Evidence for Dense and Regenerative CCK Signaling Networks amongst Mouse Cortical Pyramidal Cells

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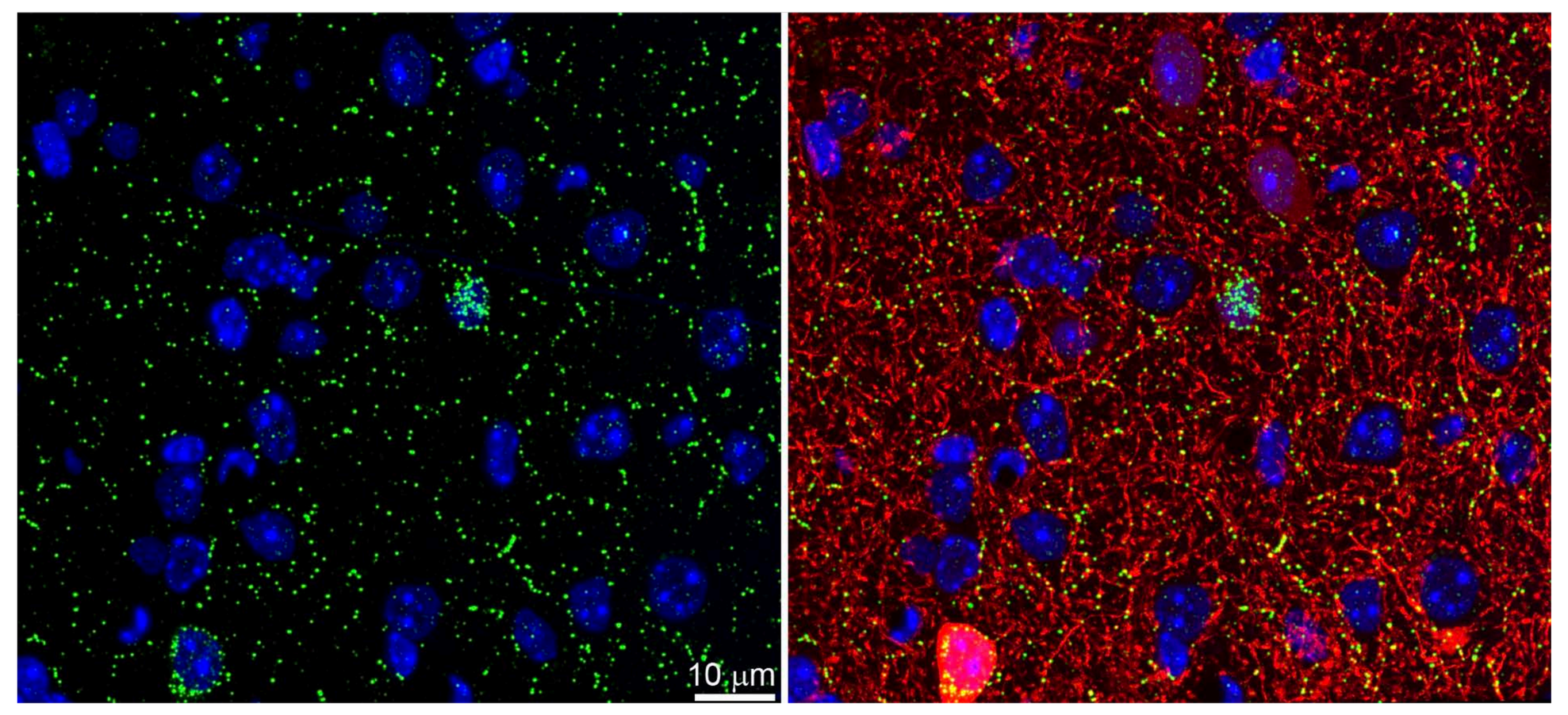
Peptidergic Networks and Transcriptomic Data Resources

The last common ancestor of all animals with nervous systems lacked neurons and synapses, but almost surely coordinated numerous sensory, motor and digestive cell types via diffusible ligands homologous to today's neuropeptides and conjugate receptors homologous to today's neuropeptide-selective GPCRs. These "wireless" peptidergic networks never went away. As pressures for speed and size drove the evolution of ever more extensive synaptic networks, peptidergic networks have taken on modulatory roles, governing adaptive plasticity of the synaptic newcomers.

The tables below list the neuropeptide precursor and receptor genes and the Allen Institute transcriptomic data resources upon which the present analysis and conjectures are based.

