neuroscience* **69**:89–98.
- Makino H, Malinow R (2011) Compartmentalized versus global synaptic plasticity on dendrites controlled by experience. *Neuron* **72**:1001–1011.
- Maletic-Savatic M, Malinow R, Svoboda K (1999) Rapid dendritic morphogenesis in CA1 hippocampal dendrites induced by synaptic activity. *Science* **283**:1923–1927.

- Maret S, Faraguna U, Nelson AB, Cirelli C, Tononi G (2011) Sleep and waking modulate spine turnover in the adolescent mouse cortex. *Nature Neuroscience* **14**:1418–1420.
- Matsuzaki M, Honkura N, Ellis-Davies GCR, Kasai H (2004) Structural basis of long-term potentiation in single dendritic spines. *Nature* **429**:761–766.
- Mitra R, Jadhav S, McEwen BS, Vyas A, Chattarji S (2005) Stress duration modulates the spatiotemporal patterns of spine formation in the basolateral amygdala. *Proceedings of the National Academy of Sciences of the United States of America* **102**:9371–9376.
- Mizrahi A, Katz LC (2003) Dendritic stability in the adult olfactory bulb. *Nature Neuroscience* **6**:1201–1207.
- Moczulska KE, Tinter-Thiede J, Peter M, Ushakova L, Wernle T, Bathellier B, Rumpel S (2013) Dynamics of dendritic spines in the mouse auditory cortex during memory formation and memory recall. *Proceedings of the National Academy of Sciences of the United States of America* **110**:18315–18320.
- Moser MB, Trommald M, Andersen P (1994) An increase in dendritic spine density on hippocampal CA1 pyramidal cells following spatial learning in adult rats suggests the formation of new synapses. *Proceedings of the National Academy of Sciences of the United States of America* **91**:12673–12675.
- Nikonenko I, Nikonenko A, Mendez P, Michurina TV, Enikolopov G, Muller D (2013) Nitric oxide mediates local activity-dependent excitatory synapse development. *Proceedings of the National Academy of Sciences of the United States of America* **110**:E4142–E4151.
- Nusser Z, Lujan R, Laube G, Roberts JD, Molnar E, Somogyi P (1998) Cell type and pathway dependence of synaptic AMPA receptor number and variability in the hippocampus. *Neuron* **21**:545–559.
- Nägerl UV, Eberhorn N, Cambridge SB, Bonhoeffer T (2004) Bidirectional activity-dependent morphological plasticity in hippocampal neurons. *Neuron* **44**:759–767.
- Nägerl UV, Willig KI, Hein B, Hell SW, Bonhoeffer T (2008) Live-cell imaging of dendritic spines by STED microscopy. *Proceedings of the National Academy of Sciences of the United States of America* **105**:18982–18987.
- Rall W (1978) Dendritic spines and synaptic potency. In: *Studies in Neurophysiology* (McIntyre AK, Porter R, eds), pp. 203–209. Cambridge: Cambridge University Press.
- Ramón y Cajal S (1893) Neue Darstellung vom histologischen Bau des Centralnervensystems. *Archiv für Anatomie und Entwicklungsgeschichte* **17**: 319–428.
- Ramón y Cajal S (1911) *Histologie du Systeme Nerveux de l'Homme et des Vertébrés*, Vols 1 and 2. Paris: Maloine.
- Roberts TF, Tschida KA, Klein ME, Mooney R (2010) Rapid spine stabilization and synaptic enhancement at the onset of behavioural learning. *Nature* **463**:948–952.
- Ruediger S, Vittori C, Bednarek E, Genoud C, Strata P, Sacchetti B, Caroni P (2011) Learning-related feedforward inhibitory connectivity growth required for memory precision. *Nature* **473**:514–518.
- Scheuss V, Bonhoeffer T (2014) Function of dendritic spines on hippocampal inhibitory neurons. *Cerebral Cortex* **24**:3142–3153.
- Schikorski T, Stevens CF (1999) Quantitative fine-structural analysis of olfactory cortical synapses. *Proceedings of the National Academy of Sciences of the United States of America* **96**:4107–4112.
- Svoboda K, Tank DW, Denk W (1996) Direct measurement of coupling between dendritic spines and shafts. *Science* **272**:716–719.
- Takahashi N, Kitamura K, Matsuo N, Mayford M, Kano M, Matsuki N, Ikegaya Y (2012) Locally synchronized synaptic inputs. *Science* **335**:353–356.
- Toni N, Buchs P-A, Nikonenko I, Bron CR, Muller D (1999) LTP promotes formation of multiple spine synapses between a single axon terminal and a dendrite. *Nature* **402**:421–425.
- Tønnesen J, Katona G, Rózsa B, Nägerl UV (2014) Spine neck plasticity regulates compartmentalization of synapses. *Nature Neuroscience* **17**:678–685.
- Trachtenberg JT, Chen BE, Knott GW, Feng G, Sanes JR, Welker E, Svoboda K (2002) Long-term *in vivo* imaging of experience-dependent synaptic plasticity in adult cortex. *Nature* **420**:788–794.

- Turrigiano G (2012) Homeostatic synaptic plasticity: local and global mechanisms for stabilizing neuronal function. *Cold Spring Harbor Perspectives in Biology* 4:a005736.
- Vyas A, Mitra R, Shankaranarayana Rao BS, Chattarji S (2002) Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. *Journal of Neuroscience* 22:6810–6818.
- Watanabe Y, Gould E, McEwen BS (1992) Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons. *Brain Research* 588:341–345.
- Wilbrecht L, Holtmaat A, Wright N, Fox K, Svoboda K (2010) Structural plasticity underlies experience-dependent functional plasticity of cortical circuits. *Journal of Neuroscience* 30:4927–4932.
- Woolley CS, McEwen BS (1992) Estradiol mediates fluctuation in hippocampal synapse density during the estrous cycle in the adult rat. *Journal of Neuroscience* 12:2549–2554.
- Yang G, Pan F, Gan W-B (2009) Stably maintained dendritic spines are associated with lifelong memories. *Nature* 462:920–924.
- Yang G, Lai CSW, Cichon J, Ma L, Li W, Gan W-B (2014) Sleep promotes branch-specific formation of dendritic spines after learning. *Science* 344:1173–1178.
- Zito K, Scheuss V, Knott G, Hill T, Svoboda K (2009) Rapid functional maturation of nascent dendritic spines. *Neuron* 61:247–258.

Chapter 20

Molecular signaling during plasticity of dendritic spines

Ryohei Yasuda

Summary

Intracellular signaling in the dendrites of pyramidal neurons is responsible for synaptic plasticity related to learning and memory. It is mediated by a biochemical network consisting of hundreds of signaling molecules that connect extensively to each other. The signaling processes for synaptic plasticity are initiated in a dendritic spine and targeted to multiple subcellular compartments in the stimulated spine and surrounding dendritic segment. This in turn causes a rapid modification of the structure and function of the stimulated spine. Some signals spread over much longer distances, influencing the properties of their parent dendritic branch or the nucleus to regulate gene transcription. Due to the complicated morphology of dendrites, the biochemical reaction is compartmentalized in different length scales, determining the length scales of different forms of synaptic plasticity and meta-plasticity. The recent development of optical techniques has allowed researchers to image signaling activity in dendrites at the single-spine level and this has provided many insights into how the spatio-temporal dynamics of intracellular signaling is organized during synaptic plasticity.

Introduction

Synaptic plasticity is an important mechanism in learning and memory. In particular, long-term potentiation (LTP) and long-term depression (LTD) of excitatory synapses are widely recognized as cellular models of learning and memory, since these forms of plasticity can convert a brief stimulation of synapses into changes of synaptic strength that last for at least many days (Abraham, 2003). The chemical reactions that lead to these forms of synaptic plasticity are initiated in dendritic spines, small postsynaptic protrusions on the dendritic surface (see Chapter 1). Each spine is made of a small bulbous “head” (0.1–1 µm in diameter) connected to its parent dendrite through a narrow “neck” (about 100 nm in diameter) (Colgan and Yasuda, 2014). LTP and LTD are associated with long-lasting enlargement and shrinkage, respectively, of the spine head (Matsuzaki et al., 2004; Zhou et al., 2004; Hayama et al., 2013; Oh et al., 2013). The sustained change in spine volume is correlated with changes in the postsynaptic sensitivity to glutamate, suggesting that structural plasticity of spines is an essential step for changing synaptic strength (Matsuzaki et al., 2004; Hayama et al., 2013; Oh et al., 2013). The process of structural plasticity has been particularly well studied for LTP in the hippocampus. In response to strong synaptic stimulation or repetitive two-photon glutamate uncaging at a spine, the stimulated spine undergoes a rapid volume increase of two- to four-fold, which decays to a level one and a half to two times higher than the original

volume (Matsuzaki et al., 2004). This volume change is enabled by multiple biochemical pathways that regulate membrane trafficking and actin polymerization (Colgan and Yasuda, 2014).

Hundreds of signaling molecules have been identified as being important for regulating the structure and function of dendritic spines (Colgan and Yasuda, 2014). These molecules interact extensively with each other to form an intricate signaling network within the spine (Bromberg et al., 2008). These networks are regulated in space and time by multiple layers of compartmentalization in dendrites, including dendritic branches, spines, the postsynaptic density, and endosomal compartments. This complex spatio-temporal organization plays a critical role in controlling the synapse specificity of plasticity as well as communication between potentiated spines and the rest of the neuron.

The study of molecular signaling underlying spine plasticity has been greatly aided by two-photon glutamate uncaging, which allows for the induction of LTP and LTD at a single spine (Matsuzaki et al., 2001; 2004; Hayama et al., 2013; Oh et al., 2013). Combining this with various imaging techniques, the spatio-temporal dynamics of signaling events during the functional and structural plasticity of spines have been extensively studied. This has increased our understanding of how signaling cascades are relayed from activity of one molecule to its downstream molecules from milliseconds to hours and from single spines to the whole neuron.

Temporal organization of signaling processes

Biochemical mechanisms that mediate activity-dependent plasticity of spines must translate transient synaptic activity into persistent modulation of the function and structure of dendritic spines. Upon synaptic activity, brief entry of Ca^{2+} into the dendritic spine initiates multistep, downstream signaling cascades (see also Chapter 11), which translate the millisecond calcium signal into signals on the time scale of minutes to hours (Figs 20.1 and 20.2). In turn, these biochemical cascades regulate long-lasting changes in the actin cytoskeleton and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in the spine, which mediate the persistent changes in spine structure and the function of neuronal plasticity (Fig. 20.2).

Ca^{2+} elevation: 10 – 100 ms

Ca^{2+} is a universal second messenger of cells, which activates diverse signaling pathways required for various forms of cellular plasticity and adaptive cell responses. Ca^{2+} transients in spines are essential for many forms of synaptic plasticity. Elevation of Ca^{2+} in single spines in brain slices has been monitored using Ca^{2+} -sensitive fluorophores and two-photon scanning microscopy or confocal laser scanning microscopy (Yasuda et al., 2004). It has been

Fig. 20.1 Spatio-temporal organization of signaling activity. (A) Spatio-temporal dynamics of signaling proteins during LTP and associated structural plasticity in spines induced with two-photon glutamate uncaging. Ca^{2+} is measured with the Ca^{2+} -sensitive fluorophore Fluo-4FF in combination with the red Ca^{2+} -insensitive fluorophore Alexa-594 loaded through a patch pipette (Lee et al., 2009). CaMKII, Cdc42, RhoA, Ras, and cofilin are measured with 2pFLIM (Bosch et al., 2014; Harvey et al., 2008; Lee et al., 2009; Murakoshi et al., 2011). (B) The time courses of signaling activity in spines and the nucleus at different time windows. The activities are normalized to their peak activity. Curves are from Lee et al. (2009) (Ca^{2+} and CaMKII), Oliveira and Yasuda (2014) (Ras), Murakoshi et al. (2011) (Cdc42 and RhoA), Bosch et al. (2014) (cofilin), Zhai et al. (2013) (nuclear ERK), and Meyer et al. (2014) (PSD-95).

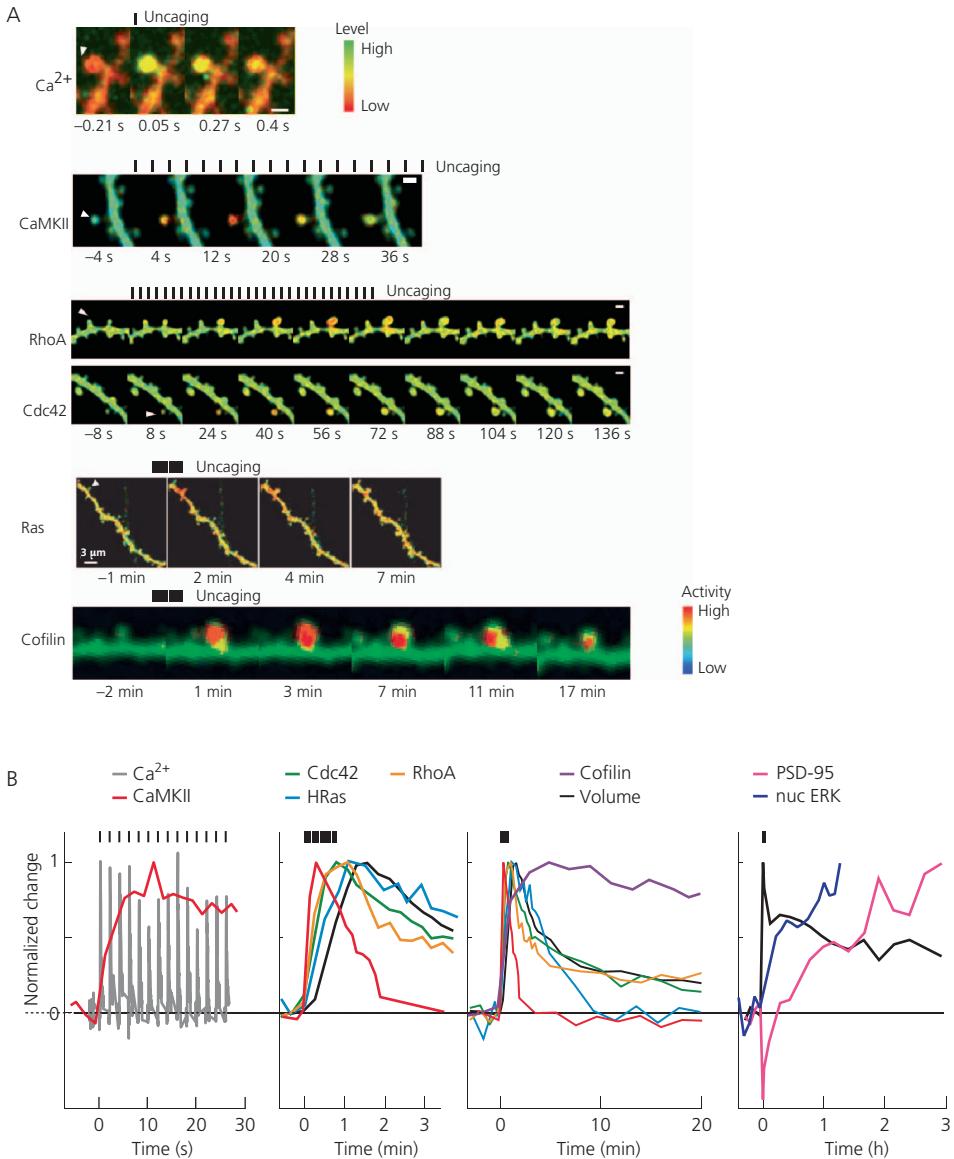


Fig. 20.1 (Continued)

Part A: Ca^{2+} and CaMKII reprinted by permission from Macmillan Publishers Ltd: *Nature*, 458(7236), Seok-Jin R. Lee, Yasmin Escobedo-Lozoya, Erzsebet M. Szatmari, and Ryohji Yasuda, Activation of CaMKII in single dendritic spines during long-term potentiation, pp. 299–304, © 2009 Nature Publishing Group; Cdc42 and RhoA reprinted by permission from Macmillan Publishers Ltd: *Nature*, 472(7341) Hideji Murakoshi, Hong Wang, and Ryohji Yasuda, Local, persistent activation of Rho GTPases during plasticity of single dendritic spines, pp. 100–104, © 2011 Nature Publishing Group; Ras reprinted from Christopher D. Harvey, Ryohji Yasuda, Haining Zhong, and Karel Svoboda, The spread of Ras activity triggered by activation of a single dendritic spine, *Science*, 321(5885) pp. 136–140, © 2008, The American Association for the Advancement of Science. Reprinted with permission from AAAS; cofilin reprinted from *Neuron*, 82(2), Miquel Bosch, Jorge Castro, Takeo Saneyoshi, Hitomi Matsuno, Mriganka Sur, and Yasunori Hayashi, Structural and molecular remodeling of dendritic spine substructures during long-term potentiation, pp. 444–459, Copyright 2014, Elsevier. With permission from Elsevier.

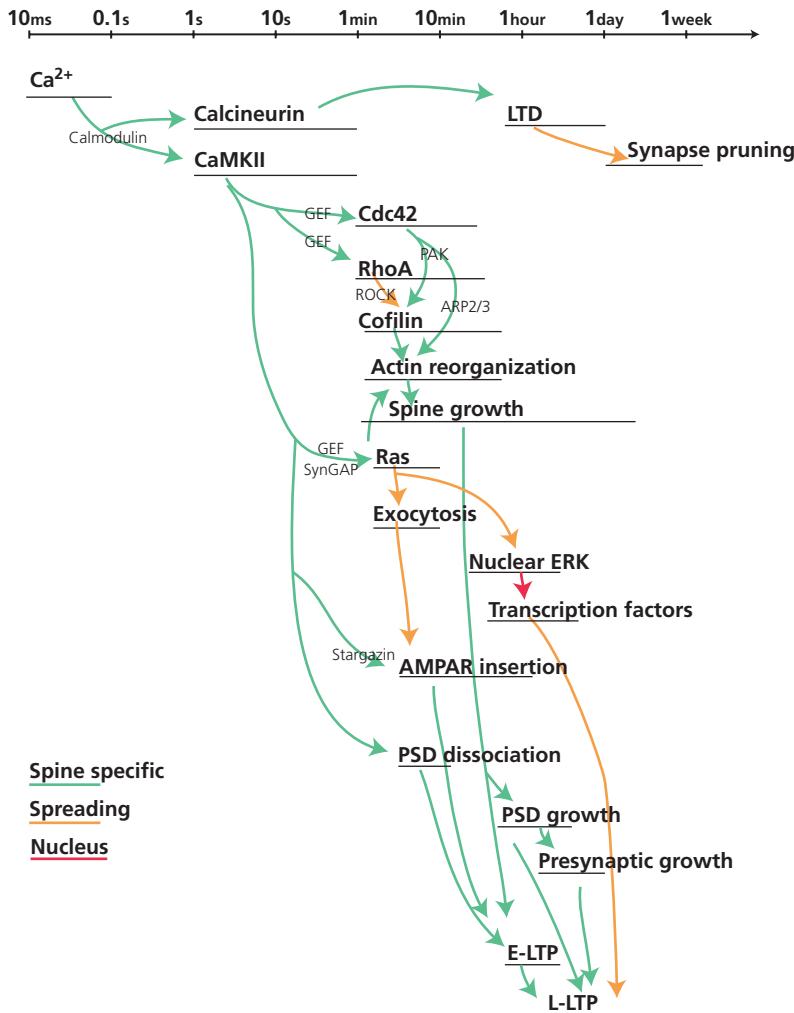


Fig. 20.2 Signaling dynamics at different temporal scales.

demonstrated that extrusion of Ca^{2+} is extremely efficient in spines and adjacent dendritic shafts, causing rapid decays in Ca^{2+} transients with a time constant of about 20 ms (Sabatini et al., 2002). In dendritic spines, major sources for activity-dependent Ca^{2+} elevations are voltage-gated Ca^{2+} (Ca_v) channels and NMDA receptors. In response to backpropagating action potentials, opening of R-type Ca_v channels produces a transient increase in Ca^{2+} lasting about 20 ms (Sabatini et al., 2002). On the other hand, synaptic stimulation to a dendritic spine causes elevation of Ca^{2+} , mainly by opening of NMDA receptors with some contribution from Ca_v channels and release from internally stored Ca^{2+} (Emptage et al., 1999; Yuste et al., 1999; Kovalchuk et al., 2000; Grunditz et al., 2008). Opening of NMDA receptors requires depolarization of cells to release Mg^{2+} blockade of their pores, serving as a coincident detector of post- and pre-synaptic activity (Spruston et al., 1995). Since NMDA receptors open for about 100 ms, the

Ca^{2+} elevation lasts for about 100 ms and remains relatively restricted to the stimulated spine (Fig. 20.1). As Ca^{2+} enters the spine it interacts with various Ca^{2+} -binding proteins which relay the signal to downstream molecules.

Both LTP and LTD depend on opening of NMDA receptors. It has been proposed that highly elevated Ca^{2+} in spines induces LTP while low Ca^{2+} induces LTD (Lisman, 1989; Malenka and Bear, 2004). However, recent studies suggested that elevated Ca^{2+} is not necessary for some forms of LTD (Nabavi et al., 2013). Instead, metabotropic function of NMDA receptors plays an important role in these cases. There are other studies suggesting that ionotropic function (Babieć et al., 2014) and Ca^{2+} elevation (Mulkey and Malenka, 1992; Hayama et al., 2013) are required for induction of LTD. Thus, this issue requires further investigation.

Integration of Ca^{2+} signaling by CaMKII and calcineurin: 1 – 60 s

Among a number of Ca^{2+} -binding proteins in spines, calmodulin is important in diverse signaling pathways. Ca^{2+} -bound calmodulin binds to a variety of downstream molecules including Ca^{2+} /calmodulin-dependent kinase II (CaMKII) and calcineurin, molecules known to be essential for LTP and LTD, respectively (Fig. 20.2).

CaMKII

CaMKII is required for LTP and hippocampus-dependent memory (Lisman et al., 2012). The holoenzyme of CaMKII is made of 12 subunits, and each subunit acts as a serine–threonine kinase (Rosenberg et al., 2005). When Ca^{2+} -bound calmodulin binds to a subunit, the subunit undergoes a conformational change from a closed state to an open state, allowing downstream substrates access to the kinase domain of CaMKII (Rosenberg et al., 2005). When two adjacent subunits are activated, they undergo trans-autophosphorylation at threonine-286. This increases the affinity of the CaMKII subunit to calmodulin by orders of magnitude and makes the kinase activity independent of Ca^{2+} /calmodulin. Because of this “autonomous” activation of CaMKII, some models predicted that the activation can persist more than hours (Zhabotinsky, 2000). If this is the case, CaMKII could serve as a biochemical memory for maintaining LTP. Biochemical analyses showed that threonine-286 phosphorylation increases in response to LTP-inducing stimuli (Barria et al., 1997). Also, the importance of the phosphorylation site was proven by knock-in mice in which the threonine-286 is mutated to alanine. These mice show impaired LTP and spatial learning (Giese et al., 1998).

Optical techniques to measure the kinetics of CaMKII activity in single dendritic spines were developed using the FRET-based¹ sensor Camui- α or its variants (Takao et al., 2005; Lee et al., 2009; Fujii et al., 2013). These sensors consist of the CaMKII α subunit fused with a pair of FRET fluorophores at both ends of the molecule. The conformation change of CaMKII α associated with its activation increases the distance between the fluorophores, decreasing FRET, which can be monitored either with ratiometric imaging or fluorescence lifetime imaging (Yasuda, 2006). In particular, two-photon fluorescence lifetime imaging (2pFLIM) provides high sensitivity and robust signal in brain

¹ Fluorescence resonance energy transfer (FRET) is a photo-physical phenomenon in which energy from an excited fluorophore (donor) is transferred to an adjacent fluorophore (acceptor) located within about 10 nm, decreasing the fluorescence of the donor and increasing that of the acceptor (Lakowicz, 2006). The efficiency of FRET depends strongly on the distance between the donor and the accepter, and thus can be used to read out molecular events such as protein–protein interaction and conformational change of proteins.

slices.² Using this method, it has been demonstrated that CaMKII activity is activated rapidly, within a few seconds, in response to glutamate uncaging at a single spine (Lee et al., 2009) (Figs 20.1 and 20.2). The decay time constant of CaMKII was determined to be 5–10 s. When repetitive uncaging stimulation is applied to induce LTP with an interval shorter than the CaMKII decay time (e.g., 0.5–1 Hz), CaMKII activation reaches a higher level by integrating each transient calcium signal.

Calcineurin

While calcineurin is also activated by binding of calmodulin, it has an opposite function from CaMKII: it is a phosphatase. The role of calcineurin in LTD and spine shrinkage has been demonstrated in several pharmacological studies (Mulkey et al., 1994; Zhou et al., 2004; Hayama et al., 2013). Moreover, calcineurin activity has been monitored with a FRET-based sensor designed similarly to the CaMKII sensors (Fujii et al., 2013). The sensor measures an activation-dependent conformational change of the molecule using a FRET pair attached to both ends of the molecule. Consistent with its known role in LTD, calcineurin is activated by much weaker stimulation than CaMKII. The temporal scale is similar to CaMKII and integrates Ca^{2+} signals over 1–10 s (Fig. 20.2). Interestingly, LTP-inducing stimulation can also result in activation of calcineurin. Under this condition, calcineurin activation becomes much more widespread and affects surrounding spines (Fujii et al., 2013).

Signals by small GTPase proteins: 1 – 30 min

During LTP, CaMKII activation is further relayed by activation of several small GTPase proteins in the Ras superfamily, including Ras, Rho, and Cdc42 (Harvey et al., 2008; Murakoshi et al., 2011) (Fig. 2). Small GTPase proteins act as molecular switches. The GTP-bound form is active and can bind to downstream signaling molecules (Takai et al., 2001). When GTP is hydrolyzed with the help of GTPase accelerating protein (GAP), small GTPase proteins become inactivated. In contrast, when a guanosine nucleotide exchange factor (GEF) exchanges GDP with GTP, the small GTPase proteins are activated. During LTP and spine enlargement, Ras, Rho, and Cdc42 are activated (i.e., they become bound to GTP and recruit downstream molecules) in a CaMKII-dependent manner (Zhu et al., 2002; Harvey et al., 2008; Murakoshi et al., 2011). While the exact mechanism linking CaMKII and small GTPase proteins is unknown, it is likely that some of GEFs in spines are regulated by CaMKII.

The spatiotemporal dynamics of small GTPases has been measured with FRET-FLIM sensors at the single spine level (Harvey et al., 2008; Murakoshi et al., 2011) (Fig. 1). The sensors for small GTPase proteins are designed by imaging the interaction between fluorophores tagged to the target small GTPase protein and the interaction domain of effector molecules. For example, the Ras

² Since FRET increases donor fluorescence and decreases accepter fluorescence, the ratio between the donor and acceptor fluorescence can be a measure of the FRET efficiency (Lakowicz, 2006; Yasuda, 2006). One drawback of this method is that the ratio depends on the concentration ratio between the donor and acceptor. Thus, it is usually used only for FRET sensors with a donor and an acceptor in the same polypeptide like the CaMKII sensor. A more robust way to measure FRET is to use fluorescence lifetime. Fluorescence lifetime is the time elapsed between fluorophore excitation and photon emission (Lakowicz, 2006; Yasuda, 2006). When a fluorophore is excited with a short laser pulse, the fluorescence emission decays in an exponential manner. FRET accelerates the decay of the donor in proportion to the FRET efficiency. The FRET efficiency measured by this method is independent of the concentration ratio of fluorophores and wavelength-dependent light scattering.

sensor is made of monomeric enhanced green fluorescent protein (mEGFP)-Ras and Ras binding domain (RBD) of Raf1 fused with monomeric red fluorescent protein (mRFP). When Ras is activated, it binds to RBD and increases FRET between mEGFP and mRFP. Unlike the unimolecular CaMKII sensor, these bimolecular sensors can be imaged only with FLIM.³ FRET sensors in combination with 2pFLIM enabled ones to image activities of Ras, Cdc42 and Rho in single dendritic spines.

Ras

Ras is known to be important for LTP and activity-dependent spinogenesis (Wu et al., 2001; Zhu et al., 2002). Activation of Ras activates the downstream Raf–MEK–ERK pathway (Thomas and Huganir, 2004) (Fig. 20.1). The pathway is required for promoting AMPA receptor exocytosis during induction of LTP and regulating activity-dependent protein synthesis and gene transcription (Thomas and Huganir, 2004). Imaging Ras activity using 2pFLIM demonstrated that Ras activity is increased within about 1 min of glutamate uncaging at a single spine. Its activity then decays over about 5 min to return to basal levels (Harvey et al., 2008). Interestingly, Ras activity is not restricted to the stimulated spine, but diffuses out of the spine and spreads along the dendritic shaft over about 10 μm (Fig. 20.1A).

Rho GTPase proteins

Rho GTPase proteins form a subfamily of small GTPase proteins that are important for actin reorganization. The roles of Rho GTPase proteins, particularly RhoA, Cdc42, and Rac1, have been extensively studied in neuronal morphogenesis (Luo, 2000; Saneyoshi et al., 2010) (Fig. 20.1A). In general, Cdc42 and Rac1 are considered to promote actin polymerization and RhoA to promote actin depolymerization. Postnatal removal from excitatory neurons in the forebrain showed that Cdc42 and Rac1 are important for regulating spine structure, synaptic plasticity, and hippocampus-dependent memory (Haditsch et al., 2009; Kim et al., 2014). 2pFLIM imaging of Cdc42 and RhoA activity showed that activity of these proteins also increased within about 1 min in a CaMKII-dependent manner. This increased activity decayed over about 5 min to a lower level (about 20% of the peak), which remained elevated for more than 30 min (Fig. 20.1B). While Cdc42 activity is restricted to the stimulated spine, RhoA activity spreads along the dendritic shaft over about 5 μm,

Actin reorganization: 1–30 min

Actin is the major component of the cytoskeleton in spines, and thus its regulation is essential for structural plasticity of spines. Also, pharmacological inhibition of actin polymerization is known to inhibit LTP and spine enlargement (Kim and Lisman, 1999; Krucker et al., 2000; Matsuzaki et al., 2004). Regulation of actin occurs downstream of Rho GTPase proteins, which activate many kinases including p21-activated kinase (PAK) and Rho-dependent kinase (ROCK) (Murakoshi et al., 2011; Bosch et al., 2014) (Fig. 20.2). Both PAK and ROCK can activate LIM kinase, which phosphorylates cofilin, a direct regulator of actin (Yang et al., 1998). In addition, Arp2/3, a protein that nucleates actin branching, is also downstream of Rho GTPase proteins, and has been found to play a critical role in structural plasticity of spines, synaptic plasticity, and hippocampus-dependent memory (Kim et al., 2013). The spatio-temporal dynamics of cofilin and actin have been measured using FRET-based methods.

³ Since ratiometric imaging depends on the concentration ratio of donor and acceptor, it is usually used only when both donor and acceptor are in the same polypeptide, as in the CaMKII sensor. Because donor and acceptor for the Ras sensor are separated, it requires the use of FLIM to measure FRET.

Cofilin

The action of cofilin is concentration dependent (Andrianantoandro and Pollard, 2006). When the concentration is low, active cofilin severs actin filaments. When the concentration is high, it decorates actin filaments to form a co-helix with actin and stabilizes the filaments. It has been demonstrated that cofilin is phosphorylated within a few minutes of LTP induction (Chen et al., 2007) and robustly accumulates near the neck of the stimulated spines (Bosch et al., 2014). The action of cofilin in spines has been analyzed using FRET–FLIM sensors measuring cofilin–actin and cofilin–cofilin interactions under 2pFLIM (Bosch et al., 2014). The sensor for cofilin–actin interaction is made of cofilin–mEGFP and mRFP–actin and that for the cofilin–cofilin interaction is made of cofilin–mEGFP and cofilin–mRFP (Fig. 20.1). For both sensors, an increase in the interaction increases FRET. According to this measurement, both cofilin–actin and cofilin–cofilin interactions increase during LTP, suggesting that cofilin–actin filaments are formed in the spine. The structure is stable for at least 30 min and is specific to the stimulated spine (Fig. 20.1), suggesting that this structure is important for the stable maintenance of LTP.

Actin polymerization/depolymerization

Regulation of actin polymerization and depolymerization is critical for the structural plasticity of spines and is required for bidirectional synaptic plasticity (Kim and Lisman, 1999; Krucker et al., 2000; Lang et al., 2004; Matsuzaki et al., 2004; Okamoto and Hayashi, 2006). Actin cycles between the monomeric form (globular actin or G-actin) and the filamentous form (F-actin). Because the equilibration of one end (barbed end) is shifted more toward F-actin than the other end (pointed end), actin monomers in the filament undergo a rapid treadmilling: they bind to the barbed end, move in the filament toward the pointed end, and then unbind from the pointed end, while maintaining the length of the filament in the steady state.

The treadmilling of actin in spines has been measured using fluorescence recovery (FRAP) after photobleaching of GFP-tagged actin (Star et al., 2002). After bleaching of actin–GFP, the fluorescence recovers by the diffusion of unbleached GFP–actin into the spine and its incorporation into the filaments. One can also use photoactivatable GFP (paGFP) instead of GFP and measure fluorescence decay after photoactivation of paGFP in spines (Honkura et al., 2008). These methods have demonstrated that F-actin in spines is organized in at least two pools: one with fast treadmilling (having a time constant of about 1 min) and the other in a more stable structure (time constant about 20 min). The stable component is located at the base of the spine (Honkura et al., 2008). Induction of LTP recruits a new pool of actin in the simulated spines within a few minutes. The component is stable and localized at the base of the stimulated spine. The formation of this pool of actin is essential for spine enlargement. This pool of actin may be stabilized by forming a co-helix with cofilin (see above).

Treadmilling has been measured at the nanometer scale with single-particle tracking photoactivated localization microscopy (sptPALM) to track single actin monomers in dissociated neurons (Tatavarty et al., 2009; Frost et al., 2010). Consistent with the unorganized actin cytoskeleton in spines observed in electron micrographs (Korobova and Svitkina, 2010), the direction of the treadmilling of each filament in a spine is relatively random with a net flow from the tip of the head toward the neck (Tatavarty et al., 2009; Frost et al., 2010). The rate of the treadmilling is highly inhomogeneous in a spine with multiple “hot spots” of high velocity (Frost et al., 2010).

The equilibration between G- and F-actin during LTP and LTD has been monitored using FRET between CFP-actin and YFP-actin (Okamoto and Hayashi, 2006). This approach demonstrated that induction of LTP shifts the equilibrium of actin toward F-actin within a few minutes, indicating rapid polymerization of actin in the stimulated spine. On the other hand, LTD induction shifts

the equilibrium in the opposite direction. The shift in the F-/G-actin ratio lasts more than 30 min, and is associated with the change in the size of the stimulated spines (Fig. 20.2). Thus, the equilibration between F- and G-actin is rapidly regulated during synaptic plasticity in a bidirectional manner during LTP and LTD.

AMPA receptor trafficking: 1 – 10 min

AMPA receptor trafficking in and out of the stimulated synapses is thought to be one of the most critical steps in long-term synaptic plasticity (Malinow and Malenka, 2002). During LTP, the trafficking is thought to be regulated in multiple steps including exocytosis, diffusion, and trapping of AMPA receptors. Different steps of trafficking appear to be regulated by different upstream signals (Fig. 20.2).

Exocytosis

Exocytosis of AMPA receptor-containing vesicles is an essential part of AMPA receptor trafficking during LTP (Lledo et al., 1998; Park et al., 2004; Jurado et al., 2013). In addition to AMPA receptor trafficking, vesicle exocytosis during LTP perhaps plays an important role in providing more membrane to the surface so that the stimulated spine can grow (Park et al., 2006). Since many recycling endosomes exist in dendritic shafts as well as spines, it is a potential target of spreading signals from the spine to the dendritic shaft (Jurado et al., 2013).

AMPA receptor exocytosis has been imaged using the GluA1 AMPA receptor subunit fused with super ecliptic-pHluorin (SEP), a pH-dependent variant of GFP, at its extracellular domain (Kopec et al., 2006; Yudowski et al., 2007; Makino and Malinow, 2009; Patterson et al., 2010). When AMPA receptors are in acidic endosomes SEP is quenched, but when exocytosed to the surface, tagged AMPA receptors become fluorescent (Miesenbock et al., 1998). During LTP, SEP-GluA1 fluorescence increases in the spine, indicating an increase in GluA1-containing receptors on the spine surface (Kopec et al., 2006; Makino and Malinow, 2009; Patterson et al., 2010). By pre-bleaching existing surface SEP-GluA1, individual exocytosis events can be imaged (Yudowski et al., 2007; Makino and Malinow, 2009; Patterson et al., 2010). Exocytosis occurs in the stimulated spine and the surrounding dendritic area within about 5 μm . The increase of exocytosis occurs within a minute of stimulation and decays rapidly in about 5 min (Patterson et al., 2010). Consistent with the spreading pattern of the upstream signaling events, the increase in exocytosis requires the Ras–Raf–MEK–ERK pathway but not CaMKII signaling (Patterson et al., 2010) (Fig. 20.2).

Diffusion and capture of AMPA receptors

Once AMPA receptors have been exocytosed, they diffuse laterally in the surface membrane before being captured by the synapse. The capture process during LTP appears to be caused by interaction between stargazin, an auxiliary subunit of the AMPA receptor, and postsynaptic density protein 95 (PSD-95) in a CaMKII-dependent manner (Opazo et al., 2010; Sumioka et al., 2011). The C-tail of unphosphorylated stargazin binds to the membrane. Phosphorylation of the C-tail of stargazin causes its dissociation from the membrane, allowing the C-tail to interact with PSD-95. Thus, the Ras–Raf–MEK–ERK and CaMKII pathways target to different steps of AMPA receptor trafficking (exocytosis and capture, respectively) (Fig. 20.2).

PSD and presynaptic reorganization: minutes to hours

During the first few minutes of LTP induction, PSD proteins undergo dramatic reorganization. This has been revealed by photoactivation of paGFP-fused PSD proteins. Upon stimulation of a target spine, the dynamics of molecular diffusion in the spine are measured (Steiner et al., 2008).

According to this measurement, PSD-95 and Shank, the major PSD components, dissociate from the PSD after induction of LTP and are then replaced within a few minutes by non-photoactivated proteins. The dissociation of the PSD proteins is found to be dependent on CaMKII. For PSD-95, the CaMKII-dependent phosphorylation site critical for the recycling is identified and found to be important for inducing LTP. After this protein recycling, the net content of these PSD proteins is unchanged for the first 30–60 min of LTP induction. SynGAP, a major PSD protein and a Ras inactivator, also rapidly dissociates from the spine (Araki et al., 2015). Unlike PSD95 and Shank, the decrease in SynGAP lasts for more than 20–30 min, contributing to activation of Ras (Fig. 20.2). Thus, PSD shows a rapid reorganization in the first 2–3 min of LTP induction.

These results are apparently inconsistent with ultrastructural analyses showing that the PSD size is proportional to the size of the spine in the steady state (Harris and Stevens, 1989). This mystery has been recently solved by monitoring GFP-tagged PSD proteins for more than several hours (Bosch et al., 2014; Meyer et al., 2014). These studies have revealed that PSD proteins slowly accumulate in potentiated spines over a few hours following LTP induction. Electron micrographs of stimulated spines also show that the growth of PSD is much slower than spine enlargement (Fig. 20.1B). In association with the PSD change, presynaptic boutons also grow over several hours (Meyer et al., 2014). Considering the time scale of the PSD growth (hours) compared with the rapid process of LTP induction (within about 10 min), PSD growth and presynaptic growth appear to be not necessary for LTP induction. The maintenance of LTP over many hours (late-phase LTP), however, may require these structural changes (Fig. 20.2).

Synapse reorganization: days

It is established that long-term plasticity can last many days, like our own long-term memories (Abraham, 2003). However, studying synaptic plasticity and spine structural plasticity at the single-spine level for such a long time has been complicated. This limitation has been overcome by using organotypic hippocampal slices, which can be maintained over many days, in combination with optogenetics. By expressing channelrhodopsin 2 (ChR2) in CA3 neurons and the calcium sensor GCaMP in CA1 pyramidal neurons, synaptic transmission in single synapses can be monitored for weeks (Wiegert and Oertner, 2013). When ChR2 is stimulated with blue light, Ca^{2+} elevation occurs in spines in CA1 neurons. By stimulating CA3 neurons with low-frequency light pulses, LTD is induced in these synapses. Both the probability of presynaptic release and the glutamate sensitivity of postsynaptic neurons appear to be depressed in this paradigm. Interestingly, after a few days significant numbers of stimulated and non-stimulated spines are pruned from the stimulated dendrites. The probability of the pruning is not correlated with the degree of LTD but with the initial release probability. While the exact mechanism is unknown, the stimulated neuron changes its mode to modify synaptic strength from regulation of the potency of each synapse to regulation of the number of synapses (Wiegert and Oertner, 2013) (Fig. 20.2). It is unknown if LTP also undergoes similar “analog” to “digital” conversion in its expression mechanisms over days.

Spatial scale of signaling and plasticity

Intracellular signaling that is required for synaptic plasticity is regulated at various spatial scales (Fig. 20.3). While synaptic plasticity is induced in a synapse-specific manner, spreading of biochemical signals is necessary to mediate phenomena such as heterosynaptic plasticity and metaplasticity, which occur in the surrounding dendrite. Moreover, long-term maintenance of synaptic plasticity relies on gene expression. Therefore, biochemical signals must spread to the nucleus to affect nuclear transcription.

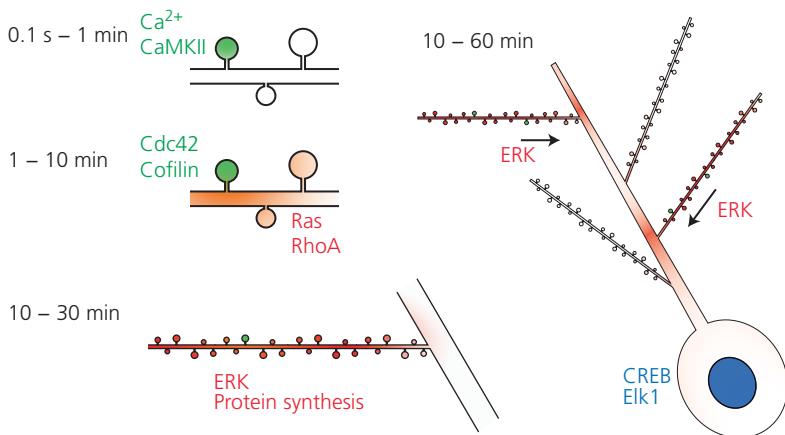


Fig. 20.3 Schematics of signaling dynamics at different spatial scales at different times. Ca^{2+} signaling starts from single spines and spreads over time, while some signals are restricted to the stimulated spines. Signals at different spatial scales are illustrated in different colors. Green, spine-specific signaling; orange, signaling spreads over a short stretch of dendritic segment ($\sim 5\text{--}10 \mu\text{m}$); red, signaling spreads over a whole dendritic branch and more; blue, nuclear signaling.

Spine-specific signaling: $0 \mu\text{m}$

LTP and spine enlargement occur in a spine-specific manner. When a single spine is stimulated with two-photon glutamate uncaging, increases in spine volume and glutamate sensitivity only occur in the stimulated spines, not in adjacent spines (Matsuzaki et al., 2004; Lee et al., 2009). Thus, some signaling events must be restricted to the stimulated spine to maintain the spine specificity of plasticity. Spine-specific signals include Ca^{2+} at the 10–100 ms window, CaMKII at the 10–60 s window (Lee et al., 2009), and Cdc42 and cofilin activation at the 10–30 min window (Murakoshi et al., 2011; Bosch et al., 2014). Thus, the Ca^{2+} –CaMKII–Cdc42–cofilin pathway covers a large temporal range for spine-specific signaling (Figs 20.1 and 20.3). LTD and associated spine shrinkage can also be induced in a spine-specific manner when a spine is stimulated weakly with two-photon glutamate uncaging. Calcineurin activity during LTD is likely to be spine specific (Fujii et al., 2013).

Signaling in a short stretch of dendritic segment: $5\text{--}10 \mu\text{m}$

In addition to spine-specific signaling, there are signals that spread along a short stretch of dendritic segment involving several synapses. As we discussed above, Ras, RhoA, and calcineurin spread over $5\text{--}10 \mu\text{m}$ in response to stimulation of a single spine (Harvey et al., 2008; Murakoshi et al., 2011) (Fig. 20.3). These spreading signals are likely to be critical for communicating with signaling compartments located in the dendritic shafts, including recycling endosomes containing AMPA receptors and protein synthesis machinery (Park et al., 2004, 2014). Downstream of Ras signaling, exocytosis of AMPA receptors appears to occur both in the stimulated spine and adjacent dendritic regions (Patterson et al., 2010). Spreading signals must also play a role in influencing synaptic plasticity in surrounding spines (Harvey and Svoboda, 2007). These phenomena may contribute to clustered plasticity, in which functional and structural plasticity of adjacent spines

tend to be potentiated together to amplify information from a specific dendritic branch (Makino and Malinow, 2011; Fu et al., 2012).

Facilitation of LTP and spinogenesis

Spreading of signaling after LTP causes facilitation of LTP in surrounding spines. It was found that induction of LTP at a single spine with glutamate uncaging lowers the threshold for induction of LTP at surrounding spines (Harvey and Svoboda, 2007). The reduction in the threshold for LTP induction lasted about 5 min and spread over about 10 μm of dendrite. This spatio-temporal pattern is similar to that of Ras activation (Harvey et al., 2008). When the MEK–ERK pathway, which is located downstream of Ras, is inhibited after the first LTP-inducing stimulation and before the second subthreshold stimulation, this facilitation was lost, suggesting that spreading of Ras–MEK–ERK signaling is required for this metaplasticity (Harvey et al., 2008). In addition, it was found that induction of LTP in a single spine increases the probability of activity-dependent spinogenesis in the surrounding area of the dendrite (Kwon and Sabatini, 2011). This facilitation is also caused by the spread of MEK–ERK signaling.

Heterosynaptic spine shrinkage

Induction of LTP in multiple spines on a dendritic segment can cause spine shrinkage and synaptic weakening of nearby unstimulated spines located within a few micrometers (Oh et al., 2015). This heterosynaptic spine shrinkage does not depend on induction of the first LTP, since inhibition of LTP by CaMKII does not inhibit heterosynaptic LTD. Like homosynaptic LTD, it depends on calcineurin activation and mGluR activation (Oh et al., 2015). Since LTP-inducing stimulation can cause spreading of calcineurin activity (Fujii et al., 2013), the spreading of calcineurin activity may be involved in the process of the heterosynaptic spine shrinkage (Fig. 20.3).

Heterosynaptic LTD and spine shrinkage can also be induced by stimulating a single spine with a specific sequence: pairing two-photon glutamate uncaging at a single spine with backpropagating action potentials and GABA uncaging near the spine (Hayama et al., 2013). LTD induced in this protocol shows spine shrinkage in the stimulated spines as well as in surrounding spines.

Branch-level signaling (10 – 100 μm)

Some signals spread beyond the local dendritic segment around the site of stimulation and can affect an entire dendritic branch. For example, membrane excitability is regulated in a branch-specific manner (Losonczy et al., 2008).

Since protein synthesis machinery is localized in dendritic shafts, it must be a target of signals that spread into dendritic shafts (Park et al., 2014). It has been known that protein synthesis-dependent LTP can be induced with repeated high-frequency stimuli (Frey and Morris, 1997). Since this lasts more than several hours, it often referred to as late-phase LTP or L-LTP. Protein synthesis-independent LTP is called early phase LTP or E-LTP. It has been found that L-LTP can be induced in a single spine by pairing two-photon glutamate uncaging with backpropagating action potentials (Tanaka et al., 2008). This form of LTP depends on brain-derived neurotrophic factor (BDNF), likely to be released from the postsynaptic neuron. L-LTP can be also induced by pairing glutamate uncaging with extracellular application of BDNF or intracellular elevation of cyclic AMP (Tanaka et al., 2008; Govindarajan et al., 2011).

Like E-LTP, L-LTP displays crosstalk between adjacent spines (Govindarajan et al., 2011). When L-LTP is induced in a single spine and a different spine in the same branch is stimulated with the E-LTP induction protocol, the second spine can undergo L-LTP. This may be because the proteins

synthesized during LTP are shared by multiple spines within the branch. This effect extends to several tens of micrometers in space and tens of minutes in time and shows branch specificity (Fig. 20.3).

Signaling from spines to the nucleus: > 100 μm

Signaling from the synapse to the nucleus is important for activity-dependent gene transcription and L-LTP lasting more than several hours (Nguyen et al., 1994). ERK is important for E-LTP but also has been shown to regulate many transcription factors important for L-LTP, including CREB and Elk (Thomas and Huganir, 2004; Adams and Dudek, 2005). Thus, ERK has been thought to be a molecule that links synaptic stimulation with the gene transcription required for L-LTP. Using controlled synaptic stimulation with two-photon glutamate uncaging and FRET imaging of nuclear ERK signaling, the spatio-temporal integration of signaling from multiple dendritic spines into the nucleus has been explored (Zhai et al., 2013). It has been revealed that structural plasticity of only a few (three to seven) spines is sufficient to activate nuclear ERK and downstream CREB and Elk. Signal transmission from the stimulated spine to the nucleus is mediated, at least partially, by translocation of ERK from the cytosol into the nucleus (Zhai et al., 2013). Notably, synapse-to-nucleus signaling requires integration of signaling from multiple dendrites (Fig. 20.3). When a cluster of spines in a single dendritic branch are activated, nuclear ERK is not activated. However, if the input is distributed over multiple dendritic branches, it is activated. Thus, neurons have the ability to integrate signaling from multiple dendritic branches.

The integration window of the synapse-to-nucleus signaling was measured by stimulating a pair of dendritic branches with different temporal and spatial spacings (Fig. 20.3) (Zhai et al., 2013). First, two spines are stimulated in one dendritic branch. This does not induce nuclear signaling. When another pair of spines in a different dendritic branch is stimulated about 30 min after the first stimulation, the nuclear signal increases. Thus, the neuron can integrate different inputs in different branches over about 30 min. The nuclear signal is larger when the two branches are spatially separated by more than about 30 μm . So, overall, the system is designed to be activated more efficiently by distributed pattern of inputs.

The mechanism by which signal propagation is enhanced by inducing plasticity in a distributed pattern is through supralinear properties of ERK signaling in a dendritic branch. Stimulation of a single spine in a branch does not cause detectable spread of ERK activity from that branch into its parent primary dendrite. However, stimulation of two spines in the dendritic branch, induces maximal propagation of ERK activity into the parent dendrite. Thus, stimulating more than two spines does not provide additional signaling from the dendrite to the primary dendrite. This therefore provides an example in which biochemical computation in a single branch affects the integration of multiple dendritic branches.

In addition to signal spreading by diffusion, active transport also plays an important role in synapse-to-nucleus signaling. For example, it has been found that a macromolecular assembly consisting of Jacob, Importin, and ERK binds to motor molecules and is transported to the nucleus in response to synaptic activation (Karpova et al., 2013). Jacob needs to be phosphorylated by ERK in order to exit synapses and be transported to the nucleus, linking ERK activation in synapses and that in the nucleus.

The mechanism of signal spreading

Diffusion of signaling molecules plays an essential role in the spatio-temporal regulation of signaling. Diffusion is extremely efficient at micrometer scales, and thus most events occurring on this scale (e.g., in a short stretch of dendrite) involve diffusion (Yasuda and Murakoshi, 2011). Even for

long-distance signals over several tens of micrometers, some events like ERK spreading may be explained by simple diffusion of the molecule (Zhai et al., 2013).

Diffusion of signaling molecules

One-dimensional diffusion of a free molecule is given by:

$$\langle x^2 \rangle = 2Dt \quad (20.1)$$

where t is the time, x is the diffusion distance, the bracket is the mean, and D is the diffusion coefficient. For example, the diffusion coefficient of free Ca^{2+} ions is about $200 \mu\text{m}^2/\text{s}$ and thus they can diffuse about $20 \mu\text{m}$ along a dendrite in a second, on average. Most Ca^{2+} binds to Ca^{2+} -binding proteins and thus effective diffusion is much slower (Neher and Augustine, 1992). For cytosolic proteins like GFP, the value is about $20 \mu\text{m}^2/\text{s}$ (Chen et al., 2002) and membrane-associated proteins like Ras diffuse more slowly, with diffusion coefficients of $0.5\text{--}1 \mu\text{m}^2/\text{s}$ (Lommerse et al., 2004; Murakoshi et al., 2004). Since the time required for spreading a given distance is proportional to the square of the distance, diffusion becomes much less efficient for longer distances. D is proportional to the diameter of the molecule or the cubic root of the mass, and thus is only weakly correlated with the size of the molecule.

ERK signaling from synapses to the nucleus occurs on a slow time scale, and thus diffusion can still play an important role (Wiegert et al., 2007). When distal spines more than about $200 \mu\text{m}$ are stimulated, the onset of the signal is delayed by around 40 min (Zhai et al., 2013). The diffusion coefficient of ERK has been measured to be $8 \mu\text{m}^2/\text{s}$ in neurons (Lidke et al., 2010), predicting that ERK can diffuse about $200 \mu\text{m}$ in 40 min (Equation 20.1). Thus, diffusion of ERK is consistent with the measured onset of ERK activation in the nucleus.

The morphology of spines plays an important role in restricting diffusion. The average time for a molecule to diffuse out of a spine (the spine neck coupling time constant), τ , can be calculated as:

$$\tau = DV / Ls \quad (20.2)$$

where V is the volume of the spine, L is the length of the spine neck, and s is the area of the cross section of the spine (Svoboda et al., 1996). For membrane proteins, V is the surface area and s is the contour length of the spine neck. For the typical volume of a spine (0.1 fL), neck length ($\sim 0.8 \mu\text{m}$), and neck diameter ($\sim 0.1 \mu\text{m}$), values of τ are predicted to be about 0.3 s for a cytosolic protein and about 5 s for a membrane-targeted protein. Thus, even with the morphological restrictions of a spine, diffusion of signaling molecules is still efficient. The value of τ can be measured using FRAP (Svoboda et al., 1996). In this approach a fluorophore (like GFP) tagged to a protein in a spine is bleached with a strong laser. The time for non-bleached GFP-tagged protein to enter the spine and recover the fluorescence is measured. One can also use photoactivation of photoactivatable proteins to make similar measurements (Fig. 20.4). These experiments have measured values that are consistent with the calculated values for GFP and GFP-fused GTPase proteins: about 0.3 s for GFP and $3\text{--}5 \text{ s}$ for small GTPase proteins Ras, Rac1, CDC42, and RhoA (Bloodgood and Sabatini, 2005; Harvey et al., 2008; Murakoshi et al., 2011) (Fig. 20.4).

The diffusion of Ca^{2+} can also be determined following glutamate uncaging at the spine by measuring the time required for equilibration of the Ca^{2+} concentration between a spine and its parent dendrite (Noguchi et al., 2005). Also, since trial-to-trial variation of Ca^{2+} elevation in response to backpropagating action potentials is larger in spines than in dendrites, time-resolved fluctuation analysis can provide the time for equilibration between the spines and the shafts (Sabatini et al., 2002). These methods provide consistent values: the spine neck coupling time constant (τ) is about

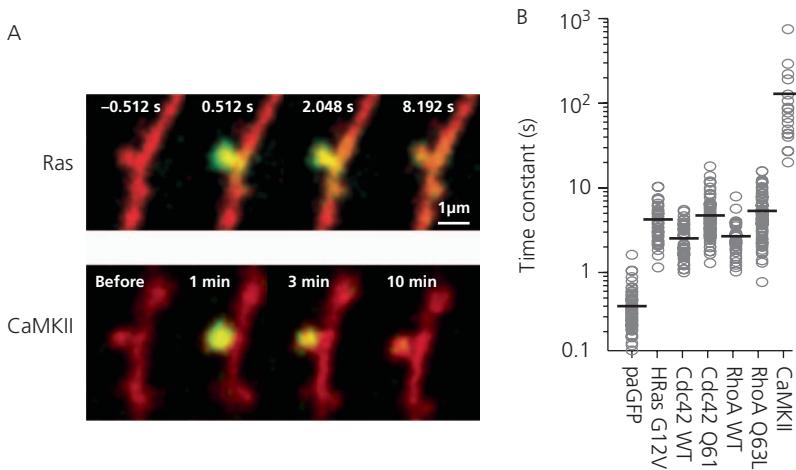


Fig. 20.4 Diffusion of signaling molecules in and out of dendritic spines. pa-GFP fused to a protein of interest is photoactivated using two-photon excitation and its diffusion was followed by the decay of pa-GFP fluorescence. (A) Representative images of photoactivation experiments for Ras and CaMKII. (B) Spine neck diffusion coupling time constant of various molecules. HRas G12V, Cdc42 Q61L, and RhoA Q63L are constitutively active mutants of HRas, Cdc42, and RhoA, respectively.

Part A reproduced from Seok-Jin R. Lee and Ryohei Yasuda, Spatiotemporal regulation of signaling in and out of dendritic spines: CaMKII and Ras, *The Open Neuroscience Journal*, 3, pp. 117–127, Figure 2a, © 2009, The Authors. Part B: data from *Nature*, 458(7236), Seok-Jin R. Lee, Yasmin Escobedo-Lozoya, Erzsebet M. Szatmari, and Ryohei Yasuda, Activation of CaMKII in single dendritic spines during long-term potentiation, pp. 299–304, 2009 and *Nature*, 472(7341) Hideji Murakoshi, Hong Wang, and Ryohei Yasuda, Local, persistent activation of Rho GTPases during plasticity of single dendritic spines, pp. 100–104, 2011.

100 ms, which corresponds to $D \sim 60 \mu\text{m}^2/\text{s}$ (Eq. 20.2). Because a large fraction of Ca^{2+} binds to immobile buffer, this value is likely to be underestimated and effective diffusion should be much slower (Sabatini et al., 2002). Still, this constant is larger than the time constant of Ca^{2+} extrusion (about 20 ms; Sabatini et al., 2002), consistent with experimental imaging results showing that Ca^{2+} is restricted to the stimulated spines (Fig. 20.5).

In many cases, experimental measures of diffusion or spine neck coupling are much slower than those expected for free diffusion. This is probably due to molecular interactions to subspine structures such as the actin cytoskeleton or PSD. For example, CaMKII is known to interact with many molecules, including Ca^{2+} channels, NMDA receptors, and the actin cytoskeleton. Indeed, the spine neck coupling time constant for CaMKII was measured to consist of two time constants, of about 1 and 20 min, much slower than that for free proteins (Okamoto and Hayashi, 2006; Lee et al., 2009). These values corresponding to effective diffusion of about $0.1 \mu\text{m}^2/\text{s}$ (the fast component) and $0.01 \mu\text{m}^2/\text{s}$ (the slow component) (Fig. 20.4). This value contrasts with CaMKII diffusion in solution, which is much faster ($\sim 25 \mu\text{m}^2/\text{s}$) (Kim et al., 2005).

In the case of actin filaments, FRAP provides the time for actin turnover by treadmilling, and again is about 1 and 20 min, representing dynamic and stable components of the actin cytoskeleton (see earlier for a discussion of treadmilling) (Star et al., 2002; Honkura et al., 2008). For PSD proteins such as PSD-95, the value is much longer: it can be hours (Gray et al., 2006; Sharma et al., 2006; Steiner et al., 2008).

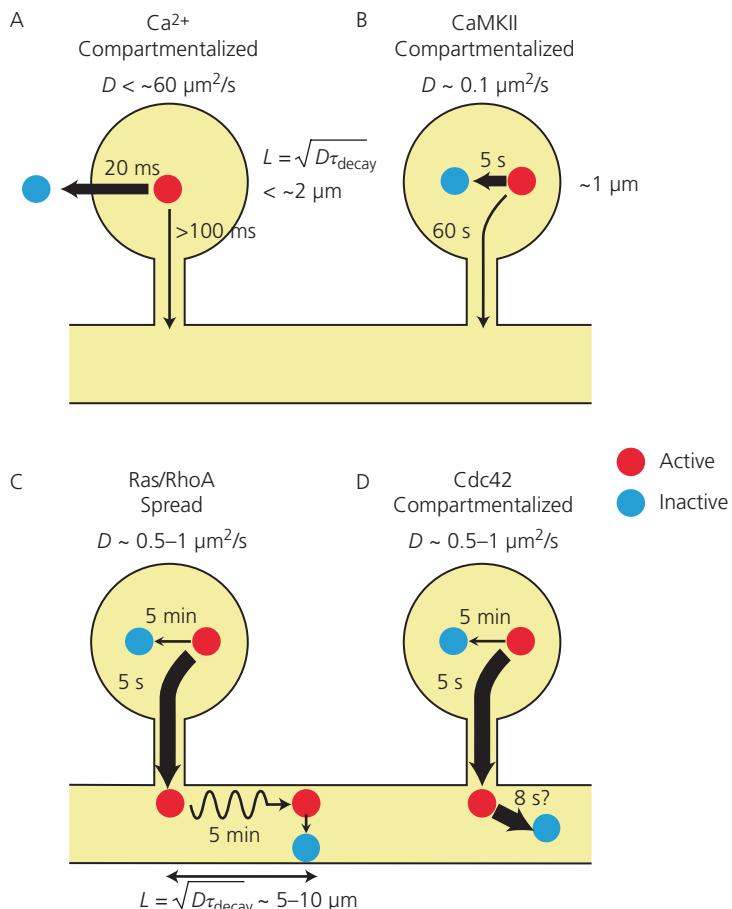


Fig. 20.5 The degree of signal compartmentalization for different signaling molecules. The balance between inactivation and diffusion determines the degree of signal compartmentalization. Red and blue circles represent active and inactive molecules, respectively. For Ca^{2+} , intracellular and extracellular Ca^{2+} is drawn in red and blue, respectively. Thicker arrows indicate faster events. The mean distance that molecules diffuse before they are inactivated is calculated as $L = \sqrt{D\tau_{\text{decay}}}$,

where D is the diffusion coefficient and τ_{decay} is the rate of inactivation. (A) Ca^{2+} extrusion (thick arrow) is fast ($\sim 20 \text{ ms}$) compared with the diffusion of Ca^{2+} through the spine neck ($\sim 100 \text{ ms}$; thin arrow), producing elevated Ca^{2+} restricted within the stimulated spine (Sabatini et al., 2001; Noguchi et al., 2005). (B) CaMKII diffuses out of a spine slowly ($\sim 1 \text{ min}$ for the fast component; thin arrow) compared with its inactivation time constant ($\sim 5 \text{ s}$; thick arrow), compartmentalizing CaMKII activity within the stimulated spines (Lee et al., 2009). (C) Ras and RhoA diffuse out of a spine rapidly ($\sim 5 \text{ s}$; thick arrow) and their activities decay slowly ($\sim 5 \text{ min}$; thin line). These molecules diffuse over about $5\text{--}10 \mu\text{m}$ (wavy arrow, diffusion) before they are inactivated (turn to blue), producing activity patterns spreading over a short stretch of dendritic segment (Harvey et al., 2008; Murakoshi et al., 2011). (D) Cdc42 diffuses out of a spine rapidly ($\sim 5 \text{ s}$) and is inactivated slowly ($\sim 5 \text{ min}$), similarly to Ras and RhoA . However, it shows an activity pattern restricted to the stimulated spines (Murakoshi et al., 2011). This may be due to fast inactivation of the molecule in the dendritic shaft (Yasuda and Murakoshi, 2011).

Competition between diffusion and inactivation determines the spatial pattern of molecular activity

Another important factor that controls the spreading of active signals is the rate of inactivation of the signaling molecule or extrusion for Ca^{2+} (Fig. 20.5). The distance over which an active molecule can diffuse before it is inactivated is determined by the competition between diffusion and inactivation (Fig. 20.5). The mean distance L can be determined as:

$$L = \sqrt{D\tau_{decay}} \quad (20.3)$$

in which τ_{decay} is the time constant of inactivation of signaling molecule. For Ca^{2+} this value can be calculated to be about 2 μm ($D \sim 200 \mu\text{m}^2/\text{s}$ and, $\tau_{decay} \sim 20 \text{ ms}$ as already discussed). It should be noted again that the effective diffusion should be much slower, and thus the length constant can be much smaller. Thus, it is expected that Ca^{2+} is mostly compartmentalized in spines, and experimental results are consistent with this hypothesis (Sabatini et al., 2002; Noguchi et al., 2005) (Fig. 20.1 and 20.5). Similarly, due to slow diffusion ($\sim 0.1 \mu\text{m}^2/\text{s}$ for a fast component) and fast decay ($\sim 5 \text{ s}$), L for CaMKII is about 1 μm (Fig. 20.5) (Lee and Yasuda, 2009). This value is consistent with the spine-specific compartmentalization observed during plasticity (Fig. 20.1) (Lee et al., 2009).

Compared with Ca^{2+} and CaMKII, the activity of small GTPase proteins decays more slowly over about 5 min (Harvey et al., 2008; Murakoshi et al., 2011). With diffusion constants of around 0.5–1 $\mu\text{m}^2/\text{s}$, the length constant for these proteins is calculated to be 5–10 μm , consistent with the measured length constants of RhoA and Ras activity but not with Cdc42 (Fig. 20.1 and 20.5). Cdc42 diffuses as fast as RhoA and Ras (Fig. 20.4) but it is compartmentalized within a single spine. One possible mechanism is that inactivation of Cdc42 is much faster in the dendritic shaft than in the spine, decreasing the effective length constant in the dendrite. In a mathematical simulation, a decay constant of about 8 s provided a spatial restriction consistent with the observed pattern (Fig. 20.5) (Yasuda and Murakoshi, 2011).

Regulation of signal decay can thus have a large effect on the signal spread, as seen from Equation 20.3. One example of particular interest is the regulation of Ras activity by neurofibromin (NF1). Loss of function of *NF1* is the known genetic cause for neurofibromatosis type 1, a disorder often associated with learning disabilities. The decay kinetics of Ras activation during spine plasticity in neurons in which NF1 is knocked down with shRNA has been recently measured (Oliveira and Yasuda, 2014). In neurons with low NF1, glutamate uncaging induces normal levels of Ras activation but almost no decay after the activation. Under this condition, Ras signaling spreads much further along dendrites, consistent with the above theory.

Overall, a relatively simple model can predict how signals spread from stimulated spines to surrounding areas. More detailed analyses, by simulating diffusion of individual molecules, may provide more information about the mechanisms underlying the spatio-temporal organization of signal transduction in dendrites (Kerr et al., 2008).

Conclusions

The biochemical computations in dendrites are regulated in a complicated manner by diffusion, molecular interactions, and their activation kinetics in multiple layers of compartments as well as across multiple time scales. Some signals are compartmentalized locally in dendritic spines and are likely to play important roles in synapse-specific information storage. Other signals are more widespread and can affect the other synapses as well as the nucleus. Spreading of signals has an important role in synaptic crosstalk, heterosynaptic plasticity, and communication between different subcellular compartments. Signals from the spine to the nearby dendrite and to the nucleus are required for orchestrating

different signals in different compartments across spatio-temporal scales. The length scale and the temporal scale of signaling are tightly coupled, and the regulation of the diffusion and inactivation of molecules plays an important role in regulating the spatial scale of signal transduction.

Recent technological advances in imaging molecular activity in neurons have provided a lot of information about the spatio-temporal regulation of signaling cascades underlying synaptic plasticity. By monitoring only a few molecules, the mechanisms of signal organization on multiple subcellular compartments have become much clearer. Since there are hundreds of molecules involved in synaptic plasticity, imaging the spatio-temporal activity profiles of many more signaling molecules is necessary to comprehend the operational principles of signaling networks in dendrites. Automation of the laborious development of protein sensors would perhaps speed up this process. Optogenetic signal control will become a powerful tool for further investigating the causal relationships among the spatio-temporal signal dynamics of different proteins.

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References

- Abraham WC (2003) How long will long-term potentiation last? *Philosophical Transactions of the Royal Society B: Biological Sciences* **358**:735–744.
- Adams JP, Dudek SM (2005) Late-phase long-term potentiation: getting to the nucleus. *Nature Reviews Neuroscience* **6**:737–743.
- Andrianantoandro E, Pollard TD (2006) Mechanism of actin filament turnover by severing and nucleation at different concentrations of ADF/cofilin. *Molecular Cell* **24**:13–23.
- Araki Y, Zeng M, Zhang M, Huganir RL (2015) Rapid dispersion of SynGAP from synaptic spines triggers AMPA receptor insertion and spine enlargement during LTP. *Neuron* **85**:173–189.
- Babiec WE, Guglietta R, Jami SA, Morishita W, Malenka RC, O'Dell TJ (2014) Ionotropic NMDA receptor signaling is required for the induction of long-term depression in the mouse hippocampal CA1 region. *Journal of Neuroscience* **34**:5285–5290.
- Barria A, Muller D, Derkach V, Griffith LC, Soderling TR (1997) Regulatory phosphorylation of AMPA-type glutamate receptors by CaMKII during long-term potentiation. *Science* **276**:2042–2045.
- Bloodgood BL, Sabatini BL (2005) Neuronal activity regulates diffusion across the neck of dendritic spines. *Science* **310**:866–869.
- Bosch M, Castro J, Saneyoshi T, Matsuno H, Sur M, Hayashi Y (2014) Structural and molecular remodeling of dendritic spine substructures during long-term potentiation. *Neuron* **82**:444–459.
- Bromberg KD, Ma'ayan A, Neves SR, Iyengar R (2008) Design logic of a cannabinoid receptor signaling network that triggers neurite outgrowth. *Science* **320**:903–909.
- Chen Y, Muller JD, Ruan Q, Gratton E (2002) Molecular brightness characterization of EGFP in vivo by fluorescence fluctuation spectroscopy. *Biophysical Journal* **82**:133–144.
- Chen LY, Rex CS, Casale MS, Gall CM, Lynch G (2007) Changes in synaptic morphology accompany actin signaling during LTP. *Journal of Neuroscience* **27**:5363–5372.
- Colgan LA, Yasuda R (2014) Plasticity of dendritic spines: subcompartmentalization of signaling. *Annual Review of Physiology* **76**:365–385.
- Emptage N, Bliss TV, Fine A (1999) Single synaptic events evoke NMDA receptor-mediated release of calcium from internal stores in hippocampal dendritic spines. *Neuron* **22**:115–124.
- Frey U, Morris RG (1997) Synaptic tagging and long-term potentiation. *Nature* **385**:533–536.
- Frost NA, Shroff H, Kong H, Betzig E, Blanpied TA (2010) Single-molecule discrimination of discrete perisynaptic and distributed sites of actin filament assembly within dendritic spines. *Neuron* **67**:86–99.

- Fu M, Yu X, Lu J, Zuo Y (2012) Repetitive motor learning induces coordinated formation of clustered dendritic spines in vivo. *Nature* **483**:92–95.
- Fujii H, Inoue M, Okuno H, Sano Y, Takemoto-Kimura S, Kitamura K, Kano M, Bito H (2013) Nonlinear decoding and asymmetric representation of neuronal input information by CaMKIIalpha and calcineurin. *Cell Reports* **3**:978–987.
- Giese KP, Fedorov NB, Filipkowski RK, Silva AJ (1998) Autophosphorylation at Thr286 of the alpha calcium-calmodulin kinase II in LTP and learning. *Science* **279**:870–873.
- Govindarajan A, Israely I, Huang SY, Tonegawa S (2011) The dendritic branch is the preferred integrative unit for protein synthesis-dependent LTP. *Neuron* **69**:132–146.
- Gray NW, Weimer RM, Bureau I, Svoboda K (2006) Rapid redistribution of synaptic PSD-95 in the neocortex in vivo. *PLoS Biology* **4**:e370.
- Grunditz A, Holbro N, Tian L, Zuo Y, Oertner TG (2008) Spine neck plasticity controls postsynaptic calcium signals through electrical compartmentalization. *Journal of Neuroscience* **28**:13457–13466.
- Haditsch U, Leone DP, Farinelli M, Chrostek-Grashoff A, Brakebusch C, Mansuy IM, McConnell SK, Palmer TD (2009) A central role for the small GTPase Rac1 in hippocampal plasticity and spatial learning and memory. *Molecular and Cellular Neurosciences* **41**:409–419.
- Harris KM, Stevens JK (1989) Dendritic spines of CA 1 pyramidal cells in the rat hippocampus: serial electron microscopy with reference to their biophysical characteristics. *Journal of Neuroscience* **9**:2982–2997.
- Harvey CD, Svoboda K (2007) Locally dynamic synaptic learning rules in pyramidal neuron dendrites. *Nature* **450**:1195–1200.
- Harvey CD, Yasuda R, Zhong H, Svoboda K (2008) The spread of Ras activity triggered by activation of a single dendritic spine. *Science* **321**:136–140.
- Hayama T, Noguchi J, Watanabe S, Takahashi N, Hayashi-Takagi A, Ellis-Davies GC, Matsuzaki M, Kasai H (2013) GABA promotes the competitive selection of dendritic spines by controlling local Ca^{2+} signaling. *Nature Neuroscience* **16**:1409–1416.
- Honkura N, Matsuzaki M, Noguchi J, Ellis-Davies GC, Kasai H (2008) The subspine organization of actin fibers regulates the structure and plasticity of dendritic spines. *Neuron* **57**:719–729.
- Jurado S, Goswami D, Zhang Y, Molina AJ, Sudhof TC, Malenka RC (2013) LTP requires a unique postsynaptic SNARE fusion machinery. *Neuron* **77**:542–558.
- Karpova A, et al. (2013) Encoding and transducing the synaptic or extrasynaptic origin of NMDA receptor signals to the nucleus. *Cell* **152**:1119–1133.
- Kerr RA, Bartol TM, Kaminsky B, Dittrich M, Chang JC, Baden SB, Sejnowski TJ, Stiles JR (2008) Fast Monte Carlo simulation methods for biological reaction–diffusion systems in solution and on surfaces. *SIAM Journal on Scientific Computing* **30**:3126.
- Kim CH, Lisman JE (1999) A role of actin filament in synaptic transmission and long-term potentiation. *Journal of Neuroscience* **19**:4314–4324.
- Kim SA, Heinze KG, Bacia K, Waxham MN, Schwille P (2005) Two-photon cross-correlation analysis of intracellular reactions with variable stoichiometry. *Biophysical Journal* **88**:4319–4336.
- Kim IH, Racz B, Wang H, Burianek L, Weinberg R, Yasuda R, Wetsel WC, Soderling SH (2013) Disruption of Arp2/3 results in asymmetric structural plasticity of dendritic spines and progressive synaptic and behavioral abnormalities. *Journal of Neuroscience* **33**:6081–6092.
- Kim IH, Wang H, Soderling SH, Yasuda R (2014) Loss of Cdc42 leads to defects in synaptic plasticity and remote memory recall. *eLife* **3**:10.7554/eLife.02839.
- Kopec CD, Li B, Wei W, Boehm J, Malinow R (2006) Glutamate receptor exocytosis and spine enlargement during chemically induced long-term potentiation. *Journal of Neuroscience* **26**:2000–2009.
- Korobova F, Svitkina T (2010) Molecular architecture of synaptic actin cytoskeleton in hippocampal neurons reveals a mechanism of dendritic spine morphogenesis. *Molecular Biology of the Cell* **21**:165–176.

- Kovalchuk Y, Eilers J, Lisman J, Konnerth A (2000) NMDA receptor-mediated subthreshold Ca(2+) signals in spines of hippocampal neurons. *Journal of Neuroscience* **20**:1791–1799.
- Krucker T, Siggins GR, Halpain S (2000) Dynamic actin filaments are required for stable long-term potentiation (LTP) in area CA1 of the hippocampus. *Proceedings of the National Academy of Sciences of the United States of America* **97**:6856–6861.
- Kwon HB, Sabatini BL (2011) Glutamate induces de novo growth of functional spines in developing cortex. *Nature* **474**:100–104.
- Lakowicz JR (2006) *Principles of Fluorescence Spectroscopy*. New York: Plenum.
- Lang C, Barco A, Zablow L, Kandel ER, Siegelbaum SA, Zakharenko SS (2004) Transient expansion of synaptically connected dendritic spines upon induction of hippocampal long-term potentiation. *Proceedings of the National Academy of Sciences of the United States of America* **101**:16665–16670.
- Lee SJ, Yasuda R (2009) Spatiotemporal regulation of signaling in and out of dendritic spines: CaMKII and Ras. *Open Neuroscience Journal* **3**:117–127.
- Lee SJ, Escobedo-Lozoya Y, Szatmari EM, Yasuda R (2009) Activation of CaMKII in single dendritic spines during long-term potentiation. *Nature* **458**:299–304.
- Lidke DS, Huang F, Post JN, Rieger B, Wilsbacher J, Thomas JL, Pouyssegur J, Jovin TM, Lenormand P (2010) ERK nuclear translocation is dimerization-independent but controlled by the rate of phosphorylation. *Journal of Biological Chemistry* **285**:3092–3102.
- Lisman J (1989) A mechanism for the Hebb and the anti-Hebb processes underlying learning and memory. *Proceedings of the National Academy of Sciences of the United States of America* **86**:9574–9578.
- Lisman J, Yasuda R, Raghavachari S (2012) Mechanisms of CaMKII action in long-term potentiation. *Nature Reviews Neuroscience* **13**:169–182.
- Lledo PM, Zhang X, Sudhof TC, Malenka RC, Nicoll RA (1998) Postsynaptic membrane fusion and long-term potentiation. *Science* **279**:399–403.
- Lommerse PH, Blab GA, Cognet L, Harms GS, Snar-Jagalska BE, Spaink HP, Schmidt T (2004) Single-molecule imaging of the H-ras membrane-anchor reveals domains in the cytoplasmic leaflet of the cell membrane. *Biophysical Journal* **86**:609–616.
- Losonczy A, Makara JK, Magee JC (2008) Compartmentalized dendritic plasticity and input feature storage in neurons. *Nature* **452**:436–441.
- Luo L (2000) Rho GTPases in neuronal morphogenesis. *Nature Reviews Neuroscience* **1**:173–180.
- Makino H, Malinow R (2009) AMPA receptor incorporation into synapses during LTP: the role of lateral movement and exocytosis. *Neuron* **64**:381–390.
- Makino H, Malinow R (2011) Compartmentalized versus global synaptic plasticity on dendrites controlled by experience. *Neuron* **72**:1001–1011.
- Malenka RC, Bear MF (2004) LTP and LTD: an embarrassment of riches. *Neuron* **44**:5–21.
- Malinow R, Malenka RC (2002) AMPA receptor trafficking and synaptic plasticity. *Annual Review of Neuroscience* **25**:103–126.
- Matsuzaki M, Ellis-Davies GC, Nemoto T, Miyashita Y, Iino M, Kasai H (2001) Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons. *Nature Neuroscience* **4**:1086–1092.
- Matsuzaki M, Honkura N, Ellis-Davies GC, Kasai H (2004) Structural basis of long-term potentiation in single dendritic spines. *Nature* **429**:761–766.
- Meyer D, Bonhoeffer T, Scheuss V (2014) Balance and stability of synaptic structures during synaptic plasticity. *Neuron* **82**:430–443.
- Miesenbock G, De Angelis DA, Rothman JE (1998) Visualizing secretion and synaptic transmission with pH-sensitive green fluorescent proteins. *Nature* **394**:192–195.
- Mulkey RM, Malenka RC (1992) Mechanisms underlying induction of homosynaptic long-term depression in area CA1 of the hippocampus. *Neuron* **9**:967–975.

- Mulkey RM, Endo S, Shenolikar S, Malenka RC (1994) Involvement of a calcineurin/inhibitor-1 phosphatase cascade in hippocampal long-term depression. *Nature* **369**:486–488.
- Murakoshi H, Iino R, Kobayashi T, Fujiwara T, Ohshima C, Yoshimura A, Kusumi A (2004) Single-molecule imaging analysis of Ras activation in living cells. *Proceedings of the National Academy of Sciences of the United States of America* **101**:7317–7322.
- Murakoshi H, Wang H, Yasuda R (2011) Local, persistent activation of Rho GTPases during plasticity of single dendritic spines. *Nature* **472**:100–104.
- Nabavi S, Kessels HW, Alfonso S, Aow J, Fox R, Malinow R (2013) Metabotropic NMDA receptor function is required for NMDA receptor-dependent long-term depression. *Proceedings of the National Academy of Sciences of the United States of America* **110**:4027–4032.
- Neher E, Augustine GJ (1992) Calcium gradients and buffers in bovine chromaffin cells. *Journal of Physiology* **450**:273–301.
- Nguyen PV, Abel T, Kandel ER (1994) Requirement of a critical period of transcription for induction of a late phase of LTP. *Science* **265**:1104–1107.
- Noguchi J, Matsuzaki M, Ellis-Davies GC, Kasai H (2005) Spine-neck geometry determines NMDA receptor-dependent Ca^{2+} signaling in dendrites. *Neuron* **46**:609–622.
- Oh WC, Hill TC, Zito K (2013) Synapse-specific and size-dependent mechanisms of spine structural plasticity accompanying synaptic weakening. *Proceedings of the National Academy of Sciences of the United States of America* **110**:E305–312.
- Oh WC, Parajuli LK, Zito K (2015) Heterosynaptic structural plasticity on local dendritic segments of hippocampal CA1 neurons. *Cell Reports* **10**:162–169.
- Okamoto K, Hayashi Y (2006) Visualization of F-actin and G-actin equilibrium using fluorescence resonance energy transfer (FRET) in cultured cells and neurons in slices. *Nature Protocols* **1**:911–919.
- Oliveira AF, Yasuda R (2014) Neurofibromin is the major ras inactivator in dendritic spines. *Journal of Neuroscience* **34**:776–783.
- Opazo P, Labrecque S, Tigaret CM, Frouin A, Wiseman PW, De Koninck P, Choquet D (2010) CaMKII triggers the diffusional trapping of surface AMPARs through phosphorylation of stargazin. *Neuron* **67**:239–252.
- Park M, Penick EC, Edwards JG, Kauer JA, Ehlers MD (2004) Recycling endosomes supply AMPA receptors for LTP. *Science* **305**:1972–1975.
- Park M, Salgado JM, Ostroff L, Helton TD, Robinson CG, Harris KM, Ehlers MD (2006) Plasticity-induced growth of dendritic spines by exocytic trafficking from recycling endosomes. *Neuron* **52**:817–830.
- Park HY, Lim H, Yoon YJ, Follenzi A, Nwokafor C, Lopez-Jones M, Meng X, Singer RH (2014) Visualization of dynamics of single endogenous mRNA labeled in live mouse. *Science* **343**:422–424.
- Patterson MA, Szatmari EM, Yasuda R (2010) AMPA receptors are exocytosed in stimulated spines and adjacent dendrites in a Ras-ERK-dependent manner during long-term potentiation. *Proceedings of the National Academy of Sciences of the United States of America* **107**:15951–15956.
- Rosenberg OS, Deindl S, Sung RJ, Nairn AC, Kuriyan J (2005) Structure of the autoinhibited kinase domain of CaMKII and SAXS analysis of the holoenzyme. *Cell* **123**:849–860.
- Sabatini BL, Oertner TG, Svoboda K (2002) The life cycle of $\text{Ca}(2+)$ ions in dendritic spines. *Neuron* **33**:439–452.
- Saneyoshi T, Fortin DA, Soderling TR (2010) Regulation of spine and synapse formation by activity-dependent intracellular signaling pathways. *Current Opinion in Neurobiology* **20**:108–115.
- Sharma K, Fong DK, Craig AM (2006) Postsynaptic protein mobility in dendritic spines: long-term regulation by synaptic NMDA receptor activation. *Molecular and Cellular Neurosciences* **31**:702–712.
- Spruston N, Jonas P, Sakmann B (1995) Dendritic glutamate receptor channels in rat hippocampal CA3 and CA1 pyramidal neurons. *Journal of Physiology* **482**:325–352.
- Star EN, Kwiatkowski DJ, Murthy VN (2002) Rapid turnover of actin in dendritic spines and its regulation by activity. *Nature Neuroscience* **5**:239–246.

- Steiner P, Higley MJ, Xu W, Czervionke BL, Malenka RC, Sabatini BL (2008) Destabilization of the postsynaptic density by PSD-95 serine 73 phosphorylation inhibits spine growth and synaptic plasticity. *Neuron* **60**:788–802.
- Sumioka A, Brown TE, Kato AS, Bredt DS, Kauer JA, Tomita S (2011) PDZ binding of TARPGamma-8 controls synaptic transmission but not synaptic plasticity. *Nature Neuroscience* **14**:1410–1412.
- Svoboda K, Tank DW, Denk W (1996) Direct measurement of coupling between dendritic spines and shafts. *Science* **272**:716–719.
- Takai Y, Sasaki T, Matozaki T (2001) Small GTP-binding proteins. *Physiological Reviews* **81**:153–208.
- Takao K, Okamoto K, Nakagawa T, Neve RL, Nagai T, Miyawaki A, Hashikawa T, Kobayashi S, Hayashi Y (2005) Visualization of synaptic Ca^{2+} /calmodulin-dependent protein kinase II activity in living neurons. *Journal of Neuroscience* **25**:3107–3112.
- Tanaka J, Horiike Y, Matsuzaki M, Miyazaki T, Ellis-Davies GC, Kasai H (2008) Protein synthesis and neurotrophin-dependent structural plasticity of single dendritic spines. *Science* **319**:1683–1687.
- Tatavarty V, Kim EJ, Rodionov V, Yu J (2009) Investigating sub-spine actin dynamics in rat hippocampal neurons with super-resolution optical imaging. *PloS ONE* **4**:e7724.
- Thomas GM, Huganir RL (2004) MAPK cascade signalling and synaptic plasticity. *Nature Reviews Neuroscience* **5**:173–183.
- Wiegert JS, Oertner TG (2013) Long-term depression triggers the selective elimination of weakly integrated synapses. *Proceedings of the National Academy of Sciences of the United States of America* **110**:E4510–E4519.
- Wiegert JS, Bengtson CP, Bading H (2007) Diffusion and not active transport underlies and limits ERK1/2 synapse-to-nucleus signaling in hippocampal neurons. *Journal of Biological Chemistry* **282**:29621–29633.
- Wu GY, Deisseroth K, Tsien RW (2001) Spaced stimuli stabilize MAPK pathway activation and its effects on dendritic morphology. *Nature Neuroscience* **4**:151–158.
- Yang N, Higuchi O, Ohashi K, Nagata K, Wada A, Kangawa K, Nishida E, Mizuno K (1998) Cofilin phosphorylation by LIM-kinase 1 and its role in Rac-mediated actin reorganization. *Nature* **393**:809–812.
- Yasuda R (2006) Imaging spatiotemporal dynamics of neuronal signaling using fluorescence resonance energy transfer and fluorescence lifetime imaging microscopy. *Current Opinion in Neurobiology* **16**:551–561.
- Yasuda R, Murakoshi H (2011) The mechanisms underlying the spatial spreading of signaling activity. *Current Opinion in Neurobiology* **21**:313–321.
- Yasuda R, Nimchinsky EA, Scheuss V, Pologruto TA, Oertner TG, Sabatini BL, Svoboda K (2004) Imaging calcium concentration dynamics in small neuronal compartments. *Science's STKE: Signal Transduction Knowledge Environment* **2004**:l5.
- Yudowski GA, Puthenveedu MA, Leonoudakis D, Panicker S, Thorn KS, Beattie EC, von Zastrow M (2007) Real-time imaging of discrete exocytic events mediating surface delivery of AMPA receptors. *Journal of Neuroscience* **27**:11112–11121.
- Yuste R, Majewska A, Cash SS, Denk W (1999) Mechanisms of calcium influx into hippocampal spines: heterogeneity among spines, coincidence detection by NMDA receptors, and optical quantal analysis. *Journal of Neuroscience* **19**:1976–1987.
- Zhabotinsky AM (2000) Bistability in the Ca^{2+} /calmodulin-dependent protein kinase-phosphatase system. *Biophysical Journal* **79**:2211–2221.
- Zhai S, Ark ED, Parra-Bueno P, Yasuda R (2013) Long-distance integration of nuclear ERK signaling triggered by activation of a few dendritic spines. *Science* **342**:1107–1111.
- Zhou Q, Homma KJ, Poo MM (2004) Shrinkage of dendritic spines associated with long-term depression of hippocampal synapses. *Neuron* **44**:749–757.
- Zhu JJ, Qin Y, Zhao M, Van Aelst L, Malinow R (2002) Ras and Rap control AMPA receptor trafficking during synaptic plasticity. *Cell* **110**:443–455.

Chapter 21

Dendrites as transmitters

Nathaniel Urban and Troy W. Margrie

Summary

As detailed in other chapters of this book, dendrites have classically been considered as passive structures that receive signals and do little else. Beautiful in their shape but mundane in their function, dendrites have been compared with antennae, funnels, and a variety of other receivers of signals. In short, dendrites are attractive to look at, good listeners, but (it was believed) they did not have much to say. The role of chief communicator was left to the highly vocal axon that voiced (sometimes with a bit of modulation by the presynaptic context) the opinion of the neuron. Exceptions to this rule were duly noted, but little emphasized. In this chapter we focus on the role of dendrites as transmitters rather than receivers, describing the way that, through the release of neurotransmitters, dendrites can convey information about local activity across small numbers of synapses to nearby cells. We focus on the idea that by acting at a local level across many branches of a neuron's dendritic tree dendritic release has a profound impact on global neuronal function in a variety of brain areas, with special focus on the olfactory bulb.

Background

Since the end of the nineteenth century, Santiago Ramón y Cajal's decree that "a functional synapse or useful and effective contact between two neurons can only be formed between the collateral or terminal axonal ramifications of one neuron and the dendrites or cell body of another" (Ramón y Cajal, 1995) has provided the foundation for our exploration into the mechanisms of information transmission in the central nervous system (CNS). In his seminal publication *Histology of the Nervous System*, Ramón y Cajal defined classes of neurons based on "anatomical and functional characteristics." Two cell types, retinal amacrine cells (Fig. 21.1) and olfactory bulb granule cells (GCs), were placed in the class of neurons having short axons and no dendrites. For Ramón y Cajal, the processes of these cells were "undoubtedly axonal," based on his definition of an axon as an output structure.

Today it is not in question as to whether or not amacrine and GCs have dendrites. Since the pioneering light microscopy work of Ramón y Cajal and others, electron microscopy has unambiguously proven that the unmyelinated arborizations of these two cell types contain dendrite-specific postsynaptic specializations such as postsynaptic densities (Price and Powell, 1970). In addition, light microscopy studies indicate they express proteins known to be unique to dendrites, such as microtubule associated protein-2 (MAP2) (Gabriel et al., 1992; Philpot et al., 1997). These findings, together with the observation that they lack a non-tapering, often myelinated process containing varicosities, indicate these cells in fact lack any traditional type of axon. Curiously, with respect to processes underlying dendritic release, these same two cell types have become the most thoroughly studied—though they are just two of many cell types in which dendritic release is now known to occur.

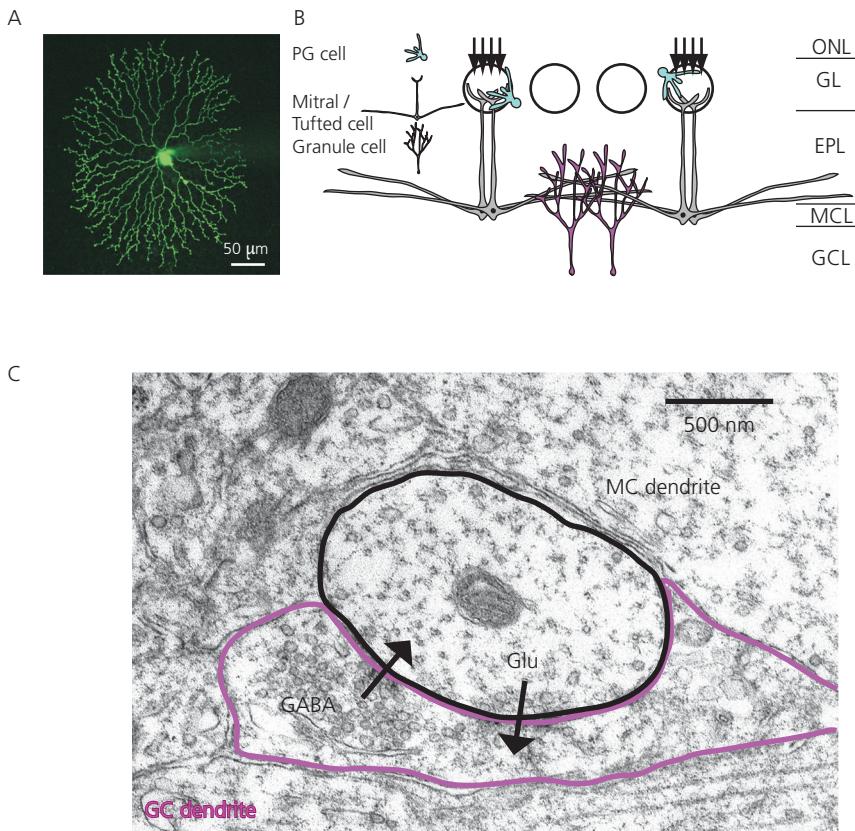


Fig. 21.1 (A) Starburst amacrine cell of the rabbit retina filled with Oregon Green 488 Bapta-1 (200 μm). (B) Anatomy of the olfactory bulb. A schematic showing the basic anatomical layout of the mammalian olfactory bulb. Olfactory nerve inputs (arrows) form glutamatergic synapses with the apical tuft of mitral/tufted (M/T) cells that in turn make dendritic synaptic contacts with periglomerular (PG) cells within the glomerulus. Dendritic synaptic contacts are also made between M/T cell lateral dendrites and granule cell dendrites within the external plexiform layer (EPL). MCL, mitral cell layer; GL, glomerulus layer; ONL, olfactory nerve layer. (C) Electron micrograph showing dendrodendritic release between a mitral cell and granule cell.

Part A provided by Susanne Hausselt and Thomas Euler. Part C provided by Charles Greer.

Introduction

The release of neurotransmitters from presynaptic axons is a complex process that serves as a final step in many examples of neuronal communication. The release of transmitters from axons most commonly proceeds via calcium-dependent exocytosis of neurotransmitter-containing vesicles, resulting in diffusion of the transmitter across the synaptic cleft and its binding to postsynaptic receptors (reviewed by Sudhof and Rothman, 2009). The observation that dendrites can release transmitters leads to questions about the extent to which dendritic release makes use of the same mechanisms as axonal release, as well as to functional questions about the consequences of dendritic release for the systems in which it is observed. In this chapter we consider these questions,

giving examples from a variety of systems in which dendritic release occurs and then focusing on a system in which dendritic release has been the subject of intense interest in the last few years—the mammalian olfactory bulb.

Examples of dendritic release

Evidence that dendrites can act as transmitters has been obtained for many cell types in a number of neural systems, using a variety of anatomical and physiological approaches. Dendritic neurotransmitter release has been observed in different types of neurons in at least ten brain regions (Ludwig and Pittman, 2003), including areas of the forebrain such as the olfactory bulb (Rall et al., 1966; Jahr and Nicoll, 1980; Schoppa and Urban, 2003), the thalamus (Cox et al., 1998; Sherman, 2004), the hippocampus (Drake et al., 1994), and the cortex (Zilberman, 2000), and mid brain areas such as the substantia nigra (Björklund and Lindvall, 1975; Drake et al., 1994), the raphe nucleus (Chazal and Ralston, 1987; Bagdy and Harsing, 1995), the hypothalamus (Ludwig, 1998), and the retina (O’Malley and Masland, 1989). All of the major neurotransmitter types including both fast classical transmitters such as glutamate (Nicoll and Jahr, 1982) and gamma-aminobutyric acid (GABA) (Nicoll, 1971), neuromodulators such as dopamine (Rice et al., 1997) and norepinephrine (Aghajanian et al., 1977), and also peptide transmitters like opioids (Drake et al., 1994) and peptide hormones (Ludwig, 1998) are released by dendrites in different systems. Some cell types, including some subtypes of retinal amacrine cells and also olfactory bulb GCs, do not have axons, meaning that in these cells dendrites are the only structures available to release neurotransmitters. However, in most cases dendritic release occurs in cells that have both axons and dendrites. In such cases, the transmitter or transmitters released from dendrites may be the same as those released from axon terminals, as in most of the above-mentioned cases, or different (Wilson and Nicoll, 2002).

Methods of studying dendritic release

At a methodological level, the study of dendritic release largely parallels the study of axonal release. As new technologies have become available they have been rapidly utilized to address questions about dendritic release. Early anatomical studies identified amacrine cells of the retina and GCs of the olfactory bulb as having a single kind of process, and these observations led to the hypothesis that these processes must be output structures. However, even Ramón y Cajal was uncertain as to whether these structures were axons or dendrites. When the development of electron microscopy allowed for the identification of neurotransmitter-containing vesicles (Palay, 1956), the application of this technique to the olfactory bulb in seminal studies by Rall and colleagues (Rall et al., 1966) allowed the identification of synaptic-like vesicles in the dendrites of mitral and GCs. Vesicles have been observed in the dendrites of many neuronal subtypes, but substantial evidence has been accumulated to indicate that not all dendritic release is vesicular (Wilson and Nicoll, 2002).

Dendritic release has been studied by direct measurement of the released neurotransmitter. This has been performed using techniques for monitoring release into bulk superfusate from slice preparations of regions that are not believed to contain axon terminals capable of releasing a given transmitter, such as substantia nigra (dopamine) and raphe nucleus (serotonin) (O’Connor and Kruk, 1991; Pineyro et al., 1995). Similar approaches have involved the monitoring of a chemoluminescence signal to detect the release of acetylcholinesterase from dendrites of neurons in the substantia nigra (Llinás and Greenfield, 1987). More recently, direct detection of neurotransmitter release has been performed at highly localized sites by cyclic voltammetry using fine-tipped

carbon fiber electrodes. This method can even be used to measure the release of a single vesicle (Rice et al., 1997) and multiple transmitters (Borisovska et al., 2013). While this method has been used to measure the calcium dependence of somato-dendritic release of several neurotransmitters, including 5-hydroxytryptamine (5-HT) and dopamine (Geffen et al., 1976; Bagdy and Harsing, 1995), unfortunately only a limited number of transmitter types can be detected because it relies on the transmitter being oxidized by the electrical field at the tip of the electrode.

Largely electrophysiological recordings have been used to study dendritic release, essentially relying on the neuronal targets of dendritic release to assay the transmitter. Early studies mostly involved the analysis of responses following bulk extracellular stimulation of neurons that released transmitter from their dendrites (Nicoll and Jahr, 1982). The increasing use of paired intracellular recordings to directly measure the properties of axonal release has also led to this method of analysis being applied to the study of dendritic release (Isaacson, 2001; Urban and Sakmann, 2002; Pimentel and Margrie, 2008). This approach is especially useful in systems in which the release of transmitter activates dendritic “autoreceptors,” because in such cases the same electrode can be used to activate release and record postsynaptic responses (Isaacson, 1999; Friedman and Stowbridge, 2000; Margrie et al., 2001; Smith and Jahr, 2002).

Other methods for visualizing release using fluorescently labeled vesicles have become available in recent years. Membrane-associated dyes such as FM1-43 have been used to measure axonal release in cultures and in slice preparations (Betz et al., 1992). Also, targeting of genetically encoded pH indicators to the vesicle lumen has allowed release to be monitored in cultures and in slices (Bozza et al., 2004). Such techniques allow analysis of vesicle dynamics at single release sites and promise to resolve many of the unanswered questions about the relationship between localized excitation and release, not only in axons but also in dendrites.

Classes and mechanisms of dendritic release

The variety of mechanisms implicated in dendritic release is greater than that seen in axonal release. In some cases, dendritic release occurs by mechanisms that appear quite similar to those thought to underlie axonal release at most CNS synapses. Calcium-dependent exocytosis of neurotransmitter-containing vesicles in many dendrites involves calcium influx through voltage-gated calcium channels (Isaacson and Stowbridge, 1998; Murphy et al., 2004; Fekete et al., 2014), elevation of cytoplasmic calcium (Isaacson, 2001), and calcium-dependent fusion of vesicles. Such axon-like release is seen at dendritic synapses made by mitral, granule, and perhaps periglomerular cells of the olfactory bulb (Isaacson and Stowbridge, 1998; Murphy et al., 2004; Fekete et al., 2014), by amacrine cells onto bipolar cells in the retina (Firth et al., 2003; Vigh and Lasater, 2004), and at F2 synapses made by interneurons onto relay cells in the thalamus (Ralston, 1971; Cox and Sherman, 2000). In GCs and thalamic interneurons the released neurotransmitter is thought to be principally GABA, whereas mitral cells release glutamate and amacrine cells are thought to release GABA, acetylcholine, and other transmitters. The diversity of olfactory bulb periglomerular cell types makes it difficult to generalize, but dopaminergic and GABAergic subpopulations of these cells are thought to exist (Kiyokane et al., 2010). Release of vesicles contained in the dendrites of these cells seems to be triggered by calcium influx, and many of these cells, even the axonless granule and amacrine cells, seem to express a variety of proteins typically associated with presynaptic release machinery (Melloni et al., 1993; Berton et al., 1997). In all of these cases, ultrastructural studies have shown that areas of the dendritic membrane have docked vesicles and often are apposed by areas having postsynaptic specializations (Price and Powell, 1970; Ralston, 1971; O’Malley and Masland, 1989).

Variants on this clearly axon-like release from dendrites are found in the case of dendritic release of several neuropeptides, including vasopressin and oxytocin by hypothalamic neurons (Ludwig, 1998) and dynorphin in dentate GCs of the hippocampus (Villacres et al., 1998). In these cells, transmitter-containing vesicles have been observed in dendrites (Drake et al., 1994), though these vesicles are often not clustered at an identifiable active zone as they are for classical fast transmitters. Such vesicles may be localized in a less precise manner, with dynorphin-containing vesicles in hippocampal GCs being localized primarily to spines (Drake et al., 1994). Release of these vesicles seems to involve calcium-dependent fusion, and this fusion may require repetitive stimulation (Simmons et al., 1995; Shibuya et al., 1998). The postsynaptic action of the peptides in these systems thus seems to be mediated by release into the extracellular space in a pericrine-like fashion (Pow and Morris, 1989; Drake et al., 1994). Similar release properties are found in axons that release peptides from dense core vesicles, and thus it seems that the major factor governing the release mechanism is not the compartment from which the release occurs but rather the type of vesicle being released.

In other cases, chemical signaling by dendrites may occur by mechanisms that are quite different from axonal release. Dendritic release of dopamine from neurons in the substantia nigra may be due to activity-induced reversal of dopamine transporters (Falkenburger et al., 2001), although data exist showing that release is quantal in nature and can be inhibited by some clostridial toxins known to cleave SNARE proteins involved in vesicular exocytosis (Bergquist et al., 2002). This may mean that multiple forms of release occur from the same dendrite. Another form of dendritic transmission involves the local activity-dependent synthesis of signaling molecules such as endocannabinoids (Wilson and Nicoll, 2001, 2002; Brown et al., 2004), arachidonic acid (Williams et al., 1989), and even nitric oxide (Schuman and Madison, 1994). Mechanistically, these processes probably have very little in common with the kinds of release that typically occur from axons, but nonetheless represent a mechanism by which local input to particular regions of the dendritic tree can result in a signal that influences the properties of nearby synapses and other neuronal processes.

In almost all cases dendritic release requires calcium influx. This requirement for calcium influx indicates that dendritic release will depend on the degree to which dendrites are active (as discussed here and in other chapters). However, the calcium channel subtypes expressed in axons and dendrites often differ, with dendrites of many cell types containing lower densities of the calcium channel types (N- and P/Q-type channels) typically involved in transmitter release from axons and higher densities of L-, R-, and T-type calcium channels. In hippocampal GCs, dendritic release of dynorphin seems to depend on influx through L-type calcium channels (Simmons et al., 1995), and in olfactory bulb GCs, blockers of T-type calcium channels block both calcium transients and release by approximately 50% (Egger et al., 2003, 2005). In contrast, dendritic release of serotonin from neurons in the raphe nucleus is believed to be regulated by N-type calcium channels (Bagdy and Harsing, 1995) and release from mitral cell dendrites seems to depend on P/Q- (Isaacson and Strowbridge, 1998) and T-type (Fekete et al., 2014) calcium channels.. It therefore seems that the requirement for particular calcium channels is less stringent for dendritic release machinery than for axonal release. The remainder of this chapter will focus mainly on the functional aspects of vesicular release of classical neurotransmitters from dendrites, with an emphasis on release of glutamate and GABA from cells of the olfactory bulb.

The mammalian olfactory bulb as a model system

In the olfactory bulb the axons of receptor neurons form excitatory connections with the dendritic tufts of the principal cells, the mitral/tufted (M/T) cells, and local interneurons, the

periglomerular (PG) cells (Fig. 21.1B). These axo-dendritic synapses are contained within glomeruli that are occupied by both M/T apical dendritic tufts and PG dendritic arborizations. Each glomerulus contains the apical dendritic tufts of approximately 50 M/T cells forming a functional column or channel of principal cells receiving the same kind of sensory information. The terminal dendritic tufts of M/T cells contain vesicles that are clustered at sites often reciprocally apposed by postsynaptic specializations and vesicles on PG cells. Immunohistochemical studies show that vesicles in M/T cells contain glutamate while those in some of the PG cells contain GABA (Ottersen and Storm-Mathisen, 1984; Quaglino et al., 1999). Electron microscope analysis also shows that PG dendrites may provide dendro-axonic feedback inhibition onto the presynaptic olfactory receptor neuron (ORN) terminals (Pinching and Powell, 1971). This anatomical arrangement seems ideally suited for intraglomerular feedback inhibition of both ORN and M/T cell activity (see later). Recent electrophysiological evidence suggests that the release of glutamate from M/T tufts results in mutual excitation of potentially all other M/T cells having tufts within that glomerulus (Aroniadou-Anderjaska et al., 1999; Schoppa and Westbrook, 2001; Urban and Sakmann, 2002; Pimentel and Margrie, 2008; Fekete et al., 2014).

Although M/T cells project a single apical tuft to a single glomerulus, they also project lateral dendrites across the external plexiform layer of the olfactory bulb (Fig. 21.1B) for distances exceeding 1 mm. Vesicles in this dendrite are clustered in locations that are apposed to spines on dendrites of GABAergic GCs (Fig. 21.1C) (Shepherd and Greer, 1998). As with PG cells, GCs contain vesicles that are often immediately adjacent to presynaptic M/T release sites (Bartel et al., 2015), thus forming a second kind of reciprocal connection. Unlike with PG cells, GCs form dendro-dendritic synapses solely with lateral dendrites of M/T cells, which project through the external plexiform layer to interact with potentially many other different “glomerular columns.” This dendro-denritic arrangement is believed to mediate lateral inhibition between columns of M/T cells having different odor-response profiles.

Activation of dendritic calcium channels may occur either via invasion of dendrites by backpropagating action potentials or as a result of local excitatory synaptic input (Margrie et al., 2001; Christie and Westbrook, 2003; Egger et al., 2003; Urban and Castro, 2005). In these two cases the spatial profiles of calcium signals are quite different, reflecting the spatial activation profile of calcium channels opened by the particular stimulus. Following an action potential, the activation of release sites will depend on the extent to which action potentials backpropagate into the dendrites, the degree of which is known to be cell and dendrite specific.

Action potential backpropagation has been examined carefully in only a few types of cells showing dendritic release. The most well-understood cells are M/T cells of the olfactory bulb. As with dopaminergic substantia nigra neurons, which also release neurotransmitter (Hausser et al., 1995), action potential backpropagation in the apical dendrite of M/T cells is non-decremental (Bischofberger and Jonas, 1997; Chen et al., 1997), indicating that the probability of transmission from these dendrites is not limited by the reliability of backpropagation of somatically initiated APs (see Fig. 21.2A). Single backpropagating action potentials evoke substantial calcium transients in all tuft branches, and this calcium influx is known to be sufficient to cause glutamate release from M/T cell tufts (Fig. 21.2A). This glutamate is known to bind to both alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartic acid (NMDA) receptors located on the same mitral cell dendrite (Fig. 21.2B), thereby evoking a form of self-excitation (Isaacson, 1999; Margrie et al., 2001; Salin et al., 2001). This release is also involved in lateral excitation between M/Ts (Fig. 21.2C)—in particular, release caused by a single action potential in one mitral cell results in an average depolarization of 1 mV in all other mitral cells having tufts in the same glomerulus (Urban and Sakmann, 2002).

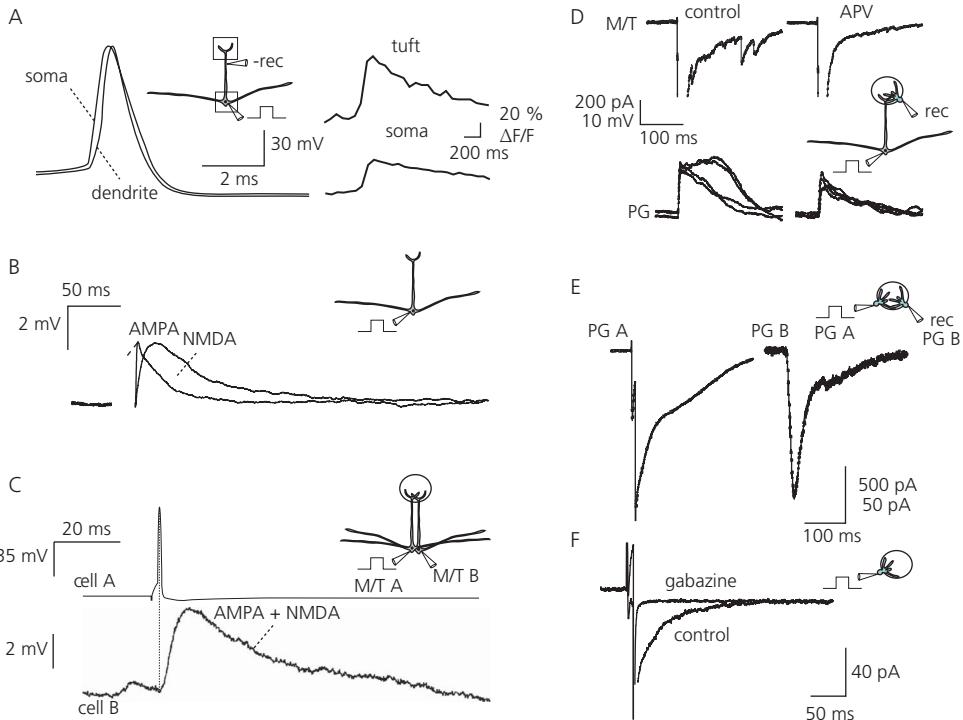


Fig. 21.2 Properties of dendritic release in olfactory bulb glomeruli. **(A)** Dual somatic and dendritic recordings show that action potentials (APs) fully backpropagate in mitral/tufted (M/T) cell apical dendrites (left). Single APs successfully evoke large calcium transients in both the soma and the dendritic tuft (right). **(B)** In the presence of GABA_A receptor antagonists, somatically initiated APs evoke M/T cell self-excitation mediated by both AMPA and NMDA receptors. **(C)** Paired recordings from M/T cells projecting their apical dendrite to the same glomerulus show that in the presence of GABA_A receptor antagonists an AP evoked in M/T cell A results in measurable EPSP waveforms in cell B. **(D)** Paired recordings from M/T and periglomerular (PG) cells projecting their dendrites to the same glomerulus similarly show glutamatergic EPSPs in PG cells and presumably consequent recurrent IPSPs in M/T cells. APV is amino-5-phosphonovaleric acid. **(E)** Paired recordings from PG cells projecting to the same glomerulus show that GABA released from a single PG cell can evoke inhibition in other PGs. **(F)** Gabazine-sensitive currents measured in PG cells reveals that dendritically released GABA also evokes self-inhibition in PGs.

Part A adapted from Action potential propagation into the presynaptic dendrites of rat mitral cells, Josef Bischofberger and Peter Jonas, *The Journal of Physiology*, 504(2), pp. 359–365, Figure 2b Copyright © 1997, John Wiley and Sons and Troy W. Margrie, Bert Sakmann, and Nathaniel N. Urban, Action potential propagation in mitral cell lateral dendrites is decremental and controls recurrent and lateral inhibition in the mammalian olfactory bulb, *Proceedings of the National Academy of Sciences of the United States of America*, 98(1), pp. 319–324, Figure 6a, Copyright © 2001, The National Academy of Sciences. Part B adapted from Troy W. Margrie, Bert Sakmann, and Nathaniel N. Urban, Action potential propagation in mitral cell lateral dendrites is decremental and controls recurrent and lateral inhibition in the mammalian olfactory bulb, *Proceedings of the National Academy of Sciences of the United States of America*, 98(1), pp.319–324, Figure 6a, Copyright © 2001, The National Academy of Sciences. Part C adapted from Reciprocal intraglomerular excitation and intra- and interglomerular lateral inhibition between mouse olfactory bulb mitral cells, Nathaniel N. Urban, Bert Sakmann, *The Journal of Physiology*, 542(2), pp.355–367, Figure 4b Copyright © 2002, John Wiley and Sons. Part E adapted with permission from Macmillan Publishers Ltd: *Nature Neuroscience*, 8(3), Gabe J Murphy, Daniel P Darcy, and Jeffry S Isaacson, Intraglomerular inhibition: signaling mechanisms of an olfactory microcircuit, pp.354–364, © 2005, Nature Publishing Group. Part F adapted with permission from Macmillan Publishers Ltd: *Nature Neuroscience*, 5(8), T. Caitlin Smith and Craig E. Jahr, Self-inhibition of olfactory bulb neurons, pp. 760–766, © 2002, Nature Publishing Group.

Acting via AMPA and NMDA receptors, glutamate released from a single M/T cell is also sufficient to evoke PG cell-mediated recurrent inhibition back onto the active M/T cell (Fig. 21.2D) (Murphy et al., 2005). M/T to PG paired recordings show that the release of GABA requires L-type channel-mediated depolarization that activates P/Q-type channels that mediate release (Murphy et al., 2005). Such recordings show that the glutamate released from M/T cells induces an L-type-mediated depolarization, while Ca influx through P/Q channels is directly coupled to the release process. As with M/T–M/T excitation, release from single M/T cells activates many PG cells specific to the individual glomerulus. The release of GABA from PG cells is also known to reciprocally act on other glomerulus-sharing PGs (Fig. 21.2E) in addition to causing self-inhibition mediated by GABA_A receptors (Fig. 21.2F) (Smith and Jahr, 2002). This is in contrast to the GABA_B receptor-mediated inhibitory feedback onto the ORN terminals evoked by PG cells (Isaacson and Vitten, 2003).

In contrast to the apical dendrite, action potential backpropagation in secondary or lateral M/T dendrites is typically decremental (Fig. 21.3A) (Margrie et al., 2001; Lowe, 2002; Christie and Westbrook, 2003; Davison et al., 2004) and can be regulated by A-type potassium channels (Fig. 21.3B) (Christie and Westbrook, 2003), GABAergic inhibition (Margrie et al., 2001; Lowe, 2002; Xiong and Chen, 2002), and neuromodulators (Davison et al., 2004). While calcium imaging is a useful tool for assessing the extent of backpropagation, it should be noted that the amplitude of calcium transients reflects many factors, including channel distribution and dendritic geometry, that are unrelated to the extent of propagation (Charpak et al., 2001). This suggests that action potential-mediated calcium elevation in dendrites, and thus release, can be independently and/or locally modulated by intrinsic properties and synaptic activity. As with backpropagation, action potential-evoked calcium transients in lateral dendrites are sensitive to 4-amino pyridine (4-AP) (Fig. 21.3C) and GABA_A receptor antagonists (Margrie et al., 2001), indicating that intrinsic and inhibitory processes may modulate release by modulating dendritic excitability. It is also important to note that substantial cell-to-cell variability in intrinsic properties of M/T cells are known to exist (Padmanabhan and Urban, 2010; Angelo and Margrie, 2011), determined by odor input to the glomerulus (Angelo et al., 2012). Finally, the glutamate released by backpropagation into M/T lateral dendrites underlies the recurrent inhibitory postsynaptic potential/current (IPSP/C) following M/T cell discharge (Fig. 21.3D), and is probably influenced by the history of sensory-evoked and local glomerular activity.