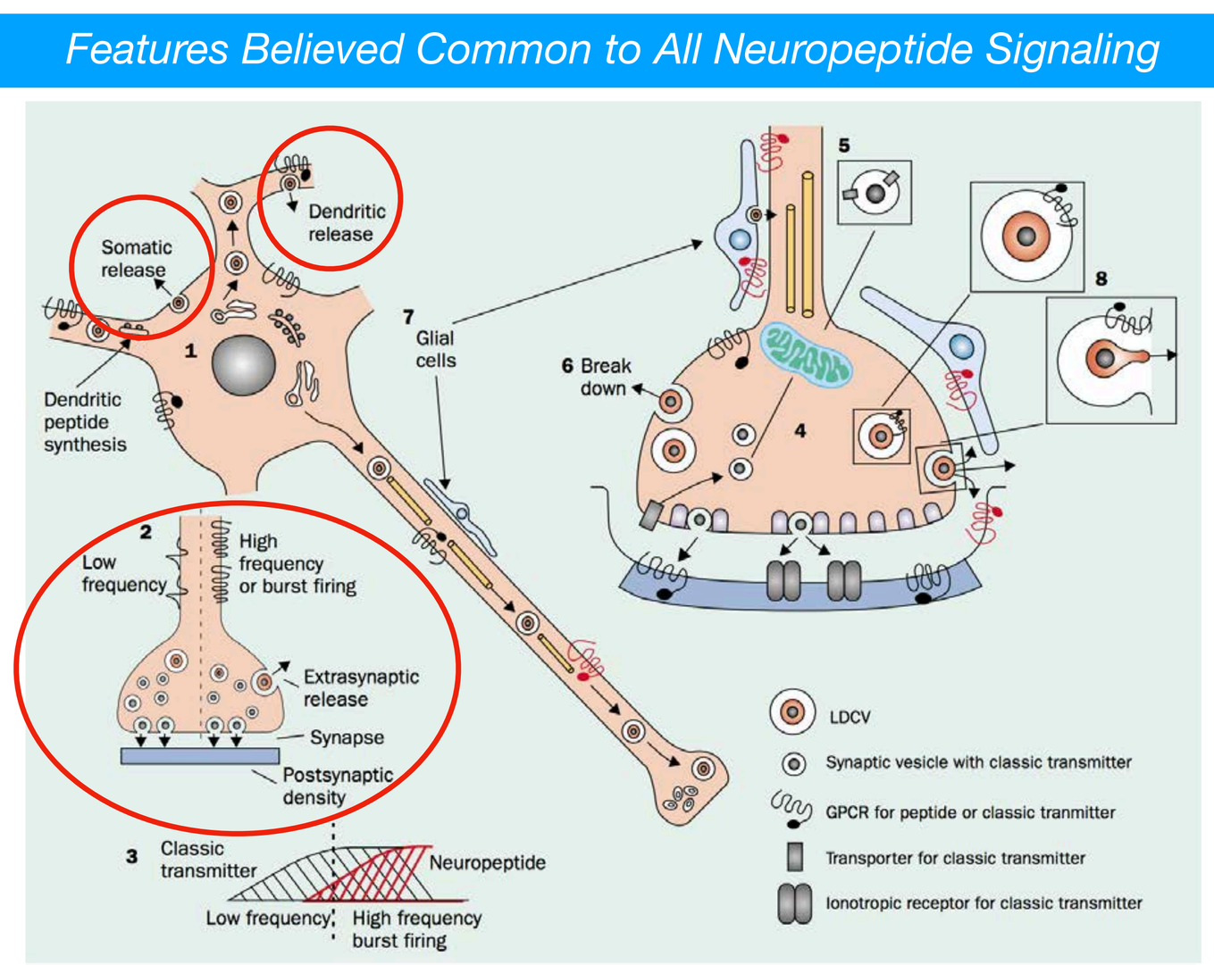
Neuropeptide-Containing Dense-Core Vesicles are All Over the Place!



DAPI Neuropeptide Y — DAPI Neuropeptide Y GABA

Kristina Micheva

Features Believed Common to All Neuropeptide Signaling



Hökfelt, Bartfai & Bloom (2003)