Non-decremental, long-range invasion of action potentials into dendrites that release transmitter is an effective means of influencing the activity of large populations of spatially distributed cells. By contrast, more localized regulation of release may provide a neuron with multiple output channels that may be independently regulated by incoming inputs. One example of localized dendritic release comes from experiments in olfactory bulb GCs. GCs have a similar dendro-dendritic arrangement of pre- and postsynaptic specializations to that of M/T and PG cells, although exhibit large spines or gemmules along their dendrites that are sites for both glutamate reception and GABA release. As with many glutamatergic axo-dendritic synapses they receive glutamate via AMPA and NMDA receptors (Fig. 21.4A). However, unlike M/T cells and many PG cells, GCs lack axons—making their dendrites the sole locus of presynaptic communication. The observation that activation of M/T cells often results in calcium transients that are restricted to single GC spines (Fig. 21.4B) is consistent with the idea that dendritic release can be compartmentalized. While it has not been shown directly that these calcium transients in isolated spines can cause release of GABA, release from GCs can occur after sodium channels have been blocked by tetrodotoxin (TTX), suggesting that some form of spatially restricted activity in these dendrites can result in release. Both the isolated spine calcium transients in GCs and the amplitude of recurrent IPSPs in

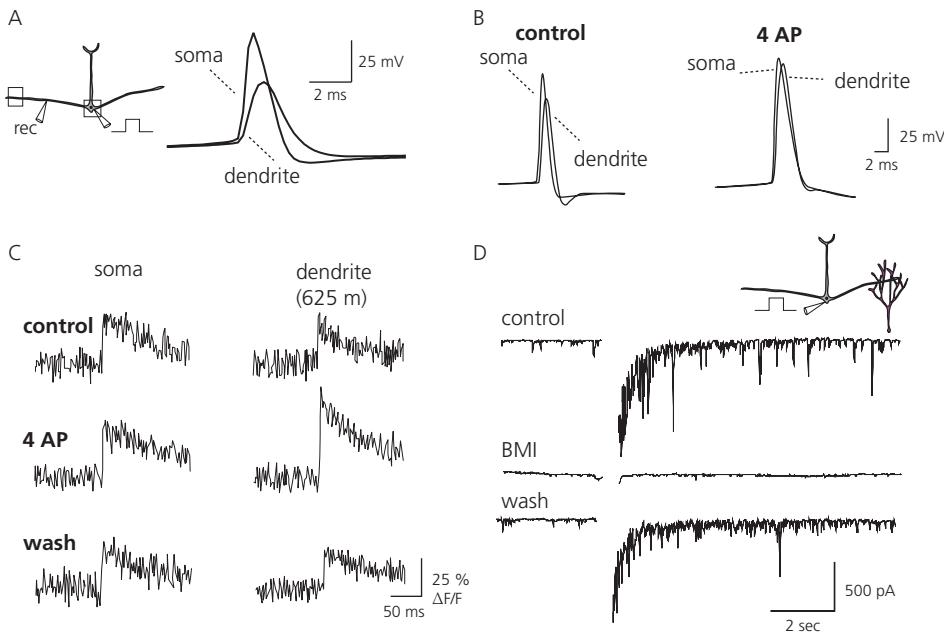


Fig. 21.3 Action potential (AP) backpropagation and release in mitral/tufted (M/T) lateral dendrites. (A) Dual somatic and dendritic recordings show attenuation of single APs in M/T cell dendrites. (B) The attenuation of APs is recovered by application of 4-AP. (C) Improving AP backpropagation by application of 4-AP boosts calcium transients in M/T cell lateral dendrites. (D) In the presence of TTX, somatic voltage steps to 0 mV induce a bicuculline-sensitive burst of inhibitory currents in M/T cells thought to result primarily from the dendritic release of GABA from granule cell interneurons.

Part A Reproduced from Troy W. Margrie, Bert Sakmann, and Nathaniel N. Urban, Action potential propagation in mitral cell lateral dendrites is decremental and controls recurrent and lateral inhibition in the mammalian olfactory bulb, *Proceedings of the National Academy of Sciences of the United States of America*, 98(1), pp.319–324, Figure 6a, Copyright © 2001, The National Academy of Sciences. Part C adapted from J. M. Christie and G. L. Westbrook, Dendritic sodium spikelets and low-threshold regulation of backpropagating action potentials in mitral cell lateral dendrites by A-type potassium currents, *Journal of Neurophysiology*, 89(5), pp.2466–2472, Figure 6a and d, Copyright © 2003, The American Physiological Society. Part D adapted from *Neuron*, 20(4), Jeffry S. Isaacson and Ben W. Strowbridge, Olfactory reciprocal synapses: dendritic signaling in the CNS, pp.749–61, Copyright 1998, Elsevier. With permission from Elsevier.

M/T cells are about 50% reduced by blockade of T-type calcium channels (Egger et al., 2003, 2005). These data have been interpreted as supporting the long-standing idea (Woolf et al., 1991) that in GCs, and perhaps in other cells having dendritic release, release may be regulated in a highly localized manner, with individual excitatory synapses made onto the dendrite controlling the release at one synapse, or a few nearby ones.

GCs also fire large all-or-none sodium spikes that propagate throughout the entire dendritic tree. These events presumably result in release from many sites, suggesting that multiple modes of dendritic excitability allow for differential regulation of dendritic output. In vitro data show that dendritic sodium spikes occur in GCs (Fig. 21.4C1) (Pinato and Midtgård, 2005), and similar spikelets have been observed in GCs during odor processing (Fig. 21.4C2). Such dendritic

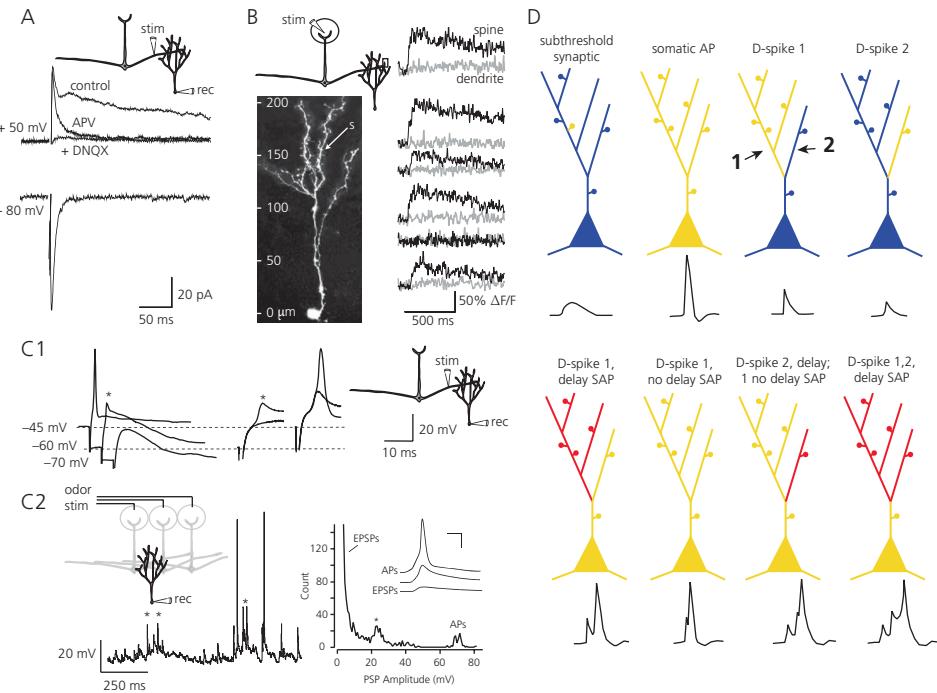


Fig. 21.4 Dendro-dendritic transmission between mitral/tufted (M/T) cells and granule cells (GCs). **(A)** Voltage clamp recordings from GCs show that at depolarized potentials extracellular stimulation in the external plexiform layer (EPL) evokes AMPA and NMDA mediate excitatory currents. Stimulation of a single glomerulus evokes calcium transients in individual GC spines. **(B)** On the left is an image of a GC filled with the calcium chelator/indicator Oregon Green BAPTA-1 (100 μ M). Traces on the right show individual all or none responses localized to the spine indicated by "s" in the image on the left. **(C1)** Recordings from GCs in turtle olfactory bulb slices show that extracellular stimulation in the EPL produces a voltage-dependent all or none event that is sensitive to sodium channel blockers. **(C2)** Classes of odor-evoked EPSP and EPSP-action potential (AP) waveforms in mouse GCs. (right) A plot of membrane potential distribution in a GC during odor-evoked activity. The inset shows averaged traces similar to those shown in C1 (scale bar represents 2 ms and 20 mV). **(D)** Schematic illustration of potentially different modes of GC release. Top row: Examples of highly localised release (yellow) due to depolarization of individual spines (left), global release due to a somatic AP fully backpropagating into all dendritic compartments (second from left), and local release in different dendritic branches (1, 2) due to dendritic spikes that fail to invade other parts of the dendritic tree or soma (third and fourth from left). Bottom row: Examples of how varying the interval between dendritic spikes (D-spike 1,2) and somatic APs (SAP) impacts on the amount and spread of calcium in different dendritic compartments. Red indicates elevation in dendritic calcium that exceeds that observed during back propagating APs (yellow).

Part A from *Neuron*, 20(4), Jeffry S. Isaacson and Ben W. Strowbridge, Olfactory reciprocal synapses: dendritic signaling in the CNS, pp.749–61, Copyright 1998, Elsevier. With permission from Elsevier. Part B adapted from Veronica Egger, Karel Svoboda, and Zachary F. Mainen, Dendrodendritic synaptic signals in olfactory bulb granule cells: local spine boost and global low-threshold spike, *The Journal of Neuroscience*, 25(14), pp. 3521–3530, Figure 2a, © 2005, The Society for Neuroscience. Part C1 Adapted from Giulietta Pinato and Jens Midtgaard, Dendritic sodium spikelets and low-threshold calcium spikes in turtle olfactory bulb granule cells, *Journal of Neurophysiology*, 93(3), pp.1285–1294, Figure 4c and d, Copyright © 2005, The American Physiological Society.

events may provide a signal for regulation of release at the level of individual dendritic branches—allowing for an intermediate degree of output between single spines and the entire dendritic tree (Fig. 21.4D). Furthermore, dendritic spikes will prevent complete backpropagation of somatic action potentials within a narrow time window that may provide a mechanism for selective disinhibition of mitral cells that are active immediately prior to GC discharge (Zelles et al., 2006).

In addition to calcium influx through voltage-gated channels, other sources of intracellular calcium may be able to elicit dendritic release in GCs (Stroh et al., 2012). Release of GABA can occur following activation of NMDA receptors, without any influx through voltage-dependent channels (Chen et al., 2000; Halabisky et al., 2000), although this mechanism may be rare under physiological conditions (Isaacson, 2001). Of perhaps greater physiological interest is the regulation of release from F2 terminals made by the dendrites of thalamic interneurons onto thalamic relay neurons. Release from these terminals is regulated by the activation of a number of different kinds of ligand-gated receptors, including metabotropic glutamate (Cox et al., 1998; Munsch et al., 2003; Govindaiah and Cox, 2004), 5-HT, and muscarinic receptors (Cox and Sherman, 2000). For example, application of metabotropic glutamate receptor agonists such as ACPD results in an increase in the frequency of asynchronous IPSPs in relay neurons of the lateral geniculate nucleus of the thalamus without altering the firing rate of thalamic interneurons, and even in the presence of TTX which blocks interneuron spiking completely. By contrast, under these same conditions, activation of muscarinic M_2 receptors results in decreased asynchronous release. Regulation of release by direct activation of receptors located on dendrites that release transmitter may be an important mechanism for regulating release in response to local inputs, independent of the total amount of excitation or inhibition received by the cell and whether or not the presynaptic cell fires somatic action potentials. Such mechanisms may be especially powerful when they do not involve calcium influx through voltage-gated channels, but rather calcium elevations that depend only on ligand binding, such as is the case for activation of transient receptor potential channels by 5-HT (Munsch et al., 2003; Stroh et al., 2012), because voltage-independent effects cannot be modulated by changes in membrane potential resulting from inhibitory inputs arriving at other synaptic sites in the cells. In this way, dendritic release may allow cells to take advantage of the relatively high degree of biochemical compartmentalization that is afforded by the dendritic morphology.

Functional role of dendritic release in the retina and olfactory bulb

Functional consideration of dendritic release from amacrine cells

Ramón y Cajal named amacrine cells for their lack of a long projecting process (*a*, without; *makros*, long; *inos*, fiber; Rodieck, 1998). In early work, he described these cells as lacking dendrites, whereas later he said that they lacked axons. The morphology of these cells, along with the fact that they are non-spiking, suggests that the release of neurotransmitters from these cells might depend on the input received by highly localized regions of the dendritic tree. In starburst amacrine cells, calcium influx in dendritic segments of about 100 μm varies as a function of the direction of motion of visual stimuli (Euler et al., 2002; Jiang et al., 2005; Li, 2005). This observation suggests that release of transmitter in these cells might reflect the output of a local dendritic computation of the direction of motion. This output is likely to depend in part on the integration of direction-selective inputs and/or on local nonlinear activation of dendritic branches.

Dendro-dendritic signal processing in olfaction

In the case of the olfactory bulb, intraglomerular activity is almost exclusively controlled by dendritic release and appears to have at least two important roles. Simultaneous whole-cell recordings from pairs of M/T cells performed *in vitro* show that, via dendritic glutamate release, a single M/T cell laterally excites many other intraglomerular M/T cells (Urban and Sakmann, 2002; Pimentel and Margrie, 2008; Maher et al., 2009; Fig. 21.5A1). Furthermore, theta burst stimulation of intraglomerular pairs induces plasticity of this connection (Pimentel and Margrie, 2008), the direction and amplitude of which is dependent on the initial strength of the M/T–M/T-evoked excitatory postsynaptic potential (EPSP) (Pimentel and Margrie, 2008). This suggests that M/T dendritic transmission can synchronize, normalize, and perhaps even modulate the gain of glomerular output. This is supported by the observation that single-shock synaptic activation of mitral cells results in ongoing synchronous activity (in the theta bandwidth) of those M/Ts projecting to the same glomerulus (Fig. 21.5A2) (Carlson et al., 2000; Schoppa and Westbrook, 2001). Such synchronization in local synaptic drive may be further promoted by electrical coupling between M/T dendrites within a glomerulus (Christie et al., 2005; Pimentel and Margrie 2008) to homotypically regulate the intrinsic biophysical properties of M/T cells belonging to the same intraglomerular network (Angelo and Margrie, 2011; Angelo et al., 2012).

Slow oscillatory synaptic activity is observed in M/T cells *in vivo*, where it is coupled to the nasal inhalation cycle (Margrie and Schaefer, 2003; Fukunaga et al., 2014; Fig. 21.5B). It ensures the precision of action potentials to the extent that minor fluctuations the patterning of synaptic input can be reliably represented by the timing of M/T cell output trains (Schaefer et al., 2006). A functional role has been proposed for this ongoing sniff-coupled theta rhythm, whereby the strength of odor activity of mitral cells within a glomerular column may be represented by the latency to firing onset relative to the sniff-coupled oscillation cycle (Margrie and Schaefer, 2003; Schaefer and Margrie, 2007; Wachowiak, 2011). Population voltage-sensitive dye (VSD) imaging and calcium imaging on ORN terminals on the dorsal surface of the bulb indicates that the sequence of activation of glomeruli is correlated with odor identity and is preserved across physiological ranges in concentration (Spors and Grinvald, 2002; Spors et al., 2006). *In vivo* whole-cell recordings shown that irrespective of the strength of activity, the firing rate of mitral cells remains constant at approximately 50 Hz (Margrie and Schaefer, 2003), though the onset to firing is concentration dependent (Cang and Isaacson, 2003). This model of odor representation in the bulb suggests that odor identity may be represented in the patterns of onset of activity of glomeruli/MT cells, and that differences in onset timing could be a further tuned or amplified by lateral inhibitory mechanisms (Schaefer and Margrie, 2007).

The extremely dense anatomical and functional interconnectivity of neurons in the glomerulus, along with highly convergent ORN input suggests that glomerular components are highly coupled. Given the distal location of the glomerulus from the M/T cell body and the degree of dendritic overlap between M/T cells and dendritic GABA/dopaminergic-releasing glomerular interneurons it seems that release of glutamate from the M/T tuft is an efficient means of engaging a rapid, but somewhat closed-loop, integrative circuit. Achieving an equivalent degree of interconnection using conventional axo-dendritic synapses would require extensive axonal outgrowth and targeting.

Dendritic release from M/T cells and GCs in the external plexiform layer is the major mechanism of both recurrent and lateral inhibition in the olfactory bulb. Recurrent inhibition down-regulates M/T cells and is thought to provide a rapid brake on odor-evoked activity. In slices, blockade of the recurrent IPSP has been shown to result in elevated levels of dendritic calcium.

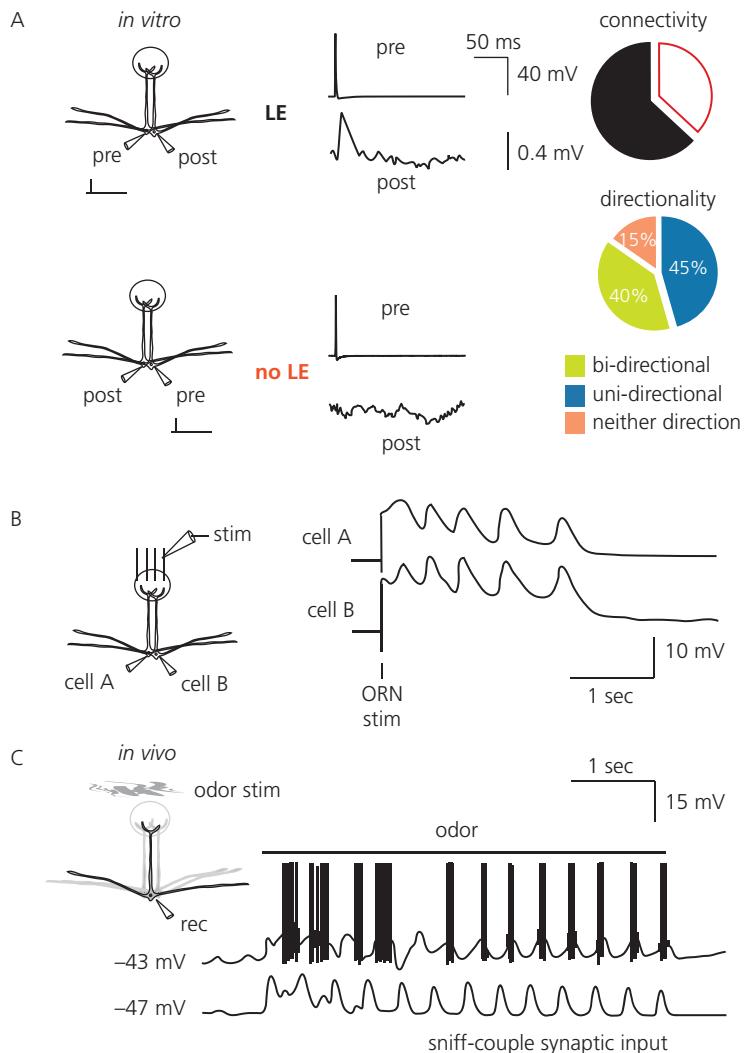


Fig. 21.5 Functional role of dendritic release from mitral cells. **(A)** Simultaneous *in vitro* whole-cell recordings from pairs of mitral cells projecting to the same olfactory bulb glomerulus show that an action potential evoked in one mitral cell often evokes lateral excitation (LE) in the other. Pie charts show the fraction and directionality of intraglomerular mitral to mitral cell connectivity based on 99 paired recordings. **(B)** In olfactory bulb slices, stimulation of olfactory receptor neuron inputs to mitral cells projecting their apical dendrites to the same glomerulus evokes synchronous rhythmic theta activity between the two recorded cells. Such synchronous activity does not occur between mitral cells projecting to different glomeruli. **(C)** *In vivo* whole-cell recordings show that subthreshold membrane potential oscillations control the patterning of odor-evoked activity in mitral cells.

Part A1 adapted from Glutamatergic transmission and plasticity between olfactory bulb mitral cells, Diogo O. Pimentel and Troy W. Margrie, *The Journal of Physiology*, 586(8), pp.2107–2119, Figure 1, © 2008 The Physiological Society. Part A2 reprinted from *Neuron*, 31(4), Nathan E. Schoppa and Gary L. Westbrook, Glomerulus-specific synchronization of mitral cells in the olfactory bulb, p. 639–51, Copyright 2001, Elsevier. With permission from Elsevier. Part B adapted from Theta oscillation coupled spike latencies yield computational vigour in a mammalian sensory system, Troy W. Margrie and Andreas T. Schaefer, *The Journal of Physiology*, 546(2), pp.363–374, Figure 1c, © 2003 The Physiological Society.

Thus recurrent inhibition may itself regulate the extent of glutamate release and therefore the degree of inhibition of M/T lateral dendrites. At the cellular level, lateral inhibition has also been shown to reduce backpropagation of action potentials in M/T lateral dendrites and controls the onset of odor-evoked firing of M/T cells (Hamilton and Kauer, 1989; Margrie et al., 2001). As global blockade of recurrent and lateral inhibition causes increases in recruitment of M/T activity in the mouse olfactory bulb (Margrie et al., 2001) it may help to tune the receptive fields of M/Ts and amplify differences in the patterns of onset latencies across populations of M/T cells.

Concluding remarks

Consequences of putting release sites next to reception sites

When dendrites release transmitter, the site of release is believed to be proximal to reception sites on the same cell. Unless the localization of postsynaptic receptors is tightly controlled, such an arrangement would seem to lead to an increased probability of the dendritically released transmitter activating receptors located on the dendrites from which it was released. At axonal release sites, activation of presynaptic autoreceptors functions to limit this. Such autoreceptor-mediated responses are thought to be involved in the rapid regulation of neurotransmitter release, usually providing a source of negative feedback. A second consequence of having dendritic release sites near sites of input is the degree to which individual inputs can regulate single outputs. Such one-to-one mapping is not possible in cases where release sites are separated from input sites by even tens of microns. This kind of local control of release bypasses the need for signals to be integrated at the soma, allowing for the possibility of small numbers of synapses, or even a single synapse, to control a neuron's output.

Is there a fundamental distinction between axons and dendrites?

Since dendrites can release transmitter and axons can receive synaptic input, one might begin to wonder whether the distinction between axons and dendrites is really as fundamental as has been thought. Recent work on the regulation of neuronal polarity has identified some key players in the specification of axons and dendrites including GSK-3 β , the expression of which regulates the development and stability of neuronal polarity in vitro (Jiang et al., 2005; Li, 2005; Yoshimura et al., 2005). In these experiments polarity is defined by the expression of MAP and tau proteins and the ability to release transmitter. These experiments may point the way toward a better understanding of the specification of neural processes and the mechanisms that underlie the targeting of the pre-synaptic machinery to specific zones within a neuron. Also, it has recently been suggested that the motor protein kinesin-1 is an early marker of the nascent axon (Jacobson et al 2006), indicating that specific motor proteins may play a role in determining neuronal polarity, perhaps by delivery of particular axon-specific cargoes. Extension of this work on early specification of neuronal polarity to cell types in which dendritic release is prominent may provide insight into why when most dendrites remain forever silent, others find a voice.

References

- Aghajanian GK, Cedarbaum JM, Wang RY (1977) Evidence for norepinephrine-mediated collateral inhibition of locus coeruleus neurons. *Brain Research* 136:570–577.
- Angelo K, Margrie TW (2011) Population diversity and function of hyperpolarization-activated current in olfactory bulb mitral cells. *Scientific Reports* 1:50 doi: 10.1038/srep00050

- Angelo K, Rancz EA, Pimentel D, Hundahl C, Hannibal J, Fleischmann Am Pichler B, Margrie TW (2012) A biophysical signature of network affiliation and sensory processing in mitral cells. *Nature* **488**:375–378.
- Aroniadou-Anderjaska V, Ennis M, Shipley MT (1999) Dendrodendritic recurrent excitation in mitral cells of the rat olfactory bulb. *Journal of Neurophysiology* **82**:489–494.
- Bagdy E, Harsing LG, Jr (1995) The role of various calcium and potassium channels in the regulation of somatodendritic serotonin release. *Neurochemical Research* **20**:1409–1415.
- Bartel DL, Rela L, Hsieh L, Greer CA (2015) Dendrodendritic synapses in the mouse olfactory bulb external plexiform layer. *Journal of Comparative Neurology* **523**:1145–1165.
- Bergquist F, Niazi HS, Nissbrandt H (2002) Evidence for different exocytosis pathways in dendritic and terminal dopamine release in vivo. *Brain Research* **950**:245–253.
- Bertoni F, Iborra C, Boudier JA, Seagar MJ, Marqueze B (1997) Developmental regulation of synaptotagmin I, II, III, and IV mRNAs in the rat CNS. *Journal of Neuroscience* **17**:1206–1216.
- Betz WJ, Mao F, Bewick GS (1992) Activity-dependent fluorescent staining and destaining of living vertebrate motor nerve terminals. *Journal of Neuroscience* **12**:363–375.
- Bischofberger J, Jonas P (1997) Action potential propagation into the presynaptic dendrites of rat mitral cells. *Journal of Physiology* **504**:359–365.
- Björklund A, Lindvall O (1975) Dopamine in dendrites of substantia nigra neurons: suggestions for a role in dendritic terminals. *Brain Research* **83**:531–537.
- Borisovska M, Bensen AL, Chong G, Westbrook GL (2013) Distinct modes of dopamine and GABA release in a dual transmitter neuron. *Journal of Neuroscience* **33**:1790–1796.
- Bozza T, McGann JP, Mombaerts P, Wachowiak M (2004) In vivo imaging of neuronal activity by targeted expression of a genetically encoded probe in the mouse. *Neuron* **42**:9–21.
- Brown SP, Safo PK, Regehr WG (2004) Endocannabinoids inhibit transmission at granule cell to Purkinje cell synapses by modulating three types of presynaptic calcium channels. *Journal of Neuroscience* **24**:5623–5631.
- Cajal S (1995) *Histology of the Nervous System of Man and Vertebrates*. Oxford: Oxford University Press.
- Cang J, Isaacson JS (2003) In vivo whole-cell recording of odor-evoked synaptic transmission in the rat olfactory bulb. *Journal of Neuroscience* **23**:4108–4116.
- Carlson GC, Shipley MT, Keller A (2000) Long-lasting depolarizations in mitral cells of the rat olfactory bulb. *Journal of Neuroscience* **20**:2011–2021.
- Charpak S, Mertz J, Beaurepaire E, Moreaux L, Delaney K (2001) Odor-evoked calcium signals in dendrites of rat mitral cells. *Proceedings of the National Academy of Sciences of the United States of America* **98**:1230–1234.
- Chazal G, Ralston HJ, III (1987) Serotonin-containing structures in the nucleus raphe dorsalis of the cat: an ultrastructural analysis of dendrites, presynaptic dendrites, and axon terminals. *Journal of Comparative Neurology* **259**:317–329.
- Chen WR, Midtgård J, Shepherd GM (1997) Forward and backward propagation of dendritic impulses and their synaptic control in mitral cells. *Science* **278**:463–467.
- Chen WR, Xiong W, Shepherd GM (2000) Analysis of relations between NMDA receptors and GABA release at olfactory bulb reciprocal synapses. *Neuron* **25**:625–633.
- Christie JM, Westbrook GL (2003) Regulation of backpropagating action potentials in mitral cell lateral dendrites by A-type potassium currents. *Journal of Neurophysiology* **89**:2466–2472.
- Christie JM, Bark C, Hormuzdi SG, Helbig I, Monyer H, Westbrook GL (2005) Connexin36 mediates spike synchrony in olfactory bulb glomeruli. *Neuron* **46**:761–772.
- Cox CL, Sherman SM (2000) Control of dendritic outputs of inhibitory interneurons in the lateral geniculate nucleus. *Neuron* **27**:597–610.
- Cox CL, Zhou Q, Sherman SM (1998) Glutamate locally activates dendritic outputs of thalamic interneurons. *Nature* **394**:478–482.

- Davison IG, Boyd JD, Delaney KR (2004) Dopamine inhibits mitral/tufted–granule cell synapses in the frog olfactory bulb. *Journal of Neuroscience* **24**:8057–8067.
- Drake CT, Terman GW, Simmons ML, Milner TA, Kunkel DD, Schwartzkroin PA, Chavkin C (1994) Dynorphin opioids present in dentate granule cells may function as retrograde inhibitory neurotransmitters. *Journal of Neuroscience* **14**:3736–3750.
- Egger V, Svoboda K, Mainen ZF (2003) Mechanisms of lateral inhibition in the olfactory bulb: efficiency and modulation of spike-evoked calcium influx into granule cells. *Journal of Neuroscience* **23**:7551–7558.
- Egger V, Svoboda K, Mainen ZF (2005) Dendrodendritic synaptic signals in olfactory bulb granule cells: local spine boost and global low-threshold spike. *Journal of Neuroscience* **25**:3521–3530.
- Euler T, Detwiler PB, Denk W (2002) Directionally selective calcium signals in dendrites of starburst amacrine cells. *Nature* **418**:845–852.
- Falkenburger BH, Barstow KL, Mintz IM (2001) Dendrodendritic inhibition through reversal of dopamine transport. *Science* **293**:2465–2470.
- Fekete A, Johnston J, Delaney KR (2014) Presynaptic T-type Ca^{2+} channels modulate dendrodendritic mitral-mitral and mitral-periglomerular connections in mouse olfactory bulb. *Journal of Neuroscience* **34**:14032–14045.
- Firth SI, Li W, Massey SC, Marshak DW (2003) AMPA receptors mediate acetylcholine release from starburst amacrine cells in the rabbit retina. *Journal of Comparative Neurology* **466**:80–90.
- Friedman D, Strowbridge BW (2000) Functional role of NMDA autoreceptors in olfactory mitral cells. *Journal of Neurophysiology* **84**:39–50.
- Fukunaga I, Herb J, Kollo M, Boyden ES, Schaefer AT (2014) Independent control of gamma and theta activity by distinct interneuron networks in the olfactory bulb. *Nature Neuroscience* **17**:1208–1216.
- Gabriel R, Wilhelm M, Straznicky C (1992) Microtubule-associated protein 2 (MAP2)-immunoreactive neurons in the retina of *Bufo marinus*: colocalisation with tyrosine hydroxylase and serotonin in amacrine cells. *Cell and Tissue Research* **269**:175–182.
- Geffen LB, Jessell TM, Cuello AC, Iversen LL (1976) Release of dopamine from dendrites in rat substantia nigra. *Nature* **260**:258–260.
- Govindaiah, Cox CL (2004) Synaptic activation of metabotropic glutamate receptors regulates dendritic outputs of thalamic interneurons. *Neuron* **41**:611–623.
- Halabisky B, Friedman D, Radojicic M, Strowbridge BW (2000) Calcium influx through NMDA receptors directly evokes GABA release in olfactory bulb granule cells. *Journal of Neuroscience* **20**:5124–5134.
- Hamilton KA, Kauer JS (1989) Patterns of intracellular potentials in salamander mitral/tufted cells in response to odor stimulation. *Journal of Neurophysiology* **62**:609–625.
- Hausser M, Stuart G, Racca C, Sakmann B (1995) Axonal initiation and active dendritic propagation of action potentials in substantia nigra neurons. *Neuron* **15**:637–647.
- Isaacson JS (1999) Glutamate spillover mediates excitatory transmission in the rat olfactory bulb [see comments]. *Neuron* **23**:377–384.
- Isaacson JS (2001) Mechanisms governing dendritic gamma-aminobutyric acid (GABA) release in the rat olfactory bulb. *Proceedings of the National Academy of Sciences of the United States of America* **98**:337–342.
- Isaacson JS, Strowbridge BW (1998) Olfactory reciprocal synapses: dendritic signaling in the CNS. *Neuron* **20**:749–761.
- Isaacson JS, Vitten H (2003) GABA(B) receptors inhibit dendrodendritic transmission in the rat olfactory bulb. *Journal of Neuroscience* **23**:2032–2039.
- Jacobson C, Schnapp B, Banker GA (2006) A change in the selective translocation of the kinesin-1 motor domains marks the initial specification of the axon. *Neuron* **16**:797–804.
- Jahr CE, Nicoll RA (1980) Dendrodendritic inhibition: demonstration with intracellular recording. *Science* **207**:1473–1475.

- Jiang H, Guo W, Liang X, Rao Y (2005) Both the establishment and the maintenance of neuronal polarity require active mechanisms: critical roles of GSK-3beta and its upstream regulators. *Cell* **120**:123–135.
- Kiyokage E, Pan YZ, Shao Z, Koboyashi K, Szabo G, Yanagawa Y, Obata K, Okano H, Toida K, Puche AC, Shipley MT (2010) Molecular identity of periglomerular and short axon cells. *Journal of Neuroscience* **30**:1185–1196.
- Li R (2005) Neuronal polarity: until GSK-3 do us part. *Current Biology* **15**:R198–R200.
- Llinas RR, Greenfield SA (1987) On-line visualization of dendritic release of acetylcholinesterase from mammalian substantia nigra neurons. *Proceedings of the National Academy of Sciences of the United States of America* **84**:3047–3050.
- Lowe G (2002) Inhibition of backpropagating action potentials in mitral cell secondary dendrites. *Journal of Neurophysiology* **88**:64–85.
- Ludwig M (1998) Dendritic release of vasopressin and oxytocin. *Journal of Neuroendocrinology* **10**:881–895.
- Ludwig M, Pittman QJ (2003) Talking back: dendritic neurotransmitter release. *Trends in Neurosciences* **26**:255–261.
- Maher BJ, McGinley MJ, Westbrook GL (2009) Experience-dependent maturation of the glomerular microcircuit. *Proceedings of the National Academy of Sciences of the United States of America* **106**:16865–16870.
- Margrie TW, Schaefer AT (2003) Theta oscillation coupled spike latencies yield computational vigour in a mammalian sensory system. *Journal of Physiology* **546**:363–374.
- Margrie TW, Sakmann B, Urban NN (2001) Action potential propagation in mitral cell lateral dendrites is decremental and controls recurrent and lateral inhibition in the mammalian olfactory bulb. *Proceedings of the National Academy of Sciences of the United States of America* **98**:319–324.
- Melloni RH, Jr, Hemmendinger LM, Hamos JE, DeGennaro LJ (1993) Synapsin I gene expression in the adult rat brain with comparative analysis of mRNA and protein in the hippocampus. *Journal of Comparative Neurology* **327**:507–520.
- Munsch T, Freichel M, Flockerzi V, Pape HC (2003) Contribution of transient receptor potential channels to the control of GABA release from dendrites. *Proceedings of the National Academy of Sciences of the United States of America* **100**:16065–16070.
- Murphy GJ, Glickfeld LL, Balsen Z, Isaacson JS (2004) Sensory neuron signaling to the brain: properties of transmitter release from olfactory nerve terminals. *Journal of Neuroscience* **24**:3023–3030.
- Murphy GJ, Darcy DP, Isaacson JS (2005) Intraglomerular inhibition: signaling mechanisms of an olfactory microcircuit. *Nature Neuroscience* **8**:354–364.
- Nicoll RA (1971) Pharmacological evidence for GABA as the transmitter in granule cell inhibition in the olfactory bulb. *Brain Research* **35**:137–149.
- Nicoll RA, Jahr CE (1982) Self-excitation of olfactory bulb neurones. *Nature* **296**:441–444.
- O'Connor JJ, Kruk ZL (1991) Frequency dependence of 5-HT autoreceptor function in rat dorsal raphe and suprachiasmatic nuclei studied using fast cyclic voltammetry. *Brain Research* **568**:123–130.
- O'Malley DM, Masland RH (1989) Co-release of acetylcholine and gamma-aminobutyric acid by a retinal neuron. *Proceedings of the National Academy of Sciences of the United States of America* **86**:3414–3418.
- Ottersen OP, Storm-Mathisen J (1984) Glutamate- and GABA-containing neurons in the mouse and rat brain, as demonstrated with a new immunocytochemical technique. *Journal of Comparative Neurology* **229**:374–392.
- Padmanabhan K, Urban NN (2010) Intrinsic biophysical diversity decorrelates neuronal firing while increasing information content. *Nature Neuroscience* **13**:1276–1282.
- Palay SL (1956) Synapses in the central nervous system. *Journal of Biophysical and Biochemical Cytology* **2**:193–202.
- Philpot BD, Lim JH, Halpin S, Brunjes PC (1997) Experience-dependent modifications in MAP2 phosphorylation in rat olfactory bulb. *Journal of Neuroscience* **17**: 9596–9604.

- Pimentel DP, Margrie TW (2008) Glutamatergic transmission and plasticity between olfactory bulb mitral cells. *Journal of Physiology* **586**:2107–2119.
- Pinato G, Midtgård J (2005) Dendritic sodium spikelets and low-threshold calcium spikes in turtle olfactory bulb granule cells. *Journal of Neurophysiology* **93**:1285–1294.
- Pinching AJ, Powell TP (1971) The neuropil of the glomeruli of the olfactory bulb. *Journal of Cell Science* **9**:347–377.
- Pineyro G, de Montigny C, Blier P (1995) 5-HT_{1D} receptors regulate 5-HT release in the rat raphe nuclei. In vivo voltammetry and in vitro superfusion studies. *Neuropsychopharmacology* **13**:249–260.
- Pow DV, Morris JF (1989) Dendrites of hypothalamic magnocellular neurons release neurohypophysial peptides by exocytosis. *Neuroscience* **32**:435–439.
- Price JL, Powell TP (1970) The synaptology of the granule cells of the olfactory bulb. *Journal of Cell Science* **7**:125–155.
- Quaglino E, Giustetto M, Panzanelli P, Cantino D, Fasolo A, Sassoe-Pognetto M (1999) Immunocytochemical localization of glutamate and gamma-aminobutyric acid in the accessory olfactory bulb of the rat. *Journal of Comparative Neurology* **408**:61–72.
- Rall W, Shepherd GM, Reese TS, Brightman MW (1966) Dendrodendritic synaptic pathway for inhibition in the olfactory bulb. *Experimental Neurology* **14**:44–56.
- Ralston HJ, III (1971) Evidence for presynaptic dendrites and a proposal for their mechanism of action. *Nature* **230**:585–587.
- Rice ME, Cragg SJ, Greenfield SA (1997) Characteristics of electrically evoked somatodendritic dopamine release in substantia nigra and ventral tegmental area in vitro. *Journal of Neurophysiology* **77**:853–862.
- Rodeck RW (1998) *The First Steps in Seeing*. Sunderland, MA: Sinauer.
- Salin PA, Lledo PM, Vincent JD, Charpak S (2001) Dendritic glutamate autoreceptors modulate signal processing in rat mitral cells. *Journal of Neurophysiology* **85**:1275–1282.
- Schaefer AT, Margrie TW (2007) Spatiotemporal representations in the olfactory system. *Trends in Neurosciences* **30**:92–100.
- Schoppa NE, Urban NN (2003) Dendritic processing within olfactory bulb circuits. *Trends in Neurosciences* **26**:501–506.
- Schaefer AT, Angelo K, Spors H, Margrie TW (2006) Neuronal oscillations enhance stimulus discrimination by ensuring action potential precision. *PLoS Biology* **4**:163–178.
- Schoppa NE, Westbrook GL (2001) Glomerulus-specific synchronization of mitral cells in the olfactory bulb. *Neuron* **31**:639–651.
- Schuman EM, Madison DV (1994) Nitric oxide and synaptic function. *Annual Review of Neuroscience* **17**:153–183.
- Shepherd GM, Greer CA (1998) Olfactory bulb. In: *The Synaptic Organization of the Brain* (Shepherd GM, ed.), pp. 415–454. New York: Oxford University Press.
- Sherman SM (2004) Interneurons and triadic circuitry of the thalamus. *Trends in Neurosciences* **27**:670–675.
- Shibuya I, Noguchi J, Tanaka K, Harayama N, Inoue U, Kabashima N, Ueta Y, Hattori Y, Yamashita H (1998) PACAP increases the cytosolic Ca²⁺ concentration and stimulates somatodendritic vasopressin release in rat supraoptic neurons. *Journal of Neuroendocrinology* **10**:31–42.
- Simmons ML, Terman GW, Gibbs SM, Chavkin C (1995) L-type calcium channels mediate dynorphin neuropeptide release from dendrites but not axons of hippocampal granule cells. *Neuron* **14**:1265–1272.
- Smith TC, Jahr CE (2002) Self-inhibition of olfactory bulb neurons. *Nature Neuroscience* **5**:760–766.
- Spors H, Grinvald A (2002) Spatiotemporal dynamics of odor representations in the mammalian olfactory bulb. *Neuron* **34**:301–315.
- Spors H, Wachowiak M, Cohen LB, Friedrich RW (2006). Temporal dynamics and latency patterns of receptor neuron input to the olfactory bulb. *Journal of Neuroscience* **26**:1247–1259.

- Stroh O, Freichel M, Kretz O, Birnbaumer L, Hartmann J, Egger V (2012) NMDA receptor-dependent synaptic activation of TRPC channels in olfactory bulb granule cells. *Journal of Neuroscience* **32**:5737–5746.
- Sudhof TC, Rothman JE (2009) Membrane fusion: grappling with SNARE and SM proteins. *Science* **323**:474–477.
- Urban NN, Castro JB (2005) Tuft calcium spikes in accessory olfactory bulb mitral cells. *Journal of Neuroscience* **25**:5024–5028.
- Urban NN, Sakmann B (2002) Reciprocal intraglomerular excitation and intra- and interglomerular lateral inhibition between mouse olfactory bulb mitral cells. *Journal of Physiology* **542**:355–367.
- Vigh J, Lasater EM (2004) L-type calcium channels mediate transmitter release in isolated, wide-field retinal amacrine cells. *Visual Neuroscience* **21**:129–134.
- Villacres EC, Wong ST, Chavkin C, Storm DR (1998) Type I adenylyl cyclase mutant mice have impaired mossy fiber long-term potentiation. *Journal of Neuroscience* **18**:3186–3194.
- Wachowiak M (2011) All in a sniff: olfaction as a model for active sensing. *Neuron* **71**:962–973.
- Williams JH, Errington ML, Lynch MA, Bliss TV (1989) Arachidonic acid induces a long-term activity-dependent enhancement of synaptic transmission in the hippocampus. *Nature* **341**:739–742.
- Wilson RI, Nicoll RA (2001) Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. *Nature* **410**:588–592.
- Wilson RI, Nicoll RA (2002) Endocannabinoid signaling in the brain. *Science* **296**:678–682.
- Woolf TB, Shepherd GM, Greer CA (1991) Serial reconstructions of granule cell spines in the mammalian olfactory bulb. *Synapse* **7**:181–192.
- Xiong W, Chen WR (2002) Dynamic gating of spike propagation in the mitral cell lateral dendrites. *Neuron* **34**:115–126.
- Yoshimura T, Kawano Y, Arimura N, Kawabata S, Kikuchi A, Kaibuchi K (2005) GSK-3 β regulates phosphorylation of CRMP-2 and neuronal polarity. *Cell* **120**:137–149.
- Zelles T, Boyd JD, Hardy AB and Delaney KR (2006). Branch-specific Ca^{2+} influx from Na^+ -dependent dendritic spines in olfactory bulb granule cells. *Journal of Neuroscience* **26**:30–40.
- Zilberter Y (2000) Dendritic release of glutamate suppresses synaptic inhibition of pyramidal neurons in rat neocortex. *Journal of Physiology* **528**:489–496.

Chapter 22

Dendritic connectomics

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Summary

Dendrites host a number of biophysical mechanisms for the complex interaction of input synapses. Some of these interactions are enhanced by the spatial patterning of active synapses on the target dendrite. To understand the nature of dendritic signal transformations, one would ideally map out all incoming synapses and determine the location and functional properties of their source neurons. It is only in the last decade that methods based on three-dimensional electron microscopy have become available to make such microscopically precise large-scale structural synaptic mapping realistic. This chapter will summarize these methodological advances, review recent discoveries of precise dendritic input mapping in the mouse retina, and outline the steps needed to search for synaptic patterning in the cerebral cortex. Obtaining a neuron's complete synaptic input map—its dendritic connectome—will provide the link between the biophysics of complex signal transformations in dendrites and their algorithmic meaning in the context of the brain's neuronal circuits.

Introduction

Most neurons in most nervous systems are highly interconnected: each of them directly and specifically interacts with hundreds to thousands of other neurons via chemical synapses. The structure of these interacting networks is suspected to contribute substantially to the algorithmic capabilities of neuronal ensembles (Hebb, 1949; Helmstaedter et al., 2008; Denk et al., 2012; Bargmann and Marder, 2013). To gain a mechanistic understanding of these computational capabilities, a major goal of neuroscience is to map the synaptic network structure of neuronal ensembles—a methodological endeavor summarized as *connectomics* (Lichtman and Denk, 2011; Denk et al., 2012).

For most neurons, more than 90% of the synapses that integrate the neuron into its network are made onto its dendrites. As described in other chapters of this book the spatial extent and biophysical setup of neuronal dendrites make them ideally suited to locally process synaptic input (see also Schiller et al., 2000; Schiller and Schiller, 2001; Branco and Häusser, 2010; Palmer et al., 2014)—implying that the spatial location of an input synapse on the dendrite affects the neuron's action potential output (see, e.g., Rall, 1967; Rall et al., 1967; Häusser et al., 2000; Segev, 2006; Gidon and Segev, 2012). However to what degree do nervous systems exploit these biophysically possible mechanisms for functionally meaningful computations? Are synaptic inputs with common functional properties spatially clustered on the postsynaptic dendrite? Do functional properties map onto the dendritic tree in some other specific way? Addressing such questions by means

of sufficiently complete synaptic input mapping on neuronal dendrites is the topic of *dendritic connectomics*—the subject of this chapter.

We first discuss the relevant level of detail required for such analysis, and then provide a brief overview of the imaging and analysis methods currently employed in connectomics. Then we describe two insights into the specificity of synaptic connections enabling computations in the sensory periphery (mouse retina) and explore dendritic connectomics in the mammalian cerebral cortex. Finally, we discuss the strategies for complete mapping of synaptic inputs in extended neuronal circuits (exhaustive dendritic connectomics).

Pairwise dendritic connectomics

When mapping a neuronal network, a convenient formalization of connectivity is that of a directed graph, in which the neurons are nodes and the synaptic connections are edges directed from the pre- to the postsynaptic cell. Such a graph can be represented by its adjacency matrix, the neuron-to-neuron connectome $C_{N,N}$ (Fig. 22.1A, B). From the point of view of the receiving neuron, however, such a description cannot reveal the potential spatial selectivity of functionally distinct synaptic inputs. Rather, a higher-resolution connectomic description is required, which can resolve single dendrites of the receiving neuron (Fig. 22.1C) or even subsegments of a given

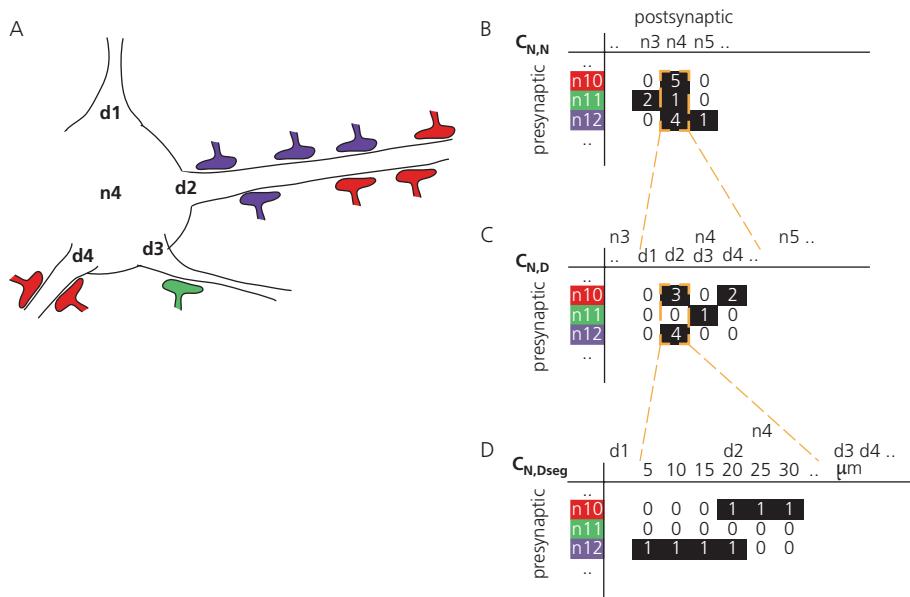


Fig. 22.1 First-order dendritic connectomics. (A) Mapping all synaptic inputs on neuronal dendrites and analyzing their functional and spatial relations is the goal of dendritic connectomics. (B) The neuron-to-neuron connectome $C_{N,N}$ reporting the number of synaptic contacts between pairs of pre-(rows) and postsynaptic (columns) neurons does not reveal dendritic input specificity. (C) The neuron-to-dendrite connectome $C_{N,D}$ can reveal innervation patterns that are specific for single-neuron dendrites. (D) Only the full-resolution neuron-to-dendrite segment connectome $C_{N,Dseg}$ allows the discovery of relevant spatial input patterns (see also Figs 22.5–22.7; note that these patterns are still based on pairwise connectivity, only—see Fig. 22.2).

dendrite (Fig. 22.1D). In the case of spatially clustered synaptic input, only a high-resolution description of the neuron-to-dendritic-segment connectivity $C_{N,Dseg}$ (Fig. 22.1D) will reveal the relevant spatial structure of the synaptic input.

Higher-order dendritic connectomics

Mapping all the synaptic inputs of one neuron corresponds to measuring one column in the neuron-to-neuron connectome $C_{N,N}$ (Fig. 22.1B). At the neuron-to-neuron level this is still a pairwise connectomic analysis.

Are there plausible higher-order connectomic patterns that we should search for? Figure 22.2 illustrates the meaning of higher-order dendritic connectomics: the analysis of synaptic input patterns across neurons. Imagine synaptic sorting across one dendrite of one neuron that is slightly varied on the dendrites of another neuron (Fig. 22.2A). Assuming that the input was sorted in time, an input signal would thus impinge in temporal order centripetally on that one dendrite, which could trigger a nonlinear response of that neuron (Branco et al., *Science* 2010). Slight variations of this temporal input pattern would be enhanced on the dendrites of the other neuron, which could be used to sharpen the response of the first neuron via inhibitory connections or to create a representation of a number of temporal input patterns in a neuronal population.

A second example is the projection of a high-dimensional input onto the dendrites of neurons with no evident local spatial sorting (Fig. 22.2B), but with distinct lower-dimensional projections on the different dendrites (Fig. 22.2B). Such dendritic connectomic patterns could be suitable for the generation of random but distinct representations of high-dimensional sensory inputs.

Both circuit patterns (Fig. 22.2) can only be revealed by concomitant mapping of synaptic inputs on multiple dendrites of multiple neurons, and are examples of higher-order dendritic connectomics.

Methods for high-resolution connectomics

For a long time the mapping of synaptic inputs was severely impaired by a lack of available imaging and reconstruction methods (for reviews see Helmstaedter et al., 2008; Briggman and Bock, 2012; Helmstaedter, 2013). Reconstructing dendritic synaptic inputs requires very high-resolution imaging since dendritic structures can have a diameter as small as 50 nm (e.g., dendritic spine necks in the cortex). However, to follow the presynaptic axon back to the cell body of the source neuron (which is usually required to identify its cell type and potential functional properties), one has to follow an equally thin axonal wire over distances of hundreds of micrometers for local circuits—or millimeters for distal inputs (Fig. 22.3). Since most dendritic and axonal arborizations are locally isotropic (i.e., they do not follow a single direction), three-dimensional (3D) high-resolution large-volume imaging techniques are required. Methods based on high-throughput electron microscopy (EM) have been developed over the last decade and are starting to be productive for dense circuit reconstructions (Denk and Horstmann, 2004; Hayworth et al., 2006; Knott et al., 2008; Bock et al., 2011; Briggman et al., 2011; Helmstaedter et al., 2013; Takemura et al., 2013; for a review see Briggman and Bock, 2012).

The main methodological steps in large-scale EM are the staining of nervous tissue, high-speed high-resolution imaging, and access to the third dimension of tissue blocks. To contrast neuronal tissue in electron-based imaging, heavy metal compounds have to be deposited in the structures of interest—cell membranes, synaptic vesicles, and postsynaptic densities. Then, the tissue is embedded in plastic—because all current 3D EM techniques still involve the imaging of successive

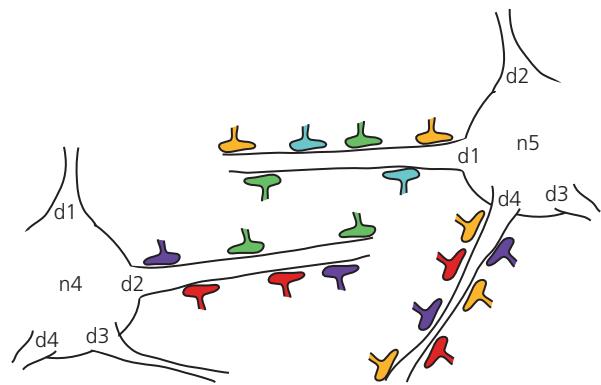
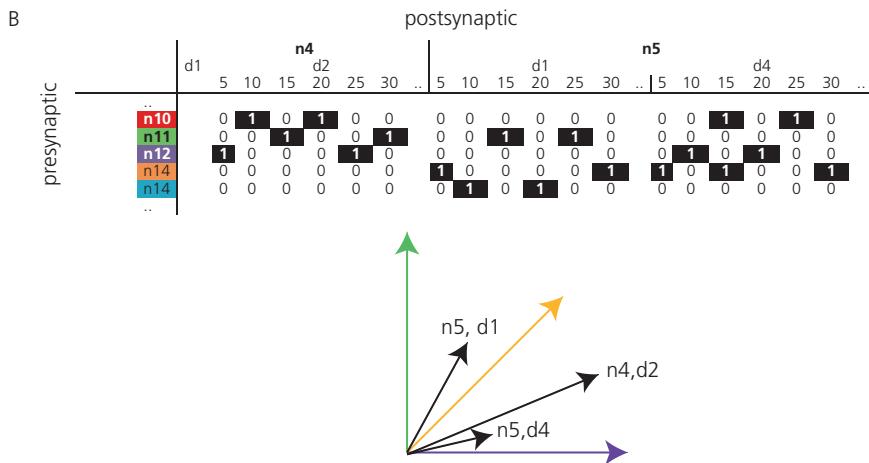
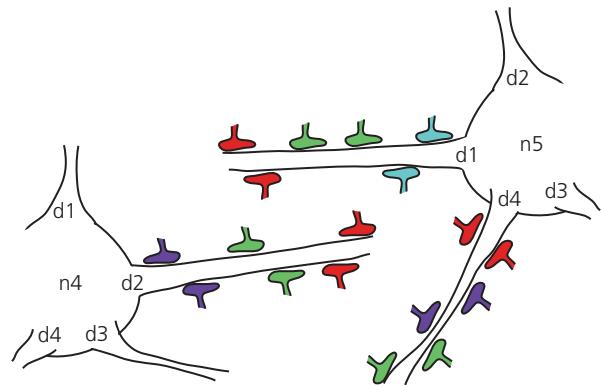
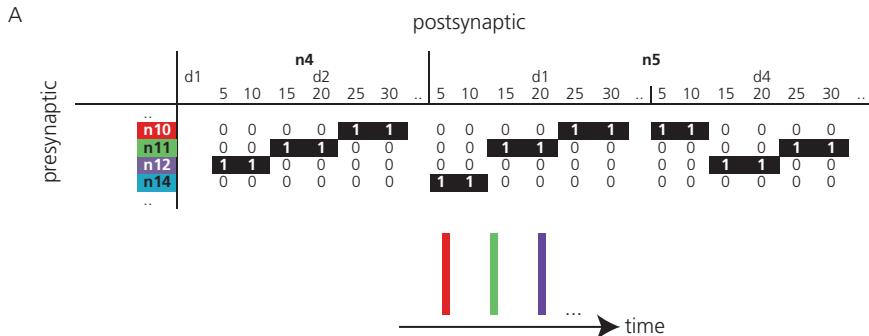


Fig. 22.2 Higher-order dendritic connectomics. Examples of higher-order dendritic innervation patterns (neuron-to-dendritic segments connectomes $C_{N,D\text{seg}}$, left, see Fig. 22.1). Such connectomic patterns can only be detected by simultaneously analyzing multiple pre- and postsynaptic neurons. **(A)** Example of an innervation sequence along multiple dendrites of different neurons. A possible temporal activation sequence is shown (see text for details). **(B)** Innervation of dendrites with overlapping but distinct input mixtures; each mixture can be interpreted as a lower-dimensional projection of the high-dimensional input space (see sketch in inset and text)

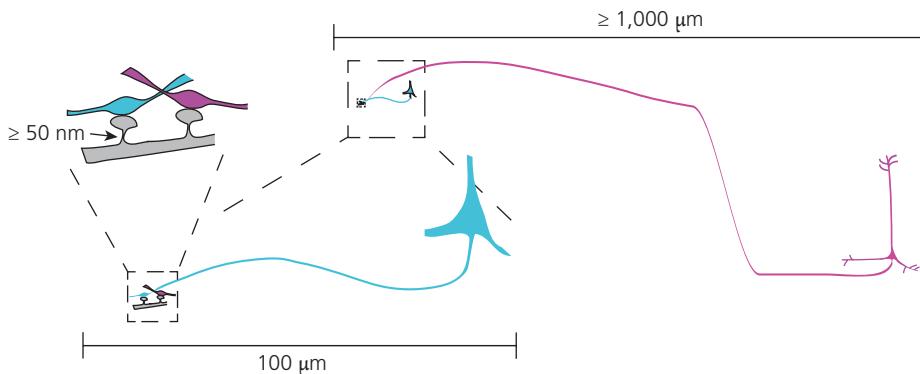


Fig. 22.3 Spatial challenges when mapping synaptic inputs to dendrites: dendritic spines have a diameter as little as 50 nm, but source neurons (magenta, cyan) can be as far as hundreds of micrometers (cyan) or millimeters (magenta) away.

slices of tissue blocks. Either sections are cut first and then exposed to high-speed transmission EM (TEMCA) (Bock et al., 2011; Fig. 22.4A), or they are automatically collected on a tape for later imaging in a scanning electron microscope (SEM)—a technique called automated tape collecting ultra-microtome (ATUM) (Hayworth et al., 2006; Fig. 22.4B). Alternatively, the entire tissue block is placed in the vacuum chamber of a SEM and the tissue at the surface of the block is removed by either a diamond-based ultramicrotome installed in the SEM chamber (serial block-face EM, SBEM) (Denk and Horstmann, 2004; Briggman et al., 2011; Fig. 22.4C) or by a focused ion beam which essentially evaporates the top-most tissue layer (FIBSEM) (Knott et al., 2008; Fig. 22.4D).

What are the relative advantages and disadvantages of these various approaches? In both block-face techniques, images are acquired while the tissue block is still intact, so that the resulting image series can be more easily aligned into a 3D data set. Alignment of images taken from thin sections can be challenging when the sections are folded or distorted. However, since the imaging and cutting processes are directly interlaced in the blockface setting, the scanning-based imaging process cannot be easily sped up by parallelization. For an extended methodological discussion of these 3D EM imaging techniques see Briggman and Bock (2012).

Even more time-consuming than volume EM imaging is the analysis of such data (see Helmstaedter, 2013, for a detailed summary). Briefly, each neuron in the imaged circuit has to be reconstructed from raw pixel values. Currently, computer analysis is nowhere near being able to faithfully reconstruct whole neurons or even whole circuits. Therefore, massive combined human-machine annotation efforts are required for connectomic data analysis. These have so far been implemented either by hiring a number of full-time annotators for segmentation proof reading (Takemura et al., 2013), involving hundreds of part-time students (Helmstaedter et al., 2011, 2013), or recruiting citizen scientists over the web (Kim et al., 2014). None of these approaches yet scales to circuits involving thousands of neurons. However, targeted circuit analysis and the reconstruction of smaller volumes are feasible today. This methodological development is ongoing and is likely to see substantial improvements in the coming years.

These methods allow for the first time high-throughput imaging and reconstruction of neuronal circuits. While still very resource-consuming, the first connectomic analyses have been published in the recent years. A set of circuit analyses were undertaken in the mouse retina (Briggman et al.,

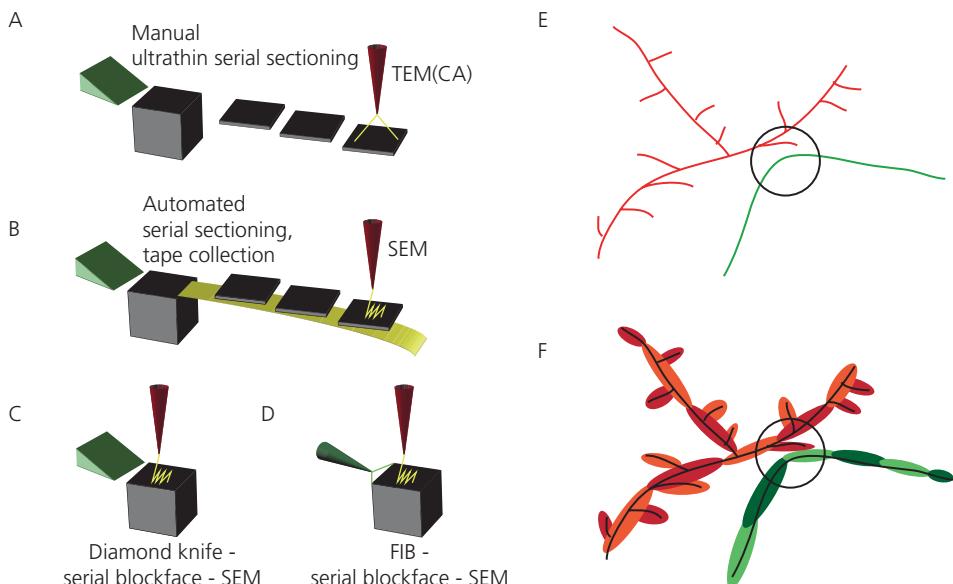


Fig. 22.4 Methods for connectomics. (A–D) Current methods for volume (3D) electron microscopy. (A) Transmission electron microscopy using camera arrays (TEM(CA)) (Bock et al., 2011). (B) Automated sectioning and tape collection, followed by scanning EM imaging (ATUM-SEM) (Hayworth et al., 2006). (C) Serial blockface scanning EM (SBEM) using in-chamber diamond-knife ultramicrotome (Denk and Horstmann, 2004). (D) Focused ion beam for surface removal (FIB-SEM) (Knott et al., 2008). (E) Reconstruction of innervation using skeleton reconstruction of dendrites and axons, followed by manual inspection of skeleton proximities (Briggman et al., 2011; Helmstaedter et al., 2011) or (F) automated contact detection based on a combination of skeleton reconstructions and locally automated volume segmentations (Helmstaedter et al. 2013; Berning et al., 2015). Alternatively, volume segmentations are computed first and proof-read by visual inspection (Takemura et al., 2013; Kim et al., 2014; not shown).

Parts A–D reprinted by permission from Macmillan Publishers Ltd: *Nature Methods*, 10(6), Moritz Helmstaedter, Cellular-resolution connectomics: challenges of dense neural circuit reconstruction, pp. 501–507, Figure 3, © 2013, Nature Publishing Group.

2011; Helmstaedter et al., 2013; Kim et al., 2014) to quantify dendritic wiring specificity, thus constituting the first examples of results in dendritic connectomics.

Dendritic wiring specificity in the mouse retina

At first glance, the retina can be compared with a charge-coupled device (CCD) camera array with its pixelated setup (CCD pixels in the camera, cone photoreceptors in the organism). The visual world is sampled at a resolution determined by the spacing of retinal photoreceptors, and these signals are further relayed via bipolar cells and ganglion cells (GCs) to the rest of the brain (in the mouse especially visual thalamus and cortex). However, in contrast to a CCD camera, the retina of mammals actually performs the first stage of visual processing: it computes at least 20 transformations of the visual world, which are encoded by the more than 20 types of GCs sending their axons via the optic nerve to downstream targets (Gollisch and Meister, 2008). One of these

transformations is the generation of direction-selective visual responses. Early on, Barlow et al. (1964) found that certain GCs in the rabbit retina are selective to the direction of movement of oriented bars in the visual field (for a recent review see Borst and Helmstaedter, 2015). Ever since this discovery, the mechanisms by which the retinal circuits compute directional selectivity have been the subject of computational and experimental research. First, based on cell-type-specific lesion experiments, it became clear that a certain type of amacrine cell, the so-called starburst amacrine cell (SAC), is required for directional selectivity (Yoshida et al., 2001). This type of cell has a very peculiar dendritic morphology: about six to eight primary dendrites extend from the cell body in an astonishingly regular radial pattern, with repeated shallow-angle branchings, which lead to a uniform circular coverage in the plane of the retina (and thus the visual field; Fig. 22.5A). Unlike most neurons in central parts of the brain, these amacrine cells lack an axon. Rather, their dendrites serve as both a postsynaptic site for incoming bipolar cell synapses and as presynaptic release sites to transmit inhibitory signals to the postsynaptic GCs.

In a landmark experiment, Euler et al. (2002) investigated how SACs contribute to the computation of directional selectivity. They used light stimulation and simultaneous two-photon imaging of calcium influx in SAC dendrites to measure the synaptic release at the tips of the SAC dendrites. They discovered that each SAC dendrite is selective for the direction of motion of the light stimulus. Specifically, radially expanding stimuli would trigger substantial Ca^{2+} transients at the dendritic release sites, but radially contracting stimuli would not. Thus, each SAC dendrite was found to be selective for outward stimulus motion.

This finding suggested that the dendrites of SACs are in a position to impose directional selectivity onto GCs, provided the dendrites were connected to the postsynaptic GCs in a very specific way (Fig. 22.5): dendrites should predominantly innervate a given direction-selective GC (DSGC) if they are pointing in the non-preferred direction (NULL direction) of the GC, but should avoid this innervation when pointing in other directions.

Connectomic analysis finally proved that in fact such highly specific dendritic innervation is implemented in the mouse retina (Briggman et al., 2011). Briggman and colleagues used SBEM (see Methods for high-resolution connectomics) to acquire a high-resolution 3D EM data set that was large enough to encompass several DSGCs and dozens of SACs. First, they used two-photon calcium imaging to measure the somatic response of GCs to moving gratings and determine their directional preference. Then they used this piece of retinal tissue for SBEM imaging, identifying the previously imaged GCs and reconstructing their dendritic arborizations. Finally, SACs in the vicinity were also reconstructed, and the appositions between SAC dendrites and DSGC dendrites were inspected. The largest fraction of these appositions turned out to be “accidental,” i.e., SAC dendrites just touched the GC dendrites without clear signs of synaptic contact. However, about 20% of the appositions had a peculiar geometry: the SAC dendrite wrapped around the postsynaptic dendrite like a claw. In a separate calibration dataset, such contacts had been shown to be synaptic, while accidental touches were not.

Intriguingly, the output synapses of a given SAC (Fig. 22.5A) were distributed according to the functionally identified directional preference of the *postsynaptic* ganglion cell (Fig. 22.5A): SAC dendrites pointing upward (plus or minus about 30°) innervated predominantly DSGCs that were selective for downward motion. Correspondingly, SAC dendrites pointing to the right innervated predominantly those DSGCs that were selective for leftward motion, and similarly for downward- and leftward-pointing SAC dendrites. When analyzing this connectivity pattern from the point of view of the *postsynaptic* GC, Briggman et al. (2011) calculated the distribution of directions of the presynaptic SAC dendrite for all input synapses to a given DSGC. Figure 22.5(B) shows their results: the angular distribution of input dendrites was almost exactly antiparallel to the functionally

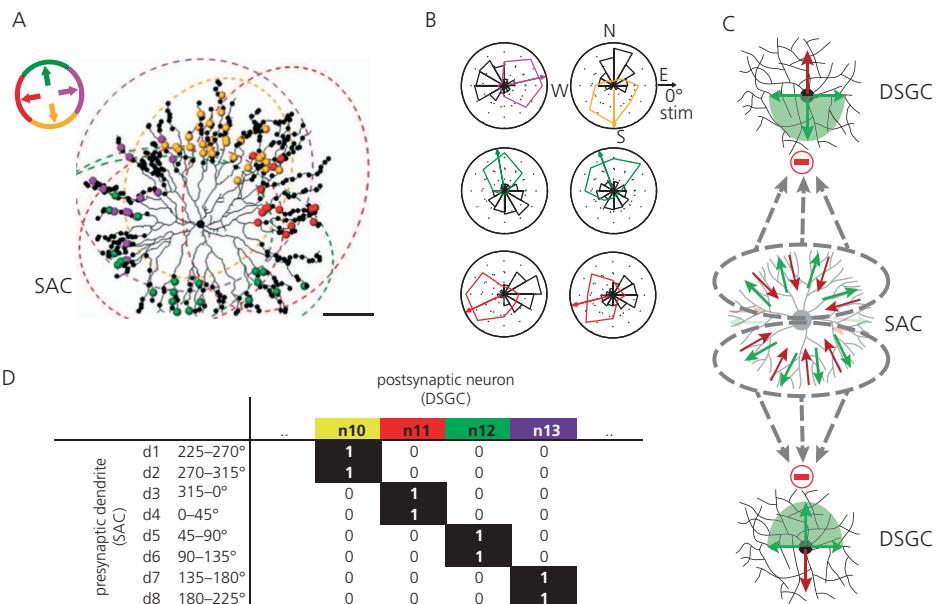


Fig. 22.5 Dendritic connectomics in the mouse retina. Proof of directionally specific neuronal wiring from starburst amacrine cell (SAC) dendrites to direction-selective ganglion cells (DSGCs). **(A)** The output synapses of one SAC (black) are color coded by the directional preference of the respective postsynaptic ganglion cells (yellow, green, red, magenta, for downward, upward, leftward, and rightward motion, respectively). These data (and the population average; Fig. 4b in Briggman et al., 2011) implied that these ganglion cells in fact inherit their directional selectivity from SACs. **(B)** Structural (black) and functional (colors) tuning curves of DSGCs. The distribution of dendritic angles of the presynaptic SAC dendrites (black) is almost exactly antiparallel to the functional tuning curve of the postsynaptic DSGCs (colors as in A). **(C)** Diagram illustrating how a range of directions is suppressed by the inhibitory effect of SAC dendrites that release GABA, generating the null direction in the postsynaptic DSGCs. **(D)** Corresponding dendrite-angle-to-neuron connectome $C_{N,D\text{Seg}}$ (cf. Fig. 22.1). Note that SAC dendrites are presynaptic.

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measured directional preference of the ganglion cells (at an angle of $165.2^\circ \pm 51.7^\circ$ to the preferred direction). Together with the previous finding that dendritic synaptic release is triggered predominantly by visual stimuli moving outward along the SAC dendrites, these data therefore provided a mechanistic explanation of the directional selectivity of DSGCs in mouse retina: SAC dendrites connect with high selectivity to DSGCs of opposite directional preference, thus imposing directional selectivity on the DSGC via their sign-inverting GABAergic release (Fig. 22.5C).

This finding constitutes a strong example of pairwise dendritic connectomics, with wiring specificity implemented at the level of dendrites and thus identifiable in the neuron-to-dendrite connectome $C_{N,D}$ (Fig. 22.5D).

However, the question remains whether the selective wiring of SAC dendrites is a result of the location of their cell bodies relative to the postsynaptic DSGCs or whether there is even higher specificity, where the connectivity of a single SAC dendrite by itself is determined by the directional selectivity wiring rule. Figure 22.6 illustrates these cases: in one case (Fig. 22.6A), only the relative position of the pre- and postsynaptic cell bodies (compared with the functional preference of the postsynaptic DSGC) dictates synaptic connectivity. SAC dendrites closest to the DSGC have the largest dendritic overlap and are thus most easily innervated, i.e., a wiring preference dependent on the relative position of somata alone is sufficient to generate directional selectivity. However, this mechanism implies that SACs positioned at 90° to the preferred direction of the DSGC should connect only weakly—although some of their appropriately oriented dendrites could still contribute to directional selectivity (Fig. 22.6B). The connectivity data (Briggman et al., 2011) (Fig. 22.6C) indeed supported a setting where relative location of the cell body matters, but in addition the preferred direction of individual dendrites for cells with a relative position of 90° is important. This finding is striking because it implies that single SAC dendrites must be able to regulate their synaptic connectivity mostly independently of each other. The mechanisms of such dendritic wiring specificity are yet to be elucidated.

Another key question is how the directionally selective calcium influx in SAC dendrites (and as a likely consequence the directionally selective release of neurotransmitter from dendritic sites) is generated in the first place. Again, a combination of functional data and dendritic connectomics analysis has recently provided substantial evidence for the following mechanistic explanation. Any

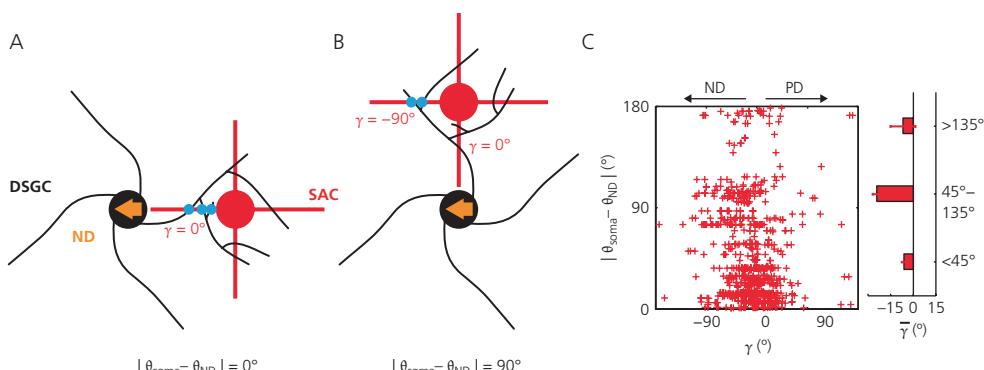


Fig. 22.6 Evidence for single-dendrite wiring specificity in the starburst amacrine cell (SAC) to direction-selective ganglion cell (DSGC) circuit of the mouse retina. (A), (B) Two extreme models of directionally specific wiring: in the soma-dependent setting (A), synaptic connectivity (blue) depends only on the angle between the SAC and DSGC somata compared with the DSGC non-preferred direction (NULL direction, ND). The example shows soma positions for which the SAC-to-DSGC soma vector is parallel to the ND (angle between these $|\theta_{\text{soma}} - \theta_{\text{ND}}| = 0^\circ$). In the dendrite-dependent setting (B) synaptic connectivity (blue) depends only on the angle between the SAC dendrite and the ND of the postsynaptic DSGC. The example shows soma positions for which the SAC-to-DSGC soma vector is orthogonal to the ND (angle between these $|\theta_{\text{soma}} - \theta_{\text{ND}}| = 90^\circ$). (C) Data on the angular wiring dependence from the mouse retina (Briggman et al., 2011): angle γ between the SAC dendrite and the SAC-to-DSGC soma vector for all synaptic contacts and average γ for three ranges of somatic positions (right). Data suggest that in addition to somatic position, the single dendritic angle biases synaptic connectivity (model A is evidenced by the higher density of innervation at around $\gamma = 0^\circ$ and $|\theta_{\text{soma}} - \theta_{\text{ND}}| = 0^\circ$, model B by the slant toward negative γ values).

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geometric position within the two-dimensional layout of the retina directly corresponds to positions in the visual world: photoreceptors collect photons from certain visual angles, which are relayed in a largely topology-preserving fashion to bipolar cells, whose axonal terminals deliver light signals to the postsynaptic ganglion and amacrine cell dendrites (Fig. 22.7A). Since the bipolar cell axons are oriented largely perpendicular to the retinal plane, the position along planar ganglion or amacrine cell dendrites (such as SAC dendrites) can be directly interpreted as a position in visual space.

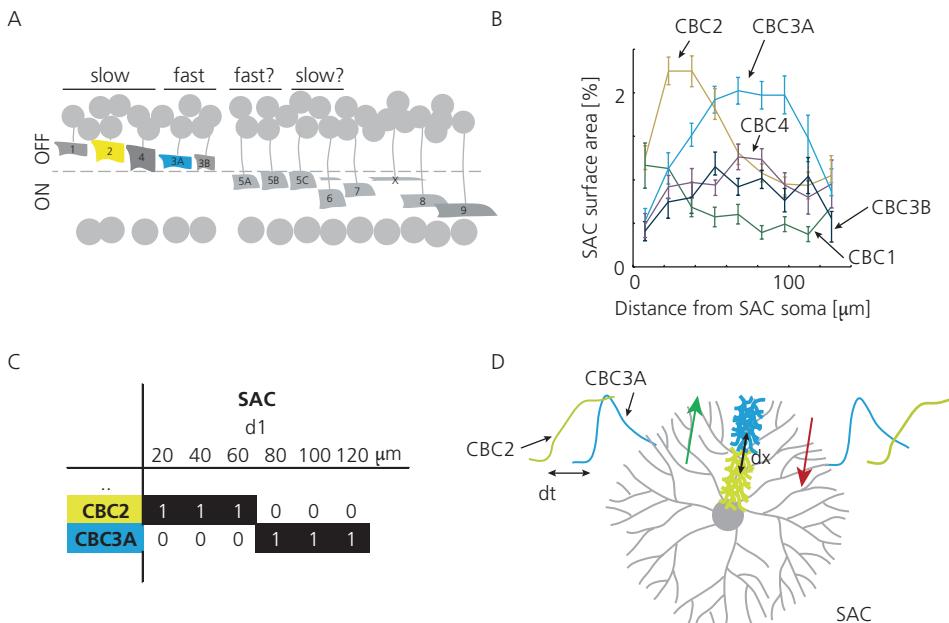


Fig. 22.7 Possible implementation of motion detection by dendritic location-specific wiring in mouse retina. **(A)** Schematic summary of the 13 cone bipolar cell (CBC) types so far known in the mouse retina (10 small-field and 3 wide-field). CBC types 2 and 3A are likely involved in the generation of directionselective OFF signals. "slow" and "fast" indicate the kinetics of ON- and OFF-bipolar cell terminals clustered into functional clusters. Based on the locations in the inner plexiform layer (IPL) at which these kinetics were found, the likely corresponding CBC types were suggested for each functional cluster (Baden et al., 2013). **(B)** Discovery of spatially inhomogeneous bipolar cell contacts along OFF starburst amacrine cell (SAC) dendrites (modified from Kim et al., 2014). **(C)** Corresponding neuron-type-to-dendrite connectome (see Fig. 22.1) $C_{N,D_{\text{seg}}}$. **(D)** Diagram illustrating that the finding of spatially offset CBC inputs (C) together with the Baden et al. data (B) instantiates a Hassenstein-Reichardt type motion detection unit at the input to the SAC dendrite: type 2 CBCs (yellow) with slower and longer-latency release innervate the SAC dendrite (gray) more proximally; while type 3A CBCs (cyan) with faster and short-latency responses innervate the SAC dendrite more distally, establishing a spatial ("dx") and temporal ("dt") offset, yielding an outward-preferred (green arrow) and inward-suppressed (red arrow) directional preference.

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Thus, the outward directional preference of SAC dendrites could be generated by a combination of bipolar cell inputs that are spatially separated on the postsynaptic SAC dendrite and additionally have appropriately distinct release kinetics. Such a model was proposed by Hassenstein and Reichardt (1956) as a basic motion detector. In this model, two signals from photoreceptors at a distance dx sum, one of which has been previously delayed (or temporally filtered with an appropriate time constant). Their sum is passed through a nonlinearity. In that way, only an input sequence that first contains the signal to be delayed, followed by the non-delayed signal, will sum to cross the nonlinearity threshold and generate an output response, while in the opposite direction the non-delayed signal would have subsided before the delayed signal arrived (Fig. 22.7D).

Support for such a motion detection mechanism in the mouse retina came from a recent study (Baden et al., 2013), which systematically sampled synaptic release kinetics by measuring Ca^{2+} -based fluorescence in axon terminals of cone bipolar cells (CBCs) in the mouse retina. This study was able to identify different types of release kinetics and relate their occurrence within the retinal layers to different bipolar cell types. In fact, bipolar cell types 1, 2, and 4 were the likely source of delayed and sustained Ca^{2+} signals, while terminals co-stratifying with the axons of bipolar cell types 3A and 3B showed short-latency but transient responses to stimulation (Fig. 22.7A). Thus, a Hassenstein-Reichardt-type motion detector could be implemented at the input level to SAC dendrites if the bipolar cells provided synaptic input in a properly ordered fashion: “slow” CBCs (such as types 1, 2, and 4) more proximally, “fast” CBCs (such as types 3A and 3B) more distally, for each SAC dendrite.

Kim et al. (2014) took up the challenge of mapping the dendritic connectome of OFF-type SAC dendrites using the same 3D EM dataset that Briggman et al. (2011) had used for identifying the dendritic wiring specificity in the SAC-to-DSGC circuit (Fig. 22.6). With impressive clarity, a differential innervation of SAC dendrites depending on the distance from the SAC soma was found (Fig. 22.7B): while most OFF bipolar cell types showed rather homogeneous contact with SAC dendrites, type 2 bipolar cells showed an inhomogeneous contact pattern with a peak around 30 μm from the soma. Type 3A bipolar cells, in contrast, were biased to more distal contacts with a peak around 80 μm from the soma (Fig. 22.7B,C). So in fact the “slower” type 2 bipolar cells contacted the SAC dendrites more proximally than the “faster” type 3A bipolar cells. Thus, a visual input moving from the branch origin to the tip of a given SAC dendrite would generate a summed input at the distal dendritic release sites, which (assuming Ca^{2+} nonlinearities) could release GABA; while an input moving from the tip to the soma would not (Fig. 22.7D).

Thus, dendritic connectomics has given a likely explanation for how directional selectivity is computed in the mouse retina. While the data still require certain caution (the mechanism is only shown for the OFF, not the ON channel of visual processing; the relationship between functional release properties and CBC types is only correlational; and the differential dendritic innervation was only analyzed by means of fractional contact area, which is correlated with but not uniquely predictive of synaptic connectivity; Helmstaedter et al., 2013), these dendritic connectomics studies illustrate the power of circuit analysis with a focus on dendrites. The study by Briggman et al. (2011) showed specificity at the level of single dendrites, whereas Kim et al. (2014) provide an example of specificity at the level of dendritic subsegments (identifiable in $C_{N,Dseg}$, cf. Fig. 22.7C).

Dendritic connectomics in the cerebral cortex

With these recent successes of dendritic connectomics in the peripheral visual system, the quest for selective dendritic innervation in central parts of the mammalian nervous system has been reinvigorated. For example, to what degree are synaptic inputs to dendrites in the primary sensory cortex spatially clustered (Fig. 22.1D)? Are there other computationally relevant dendritic connectomic patterns (Fig. 22.2)?

The challenge for dendritic connectomics in the cerebral cortex is that of spatial scale. In the mouse retina a cubic volume of about 100 μm on a side contains more than 400 complete bipolar cells and hundreds of local amacrine cells (Helmstaedter et al. 2013). Notably, no single cortical neuron would be completely contained in such a volume of cerebral cortex tissue (Fig. 22.8). Therefore, substantial methodological improvements are required to speed up imaging and data analysis for 3D EM (Helmstaedter, 2013).

In any case, a crucial prerequisite for cortical dendritic connectomics is the ability to uniquely identify synaptic contacts, since unlike in the retina a prediction from the area of touch between an axon and dendrite would not suffice in the cerebral cortex. Full en-bloc staining for large-volume samples has recently been accomplished (Mikula et al., 2012; Hua et al., 2015; Mikula et al., 2015). Figure 22.8(A) shows an example of 3D EM data from the mouse

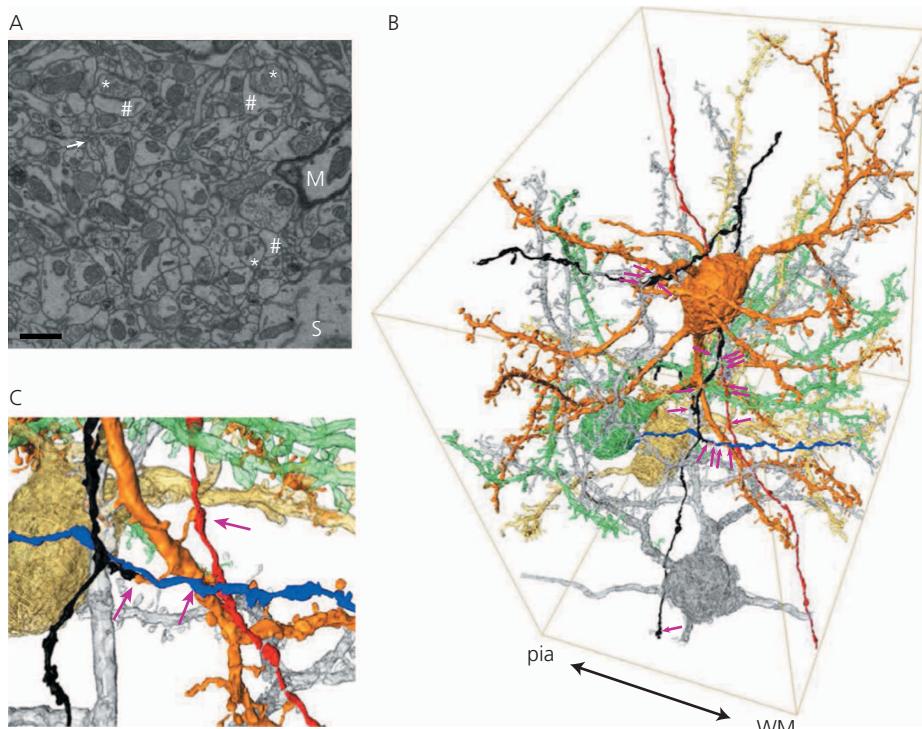


Fig. 22.8 Toward dendritic connectomics in the cerebral cortex. (A) Example data from a 3D EM dataset from L4 of mouse barrel cortex (Boergens et al., in preparation). Presynaptic axonal terminals (*) and postsynaptic dendritic spine heads (#) can be identified. Note the diameter of smallest neurite cross sections (arrow). S, soma; M, myelinated axon. Scale bar = 1 μm . (B), (C) Reconstruction of four spiny stellate neurons in the same dataset (orange, blue, yellow, gray, together with innervating axons in black, red, and blue). Magenta arrows: axon-to-dendrite contacts; part C shows a magnified cutout of three neighboring spines that were innervated by the three reconstructed axons. The combination of skeleton tracings and dense volume segmentation (see Fig. 22.4) allows automated contact detection followed by manual or automated synapse classification. Dataset bounding box in B: 93 $\mu\text{m} \times 60 \mu\text{m} \times 93 \mu\text{m}$.

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somatosensory cortex (Boergens and Helmstaedter, unpublished data), in which synapses can be clearly identified. Since the spines of dendrites are some of the thinnest processes in the cortex (as little as 50 nm in diameter), sufficient image resolution in all three dimensions is critical for cortical dendritic connectomics.

While not yet able to capture entire dendrites, a 3D EM volume of about $(100 \mu\text{m})^3$ contains a large number of substantial stretches of cortical dendrites—and allows the identification of all of their input axons. Figure 22.8(B) (Berning et al., 2015) shows an example of such an analysis, in which the local dendrites of four spiny neurons from L4 of the mouse primary somatosensory cortex were reconstructed together with a set of neighboring innervating axons (Fig. 22.2C; red, black, blue). Combined with the ability to segment fully stained EM image volumes (Berning et al., 2015), this type of dataset allows one to investigate local dendritic connectomic patterns, opening up the search for clustered or systematically distributed synaptic input motifs on cortical dendrites (cf. Figs 22.1 and 22.2). This may serve to finally resolve the conundrum of why exquisite biophysical mechanisms exist in cortical dendrites suited for the local processing of synaptic input (e.g., Schiller et al., 2000; Schiller and Schiller, 2001; Branco and Häusser, 2010; Palmer et al., 2014; and other chapters in this book), while there is conflicting evidence relating to synapse clustering (Jia et al., 2010, 2014).

Outlook: fully annotated dendritic connectomes

Where will dendritic connectomics stand a couple of years from now? Ideally, we would have identified all inputs onto the dendrites of all neurons in a circuit. This would provide us with a complete picture of the structural constraints that contribute to the network's algorithmic properties. In the meantime, the pairwise dendritic connectome of a given neuron, together with functional characterization of the mapped inputs, is essential for an exhaustive examination of its computational contribution to the circuit.

What do we mean by complete mapping? On one extreme, we would be able to identify the exact identity of each presynaptic neuron (Fig. 22.9A). A completely mapped whole-brain connectome, for example, would provide such data. However, the functional meaning of each of the synaptic inputs may still be elusive. Annotation of inputs with kinetic properties, receptive field properties, or sensory modality information may be required to make sense of the computational contribution of the postsynaptic neuron. One option for annotating synaptic inputs without the need to reconstruct an entire brain is to combine light-microscopically mapped inputs with locally dense EM-based circuit reconstruction, such that, for example, the modality of inputs can be mapped for neurons that are known to integrate multisensory inputs (Fig. 22.9B).

Alternatively, however, it may turn out that each neuron's computational meaning can only be examined in the context of its (local or entire) circuit. This would imply that only the higher-order, completely mapped dendritic connectome of many neurons will provide the required insights into the mechanisms of cortical computation.

In any case, there are likely to be decisive developments in dendritic connectomics in the coming years: the structural imaging of volumes at the cubic millimeter scale will become feasible (e.g., Eberle et al., 2015), and correlated imaging approaches that allow one to map functional properties on structural data will become more routine. Whether repeated dense circuit reconstruction combined with exhaustive functional population imaging will become a standard tool in most neuroscience laboratories within the next 5—or 15—years is an open question, which will be dictated by future methodological developments for dendritic connectomics.

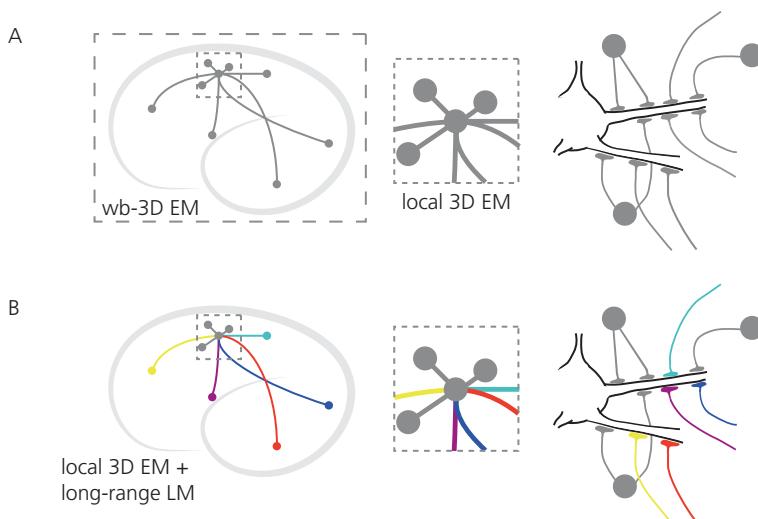


Fig. 22.9 Possible strategies for exhaustive dendritic connectomics: complete input mapping of source neuron location, functional properties, and transmitter types. (A) whole-brain (wb) connectomes contain all required dendritic input maps, but are still technically elusive. Locally dense EM-based reconstruction can map all local inputs onto a dendrite but cannot uniquely identify long-range inputs. (B) As an intermediate step, input sources or functional modalities may be labeled fluorescently. On its own, this labeling cannot elucidate dendritic innervation patterns, but it can potentially be mapped onto the locally dense EM reconstruction by post-hoc alignment, thus annotating the dendritic connectome (right).

References

- Baden T, Berens P, Bethge M, Euler T (2013) Spikes in mammalian bipolar cells support temporal layering of the inner retina. *Current Biology* **23**:48–52.
- Bargmann CI, Marder E (2013) From the connectome to brain function. *Nature Methods* **10**:483–490.
- Barlow HB, Hill RM, Levick WR (1964) Retinal ganglion cells responding selectively to direction and speed of image motion in the rabbit. *Journal of Physiology* **173**:377–407.
- Bock DD, Lee WC, Kerlin AM, Andermann ML, Hood G, Wetzel AW, Yurgenson S, Soucy ER, Kim HS, Reid RC (2011) Network anatomy and in vivo physiology of visual cortical neurons. *Nature* **471**:177–182.
- Berning M, Boergens KM, Helmstaedter M (2015) SegEM: efficient image analysis for high-resolution connectomics. *Neuron*, **87**(6):1193–1206.
- Branco T, Häusser M (2010) The single dendritic branch as a fundamental functional unit in the nervous system. *Current Opinion in Neurobiology* **20**:494–502.
- Branco, T, Clark, BA, Häusser M (2010) Dendritic discrimination of temporal input sequences in cortical neurons. *Science* **329**(5999):1671–1675.
- Briggman KL, Bock DD (2012) Volume electron microscopy for neuronal circuit reconstruction. *Current Opinion in Neurobiology* **22**:154–161.
- Briggman KL, Helmstaedter M, Denk W (2011) Wiring specificity in the direction-selectivity circuit of the retina. *Nature* **471**:183–188.
- Denk W, Horstmann H (2004) Serial block-face scanning electron microscopy to reconstruct three-dimensional tissue nanostructure. *PLoS Biology* **2**:e329.

- Denk W, Briggman KL, Helmstaedter M (2012) Structural neurobiology: missing link to a mechanistic understanding of neural computation. *Nature Reviews Neuroscience* **13**:351–358.
- Eberle AL, Mikula S, Schalek R, Lichtman JW, Tate ML, Zeidler D (2015) High-resolution, high-throughput imaging with a multibeam scanning electron microscope. *Journal of Microscopy* **259**:114–120.
- Euler T, Detwiler PB, Denk W (2002) Directionally selective calcium signals in dendrites of starburst amacrine cells. *Nature* **418**:845–852.
- Gidon A, Segev I (2012) Principles governing the operation of synaptic inhibition in dendrites. *Neuron* **75**:330–341.
- Gollisch T, Meister M (2008) Rapid neural coding in the retina with relative spike latencies. *Science* **319**:1108–1111.
- Hassenstein B, Reichardt W (1956) Systemtheroretische Analyse der Zeit-, Reihenfolgen- und Vorzeichenauswertung bei der Bewegungsperzeption des Rüsselkäfers Chlorophanus. *Zeitschrift für Naturforschung* **11b**:513–524.
- Häusser M, Spruston N, Stuart GJ (2000) Diversity and dynamics of dendritic signaling. *Science* **290**:739–744.
- Hayworth CR, Moody SE, Chodosh LA, Krieg P, Rimer M, Thompson WJ (2006) Induction of neuregulin signaling in mouse Schwann cells in vivo mimics responses to denervation. *Journal of Neuroscience* **26**:6873–6884.
- Hebb DO (1949) *The Organization of Behavior*. New York: Wiley.
- Helmstaedter M (2013) Cellular-resolution connectomics: challenges of dense neural circuit reconstruction. *Nature Methods* **10**:501–507.
- Helmstaedter M, Briggman KL, Denk W (2008) 3D structural imaging of the brain with photons and electrons. *Current Opinion in Neurobiology* **18**:633–641.
- Helmstaedter M, Briggman KL, Denk W (2011) High-accuracy neurite reconstruction for high-throughput neuroanatomy. *Nature Neuroscience* **14**:1081–1088.
- Helmstaedter M, Briggman KL, Turaga SC, Jain V, Seung HS, Denk W (2013) Connectomic reconstruction of the inner plexiform layer in the mouse retina. *Nature* **500**:168–174.
- Hua Y, Laserstein P, Helmstaedter M (2015) Large-volume en-bloc staining for electron microscopy-based connectomics. *Nat Commun.* **6**:7923, doi: 10.1038/ncomms8923.
- Jia H, Rochefort NL, Chen X, Konnerth A (2010) Dendritic organization of sensory input to cortical neurons in vivo. *Nature* **464**:1307–1312.
- Jia H, Varga Z, Sakmann B, Konnerth A (2014) Linear integration of spine Ca^{2+} signals in layer 4 cortical neurons in vivo. *Proceedings of the National Academy of Sciences of the United States of America* **111**:9277–9282.
- Kim JS, Greene MJ, Zlateski A, Lee K, Richardson M, Turaga SC, Purcaro M, Balkam M, Robinson A, Behabadi BF, Campos M, Denk W, Seung HS, EyeWirers (2014) Space-time wiring specificity supports direction selectivity in the retina. *Nature* **509**:331–336.
- Knott G, Marchman H, Wall D, Lich B (2008) Serial section scanning electron microscopy of adult brain tissue using focused ion beam milling. *Journal of Neuroscience* **28**:2959–2964.
- Lichtman JW, Denk W (2011) The big and the small: challenges of imaging the brain's circuits. *Science* **334**:618–623.
- Mikula S, Binding J, Denk W (2012) Staining and embedding the whole mouse brain for electron microscopy. *Nature Methods* **9**:1198–1201.
- Mikula S, Denk W (2015) High-resolution whole-brain staining for electron microscopic circuit reconstruction. *Nature Methods* **12**:541–546.
- Palmer LM, Shai AS, Reeve JE, Anderson HL, Paulsen O, Larkum ME (2014) NMDA spikes enhance action potential generation during sensory input. *Nature Neuroscience* **17**:383–390.

- Rall W (1967) Distinguishing theoretical synaptic potentials computed for different soma-dendritic distributions of synaptic input. *Journal of Neurophysiology* **30**:1138–1168.
- Rall W, Burke RE, Smith TG, Nelson PG, Frank K (1967) Dendritic location of synapses and possible mechanisms for the monosynaptic EPSP in motoneurons. *Journal of Neurophysiology* **30**:1169–1193.
- Schiller J, Schiller Y (2001) NMDA receptor-mediated dendritic spikes and coincident signal amplification. *Current Opinion in Neurobiology* **11**:343–348.
- Schiller J, Major G, Koester HJ, Schiller Y (2000) NMDA spikes in basal dendrites of cortical pyramidal neurons. *Nature* **404**:285–289.
- Segev I (2006) What do dendrites and their synapses tell the neuron? *Journal of Neurophysiology* **95**:1295–1297.
- Takemura SY, Bharioke A, Lu Z, Nern A, Vitaladevuni S, Rivlin PK, Katz WT, Olbris DJ, Plaza SM, Winston P, Zhao T, Horne JA, Fetter RD, Takemura S, Blazek K, Chang LA, Ogundeyi O, Saunders MA, Shapiro V, Sigmund C, Rubin GM, Scheffer LK, Meinertzhagen IA, Chklovskii DB (2013) A visual motion detection circuit suggested by *Drosophila* connectomics. *Nature* **500**:175–181.
- Yoshida K, Watanabe D, Ishikane H, Tachibana M, Pastan I, Nakanishi S (2001) A key role of starburst amacrine cells in originating retinal directional selectivity and optokinetic eye movement. *Neuron* **30**:771–780.

Chapter 23

Linking dendritic processing to computation and behavior in invertebrates

Richard B. Dewell and Fabrizio Gabbiani

Summary

The term “dendrite” originates from the Greek word for “tree.” In this chapter, we summarize results obtained in invertebrate preparations on the dendritic processing of neural information. Connecting the cellular characteristics of neurons to the information processed by neural networks can be extremely difficult, but must ultimately be accomplished in order to build a mechanistic understanding of the nervous system. In invertebrates there has been less investigation specifically into dendritic function, but this research is generally well grounded in the ecological and behavioral context of neural processing. Thus, invertebrate model systems have proven favorable for connecting the function of dendrites to neuronal networks and to behavioral input–output relations, in part because of the compactness of invertebrate nervous systems. Here, we provide a series of vignettes illustrating recent research on invertebrate dendrites and their roles in neural function. While the ecology of the animals and their neural morphology might differ from those of vertebrates, the dendritic mechanisms and neural challenges are identical to those of other animals. Thus, we argue that invertebrate models have much to teach us about how the nervous system functions, including in vertebrates.

Introduction

Trees in a wood possess a network of branches endowed with receptors for light, touch, and moisture, and have an unseen communication network based on chemical and voltage-gated channels allowing them to process information and interact with their surroundings. They can even generate action potentials (APs) (Pickard, 1973; Fromm and Lautner, 2007; Hedrich, 2012). Thus, the analogy noticed early on between trees and neuronal dendrites has turned out to be unexpectedly prescient.

This chapter deals with the dendrites of invertebrates. Giving a comprehensive review of a group comprising more than 95% of all animal species would be impossible, as would be providing a representative sample. Besides, the nervous systems of the vast majority of approximately one million invertebrate species have never been studied. So instead of an exhaustive review, we will explore a sampling of recent research on the dendrites of the 30 or so phyla not covered in other chapters in this book: a brief stroll through invertebrate arbors. While each animal touched upon is engrossing in its own right, we will start by addressing whether invertebrates have anything to teach us about brains in general.

The idea that evolution would discard hard-earned solutions to neural processing and develop entirely new ones in mammals to handle mostly the same challenges seems hard to imagine. Particularly since the components available to the nervous system remain essentially the same across the phyla. From simple Metazoa to humans there is, for instance, high conservation of ion channels, neurotransmitter receptors, and G-protein-coupled second messenger receptor pathways (Bargmann, 1998). Analogies between neural processing pathways and mechanisms across vertebrate and invertebrate species abound (e.g., Laurent et al., 2001; Sanes and Zipursky, 2010). In fact, direct swapping of genes between vertebrates and invertebrates can yield perfectly functioning neural systems (Quiring et al., 1994; Onuma et al., 2002). Due to inherent experimental advantages and the intrinsic similarities between vertebrates and many arthropod and mollusk models, a large fraction of the known neuronal mechanisms underlying nervous system function were first discovered in these “simpler” animals. In addition to the discovery of voltage-gated ion channels in squid (Hodgkin and Huxley, 1952), a long list of mechanisms governing vertebrate brains were originally discovered in invertebrates, including both synaptic facilitation (Pantin, 1936; Wiersma and van Harreveld, 1938) and presynaptic inhibition in crabs (Dudel and Kuffler, 1961), central pattern generators in the locust flight system (Wilson, 1961), lateral inhibition in horseshoe crabs (Ratliff, 1965), the Ca^{2+} dependence of neurotransmitter release in squid (Katz and Miledi, 1967), and the mechanisms of directional motion detection in beetles (Hassenstein and Reichardt, 1956)—later generalized to vertebrates by Adelson and Bergen (1985).

Much of what is known about dendritic processing in insects was expertly reviewed in the previous edition of this book (Laurent and Borst, 2008). We therefore only briefly summarize in the following paragraphs the research surveyed therein and refer the reader to that chapter for a more complete description. In the subsequent sections of this chapter we primarily focus on more recent research.

The dynamic range of a neuron’s output is generally less than that of its inputs, requiring the neuron to compress the input signal. The ability of dendrites to shift their sensitivity based on background activation levels often allows a neuron to encode information about changes in inputs rather than absolute levels, ultimately operating over a much greater dynamic range than the range of its own output. Although the light-sensing portion of photoreceptors may not strictly qualify as a dendrite, how photoreceptors deal with the widely changing light levels they experience over the course of a day provides an elegant example of a neuron shifting its sensitivity to encode information across a wide range of inputs. This problem is particularly acute in broadly active insects and is exacerbated by the fact that they do not possess two types of photoreceptors specialized for low and high light levels like the rods and cones of vertebrates. In the transition from day to night vision, blowfly photoreceptors experience over a thousand-fold decrease in light and a corresponding decrease in phototransduction-activated conductance. To compensate for this, blowflies change their light sensitivity by decreasing the K^+ conductance localized in the transducing region of their photoreceptors a hundred-fold (Laughlin, 1996; Song et al., 2012). As a result, blowfly photoreceptors can encode visual information based on relative differences in light in the environment irrespective of the mean light level. In addition, it has been clear for quite some time that differences in K^+ channels characteristics depend on fly ecology and flight behavior. Diurnal flies have prominent non-inactivating delayed rectifier K^+ channels, while night flyers possess rapidly inactivating ones (Laughlin and Weckstrom, 1993). Delayed rectifier channels open with depolarization and produce an outward current hyperpolarizing the neuron back toward rest. When these K^+ channels are active, the phototransduction currents generate smaller excitatory postsynaptic potentials (EPSPs). As a result, the extent of K^+ channel activation changes the impact of a given phototransduction current on membrane voltage. In locusts, which fly during both day and night,

this change in photoreceptor “gain” is implemented by changing the ratio of inactivating to sustained K⁺ conductances, following a circadian rhythm likely mediated by serotonin (Hevers and Hardie, 1993; Cuttle et al., 1995).

Also from the fly visual system comes an example of the inherent connection between dendritic morphology and neural function. In flies, computing a measure of image velocity depends in part on the specific dendritic morphology of lobula plate tangential cells (Single and Borst, 1998; Haag et al., 2004), as expected from theoretical considerations (Egelhaaf et al., 1989). In these neurons, dendritic gain has also been suggested to depend on the dendritic colocalization and coactivation of excitatory and inhibitory synapses (Borst et al., 1995). In an example of dendritic processing inspired by analog vision chip designs (Koch, 1989), a circuit was described for detecting objects moving relative to the background, in which three neurons with dendro-dendritic gap junctions effectively subtract a blurred image from the original to act as a spatial high-pass filter (Dürr and Egelhaaf, 1999; Haag and Borst, 2002; Cuntz et al., 2003; Farrow et al., 2003). The connection between dendritic structure and function is also exemplified within the cricket cercal system, where sensing of wind direction requires careful positioning of dendrites within the sensory afferent neuropil (Jacobs and Miller, 1985; Miller et al., 1991) (for a more recent update on this system, see Ogawa et al., 2008).

In locusts, non-spiking interneurons manage to maintain a linear relationship between synaptic current into and out of their dendrites through highly nonlinear processes. Thus, a sigmoidal relationship between membrane voltage and synaptic output is offset by a reciprocally matched relationship between membrane voltage and input resistance. This is caused at one end of the spectrum by a high density of delayed rectifier and A-type K⁺ channels that open close to the resting membrane potential, and by voltage-gated Ca²⁺ channels that open at depolarized potentials at the other end (Laurent 1990, 1993; Laurent et al., 1993).

An example of neural coincidence detection has been described in the locust olfactory system, a topic that will be further addressed in the crayfish escape circuitry later in this chapter. Locust second-order olfactory projection neurons are synchronized by common GABAergic input, allowing for fine odor discrimination (MacLeod and Laurent, 1996; Stopfer et al., 1997). This synchronization is a prerequisite for coincidence detection at the next neuronal stage, namely Kenyon cells of the mushroom body. The information extracted by Kenyon cells is then most likely conveyed to higher-order olfactory centers through the precise timing of their spikes (Gupta and Stopfer, 2014). Thus, desynchronization of the projection neurons modifies odor tuning of high-order olfactory neurons by changing the spike timing but not the spiking rate of Kenyon cells (MacLeod et al., 1998). This coincidence detection has been shown to be produced by the active dendritic properties of the Kenyon cells (Perez-Orive et al., 2002, 2004).

In the following sections we present a complementary collection of examples demonstrating dendritic processes within insects, arachnids, crustaceans, and leeches. As the task of a neural circuit is always specific to the sensory information, motor demands, and ecology of the animal, we will briefly describe this context for each case. While the ecology and the morphology of the animals might differ greatly from our own, the themes of signal filtering, information transfer, compartmentalization of voltage and Ca²⁺, and developmental plasticity undoubtedly remain common across species.

Fly motoneuron dendrites

Since the work of Ramón y Cajal (1995), neuron types have been defined by the appearance of their dendritic arborizations. This implicitly suggests that neurons must regulate dendritic morphology

and that dendritic structure is key to differences in function between neuron types. This idea is reinforced by the fact that many neurological and cognitive disorders are associated with altered dendritic morphology (Kaufmann and Moser, 2000; Kulkarni and Firestein, 2012). Yet much is still unknown about the development of dendritic arbors, and little direct evidence is available on how changes in dendritic morphology affect neural function. The combination of genetic tools and well-described identified neurons make fruit flies an attractive model for addressing these questions. A recent investigation into the development of *Drosophila* motoneurons that innervate the flight muscles and possess complex dendritic arborizations sheds some light on this issue and yields an unexpected result.

Like other holometabolous insects, fruit flies undergo a spectacular developmental change in the metamorphosis from larva to adult. This dramatic change in body plan and behavior must be accompanied by matching changes in the nervous system. Over 4 days, a larva pupates and emerges with newly formed legs and wings. One of the first noticeable features of the adult fly's nervous system is the presence of a pair of giant fibers (GFs), which, while only about 7 μm in diameter, are truly giant relative to the rest of the fly (Power, 1948). The GFs form mixed electrical and chemical synapses onto both motoneurons innervating the leg muscles used to propel the animal off the ground and an interneuron that activates flight muscles (Fig. 23.1A) (King and Wyman, 1980; Tanouye and Wyman, 1980). A single spike within a GF produces a stereotyped escape response with a jump followed by initiation of flight (Thomas and Wyman, 1983; von Reyn et al., 2014). The dorsal longitudinal wing depressor muscles (DLMs) excited by this escape circuit are innervated by motoneurons MN1–5 (Fig. 23.1A) (Coggshall, 1978; Ikeda and Koenig, 1988). The DLMs are asynchronous muscles: by contracting 5–20 times a second during flight, they produce wing beats up to 200 times per second, and are used in courtship singing in addition to flight (Levine and Wyman, 1973; Ewing, 1977).

The larval motoneurons MN1–4 innervate muscles of the dorsal mesothoracic body wall that develop into DLMs during metamorphosis (Costello and Wyman, 1986; Fernandes and Vijayraghavan, 1993), while the GFs and MN5 are differentiated but non-functional in *Drosophila* larvae (Phelan et al., 1996; Sun and Wyman, 1997; Allen et al., 1998). This requires MN1–4 to first retract larval dendrites and then MN1–5 to grow adult dendrites (Consoulas et al., 2002). Just 2 h after formation of the puparium, the larval dendrites from MN1–4 retract, and after about 20 h dendritic outgrowth of the pupal arborizations begins in MN1–5 (Consoulas et al., 2002). From this point on until eclosion, the soma of MN5 increases dramatically in size and the dendrite develops a complex and stereotyped arbor with 23 primary branches forming non-overlapping subfields with a total length of 6.5 mm and 4,000 branches (Fig. 23.1B) (Vonhoff and Duch, 2010).

In moths that do not possess GFs, MN5 is functional in larvae and follows the same developmental plan as MN1–4 (Casaday and Camhi, 1976; Duch and Levine, 2000; Duch et al., 2000). During moth metamorphosis, the increase in soma size and complex arborization of MN5 is similar to that of *Drosophila* (Liberat and Duch, 2002). The intracellular recordings from MN5 in moths have revealed that the electrical properties of the dendrites also change, with increased K^+ and Ca^{2+} conductances in the adult neuron (Duch and Levine, 2002). The active properties of the dendrites may well be different between species, however, as wing muscles act synchronously in moths, requiring that MN5 switches to faster activity (Rheuben and Kammer, 1980).

The development of new dendrites in these neurons requires activator protein 1 (AP-1) and depends on neural excitability (Hartwig et al., 2008). AP-1 is a heterodimer of Fos- and Jun-class transcription factors that binds to specific DNA sequences, thereby regulating transcription. Activating AP-1 in MN5 *in vivo* increased dendrite volume while inhibiting AP-1 decreased dendritic outgrowth, confirming a key role for this transcription factor in the increased arborization during pupation. Fos levels increased transiently with increased neural activation, while increasing

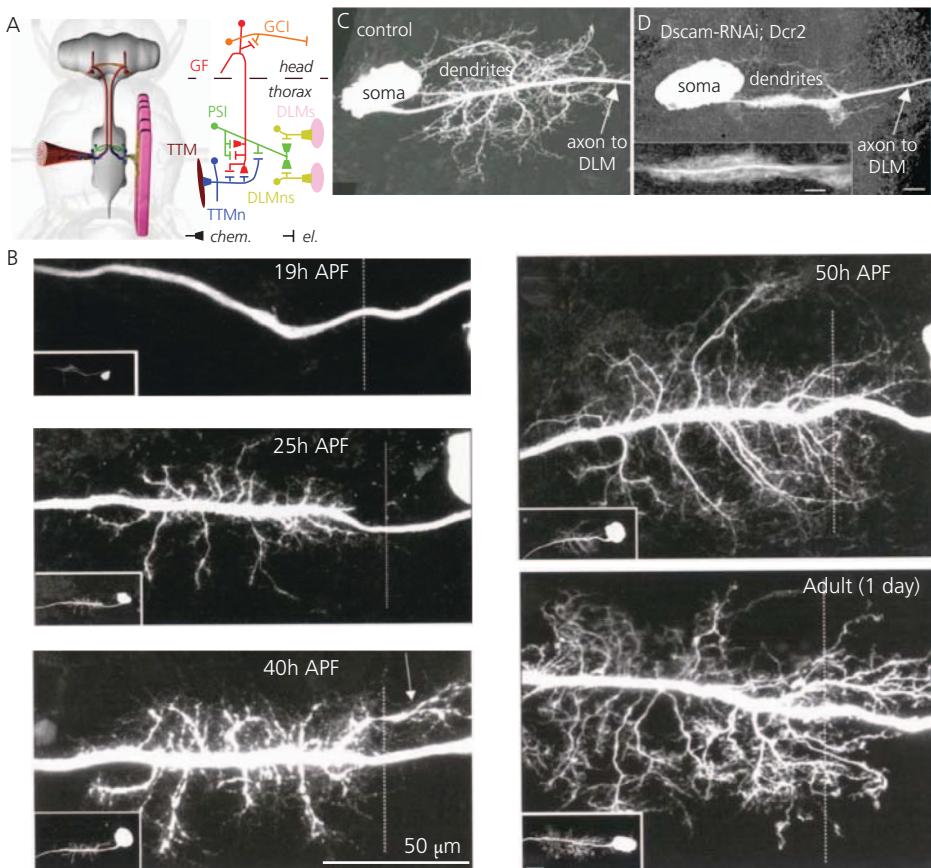


Fig. 23.1 The dorsal longitudinal motoneuron MN5, its connections, dendritic manipulation and normal development. **(A)** Left: illustration of the position of the giant fiber (GF) system within the fly's head and thorax. Colors match those on right. Right: partial schematic of the *Drosophila* GF circuitry. In the brain, the GFs form electrical synapses with the giant commissural interneurons (GCIs, orange). In the thoracic ganglion, the GFs form a mixed electrochemical synapse with the motoneurons (TTMns, blue) of the tergotorchanteral muscle (TTM) and with the peripherally synapsing interneuron (PSI, green). The PSI forms chemical synapses with the motoneurons (DLMns, MN1–5) that project to the dorsal longitudinal wing depressor muscles (DLMs, pink). Abbreviations: *chem.*, chemical synapses; *el.*, electrical synapses. **(B)** Normal developmental sequence of MN5 dendrites between the early pupa, 19 h after puparium formation (APF), and the adult (compare with C). Insets are micrographs taken at lower magnification including cell bodies. The anterior pole is at the top and the dotted line indicates the body midline. Scale bar in bottom right inset = 50 μm. **(C)** Micrograph of the dendrites of MN5 after normal development. **(D)** Disruption of MN5 dendritic development following RNAi knockdown of Dscam1. Scale bar = 10 μm and 5 μm (inset).

Part A reprinted from *Seminars in Cell & Developmental Biology*, 17(1), Marcus J. Allen, Tanja A. Godenschwege, Mark A. Tanouye, and Pauline Phelan, Making an escape: development and function of the *Drosophila* giant fibre system, p. 33, Figure 1a and b, Copyright 2006, with permission from Elsevier. Part B reproduced from Christos Consoulas, Linda L. Restifo, and Richard B. Levine, Dendritic remodeling and growth of motoneurons during metamorphosis of *Drosophila melanogaster*, *The Journal of Neuroscience*, 22(12), p. 4914, Figure 6 a–i © 2002, The Society for Neuroscience. Parts C and D reproduced from Stephanie Rglewski, Dimitrios Kadas, Katie Hutchinson, Natalie Schuetzler, Fernando Vonhoff, and Carsten Dusch, Dendrites are dispensable for basic motoneuron function but essential for fine tuning of behavior, *Proceedings of the National Academy of Sciences of the United States of America*, 111(5), p. 18,050, Figure 1, © 2014, National Academy of Sciences.

excitability of cultured motoneurons leads to increased dendrite growth (Hartwig et al., 2008). In a similar experiment, Duch et al. (2008) changed the excitability of MN5 by altering K⁺ conductance and found that increased excitability caused increased dendritic branching while decreased excitability produced longer dendritic branches.