20 Top Neuropeptide Precursor Proteins (NPPs) Brainwide and their 36 Cognate Receptors (NP-GPCRs)

NPP Gene	Neuropeptide Product Name	Cognate NP-GPCR Genes	Cognate Aggregates	Primary Transduction Pathway
Cck	cholecystokinin	*Cckar,Cckbr*	*CckRs*	$G_{q/11}$ - Phospholipase C Activation: ↑ PI(4,5)P2 Hydrolysis → ↓ PI(4,5)P2; ↑ IP3 → ↑Ca++i; ↑ DAG → Ion Channel and Synapse Impacts
Grp	gastrin releasing peptide	*Grpr*	*GrpRs*	
Pthlh	parathyroid hormone-like peptide	*Pth1r*	*PthlhRs*	
Nmb	neuromedin B	*Nmbr*	*NmbRs*	
Ghrl	ghrelin	*Ghsr*	*GhrlRs*	
Adcya	adenylate cyclase activating polypeptide 1	*Adcyap1r1,Vipr1*	*Adcyap1*	Gs - Adenylate Cyclase Activation: ↑Cyclic AMP Production → ↑Protein Kinase A Activity → ↑Ion Channel & Synaptic Protein Phosphorylation
Vip	vasoactive intestinal polypeptide	*Adcyap1r1,Vipr1,Vipr2*	*VipRs*	
Crh	corticotropin releasing hormone	*Crhr1,Crhr2*	*CrhRs*	
Adm	adrenomedullin	*Calcrl*	*AdmRs*	
Calca	calcitonin/calcitonin-related peptide	*Calcrl*	*CalcaRs*	
Penk	preproenkephalin	*Oprd1,Oprk1,Oprl1,Oprm1*	*PenkRs*	Gio - Adenylate Cyclase Inhibition: ↓Cyclic AMP Production → ↓Protein Kinase A Activity → ↓Ion Channel & Synaptic Protein Phosphorylation
Npy	neuropeptide Y	*Npy1r,Npy2r,Npy4r,Npy5r*	*NpyRs*	
Cort	cortistatin	*Sstr1,Sstr2,Sstr3,Sstr4,Sstr5*	*CortRs*	
Pnoc	prepronociceptin	*Oprl1*	*PnocRs*	
Sst	somatostatin	*Sstr1,Sstr2,Sstr3,Sstr4 Sstr5*	*SstRs*	
Pdyn	prodynorphin	*Oprd1,Oprk1,Oprl1,Oprm1*	*PdynRs*	
Agt	angiotensinogen	*Agtr1a,Agtr1b,Agtr2*	*AgtRs*	
Pomc	pro-opiomelanocortin-alpha	*Mc1r,Mc2r,Mc3r,Mc4r,Mc5r*; *Oprd1,Oprk1,Oprl1,Oprm1*	*PomcRs*	Multiple Pathways
Tac2	tachykinin 2	*Tacr2*; *Tacr3*	*Tac2Rs*	
Tac1	tachykinin 1	*Tacr2*; *Tacr1*	*Tac1Rs*	

Two Allen Institute Offerings of Single-Cell RNA-Seq Transcriptomic Data

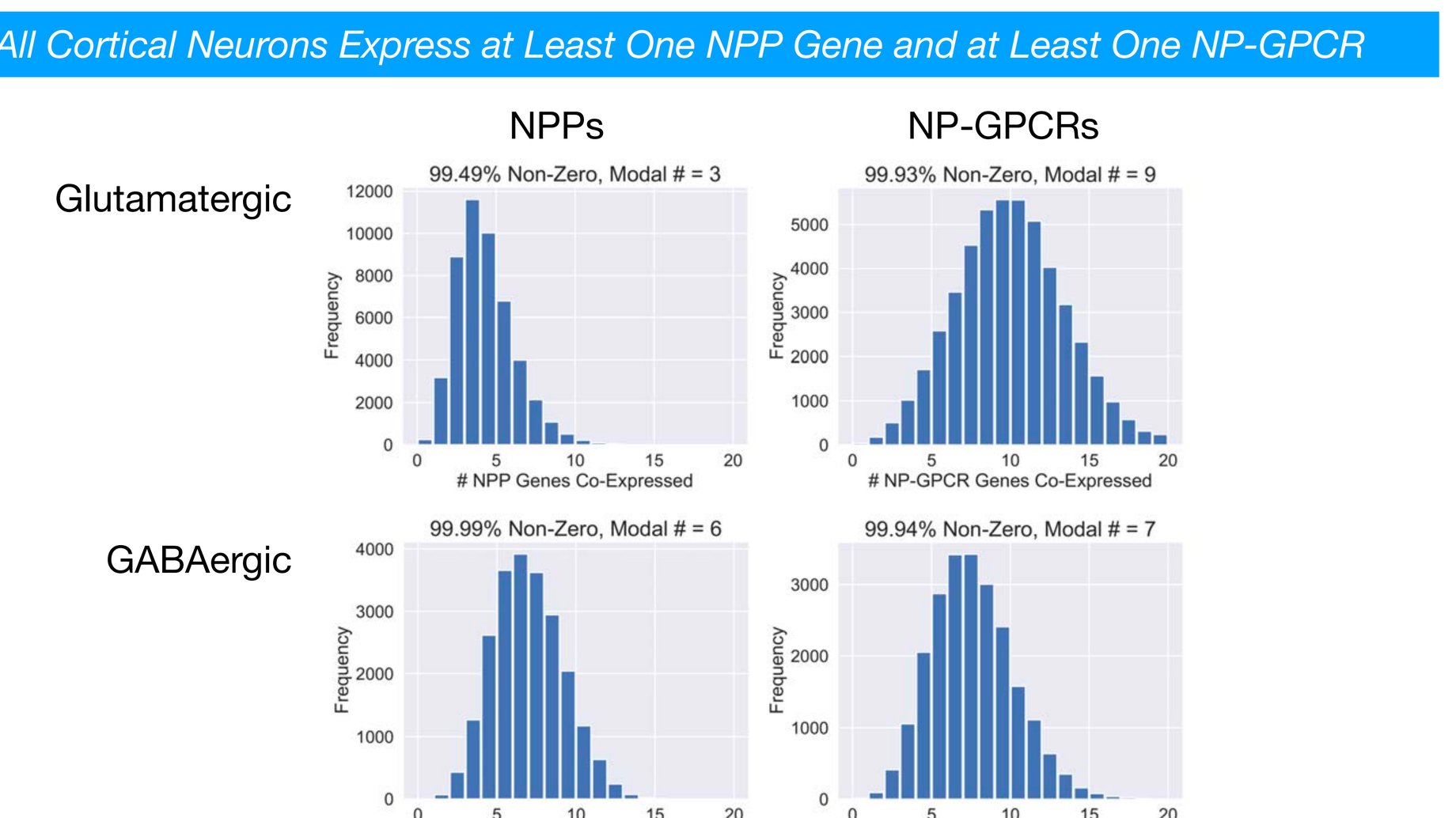
A. Droplet-Based Fast Sequencing
Average 4,106 Genes Mapped per Neuron
(Yao, et al., *Nature* 2023)
2,957,911 Neurons, 29 Regions Brainwide