The complexity and small inter-animal variability of MN1–5 dendritic structures suggest that these arborizations are key components in their function. For this reason it was surprising that in *Drosophila* GF-invoked escape, flying, singing, or courtship wing display do not require the dendritic arbors of these neurons. This could be demonstrated by RNAi knock-down of Dscam1 (Down syndrome cell adhesion molecule 1), a protein required for dendritic growth and branching in MN1–5 (Hutchinson et al., 2014). Dscam1 knock-down within MN1–5 reduced dendritic arbors by 90% (Fig. 23.1C, D), but in these animals presynaptic inputs still targeted the neurons correctly and the animals were able to perform the basic behaviors listed above, which each involve the DLMs. Further, voltage-clamp experiments revealed that the active conductances present within the motoneurons remained within a normal range despite removal of most of the dendritic arbor (Ryglewski et al., 2014). In the mouse retina, it had been previously reported that maintaining a complex dendritic architecture was not necessary for correct synaptic partner matching (Fuerst et al., 2009). Further behavioral experiments revealed that the complex arbors of MN1–5 are indispensable. RNAi knock-down of Dscam1 in flies disrupts fine motor control and optomotor flight stabilization, but the clearest effect was in courtship. Males were able to use the wing muscles to produce mating songs, but successful mating decreased markedly as the size of the dendritic arborization decreased (Ryglewski et al., 2014).

Courtship display is just one of the many differences in behavior which must at some level result from differences in the nervous system in males and females. Another recently discovered developmental mechanism in *Drosophila* demonstrated how the fruitless transcription factor in males causes changes in dendritic morphology, acting as a bi-directional circuit switch that produces differences in olfactory processing of the sex pheromone 11-cis-vaccenyl acetate (cVA; Fig. 23.2A, B). Both first- and second-order olfactory neurons in males and females respond similarly to cVA (Kurtovic et al., 2007; Datta et al., 2008), but the third-order olfactory neurons that extend dendrites to receive this olfactory input differ between the sexes (Fig. 23.2C, D). Datta et al. (2008) suggested that male-specific changes to axonal terminals of projection neurons produce these sex-specific behaviors. Expression of the male-specific transcription factor in just the higher-order olfactory neurons of females, however, was sufficient to produce a change in dendritic morphology, effectively switching which behavioral circuit the sex pheromone activated (Kohl et al., 2013). In males, activation of the cVA pheromone receptor leads to activation of motor circuitry inhibiting courtship, while in females it activates a different motor circuit that promotes mating receptivity. This provides another example of the relationship between dendritic morphology and neural function.

Dendritic growth in Kenyon cells—role in learning in social insects

The work in fruit flies described above demonstrates how changes in developmental stage- and sex-regulated dendritic morphology affect behavior, but dendritic morphology can also change in response to experience and behavioral demands. A key example comes from honeybees and other social insects that grow elaborate dendritic arbors to meet new demands for navigation and memory. The dendritic growth occurs in the mushroom bodies (MBs), which are structures involved in multimodal sensory integration and spatial orientation, as well as learning and memory (Erber

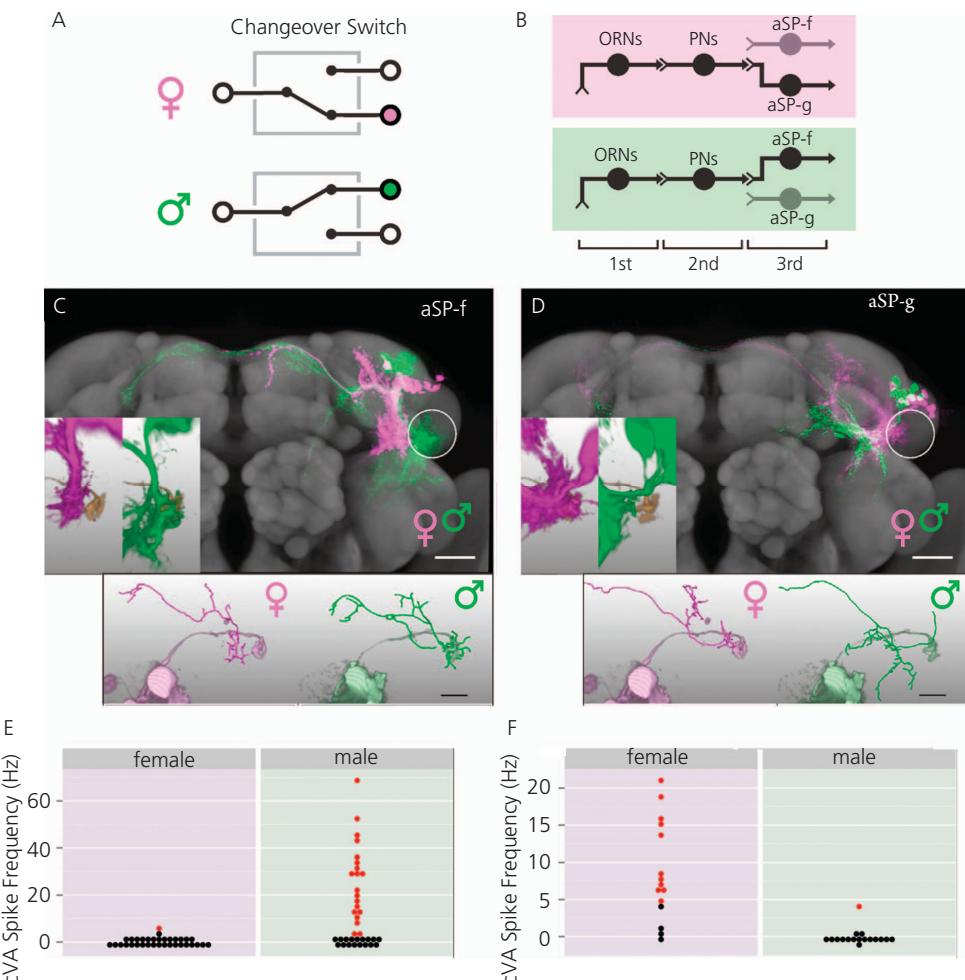


Fig. 23.2 Pheromone-mediated switch in dendritic growth pattern of third-order olfactory neurons in *Drosophila*. **(A)** Schematics of the connection switch occurring between second-order antennal lobe projection neurons (PNs) and third-order lateral horn neurons (LHNs). **(B)** Corresponding circuit model, illustrating first-order olfactory receptor neurons (ORNs) processing the male pheromone 11-cys-vaccenyl acetate (cVA) in males and females and their second- and third-order postsynaptic targets. aSP-f and aSP-g: the third-order LHNs coding for cVA in males and females, respectively. **(C–D)** Top: aSP-f and aSP-g neuron clusters with dendritic localization in the ventral lateral horn indicated by the white circle. Insets: spatial relationship between aSP-f and aSP-g dendrites in males (green) and females (magenta) and PN axonal terminals (ochre). Bottom: single aSP-f- and aSP-g-filled processes in males (green) and females (magenta) in relation to PN terminals (pale magenta or green). Scale bars: 25 μ m. **(E–F)** Responses of aSP-f (**E**) and aSP-g neurons (**F**) to cVA in males and females. Each dot represents one neuron with either significant (red) or non-significant changes in the firing rate to cVA.

Reprinted from *Cell*, 155(7), Johannes Kohl, Aaron D. Ostrovsky, Shahar Frechter, and Gregory S.X.E. Jefferis, A bidirectional circuit switch reroutes pheromone signals in male and female brains, p. 1612, Figure 1a–f, Copyright © 2013 The Authors. Published by Elsevier Inc.

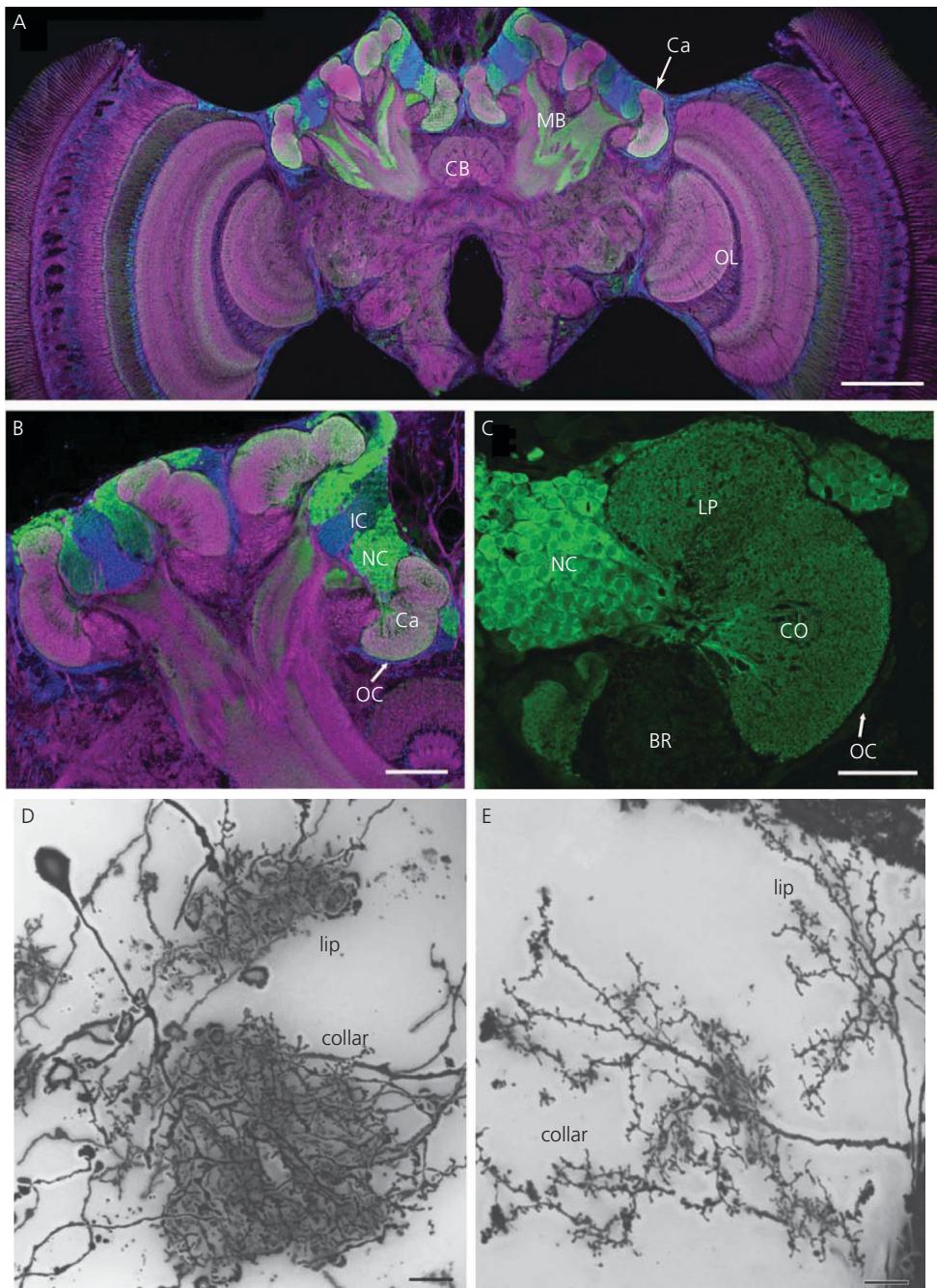


Fig. 23.3 Localization of phosphorylated CaMKII (pCaMKII) and dendritic remodeling in hymenopteran mushroom bodies. **(A)** Frontal section through the honeybee brain showing the location of the mushroom bodies (MBs), central body (CB) and optic lobes (OLs). Staining for pCaMKII is shown in green, cell nuclei are marked in blue and neuropils in magenta. Scale bar = 300 µm. **(B)** Close-up view of the MB showing that the three types of Kenyon cell (KC) bodies stain differentially

and Menzel, 1980; Heisenberg et al., 1985; Heisenberg, 1989; Davis, 1993; Zars, 2000; Menzel and Giurfa, 2006). The MBs in Hymenoptera, including bees, wasps, and ants, receive strong olfactory and visual projections, along with other sensory input (Mobbs, 1982; Gronenberg, 2001). In honeybees, the MBs contain more than 40% of all neurons in the brain and have been shown to be key to behavioral plasticity (Chapman, 1998; Strausfeld, 2002; Rössler and Groh, 2012). In some insect species, neurogenesis continues into adulthood, but this is not the case for bees.

Each MB possesses a calyx subdivided into three regions, the lip, collar, and basal ring (Fig. 23.3A–C). Within each region, neurons intrinsic to the MB, called Kenyon cells (KCs), receive different inputs (Laurent and Naraghi, 1994). The lip processes olfactory information originating from second-order olfactory neurons called projection neurons in the antennal lobe (Gronenberg, 2001; Fahrbach, 2006). The collar receives bilateral visual information from the optic lobes (Mobbs, 1982; Gronenberg, 2001). Both the antennal and optic lobes innervate the basal ring (Gronenberg, 2001). KCs are small and numerous, with a bilateral total of about 5,000 in *Drosophila*, 100,000 or more in locusts and cockroaches, and about 500,000 in the honeybee. KCs fire sparsely with high specificity, which is believed to underlie their role in memory formation.

Many social hymenopterans spend the initial period of adulthood inside the colony before transitioning to an outside foraging role. Honeybees, for example, spend the first 2–3 weeks of adult life inside the dark hive acting as nurse bees, followed by a transition to foraging for food outside (Lindauer, 1961; Seeley, 1982). A brief period of short orientation flights occurs at the start of the behavioral transition (Winston, 1987; Robinson, 1992). The transition from nursing to foraging duties requires a completely different behavioral repertoire, producing major changes in sensory input, in particular visual and olfactory stimuli. In addition, the animals must quickly become skilled in novel learning and memory tasks. Honeybee foragers become able to navigate to feeding sites up to 4 km away and remember those sites, communicating the site locations to other bees through a characteristic dance.

This behavioral transition brings about large increases in neuropil volume within the regions of the MBs that process the new sensory information. Social insects with demonstrated increases in MB volume include honeybees (Withers et al., 1993; Durst et al., 1994; Fahrbach et al., 1998), carpenter ants (Gronenberg et al., 1996), desert ants (Kühn-Bühlmann and Wehner, 2006; Stieb et al., 2010), and paper wasps (O'Donnell et al., 2004). The increase in volume can be attributed

◀
Fig. 23.3 (Continued)

for pCaMKII: while non-compact (NC) KC bodies are brightly stained, inner compact (IC) and outer compact (OC) cells bodies are only weakly stained. Ca, calyx of the MB. Scale bar = 100 µm. (C) MB calyx subcompartments lip (LP), collar (CO), and basal ring (BR), as well as adjacent NC KC cell bodies stained for pCaMKII (compare with B). Note, the BR is unstained for pCaMKII. Scale bar = 40 µm. (D–E) Golgi impregnated KCs from the lip and collar MB region in a forager (D) and worker wasp (E), respectively. Scale bars = 10 µm.

Parts (A–C) adapted from CaMKII is differentially localized in synaptic regions of kenyon cells within the mushroom bodies of the honeybee brain, Elisabeth Pasch, Thomas Sebastian Muenz, and Wolfgang Rössler, *Journal of Comparative Neurology*, 519(18), p. 3706, Figure 3, Copyright © 2011 Wiley-Liss, Inc. Parts D and E reprinted from *Neurobiology of Learning and Memory*, 92(4), Theresa A. Jones, Nicole A. Donlan, and Sean O'Donnell, Growth and pruning of mushroom body Kenyon cell dendrites during worker behavioral development in the paper wasp, *Polybia aequatorialis* (Hymenoptera: Vespidae), p. 487, Figure 1a–d, Copyright 2009, with permission from Elsevier.

to growth of KC dendrites, resulting in dramatically increased length and branching in the visual collar and olfactory lip regions of the MB calyx of bees and wasps (Fig. 23.3D, E) (Farris et al., 2001; Jones et al., 2009; Groh et al., 2012). In wasps, dendritic branching increased by about 100% in the lip region and about 50% in the collar region from in-nest animals to foragers. Remarkably, a single orientation flight is able to increase KC dendritic spine volume in honeybees (Brandon and Coss, 1982). The overall density of dendritic spines, however, remains unchanged after the dendritic growth (Farris et al., 2001; Jones et al., 2009).

This dendritic growth depends in part on the new sensory information gained by leaving the hive or nest, and is not just triggered by aging. Bees induced to forage precociously undergo an expansion in MB volume comparable to that observed in foragers of normal age (Withers et al., 1993; Durst et al., 1994). Comparison of age-matched bees revealed foraging experience-dependent growth of collar dendrites in addition to a marked age-dependent increase in dendritic growth (Farris et al., 2001). In desert ants, light exposure led to an increased neuropil volume in the visual collar region of the MB, even in young ants long before the natural transition to outside foraging (Stieb et al., 2010, 2012). Using honeybees, Scholl et al. (2014) recently examined the relative influence of light exposure and hormones that are associated with the behavior transition (Bloch et al., 2002). They found that early light exposure produced increased neural plasticity, both directly and through a putative positive feedback on hormone-induced changes. Plasticity in MBs dependent on sensory activity is not limited to foraging Hymenoptera. In *Drosophila*, the activity level of olfactory projection neurons drives structural changes in the olfactory lip region of the MB (Kremer et al., 2010).

Sensory projection neurons, which provide the only cholinergic input to the MB calyx, appear to cause the increased dendritic growth after bees leave the nest by activating muscarinic acetylcholine receptors (mAChRs) (Huang and Knowles, 1990; Yasuyama et al., 1995). Honeybees with 1 week of foraging experience that were then given either a second week of foraging or treated with the muscarinic receptor agonist pilocarpine displayed equal increases of MB neuropil (Ismail et al., 2006). Increased dendritic complexity of collar KCs similar to that produced by foraging experience was also induced by pilocarpine, and inhibited by blocking muscarinic receptors with scopolamine (Dobrin et al., 2011). Cholinergic antagonists in the MB can also produce memory impairment (Lozano et al., 2001).

Although, the signaling pathway that connects activation of mAChRs to dendritic growth is uncertain, it is likely that calcium/calmodulin-dependent kinase II (CaMKII) is involved. CaMKII has been associated with learning in vertebrates (Soderling, 1993) and invertebrates (Kamikouchi et al., 2000). CaMKII constitutes 2% of the total protein mass in the rat hippocampus (Erondu and Kennedy, 1985). In honeybees, Ca^{2+} is necessary for protein-synthesis-dependent long-term memory (Perisse et al., 2009), possibly through a CaMKII pathway as is thought to be the case for mammals (Mayford, 2007). While it is found throughout the pupal brain, in adult honeybees the active, phosphorylated form of CaMKII (pCaMKII) is localized within the dendrites of lip and collar KCs, matching where the foraging transition-evoked dendritic growth occurs. Conversely, pCaMKII protein is absent from the inner compact KCs associated with the multimodal basal ring, which do not exhibit the dramatic increase in dendritic branching accompanying foraging experience (Pasch et al., 2011) (Fig. 23.3A–C). CaMKII is also likely to be involved in large-scale dendritic remodeling during metamorphosis in the holometabolous moth *Manduca sexta* (Burkert and Duch, 2006).

Thus, within social insects a picture emerges whereby a sharp behavioral transition to foraging requires the ability to navigate long distances and remember precise locations of home and food sources. This requirement is met with an increased dendritic complexity within the cells that

integrate the visual and olfactory information being learned. The changes are induced by the new sensory input itself through cholinergic activation of CaMKII-regulated plasticity, thus ensuring that dendritic growth occurs in synchrony with the need for the new cognitive abilities.

Dendritic processes enhance auditory discrimination in Orthoptera

A primary auditory discrimination task for singing orthopteran insect species like crickets or katydids consists in recognizing the mating calls of conspecifics and distinguishing them from self-generated calls. In addition, orthopteran insects such as grasshoppers need to detect predatory calls, primarily bat ultrasound. Here, we describe some recent research on the roles of dendritic processes in these auditory tasks. Specifically, the role of tuning neuronal frequency selectivity to species-specific calls, the use of corollary discharge to discriminate self-generated from extrinsic calls, and stimulus-specific adaptation that could attenuate responses to conspecific calls without any attenuation to predatory calls.

The auditory system of orthopterans is among the best-studied invertebrate sensory systems, especially in crickets and grasshoppers (for reviews see Boyan, 1984; Schildberger, 1988; Lewis, 1992). Crickets communicate with stereotyped patterns of sound pulses (Gerhardt and Huber, 2002), with each species being recognizable by the temporal pattern of their calling songs (Pollack and Hoy, 1979). Males “sing” or stridulate by rubbing their forewings together. Cricket song can be produced continuously for hours with a high sound intensity (Nocke, 1972). Crickets’ ears are located on their forelegs and are therefore exposed fully to the self-generated sounds (Fig. 23.4A). About 60 auditory afferent neurons project to local and ascending interneurons in the auditory neuropil of the prothoracic ganglion (Michel, 1974). One of these identified auditory local interneurons is the omega neuron 1 (ON1) (Schildberger et al., 1989).

There are two ON1 neurons, one for each ear (they are named for their characteristic shape; Fig. 23.4B). Each ON1 receives inputs from ipsilateral auditory afferents and mutually inhibits the contralateral ON1 (Imaizumi and Pollack, 2005; Selverston et al., 1985). Additionally, ON1s inhibit contralateral ascending auditory interneurons (Selverston et al., 1985; Horseman and Huber, 1994; Faulkes and Pollack, 2000), which transmit auditory information to the brain (Wohlers and Huber, 1982). This inhibition is hypothesized to enhance bilateral contrast to improve sound localization (Wiese and Eilts, 1985; Nabatianyan et al., 2003). The dendritic processing within these neurons was recently examined by Baden and Hedwig (2006).

Fast Ca^{2+} imaging allowed for an analysis of the spatio-temporal pattern of Ca^{2+} activation resulting from acoustic stimulation in ON1 (Fig. 23.4C). Earlier work (reviewed in the previous edition of this book; Laurent and Borst, 2008) described a Ca^{2+} -dependent hyperpolarization through a Ca^{2+} -activated K^+ conductance (see Sah and Faber, 2002) that resulted in “forward masking,” a phenomenon by which loud stridulation calls inhibit responses in ON1 to quieter sounds (Sobel and Tank, 1994). Acoustic stimulation caused rapid influx of Ca^{2+} within both the dendritic and axonal arbors in synchrony with chirp pulses, but not in the axon segment connecting these arbors, suggesting independent sites of Ca^{2+} entry. Particularly strong Ca^{2+} signals at the spike generation zone are the most likely source of activation for the Ca^{2+} -dependent K^+ channels responsible for forward masking and noise filtering (Baden and Hedwig, 2006). A tonotopic map within the dendrites was revealed by Ca^{2+} gradients to acoustic stimuli of different frequencies, with higher-frequency sound generating inputs on the most distal dendrites. Ca^{2+} within the axonal arbor had no such mapping, with the largest Ca^{2+} signals produced by a narrow range of sound centered at about 5 kHz, the carrier frequency of the species’ song.

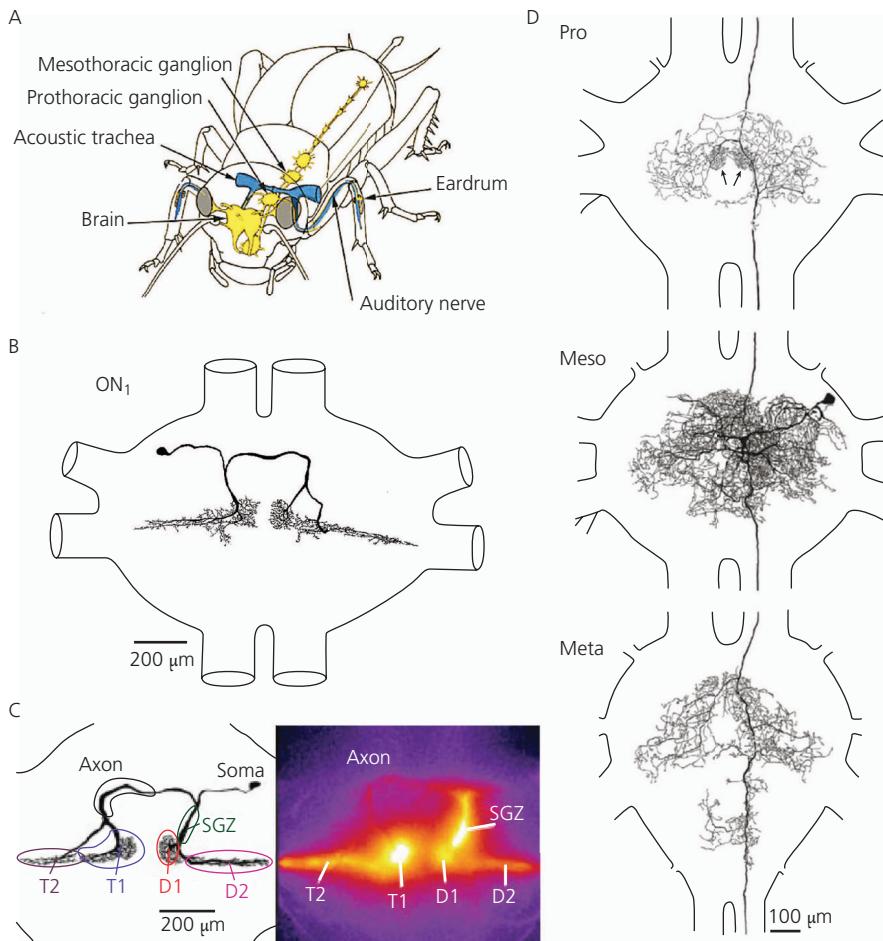


Fig. 23.4 Processing of auditory information in crickets. (A) Schematics of a female cricket with its central nervous system in yellow and acoustic trachea in blue. The main components of the auditory system are indicated by arrows. (B) Reconstruction of the omega neuron 1 (ON1) in the prothoracic ganglion. (C) Left: functional organization of the ON1. Regions highlighted are the two main branches of the axonal terminals (T1 and T2), the two branches of the dendrite (D1 and D2), the spike-generating zone (SGZ), the axon, and the soma. Right: relative distribution of Ca²⁺ fluorescence changes after 1 s of acoustic stimulation. (D) Reconstruction of one of the corollary discharge interneurons (CDIs) in the thoracic ganglia of the cricket. In the prothoracic ganglion (Pro), the CDI makes axonal arborizations where the terminals of primary auditory afferent axons and the dendrites of auditory interneurons, including the ON1, are located (arrows).

Parts A and D reprinted from *Trends in Neurosciences*, 30(1), James F.A. Poulet, and Berthold Hedwig, New insights into corollary discharges mediated by identified neural pathways, p. 16, Figure 2d, Copyright 2006, with permission from Elsevier. Part B adapted *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, 146(2), Processing of sound signals by six types of neurons in the prothoracic ganglion of the cricket, *Gryllus campestris* L., David W. Wohlers and Franz Huber, pp. 161–173, Figure 2, Copyright © 1982, Springer-Verlag. With permission from Springer Science and Business Media. Part C reproduced from Neurite-specific Ca²⁺ dynamics underlying sound processing in an auditory interneurone, T. Baden and B. Hedwig, *Journal of Neurobiology*, 67(1), pp. 68–80, Figure 1a-f, Copyright © 2006 Wiley Periodicals, Inc.

Crickets' ears remain fully sensitive to external sounds during singing (Poulet and Hedwig, 2001), and therefore the central nervous system receives a massive influx of auditory, proprioceptive, and mechanoreceptive information. Crickets maintain auditory sensitivity during singing by inhibiting their central auditory pathway with a corollary discharge in phase with sound production (Poulet and Hedwig, 2003). This corollary discharge guarantees that auditory inputs to ON1 originating from the cricket's own song (invariably the loudest) do not produce forward masking of other crickets' songs. A single neuron for each ear, named the corollary discharge interneuron (CDI), prevents the Ca^{2+} influx in ON1 which generates the masking by providing inhibitory input to both the ON1 dendrites and the presynaptic terminals of auditory afferents (Poulet and Hedwig, 2007). In support of its task, the CDI has remarkably elaborate dendritic and axonal processes spanning a large part of the animal's nerve cord (Fig. 23.4D).

At least two of the auditory ascending neurons, AN2 and TN1, that bring information to the brain and help signal approaching predators, are also sensitive to stridulation calls. These neurons remain sensitive to ultrasound, used to detect predatory bats, during ongoing bouts of mate calling, while their responses to mate calls show quick adaptation. This phenomenon is a form of stimulus-specific adaptation (SSA)—adaptation to repeated stimuli that does not generalize to distinct or rare stimuli. SSA has also been described in the context of mammalian auditory processing (Ulanovsky et al., 2003; Malmierca et al., 2009). For AN2 in crickets, SSA might involve divisive gain control through presynaptic inhibition (Hildebrandt et al., 2011). A different, very interesting, mechanism has been suggested for TN1, however, based on work in katydids.

The TN1 neuron in these animals conveys information to the brain on katydid songs and predator approach (Greenfield, 1990). Katydid songs consist of about 140 chirps per second with lower intrachirp frequencies (15 kHz) than bat search echolocation which uses about 10 chirps per second of high-frequency sound (~40 kHz). Given acoustic stimuli matching both sounds together, TN1 responses are attenuated to the katydid call while remaining sensitive to the ultrasounds of bats (Schul et al., 2012). Experiments with intracellular injection of a fluorescent form of the fast Ca^{2+} chelator BAPTA revealed a Ca^{2+} -dependent hyperpolarizing conductance producing spike-frequency adaptation. Additionally, SSA required a Na^+ -dependent mechanism, blocked by LiCl (Triblehorn and Schul, 2013). The proposed mechanism involves activation of a Na^+ -dependent K^+ conductance within the tonotopically arranged dendrites. Steady sound at one frequency would turn on this sustained K^+ conductance within that region of the dendritic arbor, thus locally decreasing EPSPs, while EPSP amplitude in other parts of the arbor would remain high.

The auditory system of Orthoptera might be fairly simple, designed primarily to detect the location and strength of songs from other members of the species and to detect potential predators. Yet, sophisticated active processing within dendrites allows a relatively small number of auditory interneurons to accomplish these tasks. The ON1 in crickets, which is integral in locating song sources, uses Ca^{2+} compartmentalization and Ca^{2+} -dependent K^+ conductances to attend to the loudest song while filtering out background noise and self-generated songs. The TN1 in katydids appears to use Na^+ -dependent K^+ channels within a tonotopic dendritic arbor to filter out ongoing background sounds while remaining sensitive to sounds at different frequencies, which could indicate an approaching predator.

The role of Ca^{2+} in the dendrites of leech neurons

Leeches are annelid worms with a central nervous system containing a chain of 21 segmental ganglia, each consisting of about 400 neurons (Nichols and Baylor, 1968; Muller et al., 1981). There are many identified neurons within each ganglion, including seven pairs of mechanosensory neurons, single pairs of serotonergic Retzius and octopaminergic Leydig neurons, NS (non-spiking)

interneurons, and several motoneurons (Nicholls and Baylor, 1968). Generally, dendrites of leech neurons possess both presynaptic and postsynaptic structures (Coggeshall and Fawcett, 1964; Muller et al., 1981). While the function of many leech neurons remains unknown, the ability to culture leech cells and the ease of electrophysiological experiments has led to substantial research into their cellular properties. Here, we will briefly summarize recent experiments on the Ca^{2+} dynamics within dendrites.

As they are the two largest neurons within the segmental ganglion, the electrically coupled Retzius cells have long been a focus of attention (Hagiwara and Morita, 1962; Eckert, 1963). Retzius activity is increased by both mechanosensory inputs from the body wall (Bagnoli and Magni, 1975) and stimuli at the anterior end of the body (Brodfuehrer and Friesen, 1986a,b). Application of serotonin to and electrical stimulation of serotonergic Retzius cells both increase the probability that swimming activity will be initiated (Willard, 1981; Kristan and Nusbaum, 1982; Brodfuehrer et al., 1995). Oscillatory activity during sustained fictive swimming also excites Retzius neurons (Willard, 1981). In addition to having gap junctions between the dendrites of Retzius pairs (Mason and Leake, 1978), the dendrites also receive common synaptic inputs, increasing their coupling (De-Miguel et al., 2001).

Calcium imaging of Retzius cells revealed high compartmentalization, with voltage-activated Ca^{2+} conductances in both axons and dendrites (Beck et al., 2001). Somewhat surprisingly, Ca^{2+} fluorescence was as high in the distal axon as in the dendrites. Retzius cells are the primary source and target of serotonin in the segmental ganglia. Activation of serotonin autoreceptors evokes a Cl^- current (Walker and Smith, 1973; Lessmann and Dietzel, 1991) and modulates two different K^+ conductances (Acosta-Urquidi et al., 1989). Beck et al. (2002) demonstrated that activation of Retzius cells by serotonin produced influx of Ca^{2+} through G-protein-coupled receptors, and reduced the electrical coupling between the Retzius cell pairs. It is unclear whether this reduced coupling is directly due to the influx of Ca^{2+} .

As in Retzius cells, Ca^{2+} is also highly compartmentalized within the anterior pagoda neuron (APN) (Andjelic and Torre, 2005). Activation of pressure-sensing sensory neurons triggers EPSPs in APNs. These EPSPs summate supralinearly in APN dendrites, despite their attenuation by Ca^{2+} -activated K^+ channels (Wessel et al., 1999b). This supralinear summation is caused by the closing of inward-rectifying K^+ (Kir) channels (Wessel et al. 1999a). Closing Kir channels between -80 and -60 mV increase the input resistance, producing the increase in summation, estimated to be around 40% greater than if the K^+ channels remained open. Both EPSPs and backpropagating APs induce influx of Ca^{2+} through low-voltage-activated Ca^{2+} channels located throughout these neurons, which in turn activates Ca^{2+} -activated K^+ channels (Wessel et al., 1999b). This forms a negative-feedback loop as depolarization also activates Ca^{2+} -activated K^+ channels, which act to decrease EPSP amplitude. This is in contrast to the role of low-voltage-activated Ca^{2+} channels in NS interneurons, where they amplify dendritic depolarization and generate graded Ca^{2+} events that propagate throughout the dendritic arbor (Yang et al., 2013).

Electrical excitability and GABAergic modulation in spider mechanoreceptors increase high-frequency information

The wandering spider *Cupiennius salei*, a venomous ambush predator, has become a common model animal since banana shipments brought it accidentally to Europe (Fig. 23.5A). Due to its large size and willingness to breed in the laboratory, it has been used in research on a range of topics, including its courtship behaviors (Fig. 23.5B), pheromone responses, neurophysiology, and neurotoxic venom. These spiders are nocturnal predators that perch on leaves and

sense vibrations to detect and discriminate between approaching prey and predators. Like other arthropods, spiders possess bipolar mechanoreceptors implementing mechanotransduction within distinct dendrites, demarcated from the soma and axon by differences in glial sheath, microtubules, and channel types (Foelix and Chu-Wang, 1973; Keil, 1997). Recently, researchers have demonstrated that a type of mechanoreceptor neuron in these spiders can very reliably encode high-frequency vibrations (Pfeiffer and French, 2009), due in part to dendritic spike generation (Gingl and French, 2003) and GABAergic modulation of excitability (Panek et al., 2008).

Spiders uniquely possess a type of cuticular mechanoreceptor called slit sense organs that are widely distributed in their exoskeleton (Fig. 23.5C) (Barth and Libera, 1970; Barth, 1985, 2002). Some of these organs contain multiple slits, named lyriform organs, and are sensitive to a range of stimuli created by muscle contraction, predators, prey, and mates. The high sensitivity and wide frequency range of these unique organs has recently inspired the design of biomimetic “crack-based” sensors (Fratzl and Barth, 2009; Kang et al., 2014).

The mechanoreceptors within these organs are large bipolar neurons accessible for intracellular recording during mechanical stimulation (French et al., 2002), making them ideal for studying the many unanswered questions about mechanoreceptor function. The most detailed study of physiological properties and dendritic processing within this class of mechanoreceptors has focused on the VS-3 lyriform organ on the patella of the anterolateral leg of the spider. Sensory transduction in VS-3 neurons occurs at the tip of a long ($>100\text{ }\mu\text{m}$) dendrite heavily wrapped by glia (Fig. 23.5D) (Seyfarth et al., 1995). Despite their classical bipolar neuronal anatomy, voltage-gated channels and postsynaptic receptors are distributed throughout VS-3 neurons. Immunohistochemistry has revealed that Na^+ channels are as densely located in the dendrites as in the axon, more so than in the soma (Seyfarth et al., 1995), suggesting that receptor potentials actively propagate along the dendrite to the soma.

Although the proximal axon can generate APs, APs recorded from the soma occur sooner after mechanical stimulation to the dendrites than following direct electrical stimulation, suggesting that APs are initiated in the dendrites (French et al., 2001). Dendritic excitability has been suggested in insect mechanoreceptors (Guillet et al., 1980; Erler and Thurm, 1981; Field and Matheson, 1998), but the challenge of recording directly from these dendrites makes this difficult to test. Mammalian Pacinian corpuscles (Pawson and Bolanowski, 2002), frog muscle spindles (Querfurth, 1985), and mammalian thermoreceptors (Carr et al., 2009) similarly generate APs at their receptors, although they might not be properly classified as dendrites. Gingl and French (2003) confirmed that the mechanically induced APs were initiated at the dendritic terminals by mechanically stimulating the slit organ while voltage clamping the soma of VS-3 neurons (Fig. 23.5E). One advantage of generating APs at the tip of the dendrites may lie in minimizing the time for sensory detection to reach the central nervous system. Furthermore, the perception of rapidly changing mechanical signals such as vibrations, and spike-timing reliability would likely be increased.

In addition to standard inactivating Na^+ and K^+ channels involved in AP conduction, the dendrites of VS-3 neurons contain low-voltage-activated (LVA) Na^+ and Ca^{2+} channels and Ca^{2+} -dependent K^+ channels. Depolarization from Na^+ influx through receptor channels produces additional Na^+ and Ca^{2+} influx through LVA channels, and forms a negative feedback loop. A 10–15% increase in intracellular $[\text{Ca}^{2+}]$ decreases the receptor potential by about 40%, apparently by decreasing the opening probability of mechanotransduction channels in the tips of the dendrites (Fig. 23.5F) (Höger et al., 2010).

VS-3 neurons receive GABAergic input on dendrites, somata, and axons (Fabian-Fine et al., 1999), which express both ionotropic GABA_A and metabotropic GABA_B receptors (Fig. 23.5G)

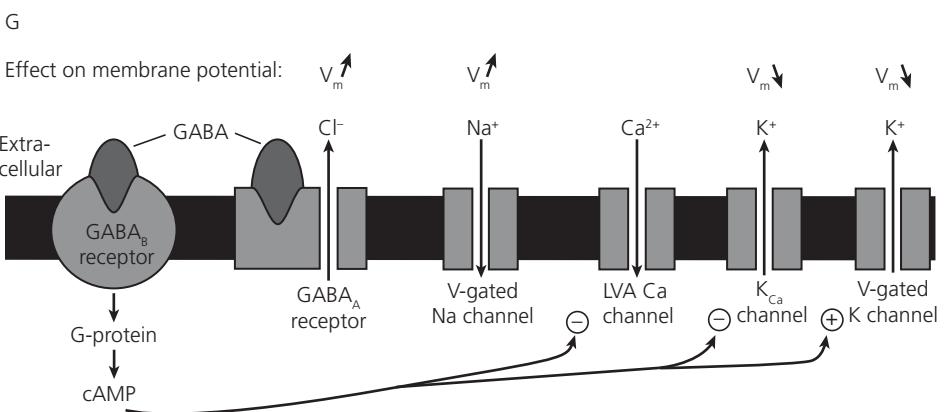
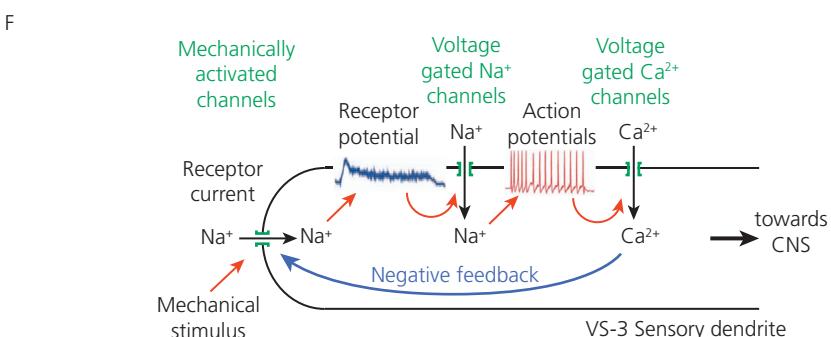
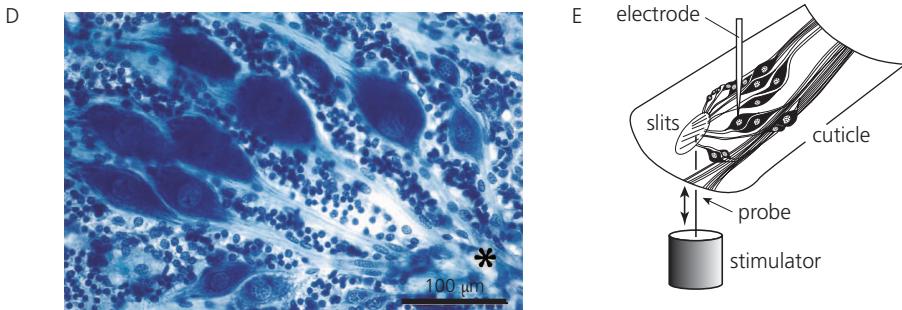
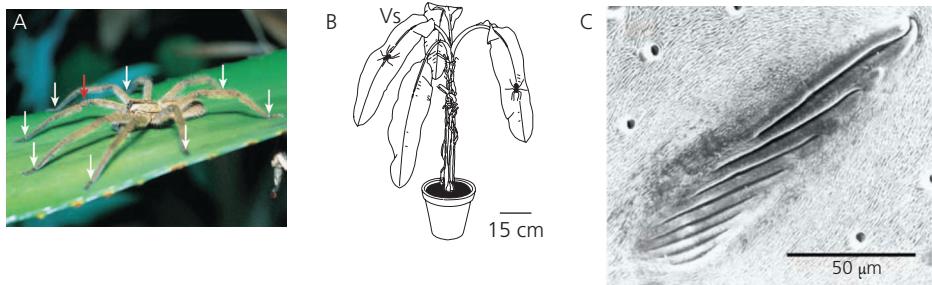


Fig. 23.5 **(A)** Picture of *Cupiennius salei*. White arrows point to the approximate location of vibration sensors HS-10, while the red arrow points to the location of one VS-3 organ. **(B)** Experimental setup used to study communication in tropical wandering spiders. The male (left) initiates courtship on a pheromone-covered leaf blade and must then select the correct leaf stalk to reach the female (right). Vs, vibration sensor. **(C)** Scanning electron micrograph of eight cuticular slits. **(D)** Inside view of a stained VS-3 organ revealing pairs of fusiform neuronal somata and their dendrites extending toward the slit region (asterisk, lower right). **(E)** Stimulation and recording of spider VS-3 neurons. A piece

(Panek et al., 2003; Gingl et al., 2004). GABA_A receptors are located primarily near the soma, while GABA_B receptors are mostly located at the dendrites (Panek et al., 2003; Gingl et al., 2004). Panek et al. (2008) combined ratiometric Ca²⁺ imaging experiments with intracellular recordings to study the effect of GABA_A receptor activation by muscimol. Activation of GABA_A receptors leads to an efflux of Cl⁻ out of the neuron, producing depolarization of more than 10 mV and a complex series of indirect effects (Fig. 23.5G). Thus, this depolarization initially activates the LVA Na⁺ and Ca²⁺ channels, increasing the depolarization. Then depolarization inactivates Na⁺ channels and, with the Ca²⁺ influx, opens Ca²⁺-dependent K⁺ channels (Panek et al., 2008). Additionally, the influx of Ca²⁺ would decrease receptor currents via the negative-feedback mechanism described above (Höger et al., 2010).

The net effect of GABAergic inputs was initially believed to be inhibitory due to its reduction of axonally generated APs (Panek et al., 2002; Gingl et al., 2004). Subsequent stimulation of VS-3

Fig. 23.5 (Continued)

of patellar cuticle containing the slits and associated neurons is mounted in a fixed holder. The slits are displaced by a glass probe driven by a piezoelectric stimulator during simultaneous intracellular recordings from VS-3 neurons. (F) Ca²⁺ feedback regulation of mechanotransduction in VS-3 neurons. Mechanical stimulation opens sodium-selective channels, producing a receptor current and potential across the membrane. A receptor potential of sufficient amplitude produces action potentials (APs) driven by voltage-activated sodium channels. The APs result in voltage-activated calcium channels opening and an increase in intracellular Ca²⁺, which reduces receptor current and receptor potential, completing a negative-feedback loop. (G) Model of GABA action on VS-3 neurons. GABA binding to GABA_A receptors induces Cl⁻ efflux and depolarization. The depolarization is further increased by a slow Na⁺ influx via voltage-gated Na⁺ channels. During the depolarization, low-voltage-activated (LVA) Ca²⁺ channels open enabling influx of Ca²⁺. Increase in intracellular [Ca²⁺] leads to opening of K_{Ca} channels, K⁺ efflux, and hyperpolarization. K⁺ also exits via voltage-gated K⁺ channels, hyperpolarizing the neuron to the resting level. GABA_B receptors act by activating G-proteins, leading to an increase in intracellular cAMP concentration. This cascade is followed by a larger outward current via voltage-gated K⁺ channels and reduced inward current via LVA Ca²⁺ channels.

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neurons with naturalistic vibrations, however, revealed that GABA also causes a profound excitatory effect (Pfeiffer et al., 2009; Pfeiffer and French, 2009). Most recently, it has been shown by Pfeiffer et al. (2012) that GABA_A activation shifts the frequency sensitivity of the mechanoreceptors. Thus, activation of GABA_A receptors produced increased firing rates, made receptor currents more selective to high-frequency stimulation, and decreased low-pass attenuation of receptor currents propagating through the dendrites (Pfeiffer et al., 2012). In addition, octopaminergic synapses on the soma and proximal axon in these cells have also been shown to increase the sensitivity and information capacity of these neurons through a CaMKII-dependent mechanism (Widmer et al., 2005; Torkkeli et al., 2011).

The overall effect of dendritic GABA_B receptors remains unclear (Gingl et al., 2004), but application of a GABA_B agonist activates a cyclic AMP signaling pathway, and decreases Ca²⁺ influx, leading to a delayed decrease of Ca²⁺-dependent K⁺ conductance (Fig. 23.5G) (Panek et al., 2003, 2008). This decreased dendritic Ca²⁺ produced by GABA_B activation might have opposite effects to GABA_A activation, decreasing the high-frequency sensitivity of the neuron. The decrease in [Ca²⁺] from GABA_B activation could also prevent the decrease in receptor currents triggered by LVA Ca²⁺ influx.

In summary, a complex combination of dendritic excitability (Gingl and French, 2003) and GABAergic modulation (Panek et al., 2008) in VS-3 mechanoreceptors leads to minimally delayed detection of tactile stimuli through dendritic APs, highly reliable spike timing (Pfeiffer and French, 2009), the ability to encode high-frequency signals (Pfeiffer et al., 2012), and an overall high information capacity (Pfeiffer et al., 2012). Although, the physiological properties of the lyriform mechanoreceptors are best studied in the VS-3 organ, the biomechanical properties and ecological roles have been primarily investigated in other lyriform organs, including HS-10 (Barth, 2002). *Cupiennius salei* spiders use HS-10 mechanoreceptors to sense mechanical vibrations over 100 Hz in communication during courtship (Fig. 23.5B) (Rovner and Barth, 1981; Barth, 2002). No extensive comparison has been made between the electrophysiology of mechanosensory neurons in HS-10 and VS-3, but they are superficially similar, with the same morphology and dendritic AP generation (Gingl et al., 2006). This suggests that the dendritic properties that enhance information from high-frequency signals may play an important ecological role in the spiders' vibratory communication.

Dendritic properties influence crayfish escape

Crayfish possess two pairs of bilateral command neurons, the medial giant (MG) and lateral giant (LG) interneurons, that generate fast, powerful escape movements with a single AP. These two neurons have been studied for the past 70 years (Wiersma, 1947, 1961). Extensive investigation of the LG circuit aided by its accessibility has led to it being one of the best-understood neural circuits in the entire animal kingdom (Edwards et al., 1999; Krasne and Edwards, 2002a,b). Attack from the rear of the animal activates the LG, producing a flexion of the abdomen that propels the animal up and forward (Edwards et al., 1999; Herberholz et al., 2004). Primary afferents excited by the movement of sensory hairs and mechanical displacement of tail segments excite the LG directly through a somatotopic mapping onto its dendritic tree (Calabrese, 1976; Antonsen and Edwards, 2003) and indirectly through mechanosensory interneurons (Zucker et al., 1971; Araki and Nagayama, 2003). LG excitation occurs through a mix of rectifying electrical and chemical synapses (Zucker, 1972; Edwards et al., 1991; Araki and Nagayama, 2003). As the LG-mediated escape propels the animal upward, making it more visible to predators, appropriate gain setting of the circuit is important to produce escape only when necessary.

The LG can act as a precise coincidence detector: highly synchronous activation of rectifying electrical synapses onto LG dendrites summate substantially more than inputs delayed even slightly in time (about a 30% increase; Edwards et al., 1998). The cause of this sharp coincidence tuning lies in the fact that electrical synapses from primary afferents are voltage gated and produce a biphasic current in LG dendrites. Thus, if dendritic inputs arrive separated in time, the second synaptic activation will occur during the outward current phase of the initial synaptic current, reducing the postsynaptic voltage (Edwards et al., 1998). The temporal tuning is very tight, and summation only occurs if the second input arrives during the 0.1 ms of the inward current phase. As explained next, the bidirectional current flow through these synapses has also been revealed to produce a form of dendritically mediated lateral excitation of primary afferents (Herberholz et al., 2002; Antonsen et al., 2005).

When LG dendrites depolarize, current passes back to presynaptic primary afferents. This current flowing from postsynaptic dendrites to presynaptic axonal terminals causes a temporary positive-feedback loop between the LG and its presynaptic afferents. As a result, mechanosensory afferent neurons are more sensitive to tail stimulation following excitation from nearby tail afferents connected to the LG (Herberholz et al., 2002; Antonsen et al., 2005). This allows for excitation to spread between the afferent nerve fibers despite few direct connections (Antonsen and Edwards, 2003). This positive feedback loop only lasts as long as activation of the LG is subthreshold because short-latency recurrent inhibition evoked by a LG spike inhibits the network (Roberts, 1968; Kennedy et al., 1974, 1980). Similar mechanisms of lateral excitation increasing a circuit's sensitivity have been found in vertebrates. The light sensitivity in cone photoreceptors is increased through the spread of photoresponses of neighbors through gap junctions (DeVries et al., 2002; Laughlin, 2002). Mauthner neurons in teleost fish also generate similar "antidromic" synaptic potentials in presynaptic afferents that enhance their excitability (Pereda et al., 1995; Curti and Pereda, 2004).

In addition to modulation of afferent sensitivity by electrical synapses onto LG dendrites, the gain of the circuit can be regulated by serotonin through cyclic AMP signaling (Araki et al., 2005) and when octopamine activates an inositol 1,4,5-trisphosphate (IP₃) signaling pathway (Araki and Nagayama, 2012). Serotonin injection modulates the circuit in an application-dependent manner. Slowly increasing the quantity of serotonin injected over about 20–30 min produces transient facilitation of LG circuitry in socially dominant animals, transient inhibition in subordinate crayfish, and persistent facilitation in socially isolated animals (Yeh et al., 1996, 1997; Teshiba et al., 2001). Further, LG circuits do not facilitate and are transiently inhibited in isolated animals when serotonin is applied suddenly (Teshiba et al., 2001). Antonsen and Edwards (2007) investigated the mechanisms for these seemingly contradictory effects of serotonin on LG circuitry. They discovered that at least three different mechanisms are involved. Serotonin can increase the input resistance of LG distal dendrites thus increasing EPSP amplitude, but it can also increase the coupling of the electrical synapses of afferents onto the LG. The increased electrical coupling would not only increase synaptic currents, but would also increase the strength of the dendrite-mediated lateral excitation between afferents. Thirdly, serotonin can also increase the input resistance near the initial segment of the LG neuron (Antonsen and Edwards, 2007). Different serotonin application procedures can presumably initiate these effects independently, explaining the different responses observed.

Although the sources of activation for the serotonin-dependent mechanisms outlined above are not yet known, serotonin can arise from synaptic, paracrine, and hormonal sources that would each provide serotonin input at different time scales (Beltz and Kravitz, 2002). This relatively simple circuit is therefore far from static, and exhibits dynamic gain control both intrinsically from the active integration of signals within the LG dendrites and extrinsically from neuromodulatory

input that can change with social status or experience. Recently, the LG neurons were discovered to receive descending excitation from rostral mechanical stimulation of the animal. While this input is not strong enough to initiate escape, the descending signal depolarizes the LG neuron, increasing sensitivity to tail stimulation (Liu and Herberholz, 2010).

Larval crayfish are subject to predation from a variety of animals, and use the LG circuitry to escape potential predators as quickly as possible. The electrical synapses of larval crayfish have a high gain and generate reliable spikes in the LG neuron and subsequent escape. As the crayfish grows, the LG neuron grows isometrically and the dendrites expand electrotonically, increasing the influence of chemical synapses and decreasing the circuit gain (Edwards et al., 1994; Hill et al., 1994). As with growth of the animal, ascent in social dominance also leads to a decreased gain of the LG circuit (Araki et al., 2013). Thus changes in the dendritic properties of the LG neuron accompany each of the developmental and social changes that alter a crayfish's escape behavior.

Collision detection and mechanisms of multiplication within a single locust neuron

We have already described the escape circuitry in the crayfish and fly, both of which used giant fibers acting as command neurons. These systems are optimized for response speed, often producing immediate escape from an abrupt stimulus. It would be even more advantageous, though, to be able to anticipate an approaching predator while it was still at a distance. For a crayfish in a muddy marsh, that may not be feasible, but the situation is different for animals living in open fields or beaches, for instance. For this reason, nearly all animals endowed with spatial vision exhibit avoidance behaviors in response to approaching predators (Fotowat and Gabbiani, 2011). The neural computations required for timely escape include distinguishing approaching objects from those passing by, possibly estimating the time until impact, not to mention having to segment the threat from other objects in the visual world. It is not evident how these different computations can be accomplished within a single neuron.

An object on direct collision course expands across the retina in all directions. If the object approaches with constant velocity then the angular velocity of its edges increases nearly exponentially. The same pattern of expansion is produced by the silhouette of the moving object, or equivalently its two-dimensional projection on a screen, called a looming stimulus. For monocular stimulation, the temporal dynamics of the approach is fully characterized by the ratio of the object's radius or half-size, l , to the approach speed, v , and given by $l/|v|$ ($v < 0$ for an approaching object; Gabbiani et al., 1999).

Locusts exhibit visually evoked escape behavior to objects approaching on a collision course, but not to objects on non-colliding trajectories or spatially incoherent stimuli. Escape does not occur at a fixed time before collision, but instead varies linearly with $l/|v|$, so escape occurs a set delay after the approaching object reaches a fixed angular size on the retina (Fotowat and Gabbiani, 2007). The initiation of this escape depends on the activity of a pair of descending neurons, which perfectly relay the firing profile of an identified neuron within the locusts' optic lobe, called the lobula giant movement detector (LGMD) (O'Shea and Williams, 1974; Fotowat et al., 2011).

The LGMD lies in the lobula, the third neuropil of the optic lobe, with inputs from the retina passing through the lamina and medulla before reaching the lobula. With a dendritic arbor separated into three distinct fields (A, B, and C), the LGMD receives spatially separated inhibition and excitation (Fig. 23.6A). All three fields integrate inputs from the entire eye, which spans half of the visual field (about 180° in azimuth and elevation). Approximately 500 inputs convey feedforward inhibition for increasing and decreasing luminance channeled to fields B and C, respectively

(Gabbiani et al., 2005). The feedforward inhibitory input conveys information about stimulus size (Gabbiani et al., 2005). Excitation from luminance changes of either polarity result in activation of half of the approximately 15,000 inputs mapped onto field A (Strausfeld and Nässel, 1981; Krapp and Gabbiani, 2005). In addition, this excitatory pathway is endowed with a lateral inhibitory network activated by wide-field motion that protects the LGMD's responses from habituation and makes the neuron insensitive to self-motion-induced optic flow (O'Shea and Rowell, 1975). Examination of excitatory inputs onto the LGMD shows that the strength and timing of inputs depends on the angular speed of the stimulus. No evidence has been found for elementary motion detector circuits like those found in flies, or the equivalent ones of mammals (Hassenstein and Reichardt, 1956; Adelson and Bergen, 1989), in the excitatory presynaptic pathway to the LGMD (Krapp and Gabbiani, 2005; Jones and Gabbiani, 2010).

Within field A, excitatory inputs are mapped with a precise retinotopic arrangement (Peron et al., 2009; Zhu and Gabbiani, unpublished observations). The morphology of dendritic field A matches that of the eye, spanning a curved ellipse, with the dorso-ventral axis of the eye mapped to the dorso-ventral axis of the dendrites. As the eye grows during larval development, new branches within dendritic field A grow in proportion to the increasing number of ommatidia in the compound eye (Sztarker and Rind, 2014). The arbor is rotated orthogonally relative to the eye, with the eye's antero-posterior axis mapping to the medio-lateral axis of the LGMD's dendrites. This retinotopy is preserved down to the level of single ommatidia (Fig. 23.6B).

When presented with looming stimuli, the firing rate of the LGMD increases as the angular size of the stimulus increases, but then peaks and declines as the stimulus exceeds an angular threshold (Fig. 23.6C). This would imply that early during approach the excitation (conveying angular speed) dominates, but later in the approach the size-dependent inhibition overtakes excitation. More specifically, the firing rate of the LGMD can be described as a function of the angular speed of stimulus expansion multiplied by a negative exponential of its angular size (Fig. 23.6C) (Hatsopoulos et al., 1995; Gabbiani et al., 2002).

The LGMD dendrites are electrotonically large, and contain active conductances (Gabbiani and Krapp, 2006), but how can dendritic processing lead to the stimulus selectivity apparent in the animal's behavior? One key discrimination required by the animal consists in distinguishing between approaching versus translating objects. Selectivity of the LGMD responses for looming stimuli approaching the animal versus translating stimuli passing by is aided by spike frequency adaptation mediated by SK-like potassium channels localized in the main dendritic trunk (Gabbiani and Krapp, 2006; Peron and Gabbiani, 2009). These Ca^{2+} -dependent K^+ channels act as a damping mechanism, preventing sustained spiking in response to steady excitation. The opening of voltage-activated Ca^{2+} channels whose localization is limited to the immediate vicinity of the LGMD's spike initiation zone activates this Ca^{2+} -dependent conductance that can in turn act specifically on attenuating firing rates. In terms of synaptic activation, translating stimuli with a fixed angular size and speed would provide a constant level of excitation, unlike approaching stimuli with steadily increasing angular size and speed. Translating stimuli thus initiate spiking that then opens voltage-gated Ca^{2+} and Ca^{2+} -dependent K^+ channels at the spike initiation zone, which reduce spiking, resulting in a transient response. After intracellular application of the fast calcium chelator BAPTA, the LGMD produced continuous firing to translating stimuli, but responses to looming stimuli were qualitatively unchanged, confirming the specific role of the SK-like conductance in implementing the selectivity for approaching versus translating objects (Fig. 23.6D) (Peron and Gabbiani, 2009).

While the SK-like conductance can explain the preference for approach over translation, it does not explain the LGMD's characteristic response dynamics to approaching objects. As mentioned

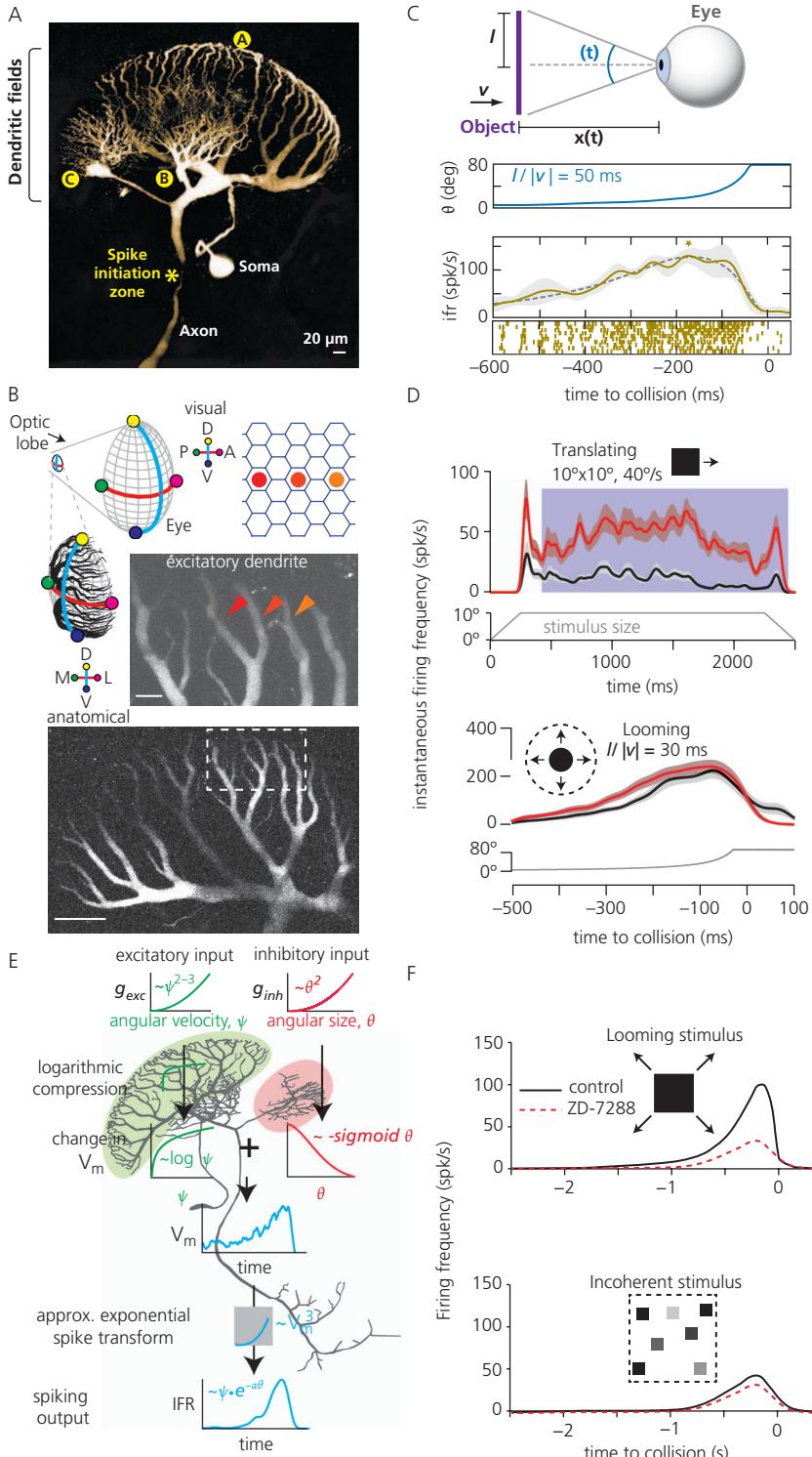


Fig. 23.6 Collision detection in the locust. **(A)** Stain of the lobula giant movement detector (LGMD) neuron, showing its three dendritic fields, A, B and C, the spike initiation zone, axon, and soma. **(B)** Top left: schematics illustrating the eye and the relative position of the LGMD in the optic lobe

above, LGMD firing patterns can be well modeled by the multiplication of angular speed and the negative exponential of angular size. If the excitatory input were proportional to the logarithm of angular speed, and the inhibitory input proportional to angular size, these two signals could be added together and then exponentiated within the LGMD (Fig. 23.6E). The firing rate of the LGMD is indeed nearly an exponentiation of the membrane potential at the spike initiation zone



Fig. 23.6 (Continued)

(magnified below). The matching color lines and circles indicate the retinotopic mapping of the eye surface onto dendritic field A of the LGMD. Abbreviations: A, anterior; V, ventral; P, posterior; D, dorsal; M, medial; L, lateral. Bottom: two-photon scan of field A of the LGMD with a magnified inset (dashed rectangle) shown immediately above. Color-coded arrowheads indicate the principal dendritic branches activated when three facets are individually stimulated as illustrated on the top right. Functional imaging obtained with the Ca^{2+} -sensitive dye Oregon green BAPTA-1. Bottom and middle scale bars = 100 and 10 μm , respectively. (C) Top: a looming stimulus mimics the approach of an object with half size, l , approaching at constant speed, v . The time course of the angular size subtended by the object is determined by the ratio of the half size to the distance from the eye, $x(t)$, and depends only on l/v ($v < 0$ for an approaching object). Middle: time course of the angular size subtended by a looming stimulus with $l/v = 50$ ms as a function of time to collision. Bottom: mean firing rate of the LGMD and individual spike rasters (10 trials) for the same looming stimulus. * indicates the peak firing rate; the dashed line shows a fit obtained by multiplying a negative exponential of angular size by angular speed. (D) Top: LGMD mean instantaneous firing rate response to a small translating black square (top inset) in control condition (black) and after intracellular injection of BAPTA to block spike frequency adaptation (red). Bottom: the same experimental protocol, but for a looming stimulus. (E) Summary of the current model for how the LGMD implements the multiplication of angular speed with a negative exponential of size. Excitatory and inhibitory synaptic inputs encode speed and size by power-law functions. The logarithm of angular speed is taken in the excitatory dendrites by a compression step related to the reversal potential of excitatory synaptic input, while the effect of inhibition on membrane potential is close to linear (sigmoidal). The resulting membrane potential at the spike initiation zone is approximately exponentiated (third-order power law), leading to a spiking output that is well approximated by multiplying angular speed with a negative exponential of angular size. (F) Schematic illustration of the effect of I_h on responses to looming stimuli and stimuli with a temporally matched luminance time course but randomly distributed in space. Top: in control conditions, the LGMD responds strongly to looming stimuli (inset) and the response is largely suppressed when I_h is blocked by ZD-7288. Bottom: in contrast, the response to a spatially incoherent stimulus (inset) is nearly unaffected by blocking of I_h . As a result, the response of the LGMD to a looming stimulus when I_h is blocked is similar to its response to an incoherent stimulus under control conditions.

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(Gabbiani et al., 2002), and inhibition depends on stimulus size (Rowell et al., 1977; Gabbiani et al., 2005), although the exact relationship has not yet been measured. Excitatory inputs to the LGMD are proportional to a power of angular speed, not its logarithm (Jones and Gabbiani, 2010), so for this model to be supported requires a logarithmic compression of excitatory inputs within dendritic field A. Jones and Gabbiani (2012) used a mix of physiology and computational modeling to confirm this compression. During looming stimuli a massive barrage of synaptic inputs impinges on the LGMD, leading to a strongly saturating response. As excitatory current increases, the membrane depolarizes, decreasing the driving force for Na^+ and Ca^{2+} influx that generates the EPSPs. Additionally, the K^+ driving force increases. The large conductance change during a looming stimulus causes an approximately logarithmic saturation of the synaptic response as the membrane approaches the net reversal potential of synaptic and non-synaptic channels open in the dendrites.

Throughout this description of the computations carried out by the LGMD as it tracks approaching objects, it has been presumed that the visual system is able to segment approaching objects from other distinct objects present in the visual scene. Indeed, the addition of spatially random background movement only slightly alters the LGMD firing rates in response to looming stimuli (Silva et al., 2015). Although segmenting a single threatening approaching object from its surroundings has a clear ecological value, currently there are no known physiological mechanisms for accomplishing this. Recent evidence suggests that hyperpolarization-activated and cyclic nucleotide-gated (HCN) channels localized to dendritic field A may help accomplish this segmentation process. When presented with a spatially incoherent version of a looming stimulus whose overall luminance time course is identical to that of looming stimuli, LGMD responses are greatly reduced and the animals do not escape (Dewell et al., in production). On the other hand, blocking the HCN channels within dendritic field A greatly reduces responses to looming stimuli, but has no effect on responses to spatially incoherent stimuli (Fig. 23.6F). This HCN-dependent increase in responses to looming stimuli relative to spatially incoherent ones effectively results in a behavioral discrimination between single coherent approaching objects and identical luminance patterns generated by spatially incoherent sources.

Despite over 40 years of research into the LGMD and its associated network of about 50,000 presynaptic neurons, much of the processing that goes on within this nerve cell remains to be understood. What has been characterized to date shows us the tremendous computational power that can be implemented within a dendritic arborization. The combination of morphological separation of excitation and inhibition, the fine-scale retinotopic arrangement of the excitation, and the localization of active conductances all allow for multiple computational problems to be simultaneously solved within a single neuron.

Conclusions

The above examples hopefully demonstrate the wide array of dendritic properties found within invertebrates, but the question of the homology of vertebrate and invertebrate dendrites remains unanswered. Recent experiments, particularly in *Drosophila*, have compared dendritic homology between insects and mammals at the genetic level. The morphological differences are clear (see example images in Figs 23.1–23.4 and 23.6). Insect neurons have somata that are often electrically distant from their input/output regions with a single neuritic branch that separates into both dendrite and axon. Despite the obvious morphological differences, the maturation process and the cellular architecture of adult dendrites are very similar (Liberat and Duch, 2004; Sánchez-Soriano et al., 2005; Jan and Jan, 2010).

For instance, in both mammals and fruit flies microtubules in axons uniformly orient with their plus-end located distally, whereas dendritic microtubules orient in both directions (Baas et al., 1988; Rolls et al., 2007). In both taxa, axonal development comes first, followed by dendritic development (Liberat and Duch, 2004; Jan and Jan, 2010). In cultured *Drosophila* neurons, dendrites can be shifted to originate at the soma producing a bipolar neuron more like what is seen in mammalian cortex (Sánchez-Soriano, et al. 2005). Between flies and vertebrates, transcription factors, receptor–ligand interactions, signaling pathways, cytoskeletal proteins, Golgi outposts, and endosomes are conserved (Jan and Jan, 2010). This supports the view that the dendrites described above are not just analogous in their function and information-processing characteristics, but are truly homologous to those of vertebrates with both arising from a common urbilaterian ancestry (Ghysen, 2003; Sánchez-Soriano et al., 2005).

Neurons are information-processing units, and their dendrites are constrained by and optimized for the information they process. Some common elements of information processing influenced by dendritic properties include signal filtering, response speed, reliability, plasticity, compartmentalization, and connectivity. The last is perhaps the most basic: information that does not reach a cell's dendrites cannot be transmitted to its postsynaptic targets. This feature is best demonstrated by the circuit switch for pheromone processing in flies (Kohl et al., 2013). Generation of APs in the distal dendrites of spider mechanoreceptors and lateral excitation of electrical synapses in crayfish LG dendrites are examples of mechanisms for minimizing response time. In crickets and other Orthoptera, active conductances and corollary discharge allow a small number of auditory neurons to produce remarkable information filtering, inhibiting self-generated inputs as well as those that correspond to the weaker calls generated by other members of their species. Within the dendrites of the LGMD, the compartmentalization and fine-scale retinotopy of inputs, the active conductances, and compression of signals leads to complex computations within a single neuron.

Since subcellular properties and the processing challenges faced by dendrites apply across phyla, we will continue to learn fundamental aspects of neural function from invertebrates. In understanding the nervous system it is helpful to remember that it does not work in isolation, and that the behavioral and ecological context, the sensory input, motor output, and development of the system must all be examined to discover the underlying features of neural information processing, for it is only within these contexts that the information is meaningful. The high level of experimental control, presence of identifiable neurons, and more tractable circuitry in “simpler” invertebrates provide distinct advantages for such comprehensive investigations. The strong influence of neuroethology and systems neuroscience on invertebrate neuroscience facilitates grounding the existing dendritic research to the overall circuit function and behavioral context from which the dendritic processes emanate.

We would like to conclude with some ideas about how researchers could leverage these advantageous models in the coming years. Firstly, the complexity of the nervous system requires integrative and comparative investigation. Neural processing is widely integrative, continuously comparing and adding different streams of information, and so our study of it must be similarly integrative in order to unlock the characteristics of neural computation. At the same time it must be kept in mind that neurons span a tremendous range, and undoubtedly they do not all work in the same way. Just because one circuit/channel/species works in a specific way does not mean that others do as well. What we are learning about one nervous system will invariably help us understand others, but how to apply the new knowledge requires careful comparative work between systems.

New experimental methods, particularly in genetic systems like *Caenorhabditis elegans* and *Drosophila*, are dramatically refining the scale of experimental control available to investigators.

The ability to watch dendrites develop using *in vivo* time-lapse imaging (Williams and Truman, 2004) combined with optogenetic stimulation and physiological imaging promises to greatly increase our understanding of the role of activity in dendritic growth and morphology. Engineering advances, meanwhile, continually improve our ability to record and control neural activity in freely moving animals. While both genetic and physiological advances help on their own, being able to combine these different approaches within the same species will dramatically increase our understanding of dendritic function in the broader context of the nervous system. Toward this end, we need to sequence the genomes and adapt genetic tools for species currently accessible only through electrophysiology. Conversely, development of electrophysiological techniques for species and systems with existing genetic tools will also hasten our understanding of dendritic computation. With these new advances we can look forward to expanding the list of fundamental neural mechanisms originally discovered in this broad range of “simpler” animals.

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References

- Acosta-Urquidi J, Sahley CL, Kleinhaus AL (1989) Serotonin differentially modulates two K⁺ currents in the Retzius cell of the leech. *Journal of Experimental Biology* **145**:403–417.
- Adelson EH, Bergen JR (1985) Spatiotemporal energy models for the perception of motion. *Journal of the Optical Society of America A* **2**:285–299.
- Allen MJ, Drummond JA, Moffat KG (1998) Development of the giant fiber neuron of *Drosophila melanogaster*. *Journal of Comparative Neurology* **397**:519–531.
- Allen MJ, Godenschwege TA, Tanouye MA, Phelan P (2006) Making an escape: development and function of the *Drosophila* giant fibre system. *Seminars in Cell and Developmental Biology*, **17**:31–41.
- Andjelic S, Torre V (2005) Calcium dynamics and compartmentalization in leech neurons. *Journal of Neurophysiology* **94**:4430–4440.
- Antonsen BL, Edwards DH (2003) Differential dye coupling reveals lateral giant escape circuit in crayfish. *Journal of Comparative Neurology* **466**:1–13.
- Antonsen BL, Edwards DH (2007) Mechanisms of serotonergic facilitation of a command neuron. *Journal of Neurophysiology* **98**:3494–3504.
- Antonsen BL, Herberholz J, Edwards DH (2005) The retrograde spread of synaptic potentials and recruitment of presynaptic inputs. *Journal of Neuroscience* **25**:3086–3094.
- Araki M, Nagayama T (2003) Direct chemically mediated synaptic transmission from mechanosensory afferents contributes to habituation of crayfish lateral giant escape reaction. *Journal of Comparative Physiology A* **189**:731–739.
- Araki M, Nagayama T (2012) IP3 mediated octopamine-induced synaptic enhancement of crayfish LG neurone. *Journal of Comparative Physiology A* **198**:607–615.
- Araki M, Nagayama T, Sprayberry J (2005) Cyclic AMP mediates serotonin-induced synaptic enhancement of lateral giant interneuron of the crayfish. *Journal of Neurophysiology* **94**:2644–2652.
- Araki M, Hasegawa T, Komatsuda S, Nagayama T (2013) Social status-dependent modulation of LG-flip habituation in the crayfish. *Journal of Experimental Biology* **216**:681–686.
- Baas PW, Deitch JS, Black MM, Banker GA (1988) Polarity orientation of microtubules in hippocampal neurons: uniformity in the axon and nonuniformity in the dendrite. *Proceedings of the National Academy of Sciences of the United States of America* **85**:8335–8339.

- Baden T, Hedwig B (2006) Neurite-specific Ca^{2+} dynamics underlying sound processing in an auditory interneurone. *Developmental Neurobiology* **67**: 68–80.
- Bagnoli P, Magni F (1975) Synaptic inputs to Retzius' cells in the leech. *Brain Research* **96**:147–152.
- Bargmann CI (1998) Neurobiology of the *Caenorhabditis elegans* genome. *Science* **282**:2028–2033.
- Barth FG (1985) Slit sensilla and the measurement of cuticular strains. In: *Neurobiology of Arachnids* (Barth FG, ed.), pp 162–188. Berlin: Springer.
- Barth FG (2002) *A Spider's World. Senses and Behavior*. Berlin: Springer.
- Barth FG, Libera W (1970) Ein Atlas der Spaltsinnesorgane von *Cupiennius salei* Keys. Chelicerata (Araneae). *Zeitschrift für Morphologie der Tiere* **68**:343–369.
- Beck A, Lohr C, Deitmer JW (2001) Calcium transients in subcompartments of the leech Retzius neuron as induced by single action potentials. *Journal of Neurobiology* **48**:1–18.
- Beck A, Lohr C, Berthold H, Deitmer JW (2002) Calcium influx into dendrites of the leech Retzius neuron evoked by 5-hydroxytryptamine. *Cell Calcium* **31**:137–149.
- Beltz BS, Kravitz EA (2002) Serotonin in crustacean systems: more than a half century of fundamental discoveries. In: *Crustacean Experimental Systems in Neurobiology* (Wiese K, ed.), pp. 141–163. Berlin: Springer.
- Bloch G, Sullivan JP, Robinson GE (2002) Juvenile hormone and circadian locomotor activity in the honeybee *Apis mellifera*. *Journal of Insect Physiology* **48**:1123–1131.
- Borst A, Egelhaaf M, Haag J (1995) Mechanisms of dendritic integration underlying gain control in fly motion-sensitive interneurons. *Journal of computational neuroscience* **2**:5–18.
- Boyan GS (1984) Neural mechanisms of auditory information processing by identified interneurones in Orthoptera. *Journal of Insect Physiology* **30**:27–41.
- Brandon J, Coss R (1982) Rapid dendritic spine shortening during one-trial learning: the honeybee's first orientation flight. *Brain Research* **252**:51–61.
- Brodfuehrer PD, Friesen WO (1986a) Initiation of swimming activity by trigger neurons in the leech subesophageal ganglion. I. Output connections of Tr1 and Tr2. *Journal of Comparative Physiology A* **159**:489–502.
- Brodfuehrer PD, Friesen WO (1986b) Initiation of swimming activity by trigger neurons in the leech subesophageal ganglion. III. Sensory inputs to Tr1 and Tr2. *Journal of Comparative Physiology A* **159**:511–519.
- Brodfuehrer PD, Debski EA, O'Gara BA, Friesen WO (1995) Neuronal control of leech swimming. *Journal of Neurobiology* **27**:403–418.
- Burkert P, Duch C (2006) Developmental changes of CaMKII localization, activity and function during postembryonic CNS remodelling in *Manduca sexta*. *European Journal of Neuroscience* **23**:335–349.
- Calabrese RL (1976) Crayfish mechanoreceptive interneurons. Part 1. The nature of ipsilateral excitatory inputs. *Journal of Comparative Physiology A* **105**:83–102.
- Carr RW, Pianova S, McKemy DD, Brock JA (2009) Action potential initiation in the peripheral terminals of cold-sensitive neurones innervating the guinea-pig cornea. *Journal of Physiology* **587**:1249–64.
- Casaday GB, Camhi JM (1976) Metamorphosis of flight motoneurons in the moth, *Manduca sexta*. *Journal of Comparative Physiology A* **112**:143–158.
- Chapman RF (1998) *The Insects: Structure and Function*. Cambridge: Cambridge University Press.
- Coggesshall E, Fawcett DW (1964) The fine structure of the central nervous system of the leech, *Hirudo medicinalis*. *Journal of Neurophysiology* **27**:229–289.
- Coggshall JC (1978) Neurons associated with the dorsal longitudinal flight muscle of *Drosophila melanogaster*. *Journal of Comparative Neurology* **177**:707–720.
- Consoulas C, Restifo LL, Levine RB (2002) Dendritic remodeling and growth of motoneurons during metamorphosis of *Drosophila melanogaster*. *Journal of Neuroscience* **22**:4906–4917.

- Costello WJ, Wyman RJ (1986) Development of an indirect flight muscle in a muscle-specific mutant of *Drosophila melanogaster*. *Developmental Biology* **118**:247–258.
- Cuntz H, Haag J, Borst A (2003) Neural image processing by dendritic networks. *Proceedings of the National Academy of Sciences of the United States of America* **100**:11082–11085.
- Curti S, Pereda AE (2004) Voltage-dependent enhancement of electrical coupling by a subthreshold sodium current. *Journal of Neuroscience* **24**:3999–4010.
- Cuttle MF, Hevers W, Laughlin SB, Hardie RC (1995) Diurnal modulation of photoreceptor potassium conductance in the locust. *Journal of Comparative Physiology A* **176**:307–316.
- Datta SR, Vasconcelos ML, Ruta V, Luo S, Wong A, Demir E, Flores J, Balonze K, Dickson BJ, Axel R (2008) The *Drosophila* pheromone cVA activates a sexually dimorphic neural circuit. *Nature* **452**:473–477.
- Davis RL (1993) Mushroom bodies and *Drosophila* learning. *Neuron* **11**:1–14.
- De-Miguel FF, Vargas-Caballero M, García-Pérez E (2001) Spread of synaptic potentials through electrical synapses in Retzius neurones of the leech. *Journal of Experimental Biology* **204**:3241–3250.
- DeVries SH, Qi X, Smith R, Makous W, Sterling P (2002) Electrical coupling between mammalian cones. *Current Biology* **12**:1900–1907.
- Dobrin SE, Herlihy JD, Robinson GE, Fahrbach SE (2011) Muscarinic regulation of Kenyon cell dendritic arborizations in adult worker honey bees. *Arthropod Structure and Development* **40**:409–419.
- Duch C, Levine RB (2000) Remodeling of membrane properties and dendritic architecture accompanies the postembryonic conversion of a slow into a fast motoneuron. *Journal of Neuroscience* **20**:6950–6961.
- Duch C, Levine RB (2002) Changes in calcium signaling during postembryonic dendritic growth in *Manduca sexta*. *Journal of Neurophysiology* **87**:1415–1425.
- Duch C, Bayline RJ, Levine RB (2000) Postembryonic development of the dorsal longitudinal flight muscle and its innervation in *Manduca sexta*. *Journal of Comparative Neurology* **422**:1–17.
- Duch C, Vonhoff F, Ryglewski S (2008) Dendrite elongation and dendritic branching are affected separately by different forms of intrinsic motoneuron excitability. *Journal of Neurophysiology* **100**:2525–2536.
- Dudel J, Kuffler SW (1961) Presynaptic inhibition at the crayfish neuromuscular junction. *Journal of Physiology* **155**:543–562.
- Dürr V, Egelhaaf M (1999) In vivo calcium accumulation in presynaptic and postsynaptic dendrites of visual interneurons. *Journal of Neurophysiology* **82**:3327–3338.
- Durst C, Eichmüller S, Menzel R (1994) Development and experience lead to increased volume of subcompartments of the honeybee mushroom body. *Behavioral and Neural Biology* **62**:259–263.
- Eckert R (1963) Electrical interaction of paired ganglion cells in the leech. *Journal of General Physiology* **46**:573–587.
- Edwards DH, Heitler WJ, Leise EM, Fricke RA (1991) Postsynaptic modulation of rectifying electrical synaptic inputs to the LG escape command neuron in crayfish. *Journal of Neuroscience* **11**:2117–2129.
- Edwards DH, Yeh SR, Barnett LD, Nagappan PR (1994) Changes in synaptic integration during the growth of the lateral giant neuron of crayfish. *Journal of Neurophysiology* **72**:899–908.
- Edwards DH, Yeh SR, Krasne FB (1998) Neuronal coincidence detection by voltage-sensitive electrical synapses. *Proceedings of the National Academy of Sciences of the United States of America* **95**:7145–7150.
- Edwards DH, Heitler WJ, Krasne FB (1999) Fifty years of a command neuron: the neurobiology of escape behavior in the crayfish. *Trends in Neuroscience* **22**:153–161.
- Egelhaaf M, Borst A, Reichardt W (1989) Computational structure of a biological motion-detection system as revealed by local detector analysis in the fly's nervous system. *Journal of the Optical Society of America A* **6**:1070–1087.
- Erber J, Menzel R (1980) Localization of short-term memory in the brain of the bee, *Apis mellifera*. *Physiological Entomology* **5**:343–358.

- Erler G, Thurm U (1981) Dendritic impulse initiation in an epithelial sensory neuron. *Journal of Comparative Physiology* **142**:237–249.
- Erondu NE, Kennedy MB (1985) Regional distribution of type II Ca^{2+} /calmodulin-dependent protein kinase in rat brain. *Journal of Neuroscience* **5**:3270–3277.
- Ewing A (1977) The neuromuscular basis of courtship song in *Drosophila*: the role of indirect flight muscles. *Journal of Comparative Physiology A* **119**:249–265.
- Fabian-Fine R, Höger U, Seyfarth E-A, Meinertzhagen IA (1999) Peripheral synapses at identified mechanosensory neurons in spiders: three-dimensional reconstruction and GABA-immunoreactivity. *Journal of Neuroscience* **19**:298–310.
- Fahrbach SE (2006) Structure of the mushroom bodies of the insect brain. *Annual Review of Entomology* **51**:209–232.
- Fahrbach SE, Moore D, Capaldi EA, Farris SM, Robinson GE (1998) Experience-expectant plasticity in the mushroom bodies of the honeybee. *Learning and Memory* **5**:115–123.
- Farris SM, Robinson GE, Fahrbach SE (2001) Experience- and age-related outgrowth of intrinsic neurons in the mushroom bodies of the adult worker honeybee. *Journal of Neuroscience* **21**:6395–6404.
- Farrow K, Haag J, Borst A (2003) Input organization of multifunctional motion sensitive neurons in the blowfly. *Journal of Neuroscience* **23**:9805–9811.
- Faulkes Z, Pollack GS (2000) Effects of inhibitory timing on contrast enhancement in auditory circuits in crickets (*Teleogryllus oceanicus*). *Journal of Neurophysiology* **84**:1247–1255.
- Fernandes J, Vijayraghavan K (1993) The development of indirect flight muscle innervation in *Drosophila melanogaster*. *Development* **118**:215–227.
- Field LH, Matheson T (1998) Chordotonal organs of insect. *Advances in Insect Physiology* **27**:1–230.
- Foelix RF, Chu-Wang I-W (1973) The morphology of spider sensilla I. Mechanoreceptors. *Tissue and Cell* **5**:451–460.
- Fotowat H, Gabbiani F (2007) Relationship between the phases of sensory and motor activity during a looming-evoked multistage escape behavior. *Journal of Neuroscience* **27**:10047–10059.
- Fotowat H, Gabbiani F (2011) Collision detection as a model for sensory-motor integration. *Annual Review of Neuroscience* **34**:1–19.
- Fotowat H, Harrison RR, Gabbiani F (2011) Multiplexing of motor information in the discharge of a collision detecting neuron during escape behaviors. *Neuron* **69**:147–158.
- Fratzl P, Barth FG (2009) Biomaterial systems for mechanosensing and actuation. *Nature* **462**:442–448.
- French AS, Höger U, Sekizawa S, Torkkeli PH (2001) Frequency response functions and information capacities of paired spider mechanoreceptor neurons. *Biological Cybernetics* **85**:293–300.
- French AS, Torkkeli PH, Seyfarth EA (2002) From stress and strain to spikes: mechanotransduction in spider slit sensilla. *Journal of Comparative Physiology A* **188**:739–752.
- Fromm J, Lautner S (2007) Electrical signals and their physiological significance in plants. *Plant, Cell and Environment* **30**:249–257.
- Fuerst PG, Bruce F, Tian M, Wie W, Elstrott J, Feller MB, Erskine L, Singer JH, Burgess RW (2009) DSCAM and DSCAML1 function in self-avoidance in multiple cell types in the developing mouse retina. *Neuron* **64**:484–497.
- Gabbiani F, Krapp HG (2006) Spike-frequency adaptation and intrinsic properties of an identified, looming-sensitive neuron. *Journal of Neurophysiology* **96**:2951–2962.
- Gabbiani F, Krapp HG, Laurent G (1999) Computation of object approach by a wide-field, motion-sensitive neuron. *Journal of Neuroscience* **19**:1122–1141.
- Gabbiani F, Krapp HG, Koch C, Laurent G (2002) Multiplicative computation in a visual neuron sensitive to looming. *Nature* **420**:320–324.
- Gabbiani F, Cohen I, Laurent G (2005) Time-dependent activation of feed-forward inhibition in a looming-sensitive neuron. *Journal of Neurophysiology* **94**:2150–2161.

- Gerhardt HC, Huber F** (2002) *Acoustic Communication in Insects and Anurans: Common Problems and Diverse Solutions*. Chicago: University of Chicago Press.
- Ghysen A** (2003) The origin and evolution of the nervous system. *International Journal of Developmental Biology* **47**:555–562.
- Gingl E, French AS** (2003) Active signal conduction through the sensory dendrite of a spider mechano-receptor neuron. *Journal of Neuroscience* **23**:6096–6101.
- Gingl E, French AS, Panek I, Meisner S, Torkkeli PH** (2004) Dendritic excitability and localization of GABA-mediated inhibition in spider mechanoreceptor neurons. *European Journal of Neuroscience* **20**:59–65.
- Gingl E, Burger AM, Barth FG** (2006) Intracellular recording from a spider vibration receptor. *Journal of Comparative Physiology A* **192**:551–558.
- Greenfield MD** (1990) Evolution of acoustic communication in the genus *Neoconocephalus*: discontinuous songs, synchrony, and interspecific interactions. In: *The Tettigoniidae: Biology, Systematics and Evolution* (Bailey WJ, Rentz DCF, eds), pp. 71–97. Berlin: Springer.
- Groh C, Lu Z, Meinertzhagen IA, Rössler W** (2012) Age-related plasticity in the synaptic ultrastructure of neurons in the mushroom body calyx of the adult honeybee *Apis mellifera*. *Journal of Comparative Neurology* **520**:3509–3527.
- Gronenberg W** (2001) Subdivisions of hymenopteran mushroom body calyces by their afferent supply. *Journal of Comparative Neurology* **435**:474–489.
- Gronenberg W, Heeren S, Hölldobler B** (1996) Age-dependent and task-related morphological changes in the brain and the mushroom bodies of the ant *Camponotus floridanus*. *Journal of Experimental Biology* **199**:2011–2019.
- Guillet JC, Berard J, Coillot-Callec JJ** (1980) Electrical properties of the dendrite in an insect mechanoreceptor: Effects of antidromic or direct electrical stimulation. *Journal of Insect Physiology* **26**:755–762.
- Gupta N, Stopfer M** (2014) A temporal channel for information in sparse sensory coding. *Current Biology* **24**:2247–2256.
- Haag J, Borst A** (2002) Dendro-dendritic interactions between motion-sensitive large-field neurons in the fly. *Journal of Neuroscience* **22**:3227–3233.
- Haag J, Denk W, Borst A** (2004) Fly motion vision is based on Reichardt detectors regardless of the signal-to-noise ratio. *Proceedings of the National Academy of Sciences of the United States of America* **101**:16333–16338.
- Hagiwara S, Morita H** (1962) Electrotonic transmission between two nerve cells in leech ganglion. *Journal of Neurophysiology* **25**:721–731.
- Hartwig CL, Worrell J, Levine RB, Ramaswami M, Sanyal S** (2008) Normal dendrite growth in *Drosophila* motor neurons requires the AP-1 transcription factor. *Developmental Neurobiology* **68**:1225–1242.
- Hassenstein B, Reichardt W** (1956) Systemtheoretische Analyse der Zeit-, Reihenfolgen- und Vorzeichenauswertung bei der Bewegungsperzeption des Rüsselkäfers Chlorophanus. *Zeitschrift für Naturforschung* **11b**:513–524.
- Hatsopoulos N, Gabbiani F, Laurent G** (1995) Elementary computation of object approach by a wide-field visual neuron. *Science* **270**:1000–1003.
- Hedrich R** (2012) Ion channels in plants. *Physiological Reviews* **92**:1777–1811.
- Heisenberg M** (1989) Genetic approach to learning and memory (mnemogenetics) in *Drosophila melanogaster*. *Fortschritte der Zoologie* **37**:3–45.
- Heisenberg M, Borst A, Wagner S, Byers D** (1985) *Drosophila* mushroom body mutants are deficient in olfactory learning. *Journal of Neurogenetics* **2**:1–30.
- Herberholz J, Antonson BL, Edwards DH** (2002) A lateral excitatory network in the escape circuit of crayfish. *Journal of Neuroscience* **22**:9078–9085.

- Herberholz J, Sen MM, Edwards DH (2004) Escape behavior and escape circuit activation in juvenile crayfish during prey-predator interactions. *Journal of Experimental Biology* **207**:1855–1863.
- Hevers W, Hardie RC (1993) Serotonin modulated Shaker potassium channels in *Drosophila* photoreceptors. In: *Gene–Brain–Behavior: Proceedings of the 21st Göttingen Neurobiology Conference* (Elsner N, Heisenberg M, eds), p. 631. Stuttgart: Georg Thieme.
- Hildebrandt KJ, Benda J, Hennig RM (2011) Multiple arithmetic operations in a single neuron: the recruitment of adaptation processes in the cricket auditory pathway depends on sensory context. *Journal of Neuroscience* **31**:14142–14150.
- Hill AA, Edwards DH, Murphrey RK (1994) The effect of neuronal growth on synaptic integration. *Journal of Computational Neuroscience* **1**:239–254.
- Hodgkin AL, Huxley AF (1952) A quantitative description of membrane current and its application to conduction and excitation in nerve. *Journal of Physiology* **117**:500–544.
- Höger U, Torkkeli PH, French AS (2010) Feedback modulation of transduction by calcium in a spider mechanoreceptor. *European Journal of Neuroscience* **32**:1473–1479.
- Horseman G, Huber F (1994) Sound localisation in crickets. I. Contralateral inhibition of an ascending interneuron (AN1) in the cricket *Gryllus bimaculatus*. *Journal of Comparative Physiology A* **175**:389–398.
- Huang Z, Knowles C (1990) Nicotinic and muscarinic cholinergic receptors in honey bee (*Apis mellifera*) brain. *Comparative Biochemistry and Physiology* **97C**:275–281.
- Hutchinson KM, Vonhoff F, Duch C (2014) Dscam1 is required for normal dendrite growth and branching but not for dendritic spacing in *Drosophila* motoneurons. *Journal of Neuroscience* **34**:1924–1931.
- Ikeda K, Koenig JH (1988) Morphological identification of the motor neurons innervating the dorsal longitudinal flight-muscle of *Drosophila melanogaster*. *Journal of Comparative Neurology* **273**:436–444.
- Imaiizumi K, Pollack GS (2005) Central projections of auditory receptor neurons of crickets. *Journal of Comparative Neurology* **493**:439–447.
- Ismail N, Fahrbach SE, Robinson GE (2006) Stimulation of muscarinic receptors mimics experience-dependent plasticity in the honey bee brain. *Proceedings of the National Academy of Sciences of the United States of America* **103**:207–211.
- Jacobs GA, Miller JP (1985) Functional properties of individual neuronal branches isolated in situ by laser photoinactivation. *Science* **228**:344–346.
- Jan YN, Jan LY (2010) Branching out: mechanisms of dendritic arborization. *Nature Reviews Neuroscience* **11**:316–328.
- Jones PW, Gabbiani F (2010) Synchronized neural input shapes stimulus selectivity in a collision-detecting neuron. *Current Biology* **20**:2052–2057.
- Jones PW, Gabbiani F (2012) Logarithmic compression of sensory signals within the dendritic tree of a collision-sensitive neuron. *Journal of Neuroscience* **32**:4923–4934.
- Jones TA, Donlan NA, O'Donnell S (2009) Growth and pruning of mushroom body Kenyon cell dendrites during worker behavioral development in the paper wasp, *Polybia aequatorialis* (Hymenoptera: Vespidae). *Neurobiology of Learning and Memory* **92**:485–495.
- Kamikouchi A, Takeuchi H, Sawata M, Natori S, Kubo T (2000) Concentrated expression of Ca^{2+} /calmodulin-dependent protein kinase II and protein kinase C in the mushroom bodies of the brain of the honeybee *Apis mellifera* L. *Journal of Comparative Neurology* **417**:501–510.
- Kang D, Pikhitsa PV, Choi YW, Lee C, Shin SS, Piao L, Park B, Suh K-Y, Kim T-I, Choi M (2014) Ultra-sensitive mechanical crack-based sensor inspired by the spider sensory system. *Nature* **516**:222–226.
- Katz B, Miledi R (1967) A study of synaptic transmission in the absence of nerve impulses. *Journal of Physiology* **192**:407–436.
- Kaufmann WE, Moser HW (2000) Dendritic anomalies in disorders associated with mental retardation. *Cerebral Cortex* **10**:981–991.

- Keil TA (1997) Functional morphology of insect mechanoreceptors. *Microscopy Research and Technique* **39**:506–531.
- Kennedy D, Calabrese RL, Wine JJ (1974) Presynaptic inhibition: primary afferent depolarization in crayfish neurons. *Science* **186**:451–454.
- Kennedy D, McVittie J, Calabrese R, Fricke RA, Craelius W, Chiapella P (1980) Inhibition of mechanosensory interneurons in the crayfish. I. Presynaptic inhibition from giant fibers. *Journal of Neurophysiology* **43**:1495–1509.
- King DG, Wyman RJ (1980) Anatomy of the giant fiber pathway in *Drosophila*. I. Three thoracic components of the pathway. *Journal of Neurocytology* **9**:753–770.
- Koch C (1989) Seeing chips: analog VLSI circuits for computer vision. *Neural Computation* **1**:184–200.
- Kohl J, Ostrovsky AD, Frechter S, Jefferis GS (2013) A bidirectional circuit switch reroutes pheromone signals in male and female brains. *Cell* **155**:1610–1623.
- Krapp HG, Gabbiani F (2005) Spatial distribution of inputs and local receptive field properties of a wide-field, looming sensitive neuron. *Journal of Neurophysiology* **93**:2240–2253.
- Krasne FB, Edwards DH (2002a) Modulation of the crayfish escape reflex—physiology and neuroethology. *Integrative and Comparative Biology* **42**:705–715.
- Krasne FB, Edwards DH (2002b) Crayfish behavior: lessons learned. In: *Crustacean Experimental Systems in Neurobiology* (Wiese K, ed.), pp. 3–22. New York: Springer.
- Kremer MC, Christiansen F, Leiss F, Paehler M, Knapek S, Andlauer TFM, Förstner F, Kloppenburg P, Sigrist SJ, Tavosanis G (2010) Structural long-term changes at mushroom body input synapses. *Current Biology* **20**:1938–1944.
- Kristan WBJ, Nusbaum MP (1982) The dual role of serotonin in leech swimming. *Journal de Physiologie* **78**:743–747.
- Kühn-Bühlmann S, Wehner R (2006) Age-dependent and task-related volume changes in the mushroom bodies of visually guided desert ants, *Cataglyphis bicolor*. *Journal of Neurobiology* **66**:511–521.
- Kulkarni VA, Firestein BL (2012) The dendritic tree and brain disorders. *Molecular and Cellular Neurosciences* **50**:10–20.
- Kurtovic A, Widmer A, Dickson BJ (2007) A single class of olfactory neurons mediates behavioural responses to a *Drosophila* sex pheromone. *Nature* **446**:542–546.
- Laughlin SB (1996) Matched filtering by a photoreceptor membrane. *Vision Research* **36**:1529–1541.
- Laughlin SB (2002) Retinal function: coupling cones clarifies vision. *Current Biology* **12**:R833–R834.
- Laughlin SB, Weckström M (1993) Fast and slow photoreceptors—a comparative study of the functional diversity of coding and conductances in the Diptera. *Journal of Comparative Physiology A* **172**:593–609.
- Laurent G (1990) Voltage-dependent nonlinearities in the membrane of locust nonspiking local interneurons, and their significance for synaptic integration. *Journal of Neuroscience* **10**:2268–2280.
- Laurent G (1993) A dendritic gain control mechanism in axonless neurons of the locust, *Schistocerca americana*. *Journal of Physiology* **470**:45–54.
- Laurent G, Borst A (2008) Short stories about small brains: linking biophysics to computation. In: *Dendrites* (Stuart GJ, Spruston N, Häusser M, eds), pp. 441–464. New York: Oxford University Press.
- Laurent G, Naraghi M (1994) Odorant-induced oscillations in the mushroom bodies of the locust. *Journal of Neuroscience* **14**:2993–3004.
- Laurent G, Seymour-Laurent KJ, Johnson K (1993) Dendritic excitability and a voltage-gated calcium current in locust nonspiking local interneurons. *Journal of Neurophysiology* **69**:1484–1498.
- Laurent G, Stopfer M, Friedrich RW, Rabinovich MI, Volkovskii A, Abarbanel HDI (2001) Odor encoding as an active dynamical process: experiments, computation and theory. *Annual Review of Neuroscience* **24**:263–297.
- Lessmann V, Dietzel ID (1991) Development of serotonin-induced ion currents in identified embryonic Retzius cells from the medicinal leech (*Hirudo medicinalis*). *Journal of Neuroscience* **11**:800–809.

- Levine JD, Wyman RJ (1973) Neurophysiology of flight in wild-type and a mutant *Drosophila*. *Proceedings of the National Academy of Sciences of the United States of America* **70**:1050–1054.
- Lewis B (1992) The processing of auditory signals in the CNS of Orthoptera. In: *The Evolutionary Biology of Hearing* (Webster DB, Fay RR, Popper AN, eds) pp. 95–114. New York: Springer.
- Liberat F, Duch C (2002) Morphometric analysis of dendritic remodeling in an identified motoneuron during postembryonic development. *Journal of Comparative Neurology* **450**:153–166.
- Liberat F, Duch C (2004) Mechanisms of dendritic maturation. *Molecular Neurobiology* **29**:303–320.
- Lindauer M (1961) *Communication Among Social Bees*. Cambridge, MA: Harvard University Press.
- Liu YC, Herberholz J (2010) Sensory activation and receptive field organization of the lateral giant escape neurons in crayfish. *Journal of Neurophysiology* **104**:675–684.
- Lozano VC, Armengaud C, Gauthier M (2001) Memory impairment induced by cholinergic antagonists injected into the mushroom bodies of the honeybee. *Journal of Comparative Physiology A* **187**:249–254.
- MacLeod K, Laurent G (1996) Distinct mechanisms for synchronization and temporal patterning of odor-encoding neural assemblies. *Science* **274**:976–979.
- MacLeod K, Bäcker A, Laurent G (1998) Who reads temporal information contained across synchronized and oscillatory spike trains? *Nature* **395**:693–698.
- Malmierca MS, Cristaudo S, Pérez-González D, Covey E (2009) Stimulus-specific adaptation in the inferior colliculus of the anesthetized rat. *Journal of Neuroscience* **29**:5483–5493.
- Mason A, Leake LD (1978) Morphology of leech Retzius cells demonstrated by intracellular injection of horseradish peroxidase. *Comparative Biochemistry and Physiology A* **61**:213–216.
- Mayford M (2007) Protein kinase signaling in synaptic plasticity and memory. *Current Opinion in Neurobiology* **17**:313–317.
- Menzel R, Giurfa M (2006) Dimensions of cognition in an insect, the honeybee. *Behavioral and Cognitive Neuroscience Reviews* **5**:24–40.
- Michel K (1974) Das Tympanalorgan von *Gryllus bimaculatus* DeGeer (Saltatoria, Gryllidae). *Zeitschrift für Morphologie der Tiere* **77**:285–315.
- Miller JP, Jacobs GA, Theunissen FE (1991) Representation of sensory information in the cricket cercal sensory system. I. Response properties of the primary interneurons. *Journal of Neurophysiology* **66**:1680–1689.
- Mobbs PG (1982) The brain of the honeybee *Apis mellifera*. I. The connections and spatial organization of the mushroom bodies. *Philosophical Transactions of the Royal Society of London B, Biological Sciences* **298**:309–354.
- Muller KJ, Nicholls JG, Stent GS (eds) (1981) *The Neurobiology of the Leech*. New York: Cold Spring Harbor Laboratory Press.
- Nabatiyan A, Poulet JFA, De Polavieja GG, Hedwig B (2003) Temporal pattern recognition based on instantaneous spike rate coding in a simple auditory system. *Journal of Neurophysiology* **90**:2484–2493.
- Nicholls JG, Baylor DA (1968) Specific modalities and receptive fields of sensory neurons in CNS of the leech. *Journal of Neurophysiology* **31**:740–756.
- Nocke H (1972) Physiological aspects of sound communication in crickets (*Gryllus campestris* L.). *Journal of Comparative Physiology A* **80**:141–162.
- O'Donnell S, Donlan NA, Jones TA (2004) Mushroom body structural change is associated with division of labor in eusocial wasp workers (*Polybia aequatorialis*, Hymenoptera: Vespidae). *Neuroscience Letters* **356**:159–162.
- Ogawa H, Cummins GI, Jacobs GA, Oka K (2008) Dendritic design implements algorithm for synaptic extraction of sensory information. *Journal of Neuroscience* **28**:4592–4603.
- Onuma Y, Takahashi S, Asashima M, Kurata S, Gehring WJ (2002) Conservation of Pax 6 function and upstream activation by Notch signaling in eye development of frogs and flies. *Proceedings of the National Academy of Sciences of the United States of America* **99**:2020–2025.

- O'Shea M, Rowell CH (1975) Protection from habituation by lateral inhibition. *Nature* **254**:53–55.
- O'Shea M, Williams JLD (1974) The anatomy and output connection of a locust visual interneurone; the lobular giant movement detector (LGMD) neurone. *Journal of Comparative Physiology A* **91**:257–266.
- Panek I, French AS, Seyfarth E-A, Sekizawa S-I, Torkkeli PH (2002) Peripheral GABAergic inhibition of spider mechanosensory afferents. *European Journal of Neuroscience* **16**:96–104.
- Panek I, Meisner S, Torkkeli PH (2003) The distribution and function of GABA_B receptors in spider peripheral mechanosensilla. *Journal of Neurophysiology* **90**:2571–2580.
- Panek I, Höger U, French AS, Torkkeli PH (2008) Contributions of voltage- and Ca²⁺-activated conductances to GABA induced depolarization in spider mechanosensory neurons. *Journal of Neurophysiology* **99**:1596–1606.
- Pantin CFA (1936) On the excitation of crustacean muscle II. Neuromuscular facilitation. *Journal of Experimental Biology* **13**:111–130.
- Pasch E, Muenz TS, Rössler W. (2011) CaMKII is differentially localized in synaptic regions of Kenyon cells within the mushroom bodies of the honeybee brain. *Journal of Comparative Neurology* **519**:3700–3712.
- Pawson L, Bolanowski SJ (2002) Voltage-gated sodium channels are present on both the neural and capsular structures of Pacinian corpuscles. *Somatosensory and Motor Research* **19**:231–237.
- Pereda AE, Bell TD, Faber DS (1995) Retrograde synaptic communication via gap junctions coupling auditory afferents to the Mauthner cell. *Journal of Neuroscience* **15**:5943–5955.
- Perez-Orive J, Mazor O, Turner GC, Cassenaer S, Wilson RI, Laurent G (2002) Oscillations and sparsening of odor representations in the mushroom body. *Science* **297**:359–365.
- Perez-Orive J, Bazhenov M, Laurent G (2004) Intrinsic and circuit properties favor coincidence detection for decoding oscillatory input. *Journal of Neuroscience* **24**:6037–6047.
- Perisse E, Raymond-Delpech V, Néant I, Matsumoto Y, Leclerc C, Moreau M, Sandoz J-C (2009) Early calcium increase triggers the formation of olfactory long-term memory in honeybees. *BMC Biology* **7**:30.
- Peron S, Gabbiani F (2009) Spike frequency adaptation mediates looming stimulus selectivity in a collision-detecting neuron. *Nature Neuroscience* **12**:318–326.
- Peron SP, Jones PW, Gabbiani F (2009) Precise subcellular input retinotopy and its computational consequences in an identified visual interneuron. *Neuron* **63**:830–842.
- Pfeiffer K, French AS (2009) GABAergic excitation of spider mechanoreceptors increases information capacity by increasing entropy rather than decreasing jitter. *Journal of Neuroscience* **29**:10989–10994.
- Pfeiffer K, Panek I, Höger U, French AS, Torkkeli PH (2009) Random stimulation of spider mechanosensory neurons reveals long-lasting excitation by GABA and muscimol. *Journal of Neurophysiology* **101**:54–66.
- Pfeiffer K, Torkkeli PH, French AS (2012) Activation of GABA_A receptors modulates all stages of mechanoreception in spider mechanosensory neurons. *Journal of Neurophysiology* **107**:196–204.
- Phelan P, Nakagawa M, Wilkin MB, Moffat KG, O'Kane CJ, Davies JA, Bacon JP (1996) Mutations in *shaking-B* prevent electrical synapse formation in the *Drosophila* giant fiber system. *Journal of Neuroscience* **16**:1101–1113.
- Pickard B (1973) Action potentials in higher plants. *Botanical Review* **39**:172–201.
- Pollack GS, Hoy RR (1979) Temporal pattern as a cue for species-specific calling song recognition in crickets. *Science* **204**:429–432.
- Poulet JFA, Hedwig B (2001) Tympanic membrane oscillations and auditory receptor activity in the stridulating cricket *Gryllus bimaculatus*. *Journal of Experimental Biology* **204**:1281–1293.
- Poulet JFA, Hedwig B (2003) A corollary discharge mechanism modulates central auditory processing in singing crickets. *Journal of Neurophysiology* **89**:1528–1540.
- Poulet JF, Hedwig B (2007) New insights into corollary discharges mediated by identified neural pathways. *Trends in Neurosciences* **30**:14–21.

- Power ME (1948) The thoracico-abdominal nervous system of an adult insect, *Drosophila melanogaster*. *Journal of Comparative Neurology* **88**:347–409.
- Querfurth H (1985) Receptor potentials of isolated frog muscle spindle evoked by sinusoidal stimulation. *Journal of Neurophysiology* **53**:60–75.
- Quiring R, Walldorf U, Kloter U, Gehring WJ (1994) Homology of the eyeless gene of *Drosophila* to the small eye gene in mice and aniridia in humans. *Science* **265**:785–789.
- Ramón y Cajal S (1995) *Histology of the Nervous System of Man and Vertebrates* (Swanson N, Swanson LW, transl.). New York: Oxford University Press.
- Ratliff F (1965) *Mach Bands: Quantitative Studies on Neural Networks in the Retina*. San Francisco: Holden-Day.
- von Reyn CR, Breads P, Peek MY, Zheng GZ, Williamson WR, Yee AL, Leonardo A, Card GM (2014) A spike-timing mechanism for action selection. *Nature Neuroscience* **17**:962–970.
- Rheuben MB, Kammer AE (1980) Comparison of slow larval and fast adult muscle innervated by the same motoneuron. *Journal of Experimental Biology* **84**:103–112.
- Roberts A (1968) Recurrent inhibition in the giant-fibre system of the crayfish and its effect on the excitability of the escape response. *Journal of Experimental Biology* **48**:545–567.
- Robinson GE (1992) Regulation of division of labor in insect societies. *Annual Review of Entomology* **37**:637–665.
- Rolls MM, Satoh D, Clyne PJ, Henner AL, Uemura T, Doe CQ (2007) Polarity and intracellular compartmentalization of *Drosophila* neurons. *Neural Development* **2**:7.
- Rössler W, Groh C (2012) Plasticity of synaptic microcircuits in the mushroom body calyx of the honeybee. In: *Honeybee Neurobiology and Behavior—A Tribute to Randolph Menzel* (Giovanni Galizia C, Eisenhardt E, Giurfa M, eds), pp. 141–153. Heidelberg: Springer-Verlag.
- Rovner JS, Barth FG (1981) Vibratory communication through living plants by a tropical wandering spider. *Science* **214**:464–466.
- Rowell CH, O’Shea M, Williams JL (1977) The neuronal basis of a sensory analyser, the acridid movement detector system. IV. The preference for small field stimuli. *Journal of Experimental Biology* **68**:157–185.
- Ryglewski S, Kadas D, Hutchinson K, Schuetzler N, Vonhoff F, Duch C (2014) Dendrites are dispensable for basic motoneuron function but essential for fine tuning of behavior. *Proceedings of the National Academy of Sciences of the United States of America* **111**:18049–18054.
- Sah P, Faber ESL (2002) Channels underlying neuronal calcium-activated potassium currents. *Progress in Neurobiology* **66**:345–353.
- Sánchez-Soriano N, Bottenberg W, Fiala A, Haessler U, Kerassoviti A, Knust E, Löhr R, Prokop A (2005) Are dendrites in *Drosophila* homologous to vertebrate dendrites? *Developmental Biology* **288**:126–138.
- Sanes JR, Zipursky SL (2010) Design principles in insect and vertebrate visual systems. *Neuron* **66**:15–36.
- Schildberger K (1988) Behavioral and neuronal mechanisms of cricket phonotaxis. *Experientia* **44**:408–415.
- Schildberger K, Wohlers DW, Huber F (1989) Central auditory pathway: neural correlates of phonotactic behavior. In: *Cricket Behaviour and Neurobiology* (Huber F, Moore TE, Loher TE), pp. 423–458. Ithaca, NY: Cornell University Press.
- Scholl C, Wang Y, Krischke M, Mueller MJ, Amdam GV, Rössler W (2014) Light exposure leads to reorganization of microglomeruli in the mushroom bodies and influences juvenile hormone levels in the honeybee. *Developmental Neurobiology* **74**:1141–1153.
- Schul J, Mayo AM, Triblehorn JD (2012) Auditory change detection by a single neuron in an insect. *Journal of Comparative Physiology A* **198**:695–704.
- Seeley TD (1982) Adaptive significance of the age polyethism schedule in honeybee colonies. *Behavioral Ecology and Sociobiology* **11**:287–293.
- Silverston AI, Kleindienst HU, Huber F (1985) Synaptic connectivity between cricket auditory interneurons as studied by selective photoinactivation. *Journal of Neuroscience* **5**:1283–1292.

- Seyfarth EA, Sanders EJ, French AS (1995) Sodium channel distribution in a spider mechanosensory organ. *Brain research* **683**:93–101.
- Silva AC, McMillan GA, Santos CP, Gray JR (2015) Background complexity affects response of a looming-sensitive neuron to object motion. *Journal of Neurophysiology* **113**:218–231.
- Single S, Borst A (1998) Dendritic integration and its role in computing image velocity. *Science* **281**:1848–1850.
- Sobel EC, Tank DW (1994) In vivo Ca^{2+} dynamics in a cricket auditory neuron: an example of chemical computation. *Science* **263**:823–826.
- Soderling TR (1993) Calcium/calmodulin-dependent protein kinase II: role in learning and memory. *Molecular and Cellular Biochemistry* **127–128**:93–101.
- Song Z, Postma M, Billings SA, Coca D, Haride RC, Juusola M (2012) Stochastic, adaptive sampling of information by microvilli in fly photoreceptors. *Current Biology* **22**:1371–1380.
- Stieb SM, Muenz TS, Wehner R, Rössler W (2010) Visual experience and age affect synaptic organization in the mushroom bodies of the desert ant *Cataglyphis fortis*. *Developmental Neurobiology* **70**:408–423.
- Stieb SM, Hellwig A, Wehner R, Rössler W (2012) Visual experience affects both behavioral and neuronal aspects in the individual life history of the desert ant *Cataglyphis fortis*. *Developmental Neurobiology* **72**:729–742.
- Stopfer M, Bhagavan S, Smith BH, Laurent G (1997) Impaired odour discrimination on desynchronization of odour-encoding neural assemblies. *Nature* **390**:70–74.
- Strausfeld NJ (2002) Organization of the honey bee mushroom body: representation of the calyx within the vertical and gamma lobes. *Journal of Comparative Neurology* **450**:4–33.
- Strausfeld NJ, Nasset DR (1981) Neuroarchitectures serving compound eyes of Crustacea and insects. *Handbook of Sensory Physiology. Vol VII/6B. Vision in Invertebrates. B: Invertebrate Visual Centers and Behavior* (Astrum H, ed.), pp. 1–132. Berlin: Springer.
- Sun YA, Wyman RJ (1997) Neurons of the *Drosophila* giant fiber system. I. Dorsal longitudinal motor neurons. *Journal of Comparative Neurology* **387**:157–166.
- Sztarker J, Rind FC (2014) A look into the cockpit of the developing locust: looming detectors and predator avoidance. *Developmental Neurobiology* **74**:1078–1095.
- Tanouye MA, Wyman RJ (1980) Motor outputs of giant nerve fiber in *Drosophila*. *Journal of Neurophysiology* **44**:405–421.
- Teshiba T, Shamsian A, Yashar B, Yeh SR, Edwards DH, Krasne FB (2001) Dual and opposing modulatory effects of serotonin on crayfish lateral giant escape command neurons. *Journal of Neuroscience* **21**:4523–4529.
- Thomas JB, Wyman RJ (1983) Normal and mutant connectivity between identified neurons in *Drosophila*. *Trends in Neurosciences* **6**:214–219.
- Torkkeli PH, Panek I, Meissner S (2011) Ca^{2+} /calmodulin-dependent protein kinase II mediates the octopamine-induced increase in sensitivity in spider VS-3 mechanosensory neurons. *European Journal of Neuroscience* **33**:1186–1196.
- Triblehorn JD, Schul J (2013) Dendritic mechanisms contribute to stimulus-specific adaptation in an insect neuron. *Journal of Neurophysiology* **110**:2217–2226.
- Ulanovsky N, Las L, Nelken I (2003) Processing of low-probability sounds by cortical neurons. *Nature Neuroscience* **6**:391–398.
- Vonhoff F, Duch C (2010) Tiling among stereotyped dendritic branches in an identified *Drosophila* motor neuron. *Journal of Comparative Neurology* **518**:2169–2185.
- Walker RK, Smith PA (1973) The ionic mechanism for 5-hydroxytryptamine inhibition on Retzius cells of the leech *Hirudo medicinalis*. *Comparative Biochemistry and Physiology A* **45**:979–993.
- Wessel R, Kristan WB, Kleinfeld D (1999a) Supralinear summation of synaptic inputs by an invertebrate neuron: dendritic gain is mediated by an “inward rectifier” K^+ current. *Journal of Neuroscience* **19**:5875–5888.