	Region Symbol	Region Label	Neurons Sampled	Classes	Subclasses	Clusters
Neocortex	MOfp	Somatomotor-Frontal	51,714	2	13	75
	MOp	Primary Motor	242,906	3	18	173
	SSgv	Somatosensory-Gustatory-Visceral	83,171	2	18	121
	SSp	Primary Somatosensory	72,546	2	15	61
	AUD	Auditory	69,462	2	17	90
	AUDa	Auditory-Temporal Assoc-Peri-Ectorhinal	15,735	2	15	34
	VISa	Visual-Posterior Parietal Association	44,092	2	14	65
	VIS	Visual	313,282	3	18	187
	ACA	Anterior Cingulate	80,538	2	18	115
	PIO	Prelimbic-Infralimbic-Orbital	88,893	3	22	135
	AI	Agranular Insular	74,243	3	21	129
	RSP	Retrosplenial	91,124	2	15	81
	TPE	Temporal Assoc-Perirhinal-Ectorhinal	58,158	2	22	103
Allocortex	OLF	Olfactory	235,212	5	47	224
	HIP	Hippocampal	128,929	4	18	82
	RHP	Retrohippocampal	76,710	2	29	108
	ENT	Entorhinal	79,261	3	26	116
	CTXsp	Cortical subplate	75,455	3	39	143
Basal Forebrain	STRd	Dorsal Striatum	39,105	3	10	24
	STRv	Ventral Striatum	38,985	3	13	42
	LSX	Lateral Septal Complex	33,516	4	16	97
	sAMY	Striatum-like Amygdalar Nuclei	74,445	4	44	167
	PAL	Pallidum	57,890	4	47	108
	TH	Thalamus	167,357	3	28	269
	HY	Hypothalamus	183,095	7	72	609
Midbrain	MB	Midbrain	191,456	7	85	527
Hindbrain	PONS	Pons	40,572	6	42	74
	MY	Medulla	83,287	6	61	152
	CB	Cerebellum	166,772	3	9	17

B. Plate-Based Deep Sequencing
Average 7,677 Genes Mapped per Neuron
(Yao, et al., *Cell* 2021)
71,518 Neurons, 20 Cortical Regions

	Region Symbol	Region Label	Neurons Sampled	Subclasses	Clusters
Neocortex	VISp	Primary Visual	14,834	17	122
	VIS	Visual	3,384	15	50
	PTLp	Posterior Parietal Assocation	1,503	12	34
	AUD	Auditory	1,433	14	32
	SSp	Primary Somatosensory	5,640	15	60
	SSs	Secondary Somatosensory	1,797	13	29
	GU	Gustatory	1,396	16	35
	MOp	Primary Motor	6,558	14	90
	ALM	Anterolateral Motor	9,084	15	95
	PL-ILA	Prelimbic, Infralimbic	1,452	14	35
	ORB	Orbital	1,389	14	31
	AI	Agranular Insular	1,524	13	26
	ACA	Anterior Cingulate	5,085	15	61
	RSP	Retrosplenial	2,040	14	35
	TPE	Temporal Assoc-Perirhinal-	1,585	16	41
Hippocampal Formation	ENTm	Medial Entorhinal	1,564	15	31
	ENTl	Lateral Entorhinal	1,538	17	35
	PPP	Parasubiculum, Postsubiculum,	1,577	11	30
	SUBP	Subiculum, Prosubiculum	1,609	13	39
	HIP	Hippocampus	6,526	10	66

All Cortical Neurons Express at Least One NPP Gene and at Least One NP-GPCR

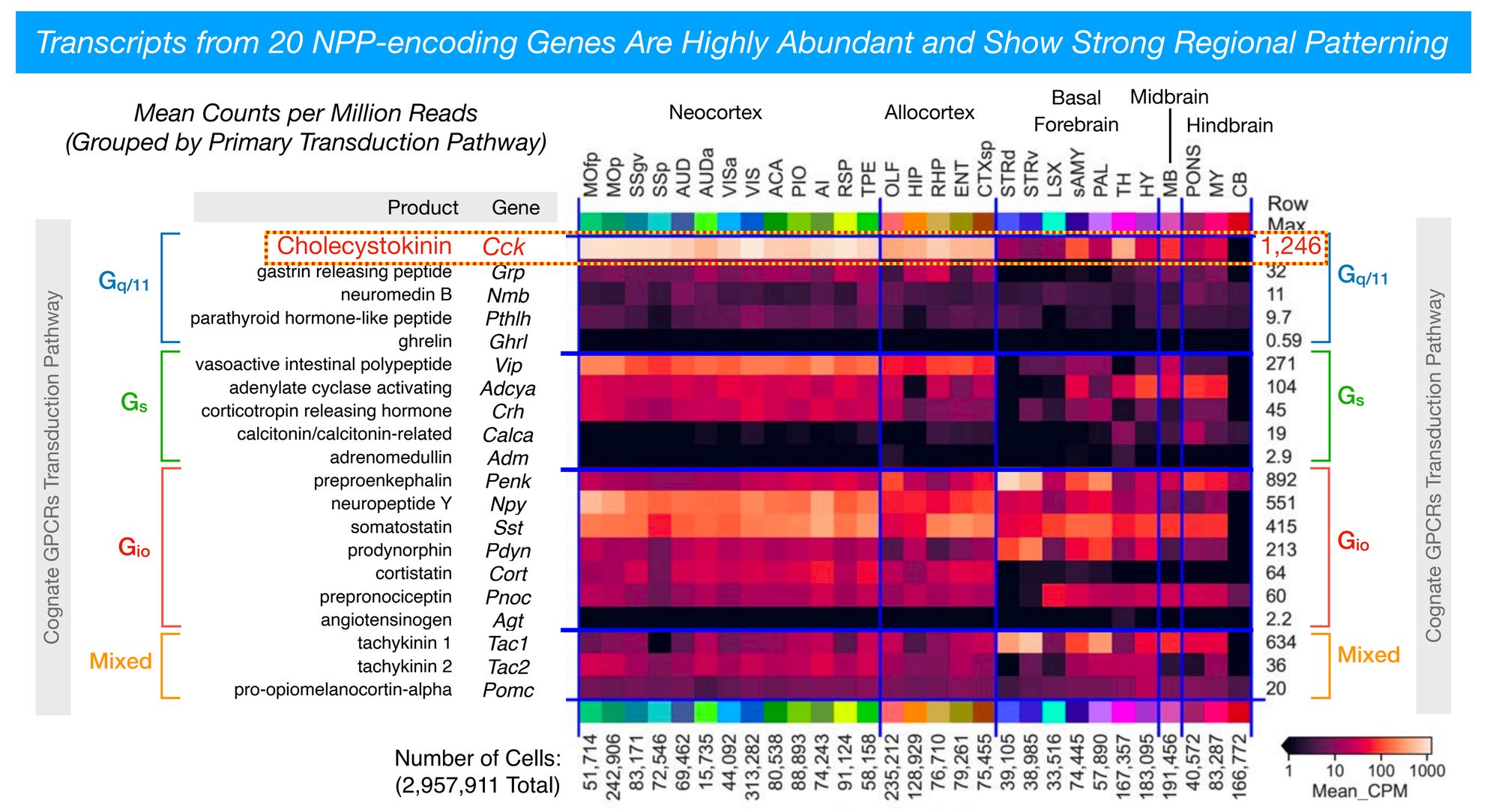


Yao, et al. (2021) Cell

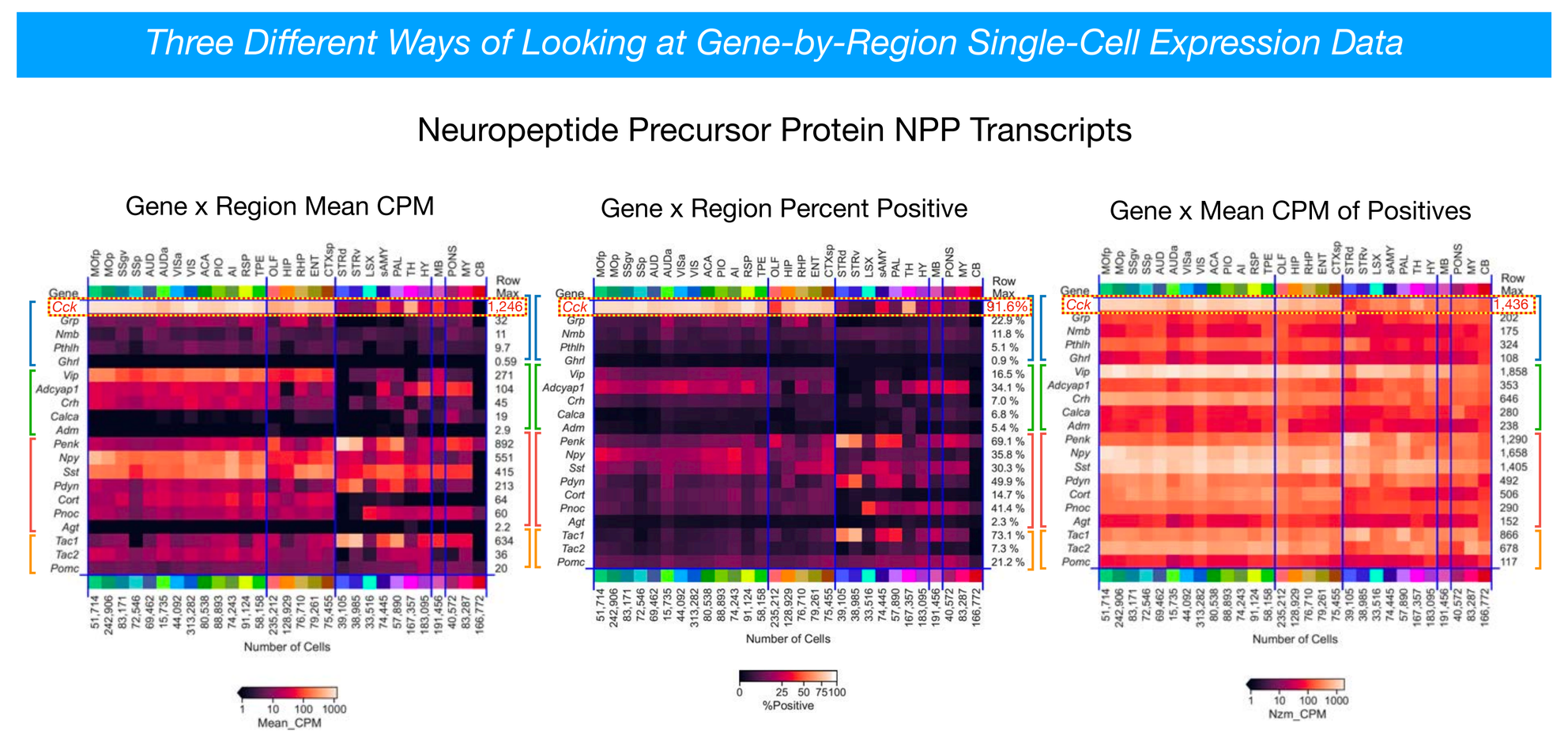
Neuropeptide Precursor and Receptor Transcripts by Region

Single-cell transcriptomes have offered the first panoramic outlines of possible peptidergic network architectures. Interestingly, the exuberant but type-specific expression of multiple precursor and receptor genes evident in the expression maps below indicate that local neuropeptide signaling may be considerably more common than previously appreciated. This prospect