- Wessel R, Kristan WBJ, Kleinfeld D (1999b) Dendritic Ca^{2+} -activated K^+ conductances regulate electrical signal propagation in an invertebrate neuron. *Journal of Neuroscience* **19**:8319–8326.
- Widmer A, Höger U, Meisner S, French AS, Torkkeli PH (2005) Spider peripheral mechanosensory neurons are directly innervated and modulated by octopaminergic efferents. *Journal of neuroscience*, **25**:1588–1598.
- Wiersma CAG (1947) Giant nerve fiber system of the crayfish: a contribution to comparative physiology of synapse. *Journal of Neurophysiology* **10**:23–38.
- Wiersma CAG (1961) Reflexes and the central nervous system. In: *The Physiology of Crustacea*, Vol. 2 (Waterman TH, ed.), pp. 241–279. New York: Academic.
- Wiersma CAG, van Harreveld A (1938) The influence of the frequency of stimulation on the slow and fast contraction in crustacean muscle. *Physiological Zoology* **11**:75–81.
- Wiese K, Eilts K (1985) Evidence for matched frequency dependence of bilateral inhibition in the auditory pathway of *Gryllus bimaculatus*. *Zoologische Jahrbücher Physiologie* **89**:181–201.
- Willard AL (1981) Effects of serotonin on the generation of the motor program for swimming by the medicinal leech. *Journal of Neuroscience* **1**:936–944.
- Williams DW, Truman JW (2004) Mechanisms of dendritic elaboration of sensory neurons in *Drosophila*: insights from in vivo time lapse. *Journal of Neuroscience* **24**:1541–1550.
- Wilson DM (1961) The central nervous control of flight in a locust. *Journal of Experimental Biology* **38**:471–490.
- Winston ML (1987) *The Biology of the Honeybee*. Cambridge, MA: Harvard University Press.
- Withers G, Fahrbach S, Robinson GE (1993) Selective neuroanatomical plasticity and division of labour in the honeybee. *Nature* **364**:238–240.
- Wohlers DW, Huber F (1982) Processing of sound signals by six types of neurons in the prothoracic ganglion of the cricket *Gryllus campestris* L. *Journal of Comparative Physiology A* **146**:161–173.
- Yang SM, Vilarchao ME, Rela L, Szczupak L (2013) Wide propagation of graded signals in nonspiking neurons. *Journal of Neurophysiology* **109**:711–720.
- Yasuyama K, Kitamoto T, Salvaterra PM (1995) Immunocytochemical study of choline acetyltransferase in *Drosophila melanogaster*: An analysis of cis-regulatory regions controlling expression in the brain of cDNA-transformed flies. *Journal of Comparative Neurology* **361**:25–37.
- Yeh SR, Fricke RA, Edwards DH (1996) The effect of social experience on serotonergic modulation of the escape circuit of crayfish. *Science* **271**:366–369.
- Yeh SR, Musolf BE, Edwards DH (1997) Neuronal adaptations to changes in the social dominance status of crayfish. *Journal of Neuroscience* **17**:697–708.
- Zars T (2000) Behavioral functions of the insect mushroom bodies. *Current Opinion in Neurobiology* **10**:790–795.
- Zucker RS (1972) Crayfish escape behaviour and central synapses. I. Neural circuit exciting lateral giant fiber. *Journal of Neurophysiology* **35**:599–620.
- Zucker RS, Kennedy D, Selverston AI (1971) Neural circuits mediating escape responses in crayfish. *Science* **173**:645–650.

Chapter 24

Dendrites and disease

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Summary

While the functional properties of dendrites in the normal brain are the main focus of this book, there is a long history of research related to changes in dendrites that are associated with certain neurological, psychiatric, and developmental disorders. Much of this research has documented morphological changes in dendritic structure, but a significant increase in work has appeared since the second edition of this book in which changes in dendritic function have been described. In this chapter we briefly review some of the research relating structural changes in dendrites to disease, sufficient to provide a beginning reference source for readers interested in pursuing the subject further. The bulk of this chapter, however, will focus on the current state of knowledge related to dendritic “channelopathies.” These are defined as genetic or acquired defects in the normal function or expression of ion channels (with a focus on voltage-gated ion channels) that are known to regulate how neuronal dendrites process and store information. The most specific and detailed information is available for epilepsy, which will be discussed at some length, but other data will be presented for certain neurodevelopmental disorders (e.g., autism, fragile X syndrome) and diseases of the adult and aging brain. Based on our knowledge of the normal properties of dendrites, we provide a framework for understanding how dendritic channelopathies can influence synaptic integration and plasticity and thus form the basis of abnormal brain function.

Introduction

Abnormalities in dendritic structure are a characteristic feature of many brain disorders. This is perhaps not surprising given the important role of dendrites as the principal site of synaptic contact for neurons. Changes in synaptic function or neuronal circuitry associated with disease might thus be expected to produce structural alterations resulting in, for example, loss of spines, changes in spine size and shape, reduced dendritic branching patterns, and shortened dendritic lengths. In fact, all of these structural changes have been described for different neurological disorders. What may be surprising, however, is how varied, extensive, and specific these structural changes are for each individually described disease state. In some cases the morphological changes in dendrites are actually used as a diagnostic fingerprint for the disorder. Unfortunately, much less is known about how these structural changes relate to the functional properties of neurons, such as synaptic integration, plasticity, excitability, and firing behaviors.

Neurodevelopmental disorders

Neurodevelopmental disorders (NDDs) are pathologies of the nervous system arising from defects in the way the nervous system grows and develops (Meredith, 2014). Such disorders may have

a plethora of consequences, including impairment in cognitive functions, speech, attention and concentration, social skills, and motor function as well as behavioral disturbances. NDDs often manifest in early childhood, leading to perturbations in developmental milestones or difficulties with socialization and integration into school. Well-known examples include autism spectrum disorders, intellectual disabilities, and attention-deficit/hyperactivity disorder (ADHD). Many NDDs have a strong genetic component, but environmental factors or trauma can also play a role (United States Environmental Protection Agency, 2013).

In 1974 Dominick Purpura published a highly influential paper in *Science* suggesting that dendritic spine abnormalities (dysgenesis) formed the basis of certain types of intellectual disability (Purpura, 1974). While this was certainly not the first time it had been suggested that changes in dendritic morphology were associated with neurological disease (cf. Marin-Padilla, 1972; Scheibel and Scheibel, 1973), it nevertheless stimulated significant further research in this area. It is now clear that there is a strong correlation between dendritic pathology and intellectual disability; in particular, this has been demonstrated for Down, Rett, fragile X (FXS), Williams, and Rubinstein-Taybi syndromes (Kaufmann and Moser, 2000), and autism spectrum disorders (Hutsler and Zhang, 2010; Tang et al., 2014).

In general, these and other developmental disorders (Nitkin, 2000) are characterized by changes in dendritic length, branching patterns, and spine number (Fiala et al., 2002). In the case of FXS and autism in particular, spines are often long and thin. While it would be surprising if the dendrites were functioning normally under these conditions, it is unclear whether the altered morphology is the primary cause of the disease or rather a compensatory or secondary change to some other primary pathology. For example, many of these same changes can occur following deafferentation (Fiala et al., 2002). Nevertheless, it would be interesting and important to better understand the physiological consequences of these pronounced and striking changes in dendritic structure that are frequently associated with mental retardation.

In addition to the well-described changes in dendritic spine morphology, alterations in the function and/or expression levels of voltage-gated ion channels are also likely to play a profound role in the pathophysiology of a number of NDDs. Although this question has, to date, received scant attention, the important role of ion channels in many aspects of dendritic physiology mean that any polymorphism/mutation affecting the targeting, modulation, or biophysical properties of ion channels are likely to impact on dendritic function. The following sections illustrate the possible and known roles that ion channel defects play in disorders of this nature.

Autism spectrum disorders

Autism spectrum disorders (ASDs) comprise a heterogeneous group of disorders that fall under the category of pervasive developmental disorders. ASDs are diagnosed by the presence of a triad of core behavioral features including defects in social interaction, communication, and repetitive or stereotyped behavior. In addition to these endophenotypes, other comorbidities are often present, such as an increased risk of epilepsy, defects in sensory information processing, or motor defects and intellectual disability. Recent estimates from the Centers for Disease Control suggest that as many as one in seventy children may be affected by this spectrum of disorders. ASDs are suggested to have a strong genetic component; however, in most cases there is not one single genetic cause (reviewed in Schmunk and Gargus, 2013). Notable exceptions include well-characterized syndromes such as Angelman, Rett, and FXS. Indeed the wide heterogeneity of ASDs is probably caused by a unique combination of gene polymorphisms, mutations, and interactions with environmental factors that underlie the etiology of individual cases (Schmunk and Gargus, 2013).

According to the SFARIgene database (a curated list of genes associated with ASD; https://gene.sfari.org/autdb/HG_Home.do) more than 600 genes have been identified as playing a putative role in ASDs, including a substantial number of ion channels of the Ca^{2+} , K^+ , and Na^+ families as well as the HCN1 isoform of the hyperpolarization-activated, cyclic nucleotide-gated (HCN) channel family.

In particular, voltage-gated Ca^{2+} channels have been suggested to play an important role in ASDs (Krey and Dolmetsch, 2007). Evidence comes from genetic studies in human patients or behavioral analysis following deletion or pharmacological ablation of specific subunit types in mice (e.g., Jinnah et al., 1999). For example, loss of function of the genes encoding the alpha 1 subunit of L-type channels ($\text{Ca}_v1.2$) or an α_2 -delta subunit 4 may arise from genomic deletion in ASDs (Smith et al., 2012). Rare missense mutations have been identified in the genes encoding the β_2 -subunit in ASDs (Breitenkamp et al., 2014). Likewise, a missense mutation in the gene encoding the T-type Ca^{2+} channel subunit $\text{Ca}_v3.2$ (Splawski et al., 2006) or polymorphism in the gene encoding $\text{Ca}_v3.3$ (Lu et al., 2012) may also contribute to the etiology of ASDs.

T-type Ca^{2+} channels are expressed in the neocortex, hippocampus, thalamus, and cerebellum and exhibit a distinct somato-dendritic pattern of expression depending on the subunit in question (McKay et al., 2006). T-type calcium channels are expressed in both the shaft and the spines of dendrites in CA1 pyramidal neurons (Christie et al., 1995; Magee and Johnston, 1995; Sabatini and Svoboda, 2000), and in dendritic spines of neocortical pyramidal neurons (Koester and Sakmann, 2000). This pattern of expression suggests an important role in normal dendritic function. L-type Ca^{2+} channels are present in the dendritic shaft of CA1 pyramidal neurons (Christie et al., 1995; Magee and Johnston, 1995), and in both dendrites and spines in neocortical pyramidal neurons (Markram et al., 1995; Koester and Sakmann, 1998). Thus, the aforementioned mutations are likely to have important consequences, which so far have not been explicitly investigated.

Direct evidence for dendritic involvement in the pathophysiology of ASDs comes from analysis of the effects of a mutant type of L-type Ca^{2+} channel in rodent and human pluripotent stem cell-derived neurons. In both cases, the mutation present in Timothy's syndrome (TS; a rare mono-genetic syndromic form of ASD) caused activity-dependent retraction of dendrites (Krey et al., 2013). Interestingly, this phenomenon was independent of Ca^{2+} signaling and instead involved ectopic activation of the RhoA signaling pathway through impaired interactions between the mutant channel and the small Ras-related proteins that inhibit VSCCs (RGK), Gem. This finding thus points to a novel role for L-type calcium channels in dendritic function. In addition, the TS mutation delays the voltage-dependent inactivation of the mutant channel, while at the same time accelerating the kinetics for Ca^{2+} -dependent inactivation (Barrett and Tsien, 2008). These alterations in gating properties could cause additional changes in excitability, which maybe contribute to the overall pathology of TS (reviewed in Szlapczynska et al., 2014).

Intellectual disability

Intellectual disability (ID; or intellectual development disorder) is a pathology encompassing a diverse group of disorders whose common feature is compromised mental abilities that impact on the individual's adaptive functioning (American Psychiatric Association, 2013). Diagnosis is based on mental function across three core domains (conceptive, social, and practical capacities) and may also incorporate a consideration of intelligence quotient (although this is no longer considered the sole criterion for diagnosis). IDs are considered to have a strong developmental component and may be classified as syndromic or non-syndromic based on the presence or absence (respectively) of accompanying comorbidities (van Bokhoven, 2011). Much of our understanding

of the mechanisms underlying ID comes from our ability to model these disorders in suitable animal models. Thus the majority of our understanding of ID comes from a somewhat limited subset of disorders (e.g., Rett syndrome, Angelman syndrome, FXS, Down syndrome). Nonetheless, in recent years, ID has been investigated in a growing number of novel animal models (e.g., Syn-GAP1; see Huang, 2009; Clement et al., 2012).

ID is often thought to arise from an underlying defect in synaptic function (e.g., Zoghbi and Bear, 2012) or from morphofunctional defects in dendrites or dendritic spines (e.g., as reviewed by Ramakers, 2002; Dierssen and Ramakers, 2006; Portera-Cailliau, 2012; Chang et al., 2013). Thus a large body of research has focused on these aspects as the causal mechanism, leading to a perhaps biased proliferation of models that assume an underlying synaptic cause. However, as mentioned above, these changes may be a consequence of altered signaling pathways rather than the causal feature of the disorder.

More than 450 genes have been associated with ID (reviewed in van Bokhoven, 2011), including a number of ion channel subunits (<http://gfuncpathdb.ucdenver.edu/iddrc/iddrc/home.php>). For example, two independent studies reported individual cases in which the deletion of *CACNAG1* (encoding the T-type calcium channel subunit, $\text{Ca}_v3.1$) was implicated in a novel form of syndromic ID disorder (Preiksaitiene et al., 2012; Harbuz et al., 2013). *KCNK9*, encoding the two-pore acid-sensitive K^+ channel, TASK3, is mutated in a rare syndromic form of ID, called Birk–Barel syndrome (Barel et al., 2008). The role of TASK3 in the brain is poorly understood, but the predominantly somato-dendritic expression of this channel (Marinc et al., 2014) suggests a role in dendritic function. In this case, the mutation resulted in a de novo inward current and an alteration in the sensitivity of the channel to a number of factors including G α q-coupled muscarinic receptor activation. Mutations in genes (*KCNQ2*, *KCNQ3*, and *KCNT1*) encoding the K^+ channel subunits $\text{K}_v7.2$, $\text{K}_v7.3$ and $\text{KCa}4.1$, respectively have also been reported in more complex disorders involving both epilepsy and ID (Heron et al., 2012; Weckhuysen et al., 2012; Miceli et al., 2014). Both $\text{K}_v7.2$ and $\text{K}_v7.3$ are subunits underlying M-type current (for review see Judy and Zandi, 2013). Although mainly expressed at the somatic and axonal level (some dendritic expression has also been reported—reviewed in Szalpczynska et al., 2014), M-type channels not only control initiation of action potentials (APs) and neuronal excitability, but can also regulate excitatory postsynaptic potential (EPSP)–spike (E–S) coupling (Brown and Passmore, 2009; Shah et al., 2011). Their alterations could therefore also affect dendritic information processing. Finally, studies involving either genetic or pharmacological ablation of a number of ion channels involved in dendritic information processing support an important role for a number of these channels in cognitive function. Moreover, a recent study combining functional brain imaging with genetics suggests a strong association between voltage-gated cation channels and cognition (Heck et al., 2014).

Fetal alcohol syndrome (FAS)

Early (in utero) exposure to alcohol is known to have profound effects on the cognitive abilities of exposed individuals. These effects are caused in part by gross-scale circuit remodeling provoked by cell death; however, a cell-dependent mechanism may also partly explain the observed changes. Early postnatal exposure of mice to alcohol (modeling the effects of FAS in humans) leads to a reduced number and duration of dendritic spikes, consistent with a defect in dendritic electogenesis in L5 neocortical pyramidal neurons (Granato et al., 2012). This effect is likely mediated through defects in the function of L-type Ca^{2+} channels, which have been shown to be important for dendritic spikes in these neurons (Almog and Korngreen, 2009).

Fragile X syndrome

In this sense FXS is perhaps the “prototypic” ID, being the most widely studied inherited ID disorder having a monogenic cause. It is also the most common inherited form of autism (Bassell and Warren, 2008). Symptoms include ADHD, anxiety and mood disorders, learning and intellectual disabilities, seizures, and autistic features. The syndrome is caused by the loss of expression of a protein called the fragile X mental retardation protein (FMRP) due to transcriptional silencing of the gene *FMR1*, which encodes FMRP. FMRP is an mRNA-binding protein that represses translation of the bound mRNA. The lack of FMRP can thus lead to enhanced translation of certain mRNAs. FMRP has multiple mRNA targets (Darnell et al., 2011), but it also can bind to other proteins and alter their function. A seminal paper by Huber et al. (2002) found an enhancement in the metabotropic glutamate receptor-mediated form of synaptic long-term depression in a mouse model of FXS in which *FMR1* was deleted. This and many subsequent studies led to the so-called mGluR theory for FXS (Bear et al., 2004), which suggested an enhancement of the mGluR signaling pathways in FXS. While very attractive, this view of FXS is probably too simplistic, and in fact several clinical trials of mGluR antagonists have failed to provide a statistically significant improvement over placebo in crossover clinical trials (reviewed in Scharf et al., 2015).

In recent years attention has focused on other FMRP-targeted mRNAs, including those associated with a number of voltage-gated ion channels that are heavily expressed in dendrites. Among the first voltage-gated ion channels identified as a target for FMRP were the L-type Ca^{2+} channel, a delayed-rectifier K^+ channel, $\text{K}_v3.1$, and Slack (sequence like a Ca^{2+} activated K^+) channel (Chen et al., 2003; Meredith et al., 2007; Brown et al., 2010; Strumbos et al., 2010). While $\text{K}_v3.1$ is a prominent channel in fast-spiking interneurons, Slack channels are heavily expressed in pyramidal neurons and dendrites in the hippocampus and parts of the cortex (Bhattacharjee and Kaczmarek, 2005). Interestingly, intracellular Na^+ (not Ca^{2+} as is the case for the many types of Ca^{2+} -dependent K^+ channels) activates Slack channels. Several recent studies have in fact explored the role of one type of Ca^{2+} -dependent K^+ channel, the BK channel, in FXS. Klyachko and colleagues found that FMRP binds directly to the $\beta 4$ regulatory subunit of BK channels in a translation-independent manner to enhance the calcium sensitivity of the BK channels. The absence of FMRP therefore leads to a broadening of the AP and increases in presynaptic transmitter release (Deng et al., 2013). In a recent report Zhang et al. (2014) found a similar defect in L2/3 and L5 pyramidal neurons of the sensory cortex, both *in vitro* and *in vivo*. In addition, the authors described a dendritic hyperexcitability phenotype in L5 neurons due to BK (and HCN) channel dysfunction (Fig. 24.1). Several core features of this dendritic hyperexcitability and changes in AP firing were rescued with a BK channel opener (Fig. 24.1E), as was hypersensitivity to sensory (auditory) stimuli. These findings provide evidence for a link between an ion channel alteration and a core symptom of FXS (Zhang et al., 2014).

One of the first predominantly dendritic channels suggested to be abnormally expressed in FXS is the fast-inactivating K^+ channel $\text{K}_v4.2$ (Gross et al., 2011; Lee et al., 2011). This channel is known to be a prominent dendritic channel in a number of cell types including CA1 pyramidal neurons in the hippocampus (Hoffman et al., 1997; Johnston et al., 2000). The first two reports suggesting a $\text{K}_v4.2$ channelopathy in the mouse model of FXS, however, were somewhat contradictory. One suggested a reduction of dendritic $\text{K}_v4.2$ (Gross et al., 2011) and the other an increase (Lee et al., 2011). A recent third study used a more functional approach by making physiological recordings from hippocampal CA1 dendrites (Routh et al., 2013) and concluded that $\text{K}_v4.2$ channels were indeed downregulated in FXS, leading to hyperexcitability of the dendrites. The reasons for the discrepancies among the studies are not clear, but more recent experiments suggesting that channelopathies in FXS are specific to both cell types and brain regions may provide some clues.

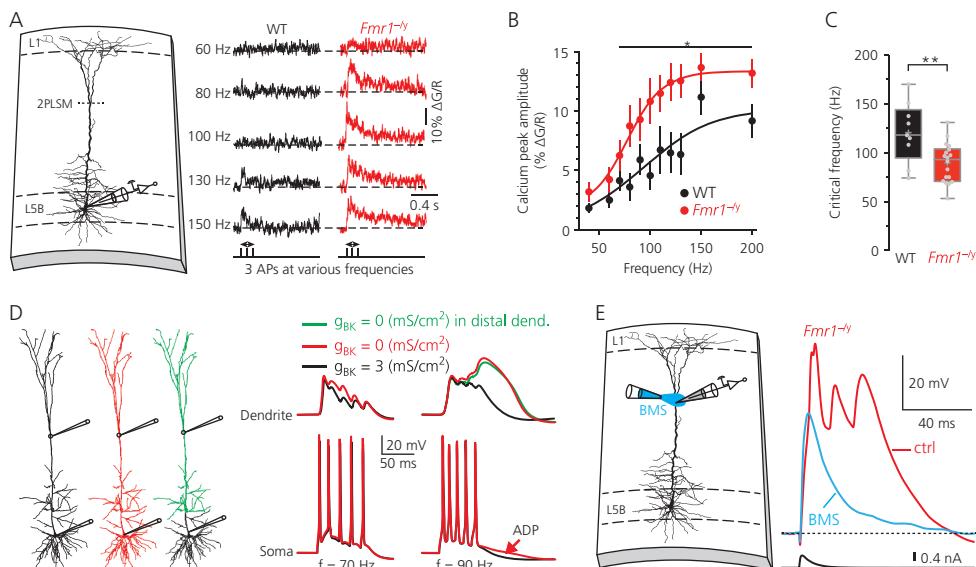


Fig. 24.1 Dysfunction of dendritic BK_{Ca} channels causes hyperexcitability of neocortical pyramidal neurons in a mouse model for fragile X syndrome. (A–C) Dendritic calcium measurements were performed near the major apical branch-points of L5B pyramidal neurons. (A) Dendritic Ca^{2+} traces accompanying trains of three backpropagating APs at various frequencies in a representative wild type (WT) and *Fmr1*^{-/-} neuron. (B) Average Ca^{2+} peak amplitudes as a function of AP train frequency (*Fmr1*^{-/-}, $n = 18$; WT, $n = 9$; $P < 0.05$ for a range from 70 to 200 Hz). (C) Average critical frequency for the generation of dendritic Ca^{2+} spikes. (D) Computer simulation of the impact of BK_{Ca} channel reduction on dendritic excitability. Left: reconstruction of the morphology of a L5B pyramidal neuron used for the NEURON® simulations. Right: model responses at the major apical branch point (upper traces) and soma (lower traces) for AP trains evoked at the soma at a frequency below 70 Hz (left trace) and around the critical frequency (90 Hz; right traces). Red traces represent stimulation in a model without BK_{Ca} conductance, and the black traces stimulation in a model with a BK_{Ca} conductance of 3 mS/cm^2 . Note that the generation of a dendritic spike was associated with an increased after-depolarization (ADP, red arrow) in the absence of a BK_{Ca} conductance. The green trace represents stimulation in a model with a BK_{Ca} conductance of 3 mS/cm^2 only in the soma and the proximal dendrites. This condition is similar to the one without BK_{Ca} conductance (red traces), indicating the role of more distal dendritic BK_{Ca} channels in the dendritic spike generation. (E) Dendritic whole-cell recordings were performed at the major branch point. Suppression of dendritic calcium spikes (evoked by current wave injections) following local puff application of the specific BK_{Ca} channel opener BMS-191,011 (100 μM) onto the dendrite of a *Fmr1*^{-/-} neuron. Data are shown as the mean \pm SEM. ** $P < 0.01$, * $P < 0.05$ (*Fmr1*^{-/-} compared with WT). Statistical significance was calculated by repeated measure two-way ANOVA (B), or by an unpaired t-test.

Parts B and C adapted with permission from Macmillan Publishers Ltd: *Nature Neuroscience*, 17(12), Yu Zhang, Audrey Bonnan, Guillaume Bony, Isabelle Ferezou, Susanna Pietropaolo, and Melanie Ginger, Dendritic channelopathies contribute to neocortical and sensory hyperexcitability in *Fmr1*^{-/-} mice, pp. 1701–1709, Figure 4b and c, © 2014, Nature Publishing Group.

As will be discussed more fully in the section on epilepsy, another prominent dendritic channel is the HCN channel, or so-called h-channel (Magee 1998, 1999), which in forebrain neurons is primarily encoded by HCN1 and HCN2 subunits. One of the first suggestions for a role of HCN1 channels in FXS came from a study by Brager et al. (2012) in which an upregulation of HCN1 channels was found to occur in the dendrites of hippocampal CA1 neurons. This was a surprising finding, because mGluR signaling has been shown to downregulate HCN channels in CA1 and other neurons. In fact, Zhang et al. (2014) and Kalmbach (in preparation) have shown downregulation of HCN channels in L5 neurons from both the sensory and prefrontal cortex. This leads to the interesting idea that FXS-related channelopathies, for the same ion channel, can have both neuron-type and brain-region specificity, and highlights the need to understand FXS and its treatment in a neuron-type/brain-region-specific manner.

Neuropsychiatric disorders

Neuropsychiatric disorders, for example schizophrenia, depression, and bipolar disorder, are complex pathologies, with etiologies that probably involve the convergence of multiple genetic and non-genetic factors (Sullivan et al., 2012). Several recent large-scale gene association studies have pointed to a role for polymorphisms in voltage-gated ion channels in the pathophysiology of these disorders (reviewed in Bhat et al., 2012; Berger and Bartsch, 2014). For example, polymorphisms within two genes encoding the L-type calcium channel subunit ($\text{Ca}_v1.2$) and the β_2 subunit of voltage-gated calcium channels have been linked with a cluster of psychiatric disorders including bipolar disorder, schizophrenia, and depression. Polymorphisms within the β_2 subunit were in addition associated with ADHD and ASD (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013). A functional cluster of voltage-gated cation channels has also been associated with working memory performance in individuals affected by schizophrenia.

Bipolar disorder has also been associated with mutations in the genes encoding $K_v7.2$ and $K_v7.3$, the major subunits responsible for the slow voltage-gated M-channel (for review see Judy and Zandi, 2013). As indicated above, although principally expressed in the soma and axons of neurons, M-channels not only control the initiation of APs and neuronal excitability but are also capable of regulating E-S coupling (Brown and Passmore, 2009; Shah et al., 2011). Thus any polymorphism/mutation that alters their function could conceivably play a role in dendritic information processing.

Lastly, BK channels might also be dysregulated in schizophrenia. Post-mortem analysis has demonstrated a reduction in BK channel mRNA in the prefrontal cortex of individuals affected by this pathology, compared with normal disease-free controls (Zhang et al., 2006). A reduction in the activity of BK channels has been associated with increased dendritic excitability (see the subsection Fragile X syndrome) thus suggesting a link between this finding and dendritic pathophysiology in schizophrenia (reviewed in Szlapczynska et al., 2014).

Neuropathic pain

While the pain neuroaxis consists of peripheral nociceptors, spinal cord, and supraspinal areas, forebrain structures are also receiving increased attention as possible sites for the central manifestations of chronic pain. In particular, the anterior cingulate cortex (ACC) appears to be consistently activated during nociception and chronic pain states (Wager et al., 2013). An interesting recent study used a sciatic nerve injury model in mice to test whether neuronal function in the ACC was altered and responsible for the development of neuropathic pain (Santello and Nevian, 2015).

The results of this study were perhaps somewhat surprising, but not for the channelopathy theme of this chapter. The authors found that following neuropathic pain induced by nerve damage there was a specific decrease in dendritic HCN channel function in L5 pyramidal neurons in the ACC. The resulting decrease in I_h led to enhanced firing in response to synaptic input, or an increase in the overall excitability of these neurons. Because serotonergic inputs, and particularly 5-HT₇ receptors, are enriched in this region of the cortex, they further found that infusion of a 5-HT₇ receptor agonist into the ACC could reverse both the decrease in I_h as well as the pain-induced hypersensitivity to mechanical touch. They suggest that the chronic pain led to HCN channel plasticity in the dendrites of these neurons, a “pain memory,” providing a potential new therapeutic target for neuropathic pain.

Neurodegenerative disorders

Neurodegenerative disorders are characterized by a progressive loss of the structure and function of neurons, and are typically associated with neuronal death. Neurodegenerative disorders include Alzheimer’s disease (AD), Parkinson’s disease (PD), Huntington’s disease (HD), and amyotrophic lateral sclerosis (ALS). The most common neurodegenerative disorders, AD and PD, have both been linked with ion channel dysfunction, implicating changes in dendritic function in these disorders. In the following we will focus our discussion on PD and AD.

Parkinson’s disease

PD is a common, debilitating, and progressive neurodegenerative condition, and is clinically characterized by motor symptoms including tremor, rigidity, postural instability, and bradykinesia (reviewed in Rivlin-Etzion et al., 2006; De Long and Wichmann, 2007; Hammond et al., 2007). In addition to these motor symptoms, PD encompasses non-motor symptoms such as cognitive and neuropsychiatric defects (Poewe and Luginger, 1999; Politis and Niccolini, 2015). The motor symptoms of PD are attributable to the degeneration of dopamine neurons of the substantia nigra pars compacta (SNc). In PD the amount of dopamine released in brain regions responsible for motor control (e.g., the striatum and globus pallidus) declines, leading to a progressive loss of movement. Other, non-dopaminergic systems such as the serotonergic (reviewed in Politis and Niccolini, 2015) and cholinergic (Hirsch et al., 1987; Rinne et al., 2008) system have also been implicated in PD.

In PD, dopaminergic neurons of the SNc display a decrease in dendritic length, a loss of dendritic spines, and several types of dendritic varicosities (Patt et al., 1991). A similar loss of dendritic spines has also been found in the medium spiny neurons of the striatum (Villalba and Smith, 2013). Several studies have provided evidence for a contribution of ion channels to the degeneration of SNc dopamine neurons and PD pathology. Among those ion channels are L-type Ca²⁺ channels (Ca_v1.2 and Ca_v1.3), T-type Ca²⁺ channels, metabolically regulated, ATP-sensitive K⁺ channels, and Ca²⁺-sensitive and voltage-gated A-type K⁺ channels (reviewed in Dragicevic et al., 2015). These and other ion channels modulate dendritic function and firing properties, and therefore dopamine release by SNc dopamine neurons (reviewed in Dragicevic et al., 2015; see Dufour et al., 2014, for a description of the somato-dendritic ion channel landscape of these neurons). Surmeier and colleagues found that during pacemaking L-type Ca²⁺ channels contribute to the generation of dendritic Ca²⁺ oscillations and that the impact of these channels increases with distance from the soma (Guzman et al., 2010; Dryanovski et al., 2013). The dendritic Ca²⁺ waves, in turn, induce waves of mitochondrial oxidant stress in SNc dopamine neurons and defensive responses (Mosharov et al., 2009; Guzman et al., 2010; Surmeier et al., 2011a). Under normal conditions

these defensive mechanisms protect SNC neurons, but in vulnerable neurons the Ca^{2+} buffering capacity is insufficient for dealing with the oscillatory Ca^{2+} load (reviewed in Dragicevic et al., 2015). Pharmacological inhibition of L-type Ca^{2+} channel function in SNC dopamine neurons promises to be a suitable neuroprotective strategy (Surmeier et al., 2010; Parkinson Study, 2013).

Although the principal target of SNC dopamine neurons is the striatum, the most prominent pathophysiology in animal models of late-stage PD and in human patients occurs in the globus pallidus (GPe). In late-stage PD the firing patterns of GPe neurons change, resulting in suppression of their autonomous activity and the emergence of rhythmic bursting activity within the GPe–subthalamic nucleus network (reviewed in Rivlin-Etzion et al., 2006; DeLong and Wichmann, 2007; Hammond et al., 2007). HCN channels are crucial for the pacemaker activity of GPe neurons (Chan et al., 2004), and a recent study implicated a downregulation of all four pore-forming HCN channel subunits (HCN1–4) as well as of the HCN trafficking protein tetratricopeptide repeat-containing Rab8b-interacting protein (TRIP8b) in the loss of this feature during PD (Chan et al., 2011). Autonomous pacemaking of GPe neurons was restored by upregulating HCN2 channel expression via viral expression (the channel subunit most affected in PD). This change in firing patterns, however, did not significantly improve motor deficits, suggesting that HCN channel downregulation is a homeostatic adaptation of the network pathology rather than a cause. Such changes in ion channel expression can act as a homeostatic mechanism (Turriagano and Nelson, 2004; Frick and Johnston, 2005). Subsequently, the authors provided evidence that calcium influx through dendritic L-type Ca^{2+} channels during burst firing activity caused HCN channel downregulation (Chan et al., 2011).

Dopamine also has immediate influences on the intrinsic excitability of target neurons by modulating their ion channels, suggesting that a decline in dopamine levels during PD has additional consequences for neuronal/dendritic function. Most of the effects of dopamine on intrinsic excitability implicate protein kinase A (PKA)-dependent modulation of voltage-gated Ca^{2+} , Na^+ , and K^+ channels (reviewed in Surmeier et al., 2011b; Tritsch and Sabatini, 2012). The outcome will depend on which dopamine receptors are present in these neurons, namely D_1 -like dopamine receptors (D_1 and D_5) or D_2 -like dopamine receptors (D_2 , D_3 , and D_4) (Svenningsson et al., 2004; Hernandez-Lopez et al., 2000). For example, spiny projection neurons of the direct pathway express primarily D_1 receptors that increase their intrinsic excitability, while those of the indirect pathway express primarily D_2 receptors that decrease their intrinsic excitability. These changes have direct consequences for synaptic plasticity (reviewed in Surmeier et al., 2011).

Alzheimer's disease

AD is a progressive and fatal disease, and the most common neurodegenerative disorder. As such, AD accounts for the majority of patients experiencing memory loss. The accumulation of amyloid- β peptide ($\text{A}\beta$), leading to the formation of so-called amyloid plaques in the brain, is believed to be the core pathophysiological mechanism of AD (Hardy and Selkoe, 2002; Ross and Poirier, 2004). Several studies have demonstrated abnormal spine and dendritic morphology, as well as aberrant dendritic signaling, in AD. The role of dendrites in AD has been highlighted in recent reviews (Nestor and Hoffman, 2012; Cochran et al., 2014). The remainder of this section on AD is devoted to a discussion of alterations in dendritic excitability due to ion channel dysfunction.

$\text{A}\beta$ causes aberrant dendritic signaling by selective binding to a variety of receptors as well as ion channels within the dendrites (reviewed in Cochran et al., 2014). In addition to this direct modulation, some of the putative $\text{A}\beta$ receptors are linked to intracellular signaling cascades such

as mitogen-activated protein kinase (MAPK) that can alter ion channel function/expression. In the following we will focus on the direct consequences of the binding of A β to dendritic ion channels.

Abnormal levels of A β cause an imbalance of excitation to inhibition (Palop and Mucke, 2010). Dysfunction of dendritic ion channels may contribute to this imbalance, in particular via the effects of A β on Ca $^{2+}$, K $^+$, and Na $^+$ channels that regulate dendritic excitability. Calcium dysregulation is a critical component of dysfunction in AD. In neuronal cultures, A β induces MAPK phosphorylation of Ca $v1$ channels and increased calcium influx through these channels, thereby causing neurotoxicity. Ca $v1.2$ channels are L-type Ca $^{2+}$ channels that are located both extrasynaptically and in dendritic spines. In an AD mouse model, Ca $v1.2$ is enriched in dendrites (Willis et al., 2010), and AD brain tissue shows increased Ca $v1$ expression in the hippocampus (Coon et al., 1999).

More recently, a role for Na $v1.1$ channels was also found in the pathophysiology of AD (Verret et al., 2012). In this study, the authors showed a decrease in Na $v1.1$ subunit expression in parvalbumin-positive interneurons of the parietal cortex in both AD patients and a mouse model of AD. In the mouse model of AD, this decrease contributed to the reduced gamma oscillations and increased network synchrony seen in the mouse model (Verret et al., 2012). Together, their results suggest that a decreased expression of Na $^+$ channels in the cortex could contribute to the epileptiform activity and seizures observed in AD patients (Verret et al., 2012; Vossel et al., 2013). Although Na $v1.1$ channels are predominantly expressed in the axon initial segment, thereby controlling the initiation and propagation of APs (Duflocoq et al., 2008), they are also expressed in the somato-dendritic compartment of neocortical and hippocampal pyramidal neurons (Gong et al., 1999), where changes in their expression would alter dendritic excitability.

Another A β -sensitive ion channel that strongly regulates dendritic excitability is the A-type K $^+$ channel (see Nestor and Hoffman, 2012). In hippocampal CA1 pyramidal neurons, these channels are highly expressed in oblique and distal apical dendrites, where they strongly regulate the efficacy of dendritic action potential backpropagation, synaptic summation, and the induction of synaptic and non-synaptic forms of plasticity (Hoffman et al., 1997; Frick et al., 2003, 2004; Chen et al., 2006; Kim et al., 2007; Losonczy et al., 2008; Makara et al., 2009). In cultured and acutely dissociated hippocampal neurons, as well as in acutely dissociated neurons from the diagonal band of Broca nucleus in the basal forebrain, A β blocks A-type K $^+$ channels (Good and Murphy, 1996; Xu et al., 1998; Jhamandas et al., 2001; Zhang and Yang, 2006). Other studies on cultured cerebellar neurons found either an increase in A-type K $^+$ currents or no effect depending on the aggregation state (Ramsden et al., 2001; Plant et al., 2006). A-type K $^+$ channels in cultured cortical neurons were either reduced or unaffected (Ramsden et al., 2001; Ye et al., 2003). From these studies, it has become clear that the brain region, culture condition, application time, and the aggregation state and peptide length of A β influence the modulation of K $^+$ channels.

The first direct evidence for an effect of A β on dendritic excitability was provided by a study by Chen (2005). A β treatment decreased A-type K $^+$ currents in dendritic membrane patches from CA1 pyramidal neurons in acute hippocampal slices. Dendritic currents were more significantly affected compared with somatic ones. The reduction in dendritic A-type K $^+$ currents caused an increase in the amplitude of backpropagating APs, consistent with their known role in regulating this phenomenon. A computational study found that the oblique dendrites would be most profoundly affected by changes in A-type K $^+$ currents (Morse et al., 2010), in agreement with experimental evidence (Frick et al., 2003). Moreover, a decrease in A-type K $^+$ current could contribute to the hyperexcitability phenotype found in hippocampal neurons in a mouse model of AD (Busche et al., 2012).

Epilepsy

Ion channelopathy in epilepsy: humans and animal models

Epilepsy, the disease state of spontaneously recurring seizures, is one of the most prevalent neurological conditions, affecting nearly 1% of the population. That ion channel dysfunction lies at the root of epilepsy has for decades seemed a self-evident truth. Ion channels, both voltage- and ligand-gated, mediate the excitable behavior of neurons, and since seizures result from neuronal hyperexcitability, altered biophysical properties or expression of ion channels must form the final common pathway of pathological hyperexcitability. This idea has been proven in human epilepsy by the discovery of a number of ion channel mutations underlying Mendelian genetic epilepsy, beginning with the finding that a mutation in nicotinic acetylcholine receptors causes autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) (Steinlein et al., 1995). Since that first demonstration in 1995, a number of human genetic epilepsy syndromes have been conclusively linked to single gene mutations (Mantegazza et al., 2010). Notably, most of these genes code for voltage-gated or ligand-gated ion channel subunits or presumptive accessory subunits. The importance of these findings cannot be overstated: they prove that defects in the expression and function of individual ion channel subtypes can produce human genetic epilepsy syndromes.

However, most human epilepsy is of undetermined origin, and not clearly inherited. Are dysfunctional ion channels involved in the pathophysiology of these “idiopathic” epilepsies? A large-scale candidate gene sequencing study suggests that ion channel mutation is not responsible. Sequencing of 237 different ion channel genes found no increase in single nucleotide polymorphisms (SNPs) (i.e., putative mutations) when the genomes of people with sporadic epilepsy were compared with normal controls (Klassen et al., 2011). This still leaves the possibility that modification of the expression of those ion channel genes by any of a number of other mechanisms—transcriptional, translational, or post-translational—may affect neuronal excitability and produce epilepsy. Such ion channel modifications may be the final result of signaling cascades set into motion by any of the acquired insults to the brain that have a high probability of producing epilepsy, such as head injury, brain tumors, or cerebral hemorrhage. This “acquired channelopathy” hypothesis has attracted increasing amounts of experimental support in recent years. And interestingly, some of the ion channels most implicated are highly expressed in the dendrites of pyramidal neurons. In this section, we will discuss some of the evidence for dendritic channelopathy in epilepsy, in both animal models and humans.

One difficulty in proving that acquired channelopathy plays a causal role in human epilepsy is the relative inaccessibility of human brain tissue from patients with epilepsy. Unlike the case with genetic epilepsy where a tube of blood is all that is needed to survey a patient’s genome, patients with acquired epilepsy do not give up brain tissue for analysis, with the exception of the most severely affected who present for brain surgery to resect the epileptogenic focus. Another equally significant problem is the lack of control tissue with which to make comparisons. For the most part, our understanding of acquired channelopathies has relied on animal modeling. Animal models provide the benefits of being able to study controls, as well as the ability to follow the time course of changes in ion channel expression after a neural insult, before spontaneous seizures have arisen.

The most widely used animal models of epilepsy are the post-status epilepticus (SE) models involving induction of SE by pilocarpine or kainic acid. With these protocols, pilocarpine or kainate are injected to produce a period of SE lasting about an hour, followed by termination with sedative drugs. This period of SE appears to produce foci of excitotoxic damage, particularly in the temporal lobe. The result is a very obvious epileptic phenotype in rodents, with spontaneously recurring convulsive seizures that originate in hippocampal and peri-hippocampal structures (Toyoda

et al., 2013). Because seizure onset is from the temporal lobe, the post-SE models appear to best replicate temporal lobe epilepsy (TLE), which is the most common epileptic syndrome in adult humans. And as in human TLE, the post-SE models show a “latent period” between the insult and the development of spontaneous seizures, as well as hippocampal pathology very similar to that seen in humans (known as “mesial temporal sclerosis”). A significant drawback to these models is the fact that they do not replicate a naturalistic insult: humans are rarely exposed to chemical convulsants. However, it has been argued that post-SE models are relevant because some human cases of adult TLE are associated with a history of febrile SE as a child. Additional models of epilepsy exist that attempt to reproduce human brain insults such as traumatic injury, stroke, or infection but vary in the robustness of their epileptic phenotypes (White, 2002).

There are multiple ways in which acquired alteration of ion channel function might contribute to epileptogenesis, either as a fundamental cause of hyperexcitability or as a mediator of the process in which spontaneous seizures progressively worsen the course of epilepsy (Ben-Ari et al., 2008). It is also possible that some ion channel alterations act to retard the development of epilepsy. When interpreting studies of changes in ion channel properties in epilepsy models, it is important to consider whether the changes seen reflect a seizure-dependent process or are set into motion by the initial insult and precede the onset of seizures. A further question is whether the changes seen are pro-convulsive or homeostatic: do they demonstrably increase the excitability of neurons, or do they diminish intrinsic excitability in response to recurrent seizures? With these thoughts in mind, we can consider recent evidence for both inherited and acquired ion channel changes in animal models of epilepsy, focusing on those species that are highly represented in the dendrites of pyramidal neurons found in the cortex and hippocampus. Whenever possible, we will seek correlation with findings in human epilepsy.

Specific dendritic ion channelopathies in epilepsy: HCN channels

HCN channels were originally identified in the sinoatrial node as a key regulator of heart rate, but have since been found widely in the brain, with the HCN1 subtype the predominant isoform in the neocortex and hippocampus, while the HCN2 subtype, although also found in the neocortex and hippocampus, is most highly expressed in subcortical regions such as the thalamus. The biophysical properties of these channels, and their contribution to neuronal excitability, are described elsewhere in this book. In brief, HCN channels are expressed predominantly in the dendrites of pyramidal neurons, including those of the CA1 hippocampus, entorhinal cortex, and neocortex. They principally act to diminish the excitability of pyramidal neurons by inhibiting the impact of excitatory postsynaptic potentials in distal dendrites. Thus, it would be predicted that epilepsy would be associated with loss of expression or function of HCN channels. Of the ion channels with predominantly dendritic expression, changes in HCN channels provide some of the most compelling evidence linking altered expression with epilepsy, particularly in animal models. In humans, the evidence linking HCN channelopathy with epilepsy is growing, but somewhat conflicting.

A recent study associated HCN1 channel mutations with a syndrome of catastrophic early childhood epilepsy characterized by unremitting seizures, intellectual disability, and autism (Nava et al., 2014). Six *HCN1* missense mutations were identified that were *de novo* mutations. When recombinant HCN1 channels with each of the mutations were exogenously expressed, diverse functional effects were seen: three of the mutations abolished I_h , the current mediated by HCN channels, whereas in the other three mutants I_h was upregulated via a depolarizing shift in its voltage-dependent activation. While interpretation of the functional consequences of these mutations must be cautious (since they were not studied in human brain tissue), this study appears to clearly link HCN1 channels and epilepsy. Another report linked a recessive mutation in the human

HCN2 gene with a phenotype of generalized epilepsy (DiFrancesco et al., 2011). Together, these results suggest that while HCN channels are not a well-recognized cause of Mendelian epilepsy, there are links between HCN channel dysfunction and genetic epilepsy in humans.

In acquired epilepsy in humans there is suggestive but inconclusive evidence that HCN channel expression is altered. HCN channel mRNA expression from temporal lobe resections overall showed no significant change in comparison with autopsy controls; however, a subgroup of patients with the greatest degree of mesial temporal sclerosis had an increase in HCN1 expression in the dentate gyrus (DG) (Bender et al., 2003). This finding was surprising, since DG neurons normally demonstrate very little I_h ; thus this could be interpreted as “compensatory” upregulation of HCN1 expression in the most severely affected patients. Other investigators found that the magnitude of I_h measured in neocortical neurons from brain tissue acutely removed during epilepsy surgery inversely depended on the pre-surgical baseline frequency of seizures, suggesting that more severe epilepsy was associated with a loss of neocortical HCN channel function (Wierschke et al., 2010). As mentioned above, the lack of control comparisons makes these findings difficult to interpret. Also, whether the changes in HCN channel expression and the magnitude of I_h are a cause or a consequence of epilepsy is impossible to establish in these studies.

The above human studies provide suggestive evidence for genetic and acquired HCN channelopathies in epilepsy in humans. Studies in animal models with genetic deletions of HCN channels provide a more compelling case that this ion channel may be relevant to epilepsy. Constitutive knock-out of the mouse *hcn2* gene produces a phenotype of generalized epilepsy, consisting of spontaneous absence seizures and generalized 5-Hz spike-wave EEG discharges, most likely resulting from the loss of thalamic HCN2 expression (Ludwig et al., 2003).

Deletion of the *hcn1* gene in rodents does not produce epilepsy, as shown in two studies (Huang et al., 2009; Santoro et al., 2010). Neither of these studies detected spontaneous seizures in knock-out animals. However, both studies demonstrated that *hcn1* deletion increased the severity of SE acutely induced by kainic acid. In addition, the latency period from SE to the occurrence of the first spontaneous seizure was shortened to one-sixth of that in wild-type animals, thus demonstrating an effect of HCN1 channels on epileptogenesis after a brain insult. This study went one step further to examine pyramidal neuron excitability in *hcn1* knock-out mice. As expected, pyramidal neurons lacking the HCN1 subunit demonstrated both increased intrinsic excitability and prolonged excitatory responses to synaptic stimulation. This effect on synaptic transmission may reflect the loss of presynaptic HCN1 channels that normally constrain excitatory transmission (Huang et al., 2011). Thus, genetic deletion of HCN channels confirms that they exert an inhibitory and even anticonvulsant role on cortical and hippocampal excitability under control conditions. However, why HCN1 deletion produces cortical and hippocampal hyperexcitability while not producing epilepsy is not easily explained. One possible reason is that constitutive deletion of HCN1 channels leads to compensatory upregulation of a tonic GABA_A receptor-mediated current that partially suppresses hyperexcitability (Chen et al., 2010).

HCN1 channel expression is also altered during epileptogenesis induced in animals by pilocarpine and KA or following hyperthermia-induced SE. One of the first studies to make this association found an increase in I_h at the soma of CA1 hippocampal pyramidal neurons after hyperthermia-provoked seizures (Chen et al., 2001). Subsequent studies that measured I_h in the dendrites, where its expression is greatest, found an acute loss of dendritic expression of HCN channels within the first week post-SE (Shah et al., 2004). This loss of expression was maintained in chronic epilepsy, and was associated with a hyperpolarizing shift in voltage-dependent gating that further downregulated I_h (Jung et al., 2007). Since HCN channels are activated by hyperpolarization, a hyperpolarizing shift in activation reduces the amount of I_h present at resting potential.

Interestingly, when recurrent seizures post-SE were blocked by administration of phenobarbital, the altered HCN channel gating reverted to normal while the loss of HCN channel expression persisted. This suggested that there are separate mechanisms of HCN channel downregulation in epilepsy, some clearly dependent on ongoing seizures and some independent of seizure activity. The altered gating of dendritic HCN channels nonetheless produces pyramidal neuron hyperexcitability. This gating change was dependent in part on a loss of p38 mitogen-activated protein kinase (p38 MAPK) activity in chronically epileptic animals, suggesting a role for phosphorylation-dependent mechanisms in maintaining the HCN channelopathy (Jung et al., 2010). Interestingly, lamotrigine, a commonly-used anti-epileptic drug, upregulates the gating of HCN channels in pyramidal neuron dendrites, an action that may contribute to its anti-epileptic action (Poolos et al., 2002).

The mechanisms underlying the loss of expression of HCN1 channels in epilepsy models have been studied in great detail, and the insights derived from these investigations may prove important for understanding how the expression of other ion channels is altered during the development of epilepsy following a brain insult. Dendritic HCN1 channelopathy in post-SE models appears to depend on several interwoven processes. Within the first hour post-SE, about half of the HCN1 channels undergo internalization from the dendritic plasma membrane, and are degraded within the first day post-SE (Jung et al., 2011). This effectively reduces the number of functional dendritic ion channels. Similar alterations in ion channel trafficking to the surface membrane post-SE have been described for A-type K⁺ channels and GABA_A receptor subunits, and thus may be a common theme in acquired channelopathy (Goodkin et al., 2008; Lugo et al., 2008; Terunuma et al., 2008). These latter examples are phosphorylation dependent; a similar mechanism may underlie altered trafficking of HCN1 channels in epilepsy, as it has been recently shown that surface expression of HCN1 channels under normal conditions is modulated by the activity of protein kinase C (Williams et al., 2015). Additional possible effectors of defective HCN1 channel trafficking include two accessory scaffolding proteins, Trip8b (Lewis et al., 2011; Piskorowski et al., 2011) and filamin A (Gravante et al., 2004). Trip8b expression is important for the establishment of the HCN1 channel gradient in CA1 pyramidal neuron dendrites, while filamin A appears to increase surface membrane expression of HCN1 channels, at least in heterologous expression systems.

Within the first days post-SE, and persisting into the chronic epilepsy phase, dendritic HCN1 expression remains diminished. This appears to be a result of reduced production of HCN1 mRNA (Brewster et al., 2002). This decrease in transcription appears to depend on the upregulation of a master transcriptional regulator, neuron restrictive silencing factor (NRSF) (McClelland et al., 2011). These results suggest that the development of HCN1 channelopathy is the result of several different mechanisms, each with a distinct temporal evolution. Whether these mechanisms proceed in a serial fashion, each dependent on signaling in the preceding mechanism, or exist as parallel processes, is as yet unknown. The identification of multiple signaling processes underlying dendritic HCN channelopathy points to the possibility of therapeutic interventions that might prevent or reverse altered HCN channel expression in epilepsy.

K⁺ channels

In his classic textbook (Hille, 2001) Bertil Hille commented that K⁺ channels were like the “stops on an organ,” able to fine-tune neuronal excitability by their diverse biophysical properties. Perhaps not surprisingly, then, K⁺ channel dysfunction has been demonstrated to underlie certain genetic epilepsy syndromes. The most well-understood is the Mendelian syndrome benign familial neonatal convulsions (or seizures, BFNC/BFNS), which results from mutation of the genes

KCNQ2 and *KCNQ3* (Biervert et al., 1998). More recently, cases of catastrophic early childhood epilepsy have also been associated with *KCNQ2* mutations (Weckhuysen et al., 2012). Other human genetic syndromes with an epileptic phenotype resulting from K⁺ channel gene mutations include: episodic ataxia and epilepsy, due to *KCNA1* mutations that produce altered defective K_v1.1 channels; developmental delay, epilepsy, and neonatal diabetes (DEND), due to mutation in the inward-rectifier K⁺ channel encoded by the gene *KCNJ11* (Gloyn et al., 2006); and epilepsy, ataxia, sensorineural deafness, and tubulopathy (EAST), due to mutation of another inward rectifier, *KCNJ10* (Reichold et al., 2010). In humans, there is a single report of a gene mutation leading to loss of functional K_v4.2 expression in an individual with epilepsy, a nonsense mutation in the *KCND2* gene leading to truncation of the K_v4.2 protein (Singh et al., 2006).

Of all the human epilepsy-associated K⁺ channelopathies, only K_v4.2 has a clear association with dendritic physiology. *KCNQ2* and *KCNQ3* channels are localized primarily to axo-somatic compartments (Shah et al., 2008), and it is unclear whether the inward rectifiers implicated in human genetic epilepsy have a prominent dendritic localization in pyramidal neurons as they do in rodents (Chen and Johnston 2005). However, numerous studies in animal models of acquired epilepsy have identified downregulation of K_v4.2 and associated neuronal hyperexcitability. Interestingly, like HCN1, genetic deletion of this ion channel produces neuronal hyperexcitability but not epilepsy (Chen et al., 2006; Barnwell et al., 2009).

Like HCN1 channels, K_v4.2 channels mediating the A-type transient K⁺ current (I_A) are also expressed at a higher density in distal dendrites of hippocampal pyramidal neurons, albeit with a somewhat less extreme gradient (about a seven-fold higher density in the apical dendrites than in the soma; Hoffman et al., 1997; Chen and Johnston 2004). The high dendritic density of rapidly activating K_v4.2 channels reduces the amplitude of EPSPs, activity-evoked intracellular calcium transients, and backpropagating dendritic APs. The discovery of its role in experimental epilepsy marked the first determination of a dendritic channelopathy (Bernard et al., 2004). This work found that K_v4.2 expression in chronically epileptic rats post-SE was diminished by about a third, as quantified by both protein and mRNA expression. The electrophysiological consequence of the loss of I_A was increased AP backpropagation in the dendrites, potentially allowing increased opening of other voltage-gated channels such as Ca²⁺ channels. An interesting additional finding was that the remaining K_v4.2 channels were more phosphorylated at a site recognized by the extracellular stimulus-related kinase (ERK), thus diminishing their activity (Hoffman and Johnston 1998; Yuan et al., 2002). K_v4.2 dysfunction seen in chronic epilepsy recapitulates the theme shown for HCN1 channels: multiple mechanisms of channelopathy that result in a loss of channel number, as well as functional downregulation of the remaining channels. Indeed, subsequent studies have shown that, like HCN1 channels, I_A is acutely downregulated in the first several hours post-SE due to internalization from the surface membrane (Lugo et al., 2008). This appears to be a phosphorylation-dependent mechanism, involving hyperactivity of the ERK pathway. How the acute loss of K_v4.2 expression in the first hour post-SE ultimately develops into a persistent state of downregulated K_v4.2 protein and mRNA expression during chronic epilepsy is thus far unknown.

Ca²⁺ channels

Ca²⁺ channels hold several associations with genetic epilepsy. Mutations in the *CACNA1* gene producing the Ca_v2.1 channel yield the Mendelian syndrome of episodic ataxia, familial hemiplegic migraine, and epilepsy. Several polymorphisms in the human *CACNA1H* gene underlying Ca_v3.2 channels have been described in patients with epilepsy (Heron et al., 2004). This latter channel

mediates the low-voltage-activated transient calcium current I_T . Numerous lines of experimental evidence implicate I_T in epilepsy and as a target of anti-epileptic drugs.

Transient T-type Ca^{2+} channels, along with high-voltage-activated R-type channels, are enriched in the dendrites of pyramidal neurons compared with their somatic densities (Magee and Johnston, 1995, 1997). T-type Ca^{2+} channels have a long association with experimental epilepsy, particularly due to their ability to promote neuronal burst firing by producing a depolarizing current that activates at voltages subthreshold to AP firing. Indeed, pharmacological blockers of I_T such as ethosuximide potently inhibit generalized absence seizures (Tringham et al., 2012). Dendritic T-type Ca^{2+} channels may also be relevant to TLE. CA1 pyramidal neurons develop bursting behavior post-SE in conjunction with an increase in dendritic I_T (Su et al., 2002; Yaari et al., 2007). This increase in I_T appears to be mediated solely by $\text{Ca}_v3.2$, and begins before the onset of spontaneous seizures, demonstrating that it is not a consequence of the seizures themselves (Becker et al., 2008). $\text{Ca}_v3.2$ upregulation appears to depend on a transcriptional mechanism. Interestingly, mice with genetic deletion of $\text{Ca}_v3.2$ channels become epileptic just as wild-type mice do post-induction with pilocarpine, but have a significantly reduced seizure frequency. This suggests that inhibition of $\text{Ca}_v3.2$ channels may exert an antiepileptic action. A similar result was seen in which transient pharmacological inhibition of $\text{Ca}_v3.2$ channels post-SE produced a long-lasting anti-epileptic effect (Doeser et al., 2015). Despite these intriguing studies linking T-type channels, particularly the $\text{Ca}_v3.2$ isoform, to seizure generation, no studies to date have looked in detail at the density or voltage-dependent properties of dendritic T-type channels to determine how these are altered in epilepsy.