is particular striking for the case of CCK signaling amongst cortical pyramidal cells. As highlighted below, transcriptomes point to expression of both the precursor protein gene, *Cck,* and the cognate GPCR receptor gene, *Cckbr*, in almost all cell of this type. Local signaling is here particularly likely, given the close packing of these cells and the dense intermingling of their dendrites. Autocrine (cell-to-self) feedback signaling is also likely to be the rule.

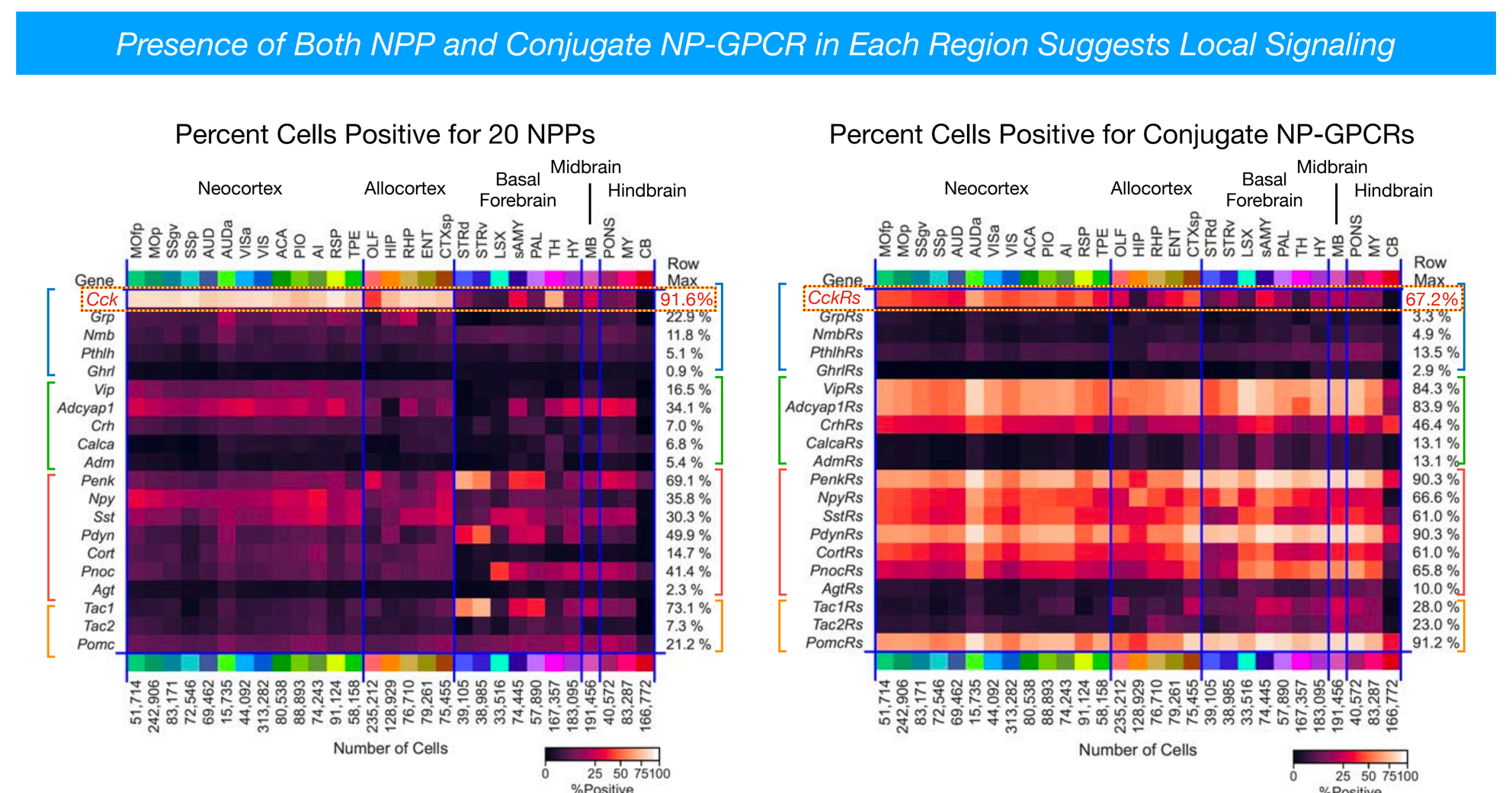
Transcripts from 20 NPP-encoding Genes Are Highly Abundant and Show Strong Regional Patterning



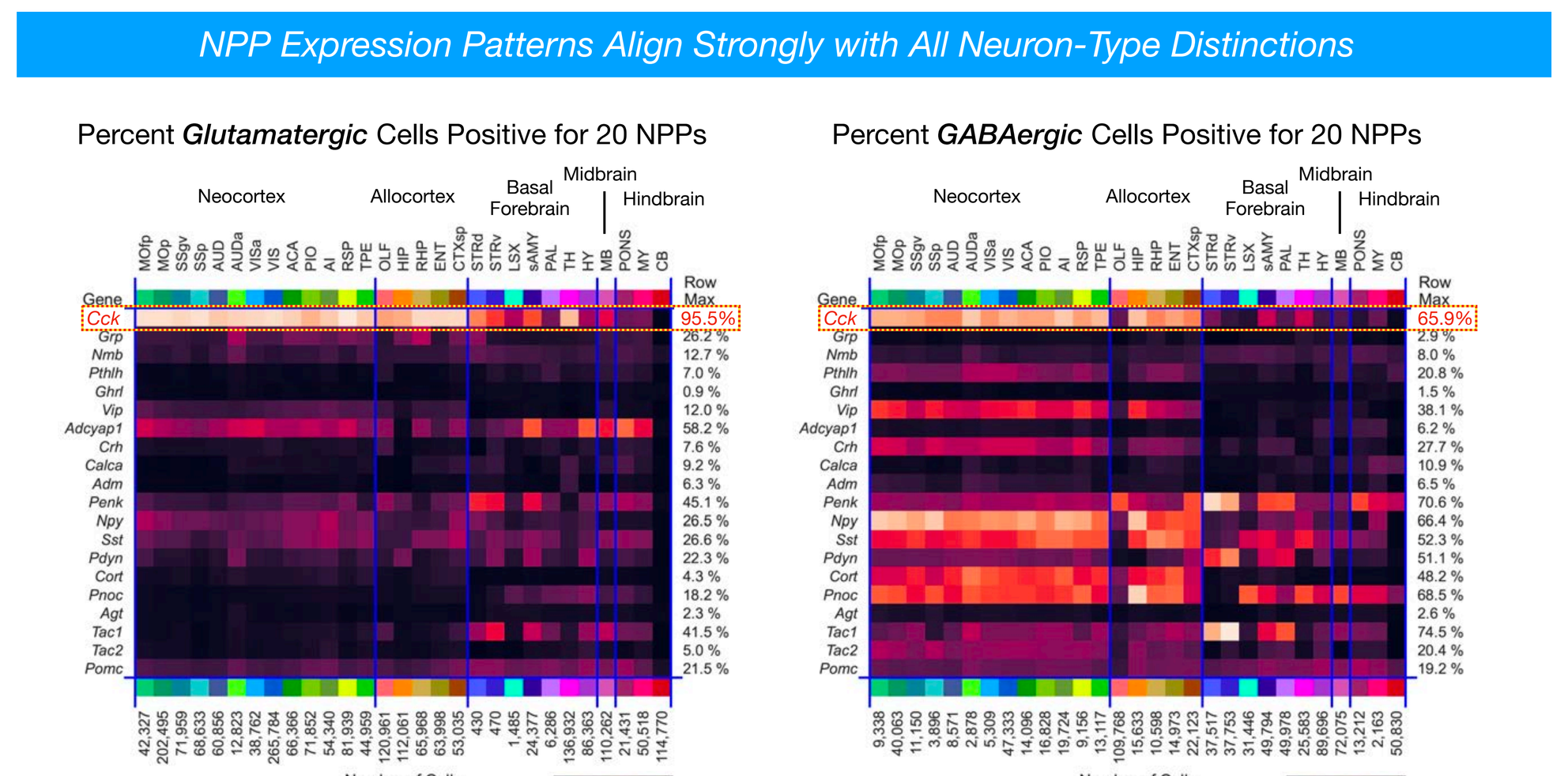
Three Different Ways of Looking at Gene-by-Region Single-Cell Expression Data