Other ion channels

Na^+ channels have become an intense focus of channelopathy research owing to their involvement in two genetic epilepsy syndromes, generalized epilepsy with febrile seizures plus (GEFS+) and severe myoclonic epilepsy of infancy (SMEI). They would seem prime suspects in any investigation of the causes of acquired epilepsy. However, even in their associated genetic syndromes, the nature of Na^+ channel dysfunction remains unclear: initial evidence from heterologous expression systems suggested that mutations in $\text{Na}_v1.1$ causing GEFS+ produced a toxic gain of function (an incompletely inactivating Na^+ current, I_{Na}), yet neurons from transgenic mice containing $\text{Na}_v1.1$ mutations appear to show a loss of I_{Na} only in interneurons, the principal neuron I_{Na} being unchanged (Martin et al., 2010). Likewise, hippocampal pyramidal neurons from mice with a truncated $\text{Na}_v1.1$, mimicking mutations seen in SMEI, show no significant alteration in I_{Na} , while hippocampal interneurons have a substantial loss of I_{Na} and decreased repetitive firing in response to stimulation (Yu et al., 2006). Thus there does not appear to be a pathological alteration of dendritic Na^+ channels in hippocampal pyramidal neurons in these relatively more common genetic epilepsies. In models of acquired epilepsy, there is little specific evidence for altered biophysical properties of Na^+ channels, with AP threshold at the soma appearing unchanged in chronic epilepsy, although dendritic Na^+ channels were not specifically examined (Sanabria et al., 2001).

Likewise, ligand-gated GABAergic channels localized to pyramidal neuron dendrites have not been specifically implicated in epilepsy. While there is good evidence that GABAergic afferents from oriens lacunosum-moleculare interneurons to pyramidal neuron dendrites are diminished in chronic epilepsy (Cossart et al., 2001), there is so far no evidence to suggest that dendritic GABA_A receptors themselves are altered in experimental or human epilepsy. However, given the studies that have shown selective up- and downregulation of GABA_A receptor subunits following the induction of epilepsy in experimental models (reviewed in Houser et al., 2012), it is possible

that significant rearrangements of pyramidal neuron dendritic GABA_A receptor subunits may occur during epileptogenesis.

Summary

The studies described here focus on one aspect of dendritic physiology implicated in epilepsy. Since dendrites comprise the majority of the surface membrane area of pyramidal neurons, it is likely that the majority of the cell's ion channel expression also occurs in dendrites. Perhaps it is no surprise then that significant alterations in dendritic ion channel expression and function occur during the development of epilepsy. Other aspects of dendritic biology, such as derangements to cytoskeletal integrity, may also contribute to neuronal hyperexcitability (Zeng et al., 2007; Casanova et al., 2012). As the signaling mechanisms underlying dendritic channelopathy become better understood, it is possible that novel treatments will emerge for reversing or preventing this common neurological disease.

Conclusions

As outlined in the early sections of this chapter, it is difficult to assess the functional significance of the changes in dendritic structure that are associated with certain disease states. Such changes could represent the primary mechanisms of the disease or merely be secondary to the loss of synaptic inputs or other brain pathology. This chapter has instead focused on the emerging field of ion channelopathies, and specifically, where known, on defects in ion channels known to be expressed in dendrites. Dendrites are highly plastic structures. Homeostatic changes in the functional properties of dendrites can occur (Turrigiano and Nelson, 2004; Frick and Johnston, 2005; Magee and Johnston, 2005) that might partially compensate for disease-related alterations in ion channels. It is critical to begin to study the physiology of dendrites directly, in both normal and animal models of disease, using dendritic recordings and imaging. As discussed above, this has been fruitful for epilepsy, FXTAS, and neuropathic pain, and we should expect significant advances in this area in the future for many of the other important and behaviorally relevant neurological and psychiatric disorders.

References

- Almog M, Korngreen A (2009) Characterization of voltage-gated Ca(2+) conductances in layer 5 neocortical pyramidal neurons from rats. *PLoS ONE* **4**:e4841.
- American Psychiatric Association (2013) *Diagnostic and Statistical Manual of Mental Disorders* (5th edn). Washington, DC: American Psychiatric Association.
- Barel O, et al. (2008) Maternally inherited Birk-Barel mental retardation dysmorphism syndrome caused by a mutation in the genetically imprinted potassium channel KCNK9. *American Journal of Human Genetics* **83**:193–199.
- Barnwell LF, Lugo JN, Lee WL, Willis SE, Gertz SJ, Hrachovy RA, Anderson AE (2009) Kv4.2 knockout mice demonstrate increased susceptibility to convulsant stimulation. *Epilepsia* **50**:1741–1751.
- Barrett CF, Tsien RW (2008) The Timothy syndrome mutation differentially affects voltage- and calcium-dependent inactivation of CaV1.2 L-type calcium channels. *Proceedings of the National Academy of Sciences of the United States of America* **105**:2157–2162.
- Bassell GJ, Warren ST (2008) Fragile X syndrome: loss of local mRNA regulation alters synaptic development and function. *Neuron* **60**:201–214.
- Bear MF, Huber KM, Warren ST (2004) The mGluR theory of fragile X mental retardation. *Trends in Neurosciences* **27**:370–377.

- Becker AJ, Pitsch J, Sochivko D, Opitz T, Staniek M, Chen CC, Campbell KP, Schoch S, Yaari Y, Beck H (2008) Transcriptional upregulation of Cav3.2 mediates epileptogenesis in the pilocarpine model of epilepsy. *Journal of Neuroscience* **28**:13341–13353.
- Ben-Ari Y, Crepel V, Represa A (2008) Seizures beget seizures in temporal lobe epilepsies: the boomerang effects of newly formed aberrant kainatergic synapses. *Epilepsy Currents* **8**:68–72.
- Bender RA, Soleymani SV, Brewster AL, Nguyen ST, Beck H, Mathern GW, Baram TZ (2003) Enhanced expression of a specific hyperpolarization-activated cyclic nucleotide-gated cation channel (HCN) in surviving dentate gyrus granule cells of human and experimental epileptic hippocampus. *Journal of Neuroscience* **23**:6826–6836.
- Berger SM, Bartsch D (2014) The role of L-type voltage-gated calcium channels Cav1.2 and Cav1.3 in normal and pathological brain function. *Cell and Tissue Research* **357**:463–476.
- Bernard C, Anderson A, Becker A, Poolos NP, Beck H, Johnston D (2004) Acquired dendritic channelopathy in temporal lobe epilepsy. *Science* **305**:532–535.
- Bhat S, Dao DT, Terrillion CE, Arad M, Smith RJ, Soldatov NM, Gould TD (2012) CACNA1C (Cav1.2) in the pathophysiology of psychiatric disease. *Progress in Neurobiology* **99**:1–14.
- Bhattacharjee A, Kaczmarek L (2005) For K channels, Na is the new Ca. *Trends in Neurosciences* **28**:422–428.
- Biervert C, Schroeder BC, Kubisch C, Berkovic SF, Propping P, Jentsch TJ, Steinlein OK (1998) A potassium channel mutation in neonatal human epilepsy. *Science* **279**:403–406.
- van Bokhoven H (2011) Genetic and epigenetic networks in intellectual disabilities. *Annual Review of Genetics* **45**:81–104.
- Brager DH, Akhavan AR, Johnston D (2012) Impaired dendritic expression and plasticity of h-channels in the fmr1(−/y) mouse model of fragile X syndrome. *Cell Reports* **1**:225–233.
- Breitenkamp AFS, Matthes J, Nass RD, Sinzig J, Lehmkul G, Nürnberg P, Herzig S (2014) Rare mutations of CACNB2 found in autism spectrum disease-affected families alter calcium channel function. *PLoS ONE* **9**:e95579.
- Brewster A, Bender RA, Chen Y, Dube C, Eghbal-Ahmadi M, Baram TZ (2002) Developmental febrile seizures modulate hippocampal gene expression of hyperpolarization-activated channels in an isoform- and cell-specific manner. *Journal of Neuroscience* **22**:4591–4599.
- Brown DA, Passmore GM (2009) Neural KCNQ (Kv7) channels. *British Journal of Pharmacology* **156**:1185–1195.
- Brown MR, et al. (2010) Fragile X mental retardation protein controls gating of the sodium-activated potassium channel Slack. *Nature Neuroscience* **13**:819–821.
- Busche MA, Chen X, Henning HA, Reichwald J, Staufenbiel M, Sakmann B, Konnerth A (2012) Critical role of soluble amyloid-β for early hippocampal hyperactivity in a mouse model of Alzheimer's disease. *Proceedings of the National Academy of Sciences of the United States of America* **109**:8740–8745.
- Casanova JR, Nishimura M, Owens JW, Swann JW (2012) Impact of seizures on developing dendrites: implications for intellectual developmental disabilities. *Epilepsia* **53**(Suppl. 1):116–124.
- Chan CS, Shigemoto R, Mercer JN, Surmeier DJ (2004) HCN2 and HCN1 channels govern the regularity of autonomous pacemaking and synaptic resetting in globus pallidus neurons. *Journal of Neuroscience* **24**:9921–9932.
- Chan CS, et al. (2011) HCN channelopathy in external globus pallidus neurons in models of Parkinson's disease. *Nature Neuroscience* **14**:85–92.
- Chang KT, Ro H, Wang W, Min K-T (2013) Meeting at the crossroads: common mechanisms in fragile X and Down syndrome. *Trends in Neurosciences* **36**:685–694.
- Chen C (2005) beta-Amyloid increases dendritic Ca^{2+} influx by inhibiting the A-type K^+ current in hippocampal CA1 pyramidal neurons. *Biochemical and Biophysical Research Communications* **338**:1913–1919.

- Chen K, Aradi I, Thon N, Eghbal-Ahmadi M, Baram TZ, Soltesz I (2001) Persistently modified h-channels after complex febrile seizures convert the seizure-induced enhancement of inhibition to hyperexcitability. *Nature Medicine* 7:331–337.
- Chen X, Johnston D (2004) Properties of single voltage-dependent K⁺ channels in dendrites of CA1 pyramidal neurones of rat hippocampus. *Journal of Physiology* 559:187–203.
- Chen X, Johnston D (2005) Constitutively active GIRK channels in dendrites of hippocampal CA1 pyramidal neurons. *Journal of Neuroscience* 25:3787–3792.
- Chen L, Yun SW, Seto J, Liu W, Toth M (2003) The fragile X mental retardation protein binds and regulates a novel class of mRNAs containing U rich target sequences. *Neuroscience* 120:1005–1017.
- Chen X, Yuan LL, Zhao C, Birnbaum SG, Frick A, Jung WE, Schwarz TL, Sweatt JD, Johnston D (2006) Deletion of Kv4.2 gene eliminates dendritic A-type K⁺ current and enhances induction of long-term potentiation in hippocampal CA1 pyramidal neurons. *Journal of Neuroscience* 26:12143–12151.
- Chen X, Shu S, Schwartz LC, Sun C, Kapur J, Bayliss DA (2010) Homeostatic regulation of synaptic excitability: tonic GABA(A) receptor currents replace I(h) in cortical pyramidal neurons of HCN1 knock-out mice. *Journal of Neuroscience* 30:2611–2622.
- Christie BR, Eliot LS, Ito K, Miyakawa H, Johnston D (1995) Different Ca²⁺ channels in soma and dendrites of hippocampal pyramidal neurons mediate spike-induced Ca²⁺ influx. *Journal of Neurophysiology* 73:2553–2557.
- Clement JP, et al. (2012) Pathogenic SYNGAP1 mutations impair cognitive development by disrupting maturation of dendritic spine synapses. *Cell* 151:709–723.
- Cochran JN, Hall AM, Roberson ED (2014) The dendritic hypothesis for Alzheimer's disease pathophysiology. *Brain Research Bulletin* 103:18–28.
- Coon AL, Wallace DR, Mactutus CF, Booze RM (1999) L-type calcium channels in the hippocampus and cerebellum of Alzheimer's disease brain tissue. *Neurobiology of Aging* 20:597–603.
- Cossart R, Dinocourt C, Hirsch JC, Merchan-Perez A, De Felipe J, Ben Ari Y, Esclapez M, Bernard C (2001) Dendritic but not somatic GABAergic inhibition is decreased in experimental epilepsy. *Nature Neuroscience* 4:52–62.
- Cross-Disorder Group of the Psychiatric Genomics Consortium** (2013) Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs. *Nature Genetics* 45:984–994.
- Darnell JC, et al. (2011) FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism. *Cell* 146:247–261.
- DeLong MR, Wichmann T (2007) Circuits and circuit disorders of the basal ganglia. *Archives of Neurology* 64:20–24.
- Deng P-Y, Rotman Z, Blundon JA, Cho Y, Cui J, Cavalli V, Zakharenko SS, Klyachko VA (2013) FMRP regulates neurotransmitter release and synaptic in- formation transmission by modulating action potential duration via BK channels. *Neuron* 78:696–711.
- Dierssen M, Ramakers GJA (2006) Dendritic pathology in mental retardation: from molecular genetics to neurobiology. *Genes, Brain, and Behavior* 5:48–60.
- DiFrancesco JC, Barbuti A, Milanesi R, Coco S, Bucchi A, Bottelli G, Ferrarese C, Franceschetti S, Terragni B, Baruscotti M, DiFrancesco D (2011) Recessive loss-of-function mutation in the pacemaker HCN2 channel causing increased neuronal excitability in a patient with idiopathic generalized epilepsy. *Journal of Neuroscience* 31:17327–17337.
- Doeser A, Dickhof G, Reitze M, Uebachs M, Schaub C, Pires NM, Bonifacio MJ, Soares-da-Silva P, Beck H (2015) Targeting pharmacoresistant epilepsy and epileptogenesis with a dual-purpose antiepileptic drug. *Brain* 138:371–387.
- Dragicevic E, Schiemann J, Liss B (2015) Dopamine midbrain neurons in health and Parkinson's disease: emerging roles of voltage-gated calcium channels and ATP-sensitive potassium channels. *Neuroscience* 284:798–814.

- Dryanovski DI, et al. (2013) Calcium entry and α -synuclein inclusions elevate dendritic mitochondrial oxidant stress in dopaminergic neurons. *Journal of Neuroscience* **33**:10154–10164.
- Duflocq A, Le Bras B, Bullier E, Couraud F, Davenne M (2008) Nav1.1 is predominantly expressed in nodes of Ranvier and axon initial segments. *Molecular and Cellular Neurosciences* **39**:180–192.
- Dufour MA, Woodhouse A, Goaillard J-M (2014) Somatodendritic ion channel expression in substantia nigra pars compacta dopaminergic neurons across postnatal development. *Journal of Neuroscience Research* **92**:981–999.
- Fiala JC, Spacek J, Harris KM. (2002) Dendritic spine pathology: cause or consequence of neurological disorders? *Brain Research Brain Research Review* **39**:29–54.
- Frick A, Johnston D (2005) Plasticity of dendritic excitability. *Journal of Neurobiology* **64**:100–115.
- Frick A, Magee J, Koester HJ, Migliore M, Johnston D (2003) Normalization of Ca^{2+} signals by small oblique dendrites of CA1 pyramidal neurons. *Journal of Neuroscience* **23**:3243–3250.
- Frick A, Magee J, Johnston D (2004) LTP is accompanied by an enhanced local excitability of pyramidal neuron dendrites. *Nature Neuroscience* **7**:126–135.
- Gloyn AL, Diatloff-Zito C, Edghill EL, Bellanne-Chantelot C, Nivot S, Coutant R, Ellard S, Hattersley AT, Robert JJ (2006) KCNJ11 activating mutations are associated with developmental delay, epilepsy and neonatal diabetes syndrome and other neurological features. *European Journal of Human Genetics* **14**:824–830.
- Gong B, Rhodes KJ, Bekele-Arcuri Z, Trimmer JS (1999) Type I and type II $\text{Na}(+)$ channel alpha-subunit polypeptides exhibit distinct spatial and temporal patterning, and association with auxiliary subunits in rat brain. *Journal of Comparative Neurology* **412**:342–352.
- Good TA, Murphy RM (1996) Effect of beta-amyloid block of the fast-inactivating K^+ channel on intracellular Ca^{2+} and excitability in a modeled neuron. *Proceedings of the National Academy of Sciences of the United States of America* **93**:15130–15135.
- Goodkin HP, Joshi S, Mtchedlishvili Z, Brar J, Kapur J (2008) Subunit-specific trafficking of GABA A receptors during status epilepticus. *Journal of Neuroscience* **28**:2527–2538.
- Granato A, Palmer LM, De Giorgio A, Tavian D, Larkum ME (2012) Early exposure to alcohol leads to permanent impairment of dendritic excitability in neocortical pyramidal neurons. *Journal of Neuroscience* **32**:1377–1382.
- Gravante B, Barbuti A, Milanesi R, Zappi I, Visconti C, DiFrancesco D (2004) Interaction of the pacemaker channel HCN1 with filamin A. *Journal of Biological Chemistry* **279**:43847–43853.
- Gross C, Yao X, Pong DL, Jeromin A, Bassell GJ (2011) Fragile X mental retardation protein regulates protein expression and mRNA translation of the potassium channel Kv4.2. *Journal of Neuroscience* **31**:5693–5698.
- Guzman JN, Sanchez-Padilla J, Wokosin D, Kondapalli J, Ilijic E, Schumacker PT, Surmeier DJ (2010) Oxidant stress evoked by pacemaking in dopaminergic neurons is attenuated by DJ-1. *Nature* **468**:696–700.
- Hammond C, Bergman H, Brown P (2007) Pathological synchronization in Parkinson's disease: networks, models and treatments. *Trends in Neurosciences* **30**:357–364.
- Harbuz R, Bilan F, Couet D, Charraud V, Kitzis A, Gilbert-Dussardier B (2013) Osteogenesis imperfecta, tricho-dento-osseous syndrome and intellectual disability: a familial case with 17q21.33-q22 (COL1A1 and DLX3) deletion and 7q32.3–q33 duplication resulting from a reciprocal interchromosomal insertion. *American Journal of Medical Genetics* **161A**:2504–2511.
- Hardy J, Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* **297**:353–356.
- Heck A, et al. (2014) Converging genetic and functional brain imaging evidence links neuronal excitability to working memory, psychiatric disease, and brain activity. *Neuron* **81**:1203–1213.
- Hernandez-Lopez S, Tkatch T, Perez-Garcia E, Galarraga E, Bargas J, Hamm H, Surmeier DJ (2000) D2 dopamine receptors in striatal medium spiny neurons reduce L-type Ca^{2+} currents and excitability via a novel PLC[β]1-IP3-calcineurin-signaling cascade. *Journal of Neuroscience* **20**:8987–8995.

- Heron SE, Phillips HA, Mulley JC, Mazarib A, Neufeld MY, Berkovic SF, Scheffer IE (2004) Genetic variation of CACNA1H in idiopathic generalized epilepsy. *Annals of Neurology* **55**:595–596.
- Heron SE, et al. (2012) Missense mutations in the sodium-gated potassium channel gene KCNT1 cause severe autosomal dominant nocturnal frontal lobe epilepsy. *Nature Genetics* **44**:1188–1190.
- Hille B (2001) *Ionic Channels of Excitable Membranes*. Boston, MA: Sinauer Press.
- Hirsch EC, Graybiel AM, Duyckaerts C, Javoy-Agid F (1987) Neuronal loss in the pedunculopontine tegmental nucleus in Parkinson disease and in progressive supranuclear palsy. *Proceedings of the National Academy of Sciences of the United States of America* **84**:5976–5980.
- Hoffman DA, Johnston D (1998) Downregulation of transient K⁺ channels in dendrites of hippocampal CA1 pyramidal neurons by activation of PKA and PKC. *Journal of Neuroscience* **18**:3521–3528.
- Hoffman DA, Magee JC, Colbert CM, Johnston D (1997) K⁺ channel regulation of signal propagation in dendrites of hippocampal pyramidal neurons. *Nature* **387**:869–875.
- Houser CR, Zhang N, Peng Z (2012) Alterations in the distribution of GABA_A receptors in epilepsy. In: *Jasper's Basic Mechanisms of the Epilepsies* (Noebels J, Avoli M, Rogawski M, Olsen R, Delgado-Escueta A, eds), pp. 532–544. New York: Oxford University Press.
- Huang K (2009) SYNGAP: bridging the gap between genetic factors and autosomal non-syndromic mental retardation. *Clinical Genetics* **76**:149–151.
- Huang Z, Lujan R, Kadurin I, Uebel VN, Renger JJ, Dolphin AC, Shah MM (2011) Presynaptic HCN1 channels regulate Cav3.2 activity and neurotransmission at select cortical synapses. *Nature Neuroscience* **14**:478–486.
- Huang Z, Walker MC, Shah MM (2009) Loss of dendritic HCN1 subunits enhances cortical excitability and epileptogenesis. *Journal of Neuroscience* **29**:10979–10988.
- Huber KM, Gallagher SM, Warren ST, Bear MF (2002) Altered synaptic plasticity in a mouse model of fragile X mental retardation. *Proceedings of the National Academy of Sciences of the United States of America* **99**:7746–7750.
- Hutsler JJ, Zhang H (2010) Increased dendritic spine densities on cortical projection neurons in autism spectrum disorders. *Brain Research* **1309**:83–94.
- Jhamandas JH, Cho C, Jassar B, Harris K, MacTavish D, Easaw J (2001) Cellular mechanisms for amyloid beta-protein activation of rat cholinergic basal forebrain neurons. *Journal of Neurophysiology* **86**:1312–1320.
- Jinnah HA, Yitta S, Drew T, Kim BS, Visser JE, Rothstein JD (1999) Calcium channel activation and self-biting in mice. *Proceedings of the National Academy of Sciences of the United States of America* **96**:15228–15232.
- Johnston D, Hoffman DA, Magee JC, Poolos NP, Watanabe S, Colbert CM, Migliore M (2000) Dendritic potassium channels in hippocampal pyramidal neurons. *Journal of Physiology* **525**:75–81.
- Judy JT, Zandi PP (2013) A review of potassium channels in bipolar disorder. *Frontiers in Genetics* **4**:105.
- Jung S, Jones TD, Lugo Jr. J, Sheerin AH, Miller JW, D'Ambrosio R, Anderson AE, Poolos NP (2007) Progressive dendritic HCN channelopathy during epileptogenesis in the rat pilocarpine model of epilepsy. *Journal of Neuroscience* **27**:13012–13021.
- Jung S, Bullis JB, Lau IH, Jones TD, Warner LN, Poolos NP (2010) Downregulation of dendritic HCN channel gating in epilepsy is mediated by altered phosphorylation signalling. *Journal of Neuroscience* **30**:6678–6688.
- Jung S, Warner LN, Pitsch J, Becker AJ, Poolos NP (2011) Rapid loss of dendritic HCN channel expression in hippocampal pyramidal neurons following status epilepticus. *Journal of Neuroscience* **31**:14291–14295.
- Kaufmann WE, Moser HW (2000) Dendritic anomalies in disorders associated with mental retardation. *Cerebral Cortex* **10**:981–991.
- Kim J, Jung S-C, Clemens AM, Petralia RS, Hoffman DA (2007) Regulation of dendritic excitability by activity-dependent trafficking of the A-type K⁺ channel subunit Kv4.2 in hippocampal neurons. *Neuron* **54**:933–947.

- Klassen T, Davis C, Goldman A, Burgess D, Chen T, Wheeler D, McPherson J, Bourquin T, Lewis L, Villasana D, Morgan M, Muzny D, Gibbs R, Noebels J (2011) Exome sequencing of ion channel genes reveals complex profiles confounding personal risk assessment in epilepsy. *Cell* **145**:1036–1048.
- Koester HJ, Sakmann B (1998) Calcium dynamics in single spines during coincident pre- and postsynaptic activity depend on relative timing of back-propagating action potentials and subthreshold excitatory postsynaptic potentials. *Proceedings of the National Academy of Sciences of the United States of America* **95**:9596–9601.
- Koester HJ, Sakmann B (2000) Calcium dynamics associated with action potentials in single nerve terminals of pyramidal cells in layer 2/3 of the young rat neocortex. *Journal of Physiology* **529**:625–646.
- Krey JF, Dolmetsch RE (2007) Molecular mechanisms of autism: a possible role for Ca^{2+} signaling. *Current Opinion in Neurobiology* **17**:112–119.
- Krey JF, Paşa SP, Shcheglovitov A, Yazawa M, Schwemberger R, Rasmusson R, Dolmetsch RE (2013) Timothy syndrome is associated with activity-dependent dendritic retraction in rodent and human neurons. *Nature Neuroscience* **16**:201–209.
- Lee HY, et al. (2011) Bidirectional regulation of dendritic voltage-gated potassium channels by the fragile X mental retardation protein. *Neuron* **72**:630–642.
- Lewis AS, Vaidya SP, Blaiss CA, Liu Z, Stoub TR, Brager DH, Chen X, Bender RA, Estep CM, Popov AB, Kang CE, Van Veldhoven PP, Bayliss DA, Nicholson DA, Powell CM, Johnston D, Chetkovich DM (2011) Deletion of the hyperpolarization-activated cyclic nucleotide-gated channel auxiliary subunit TRIP8b impairs hippocampal Ih localization and function and promotes antidepressant behavior in mice. *Journal of Neuroscience* **31**:7424–7440.
- Losonczy A, Makara JK, Magee JC (2008) Compartmentalized dendritic plasticity and input feature storage in neurons. *Nature* **452**:436–441.
- Lu AT-H, Dai X, Martinez-Agosto JA, Cantor RM (2012) Support for calcium channel gene defects in autism spectrum disorders. *Molecular Autism* **3**:18.
- Ludwig A, Budde T, Stieber J, Moosmang S, Wahl C, Holthoff K, Langebartels A, Wotjak C, Munsch T, Zong X, Feil S, Feil R, Lancel M, Chien KR, Konnerth A, Pape HC, Biel M, Hofmann F (2003) Absence epilepsy and sinus dysrhythmia in mice lacking the pacemaker channel HCN2. *EMBO Journal* **22**:216–224.
- Lugo JN, Barnwell LF, Ren Y, Lee WL, Johnston LD, Kim R, Hrachovy RA, Sweatt JD, Anderson AE (2008) Altered phosphorylation and localization of the A-type channel, Kv4.2 in status epilepticus. *Journal of Neurochemistry* **106**:1929–1940.
- McClelland S, Flynn C, Dube C, Richichi C, Zha Q, Ghestem A, Esclapez M, Bernard C, Baram TZ (2011) Neuron-restrictive silencer factor-mediated hyperpolarization-activated cyclic nucleotide gated channelopathy in experimental temporal lobe epilepsy. *Annals of Neurology* **70**:454–464.
- McKay BE, McRory JE, Molineux ML, Hamid J, Snutch TP, Zamponi GW, Turner RW (2006) $\text{Ca}(\text{V})3$ T-type calcium channel isoforms differentially distribute to somatic and dendritic compartments in rat central neurons. *European Journal of Neuroscience* **24**:2581–2594.
- Magee JC (1998) Dendritic hyperpolarization-activated currents modify the integrative properties of hippocampal CA1 pyramidal neurons. *Journal of Neuroscience* **18**:7613–7624.
- Magee JC (1999) Dendritic Ih normalizes temporal summation in hippocampal CA1 neurons. *Nature Neuroscience* **2**:508–514.
- Magee JC, Johnston D (1995) Characterization of single voltage-gated Na^+ and Ca^{2+} channels in apical dendrites of rat CA1 pyramidal neurons. *Journal of Physiology* **487**:67–90.
- Magee JC, Johnston D (1997) A synaptically controlled, associative signal for Hebbian plasticity in hippocampal neurons. *Science* **275**:209–213.
- Magee JC, Johnston D (2005) Plasticity of dendritic function. *Current Opinion in Neurobiology* **15**:334–342.

- Makara JK, Losonczy A, Wen Q, Magee JC (2009) Experience-dependent compartmentalized dendritic plasticity in rat hippocampal CA1 pyramidal neurons. *Nature Neuroscience* **12**:1485–1487.
- Mantegazza M, Rusconi R, Scalmani P, Avanzini G, Franceschetti S (2010) Epileptogenic ion channel mutations: from bedside to bench and, hopefully, back again. *Epilepsy Research* **92**:1–29.
- Marin-Padilla M (1972) Structural abnormalities of the cerebral cortex in human chromosomal aberrations. A Golgi study. *Brain Research* **44**:625–629.
- Marinc C, Derst C, Prüss H, Veh RW (2014) Immunocytochemical localization of TASK-3 protein (K2P9.1) in the rat brain. *Cellular and Molecular Neurobiology* **34**:61–70.
- Markram H, Helm PJ, Sakmann B (1995) Dendritic calcium transients evoked by single back-propagating action potentials in rat neocortical pyramidal neurons. *Journal of Physiology* **485**:1–20.
- Martin MS, Dutt K, Papale LA, Dube CM, Dutton SB, de Haan G, Shankar A, Tufik S, Meisler MH, Baram TZ, Goldin AL, Escayg A (2010) Altered function of the SCN1A voltage-gated sodium channel leads to gamma-aminobutyric acid-ergic (GABAergic) interneuron abnormalities. *Journal of Biological Chemistry* **285**:9823–9834.
- Meredith RM (2014) Sensitive and critical periods during neurotypical and aberrant neurodevelopment: a framework for neurodevelopmental disorders. *Neuroscience and Biobehavioral Reviews* **50C**:180–188.
- Meredith RM, Holmgren CD, Weidum M, Burnashev N, Mansvelder HD (2007) Increased threshold for spike-timing-dependent plasticity is caused by unreliable calcium signaling in mice lacking fragile X gene FMR1. *Neuron* **54**:627–638.
- Miceli F, et al. (2014) A novel KCNQ3 mutation in familial epilepsy with focal seizures and intellectual disability. *Epilepsia* **56**:e15–e20.
- Morse TM, Carnevale NT, Mutualik PG, Migliore M, Shepherd GM (2010) Abnormal excitability of oblique dendrites implicated in early Alzheimer's: a computational study. *Frontiers in Neural Circuits* **4**: doi: 10.3389/fncir.2010.00016.
- Mosharov EV, et al. (2009) Interplay between cytosolic dopamine, calcium, and alpha-synuclein causes selective death of substantia nigra neurons. *Neuron* **62**:218–229.
- Nava C, et al. (2014) De novo mutations in HCN1 cause early infantile epileptic encephalopathy. *Nature Genetics* **46**:640–645.
- Nestor MW, Hoffman DA (2012) Aberrant dendritic excitability: a common pathophysiology in CNS disorders affecting memory? *Molecular Neurobiology* **45**:478–487.
- Nitkin RM (2000) Dendritic mechanisms in brain function and developmental disabilities. *Cerebral Cortex* **10**:925–926.
- Palop JJ, Mucke L (2010) Amyloid-beta-induced neuronal dysfunction in Alzheimer's disease: from synapses toward neural networks. *Nature Neuroscience* **13**:812–818.
- Parkinson Study Group (2013) Phase II safety, tolerability, and dose selection study of isradipine as a potential disease-modifying intervention in early Parkinson's disease (STEADY-PD). *Movement Disorders* **28**:1823–1831.
- Patt S, Gertz HJ, Gerhard L, Cervós-Navarro J (1991) Pathological changes in dendrites of substantia nigra neurons in Parkinson's disease: a Golgi study. *Histology and Histopathology* **6**:373–380.
- Piskorowski R, Santoro B, Siegelbaum SA (2011) TRIP8b splice forms act in concert to regulate the localization and expression of HCN1 channels in CA1 pyramidal neurons. *Neuron* **70**:495–509.
- Plant LD, Webster NJ, Boyle JP, Ramsden M, Freir DB, Peers C, Pearson HA (2006) Amyloid beta peptide as a physiological modulator of neuronal "A"-type K⁺ current. *Neurobiology of Aging* **27**:1673–1683.
- Poewe W, Luginger E (1999) Depression in Parkinson's disease: impediments to recognition and treatment options. *Neurology* **52**:S2–S6.
- Politis M, Niccolini F (2015) Serotonin in Parkinson's disease. *Behavioural Brain Research* **277**:136–145.

- Poolos NP, Migliore M, Johnston D (2002) Pharmacological upregulation of h-channels reduces the excitability of pyramidal neuron dendrites. *Nature Neuroscience* 5:767–774.
- Portera-Cailliau C (2012) Which comes first in fragile X syndrome, dendritic spine dysgenesis or defects in circuit plasticity? *The Neuroscientist* 18:28–44.
- Preiksaitiene E, et al. (2012) A novel de novo 1.8 Mb microdeletion of 17q21.33 associated with intellectual disability and dysmorphic features. *European Journal of Medical Genetics* 55:656–659.
- Purpura DP (1974) Dendritic spine “dysgenesis” and mental retardation. *Science* 186:1126–1128.
- Ramakers GJA (2002) Rho proteins, mental retardation and the cellular basis of cognition. *Trends in Neurosciences* 25:191–199.
- Ramsden M, Plant LD, Webster NJ, Vaughan PF, Henderson Z, Pearson HA (2001) Differential effects of unaggregated and aggregated amyloid beta protein (1–40) on K(+) channel currents in primary cultures of rat cerebellar granule and cortical neurones. *Journal of Neurochemistry* 79:699–712.
- Reichold M, Zdebik AA, Lieberer E, Rapedius M, Schmidt K, Bandulik S, Sterner C, Tegtmeyer I, Penton D, Baukrowitz T, Hulton SA, Witzgall R, Ben-Zeev B, Howie AJ, Kleta R, Bockenhauer D, Warth R (2010) KCNJ10 gene mutations causing EAST syndrome (epilepsy, ataxia, sensorineural deafness, and tubulopathy) disrupt channel function. *Proceedings of the National Academy of Sciences of the United States of America* 107:14490–14495.
- Rinne JO, Ma SY, Lee MS, Collan Y, Röyttä M (2008) Loss of cholinergic neurons in the pedunculopontine nucleus in Parkinson’s disease is related to disability of the patients. *Parkinsonism and Related Disorders* 14:553–557.
- Rivlin-Etzion M, Marmor O, Heimer G, Raz A, Nini A, Bergman H (2006) Basal ganglia oscillations and pathophysiology of movement disorders. *Current Opinion in Neurobiology* 16:629–637.
- Ross CA, Poirier MA (2004) Protein aggregation and neurodegenerative disease. *Nature Medicine* 10(Suppl.):S10–S17.
- Routh BN, Johnston D, Brager DH (2013) Loss of functional A-type potassium channels in the dendrites of CA1 pyramidal neurons from a mouse model of fragile X syndrome. *Journal of Neuroscience* 33:19442–19450.
- Sabatini BL, Svoboda K (2000) Analysis of calcium channels in single spines using optical fluctuation analysis. *Nature* 408:589–593.
- Sanabria ER, Su H, Yaari Y (2001) Initiation of network bursts by Ca²⁺-dependent intrinsic bursting in the rat pilocarpine model of temporal lobe epilepsy. *Journal of Physiology* 532:205–216.
- Santello M, Nevian T (2015) Dysfunction of cortical dendritic integration in neuropathic pain reversed by serotonergic neuromodulation. *Neuron* 86:233–246.
- Santoro B, Lee JY, Englot DJ, Gildersleeve S, Piskorowski RA, Siegelbaum SA, Winawer MR, Blumenfeld H (2010) Increased seizure severity and seizure-related death in mice lacking HCN1 channels. *Epilepsia* 51:1624–1627.
- Scharf SH, Jaeschke G, Wettstein JG, Lindemann L (2015) Metabotropic glutamate receptor 5 as drug target for fragile X syndrome. *Current Opinion in Pharmacology* 20C:124–134.
- Scheibel ME, Scheibel AB (1973) Dendrite bundles as sites for central programs: an hypothesis. *Journal of Neuroscience* 6:195–202.
- Schmunk G, Gargus JJ (2013) Channelopathy pathogenesis in autism spectrum disorders. *Frontiers in Genetics* 4:222. doi: 10.3389/fgene.2013.00222.
- Shah MM, Anderson AE, Leung V, Lin X, Johnston D (2004) Seizure-induced plasticity of h channels in entorhinal cortical layer III pyramidal neurons. *Neuron* 44:495–508.
- Shah MM, Migliore M, Valencia I, Cooper EC, Brown DA (2008) Functional significance of axonal Kv7 channels in hippocampal pyramidal neurons. *Proceedings of the National Academy of Sciences of the United States of America* 105:7869–7874.

- Shah MM, Migliore M, Brown DA (2011) Differential effects of Kv7 (M-) channels on synaptic integration in distinct subcellular compartments of rat hippocampal pyramidal neurons. *Journal of Physiology* **589**:6029–6038.
- Singh B, Ogiwara I, Kaneda M, Tokonami N, Mazaki E, Baba K, Matsuda K, Inoue Y, Yamakawa K (2006) A Kv4.2 truncation mutation in a patient with temporal lobe epilepsy. *Neurobiology of Disease* **24**:245–253.
- Smith M, et al. (2012) Mitochondrial and ion channel gene alterations in autism. *Biochimica et Biophysica Acta* **1817**:1796–1802.
- Splawski I, Yoo DS, Stotz SC, Cherry A, Clapham DE, Keating MT (2006) CACNA1H mutations in autism spectrum disorders. *Journal of Biological Chemistry* **281**:22085–22091.
- Steinlein OK, Mulley JC, Propping P, Wallace RH, Phillips HA, Sutherland GR, Scheffer IE, Berkovic SF (1995) A missense mutation in the neuronal nicotinic acetylcholine receptor alpha 4 subunit is associated with autosomal dominant nocturnal frontal lobe epilepsy. *Nature Genetics* **11**:201–203.
- Strumbos JG, Brown MR, Kronengold J, Polley DB, Kaczmarek LK (2010) Fragile X mental retardation protein is required for rapid experience-dependent regulation of the potassium channel Kv3.1b. *Journal of Neuroscience* **30**:10263–10271.
- Su H, Sochivko D, Becker A, Chen J, Jiang Y, Yaari Y, Beck H (2002) Upregulation of a T-type Ca^{2+} channel causes a long-lasting modification of neuronal firing mode after status epilepticus. *Journal of Neuroscience* **22**:3645–3655.
- Sullivan PF, Daly MJ, O'Donovan M (2012) Genetic architectures of psychiatric disorders: the emerging picture and its implications. *Nature Reviews Genetics* **13**:537–551.
- Surmeier DJ, Guzman JN, Sanchez-Padilla J, Goldberg JA (2010) What causes the death of dopaminergic neurons in Parkinson's disease? *Progress in Brain Research* **183**:59–77.
- Surmeier DJ, Carrillo-Reid L, Bargas J (2011a) Dopaminergic modulation of striatal neurons, circuits, and assemblies. *Neuroscience* **198**:3–18.
- Surmeier DJ, Guzman JN, Sanchez-Padilla J, Schumacker PT (2011b) The role of calcium and mitochondrial oxidant stress in the loss of substantia nigra pars compacta dopaminergic neurons in Parkinson's disease. *Neuroscience* **198**:221–231.
- Svenningsson P, Nishi A, Fisone G, Girault J-A, Laird AC, Greengard P (2004) DARPP-32: an integrator of neurotransmission. *Annual Review of Pharmacology and Toxicology* **44**:269–296.
- Szlapczynska M, Bonnan A, Ginger M, Frick A (2014) Plasticity and pathology of dendritic intrinsic excitability. In: *Horizons in Neuroscience Research*, Vol. 14 (Costa A, Villalba E, eds), pp. 41–88. New York: Nova Science Publishers.
- Tang G, et al. (2014) Loss of mTOR-dependent macroautophagy causes autistic-like synaptic pruning deficits. *Neuron* **83**:1131–1143.
- Terunuma M, Xu J, Vithlani M, Sieghart W, Kittler J, Pangalos M, Haydon PG, Coulter DA, Moss SJ (2008) Deficits in phosphorylation of GABA_A receptors by intimately associated protein kinase C activity underlie compromised synaptic inhibition during status epilepticus. *Journal of Neuroscience* **28**:376–384.
- Toyoda I, Bower MR, Leyva F, Buckmaster PS (2013) Early activation of ventral hippocampus and subiculum during spontaneous seizures in a rat model of temporal lobe epilepsy. *Journal of Neuroscience* **33**:11100–11115.
- Tringham E, Powell KL, Cain SM, Kuplast K, Mezeyeva J, Weerapura M, Eduljee C, Jiang X, Smith P, Morrison JL, Jones NC, Braine E, Rind G, Fee-Maki M, Parker D, Pajouhesh H, Parmar M, O'Brien TJ, Snutch TP (2012) T-type calcium channel blockers that attenuate thalamic burst firing and suppress absence seizures. *Science Translational Medicine* **4**:121ra19.
- Tritsch NX, Sabatini BL (2012) Dopaminergic modulation of synaptic transmission in cortex and striatum. *Neuron* **76**:33–50.

- Turrigiano GG, Nelson SB (2004) Homeostatic plasticity in the developing nervous system. *Nature Reviews Neuroscience* 5:97–107.
- United States Environmental Protection Agency (2013) *America's Children and the Environment*, 3rd edn. Washington, DC: US Environmental Protection Agency.
- Verret L, et al. (2012) Inhibitory interneuron deficit links altered network activity and cognitive dysfunction in an Alzheimer model. *Cell* 149:708–721.
- Villalba RM, Smith Y (2013) Differential striatal spine pathology in Parkinson's disease and cocaine addiction: a key role of dopamine? *Neuroscience* 251:2–20.
- Vossel KA, et al. (2013) Seizures and epileptiform activity in the early stages of Alzheimer disease. *JAMA Neurology* 70:1158–1166.
- Wager TD, Atlas LY, Lindquist MA, Roy M, Woo CW, Kross E (2013) An fMRI-based neurologic signature of physical pain. *New England Journal of Medicine* 368:1388–1397.
- Weckhuysen S, et al. (2012) KCNQ2 encephalopathy: emerging phenotype of a neonatal epileptic encephalopathy. *Annals of Neurology* 71:15–25.
- Wierschke S, Lehmann TN, Dehnicke C, Horn P, Nitsch R, Deisz RA (2010) Hyperpolarization-activated cation currents in human epileptogenic neocortex. *Epilepsia* 51:404–414.
- Williams A, Jung S, Poolos NP (2015) Protein kinase C bidirectionally modulates Ih and HCN1 channel surface expression in hippocampal pyramidal neurons. *Journal of Physiology* 593:2779–2792.
- Willis M, et al. (2010) L-type calcium channel CaV 1.2 in transgenic mice overexpressing human AbetaPP751 with the London (V717I) and Swedish (K670M/N671L) mutations. *Journal of Alzheimer's Disease* 20:1167–1180.
- Xu C, Qian C, Zhang Z, Wu C, Zhou P, Liang X (1998) Effects of beta-amyloid peptide on transient outward potassium current of acutely dissociated hippocampal neurons in CA1 sector in rats. *Chinese Medical Journal* 111:492–495.
- Yaari Y, Yue C, Su H (2007) Recruitment of apical dendritic T-type Ca^{2+} channels by backpropagating spikes underlies de novo intrinsic bursting in hippocampal epileptogenesis. *Journal of Physiology* 580:435–450.
- Ye CP, Selkoe DJ, Hartley DM (2003) Protofibrils of amyloid beta-protein inhibit specific K^+ currents in neocortical cultures. *Neurobiology of Disease* 13:177–190.
- Yu FH, Mantegazza M, Westenbroek RE, Robbins CA, Kalume F, Burton KA, Spain WJ, McKnight GS, Scheuer T, Catterall WA (2006) Reduced sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in infancy. *Nature Neuroscience* 9:1142–1149.
- Yuan, LL, Adams, JP, Swank, M, Sweatt, JD, Johnston, D. (2002). Protein kinase modulation of dendritic K^+ channels in hippocampus involves a mitogen-activated protein kinase pathway. *The Journal of Neuroscience*, 22:4860–4868.
- Zeng LH, Xu L, Rensing NR, Sinatra PM, Rothman SM, Wong M. (2007) Kainate seizures cause acute dendritic injury and actin depolymerization in vivo. *Journal of Neuroscience* 27:1604–11613.
- Zhang C-F, Yang P (2006) Zinc-induced aggregation of Abeta (10–21) potentiates its action on voltage-gated potassium channel. *Biochemical and Biophysical Research Communications* 345:43–49.
- Zhang L, Li X, Zhou R, Xing G (2006) Possible role of potassium channel, big K in etiology of schizophrenia. *Medical Hypotheses* 67:41–43.
- Zhang Y, et al. (2014) Dendritic channelopathies contribute to neocortical and sensory hyperexcitability in Fmr1. *Nature Neuroscience* 17:1701–1709.
- Zoghbi HY, Bear MF (2012) Synaptic dysfunction in neurodevelopmental disorders associated with autism and intellectual disabilities. *Cold Spring Harbor Perspectives in Biology*, 4(3) doi: 10.1101/cshperspect. a009886.

Chapter 25

The future of dendrite research

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Summary

This chapter briefly summarizes the progress made in the field of dendrites since the first two editions of the book, and speculates about areas of research in which significant future progress is anticipated. It highlights seven “known unknowns,” ranging from subcellular distributions of proteins, through dendritic input maps, to the role of dendrites in disease. The chapter concludes by emphasizing the importance of theory for synthesizing experimental observations and for guiding new experimental work.

Introduction

These are exciting times for dendrite research. Our understanding of dendrites has progressed remarkably over the past decade, and dendrite research has developed in many new directions, as exemplified by the growth of this book since the last edition. From its origins in neuroanatomy and neurophysiology, dendrite research has become a highly interdisciplinary endeavor, requiring links between investigations at all levels of central nervous system function, from the molecular, anatomical, and cell biological level, through to the level of neural circuits and ultimately behavior and cognition. Perhaps because dendrites represent such a remarkable “meeting ground” for these many different approaches, there is increasing awareness that dendrite research can be recognized as a distinct field—exemplified not only by this book, but also by the Gordon Research Conference on Dendrites, which started in 2007 and is now in its sixth iteration.

Looking back at the concluding chapter in the first and second editions of this book, many of the predictions made in those pages have come to fruition. Particularly notable developments have been the remarkable advances in our understanding of the genetic and molecular determinants of dendritic morphology (see Chapters 3 and 4), the probing of the function and dynamics of single spines (Chapters 10, 11, and 20), and new approaches for imaging and recording the activity of dendrites *in vivo* (Chapters 12 and 13). Nevertheless, there is general agreement that the most exciting discoveries relevant to our understanding of dendrites still lie ahead of us, and in this final chapter we briefly summarize where we anticipate the biggest strides will be made. While of course it is difficult to make accurate predictions in science, particularly for such a dynamic and rapidly moving field, here we outline several directions in which the field is moving. We first focus on areas where knowledge is lacking, then discuss technical milestones which may help to overcome these gaps, and finally highlight how theoretical work could synthesize new experimental observations to provide a deeper understanding of dendritic function.

Known unknowns and potential solutions

Quantitative protein maps

The functional properties of dendrites depend critically on the subcellular distribution of voltage-gated channels in the dendritic membrane. We still lack a method for the quantitative determination of the distribution of membrane proteins in dendrites across an entire dendritic tree. The use of immunogold techniques can provide extremely high-resolution localization of particular ion channels, but only on a small spatial scale. Patch-clamp recording has single-channel sensitivity, but also can only sample a few locations on each neuron. A high-throughput method is urgently needed to provide a quantitative map of the relevant channels, which would be extremely useful for understanding dendritic physiology and for constraining accurate models of neural function. The new technique of array tomography (Micheva et al., 2007) can in principle provide a map of the distribution of multiple molecules across the dendritic surface of a neuron in fixed tissue with nanoscale resolution. Finally, super-resolution microscopy - such as stimulated emission depletion (STED) microscopy (Hell, 2009), photoactivated localization microscopy (PALM) (Betzig et al. 2006), sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM) (Rust et al., 2006), and newer variants such as image reconstruction by integrating exchangeable single-molecule localization (IRIS) microscopy (Kiuchi et al., 2015) and structured illumination microscopy (SIM) (Li et al., 2015) - may provide a means for examining the distribution and function of these molecules on the nanoscale in living tissue, although these approaches still have serious practical limitations.

Spine voltage

There remains significant controversy about the extent to which spines can act as filters of electrical signals (see Chapters 12, 15, and 18). This problem could be directly addressed if it were possible to accurately monitor the voltage at the spine head, and ideally also the spine shaft. While the use of spine imaging with organic voltage-sensitive dyes (Palmer and Stuart, 2006; Acker et al., 2011), the combination of dendritic patch-clamp recordings and two-photon glutamate uncaging (Hartnett et al., 2012), and most recently the combination of super-resolution microscopy and fluorescence recovery after photobleaching (Tønnesen et al., 2014) have provided important insights, we still lack a reliable way of quantifying spine voltage. The advent of new ratiometric, highly sensitive genetically encoded voltage sensors may provide a solution to this problem. Furthermore, the development of novel recording techniques, such as using carbon fiber nanotubes (Keefer et al., 2008; Schrlau et al., 2009) may allow even the smallest cellular compartments such as spines to become accessible to direct investigation. These methods will also be broadly useful for recording voltage in small dendritic compartments, such as apical oblique branches or distal apical tuft terminal branchlets, which are currently not accessible to patch clamping. Improvements on existing voltage sensors will ultimately allow us to assemble a complete picture of the distribution of voltage across the dendritic tree during physiological patterns of activity.

Dendritic taxonomy

How many different types of dendrites are there? Do L5 pyramidal cell dendrites operate in a fundamentally different way from those of CA1 pyramidal cells, or are they simply neighbors along a continuum? Addressing these issues will require a combination of anatomical analysis of different cell types (quantifying the range of branching patterns within a cell class), quantitative analysis of the distribution of voltage-gated channels and synaptic receptors, functional mapping of the properties and interactions of the different channel types, and modeling work to ultimately provide a quantitative definition—and ideally a simplified representation—of the input–output

function of individual cell types. The availability of high-throughput techniques for each of these steps (e.g., see Chapters 17 and 22)—anatomical analysis, quantifying protein distributions, measuring functional signals—will greatly accelerate this process.

Dendritic input maps

Understanding synaptic integration requires knowledge of how the different incoming inputs are distributed across the dendritic tree, and how they are engaged during behavior. Are synaptic contacts made randomly onto dendrites, or can specific inputs relaying similar or related information be targeted to particular regions of the tree? We are only just starting to address this issue, and it will be a challenging journey. First, we need to know the anatomical distribution of individual synaptic inputs along the dendritic tree with respect to their origin. This will require detailed three-dimensional reconstruction of entire neural circuits, from the neurons of origin to the synaptic contacts made by their axons with the target dendrites, using the high-throughput connectomics techniques described in Chapter 22. Next, we need to visualize the functional engagement of individual synaptic inputs and their distribution across the dendritic tree, ideally during behavior. While two-photon imaging techniques have recently yielded maps of synaptic activation with sensory stimulation (Jia et al., 2010), this approach remains to be extended to awake animals and inhibitory synapses. Finally, to fully understand the impact of different spatio-temporal patterns of synaptic inputs on synaptic integration during behavior we need simultaneous pre- and postsynaptic imaging using calcium- or voltage-sensitive dyes operating at different wavelengths.

Dendritic activity during behavior

One of the great challenges facing dendritic research is investigating how dendritic mechanisms might be engaged during specific behaviors. The use of genetically encoded calcium sensors, in combination with two-photon microscopy, now allows dendritic activity to be imaged even in awake animals (Chen et al., 2013). Although it is possible to image at two different locations near-synchronously (Sheffield and Dombeck, 2015), new technologies will be required in order to visualize dendritic activity across an entire neuron during behavior with high temporal resolution, which is necessary to pinpoint the origin and spread of dendritic events. Fast acousto-optical scanning (e.g., Grewe and Helmchen, 2014) may provide the necessary temporal and spatial resolution to solve this problem. Finally, the development of new probes signaling activation of particular proteins, such as voltage-gated calcium and sodium channels, or synaptic proteins such as GABA or NMDA receptors, may provide a way to visualize how individual elements of the dendritic “toolkit” are engaged during behavior.

Causal dendritic physiology

Ultimately it will be necessary to make causal links between particular dendritic features and behaviors. Thus far, the optogenetic revolution has only had a limited influence on the field of dendrites. This is in part because it has proven difficult to selectively target optogenetic probes to particular cellular compartments such as spines and dendritic branches. The availability of optogenetic probes with adequate two-photon cross sections (e.g., Packer et al., 2012; Prakash et al., 2012) should allow optogenetic activation and silencing to be targeted to small compartments such as individual dendritic branches. Alternatively, local optogenetic control of organelle transport and positioning (van Bergeijk et al., 2015) or molecular targeting techniques may provide a way to “steer” optogenetic probes to individual branches, for example depending on their position in the dendritic tree or their level of activity.

Dendritic disease and therapy

As described in Chapter 24, an increasing number of diseases, or disease models, exhibit disorders of dendritic structure and function. How do these changes impact dendritic computation? Are these changes central to the symptoms of the disease, or are they side effects, or even compensatory effects that mitigate the ravages of the disease? Addressing these issues will require integration of all of the approaches described above, ultimately in human tissue, and may lead to novel therapeutic interventions aimed at manipulating specific dendritic mechanisms.

The importance of theory

Since the pioneering work of Wilfrid Rall (see Chapter 14), theory has been an essential tool for interpreting dendritic experiments, providing a quantitative framework for linking different levels of understanding, and for making new experimentally testable predictions. Theory offers us perhaps the best hope of providing a rigorous intellectual framework for understanding how dendrites contribute to brain function. In particular, with the advent of ever more complete and detailed models of neural circuits, this provides us with the opportunity to test which features of dendrites confer computational advantages from the point of view of network performance. Real neurons are vastly more complex than the simple, isopotential units currently employed in most large-scale neural network models. The challenge is to identify the necessary level of complexity to incorporate into more realistic neuronal models for use in network simulations. This will require the testing of both simplified models, incorporating individual computational features, and of highly detailed models in which multiple features interact, in order to identify which features (or combination of features) can substantially enhance computation on the network level. Ultimately, a close combination of experimental and theoretical work will be required to make causal links between dendritic function and behavior, which is essential for a deeper understanding of how our brains work.

References

- Acker CD, Yan P, Loew LM (2011) Single-voxel recording of voltage transients in dendritic spines. *Biophysical Journal* **101**:L11–L13.
- van Bergeijk P, Adrian M, Hoogenraad CC, Kapitein LC (2015) Optogenetic control of organelle transport and positioning. *Nature* **518**:111–114.
- Betzig E, Patterson GH, Sougrat R, Lindwasser OW, Olenych S, Bonifacino JS, Davidson MW, Lippincott-Schwartz J, Hess HF (2006). Imaging intracellular fluorescent proteins at nanometer resolution. *Science* **313**:1642–1645.
- Chen TW, Wardill TJ, Sun Y, Pulver SR, Renninger SL, Baohan A, Schreiter ER, Kerr RA, Orger MB, Jayaraman V, Looger LL, Svoboda K, Kim DS (2013) Ultrasensitive fluorescent proteins for imaging neuronal activity. *Nature* **499**:295–300.
- Grewé BF, Helmchen F (2014) High-speed two-photon calcium imaging of neuronal population activity using acousto-optic deflectors. *Cold Spring Harbor Protocols* **2014**:618–629.
- Harnett MT, Makara JK, Spruston N, Kath WL, Magee JC (2012) Synaptic amplification by dendritic spines enhances input cooperativity. *Nature* **491**:599–602.
- Hell SW (2009) Microscopy and its focal switch. *Nature Methods* **6**(1):24–32.
- Jia H, Rochefort NL, Chen X, Konnerth A (2010) Dendritic organization of sensory input to cortical neurons in vivo. *Nature* **464**:1307–1312.
- Keefer EW, Botterman BR, Romero MI, Rossi AF, Gross GW (2008) Carbon nanotube coating improves neuronal recordings. *Nature Nanotechnology* **3**:434–439.

- Kiuchi T, Higuchi M, Takamura A, Maruoka M, Watanabe N (2015). Multitarget super-resolution microscopy with high-density labeling by exchangeable probes. *Nature Methods* **12**:743–746.
- Li D, Shao L, Chen BC, Zhang X, Zhang M, Moses B, Milkie DE, Beach JR, Hammer JA 3rd, Pasham M, Kirchhausen T, Baird MA, Davidson MW, Xu P, Betzig E (2015) Extended-resolution structured illumination imaging of endocytic and cytoskeletal dynamics. *Science* **349**(6251):aab3500.
- Micheva KD, Smith SJ (2007) Array tomography: a new tool for imaging the molecular architecture and ultrastructure of neural circuits. *Neuron* **55**(1):25–36.
- Packer AM, Peterka DS, Hirtz JJ, Prakash R, Deisseroth K, Yuste R (2012) Two-photon optogenetics of dendritic spines and neural circuits. *Nature Methods* **9**:1202–1205.
- Palmer LM, Stuart GJ (2006) Site of action potential initiation in layer 5 pyramidal neurons. *Journal of Neuroscience* **26**:1854–1863.
- Prakash R, Yizhar O, Grewe B, Ramakrishnan C, Wang N, Goshen I, Packer AM, Peterka DS, Yuste R, Schnitzer MJ, Deisseroth K (2012) Two-photon optogenetic toolbox for fast inhibition, excitation and bistable modulation. *Nature Methods* **9**:1171–1179.
- Rust MJ, Bates M, Zhuang X (2006). Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM). *Nature Methods* **3**:793–795.
- Schrlau MG, Dun NJ, Bau HH (2009) Cell electrophysiology with carbon nanowires. *ACS Nano* **3**:563–568.
- Sheffield ME, Dombeck DA (2015) Calcium transient prevalence across the dendritic arbor predicts place field properties. *Nature* **517**:200–204.
- Tønnesen J, Katona G, Rózsa B, Nágerl UV (2014) Spine neck plasticity regulates compartmentalization of synapses. *Nature Neuroscience* **17**:678–685.

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