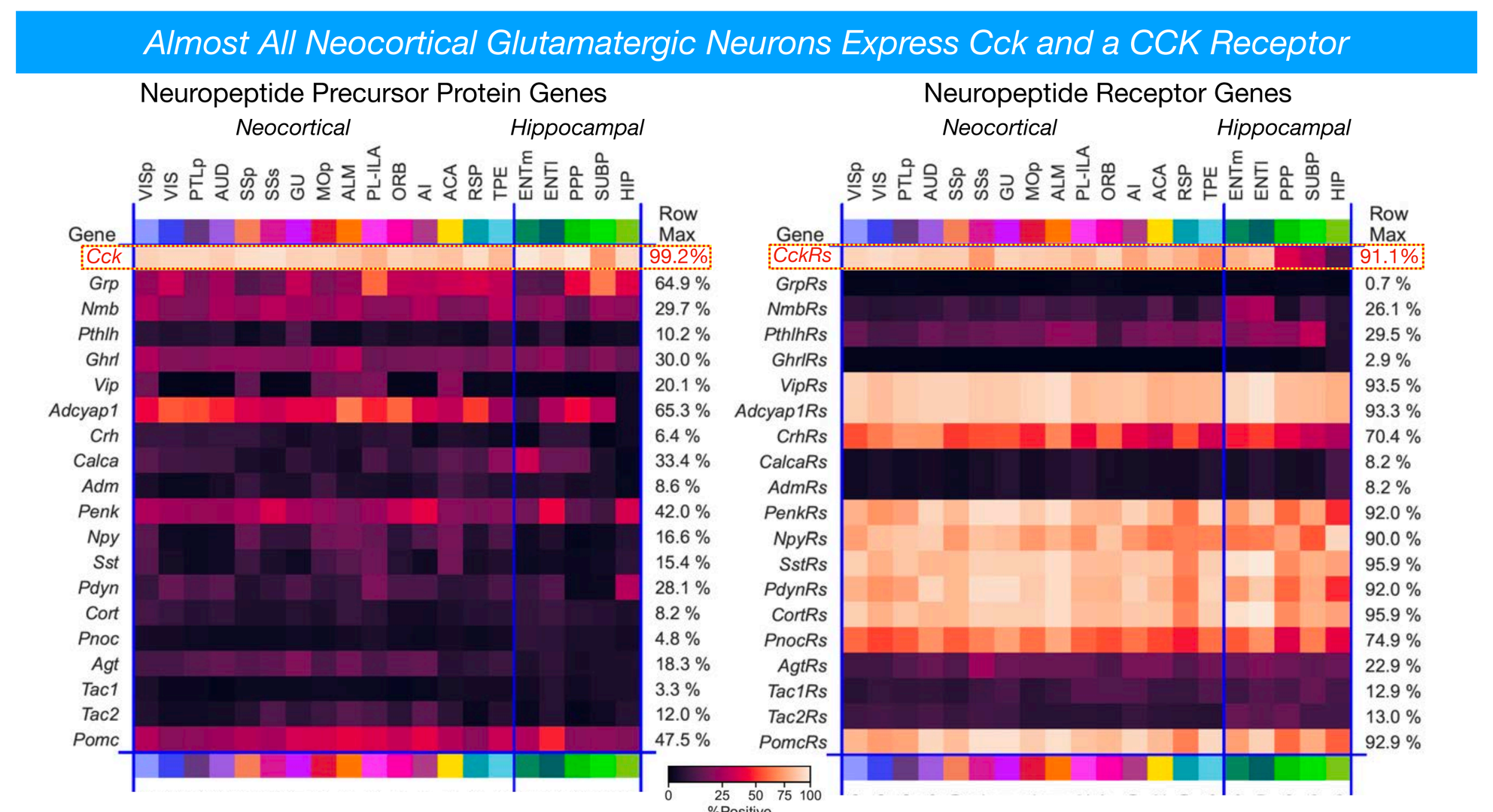
Presence of Both NPP and Conjugate NP-GPCR in Each Region Suggests Local Signaling



NPP Expression Patterns Align Strongly with All Neuron-Type Distinctions



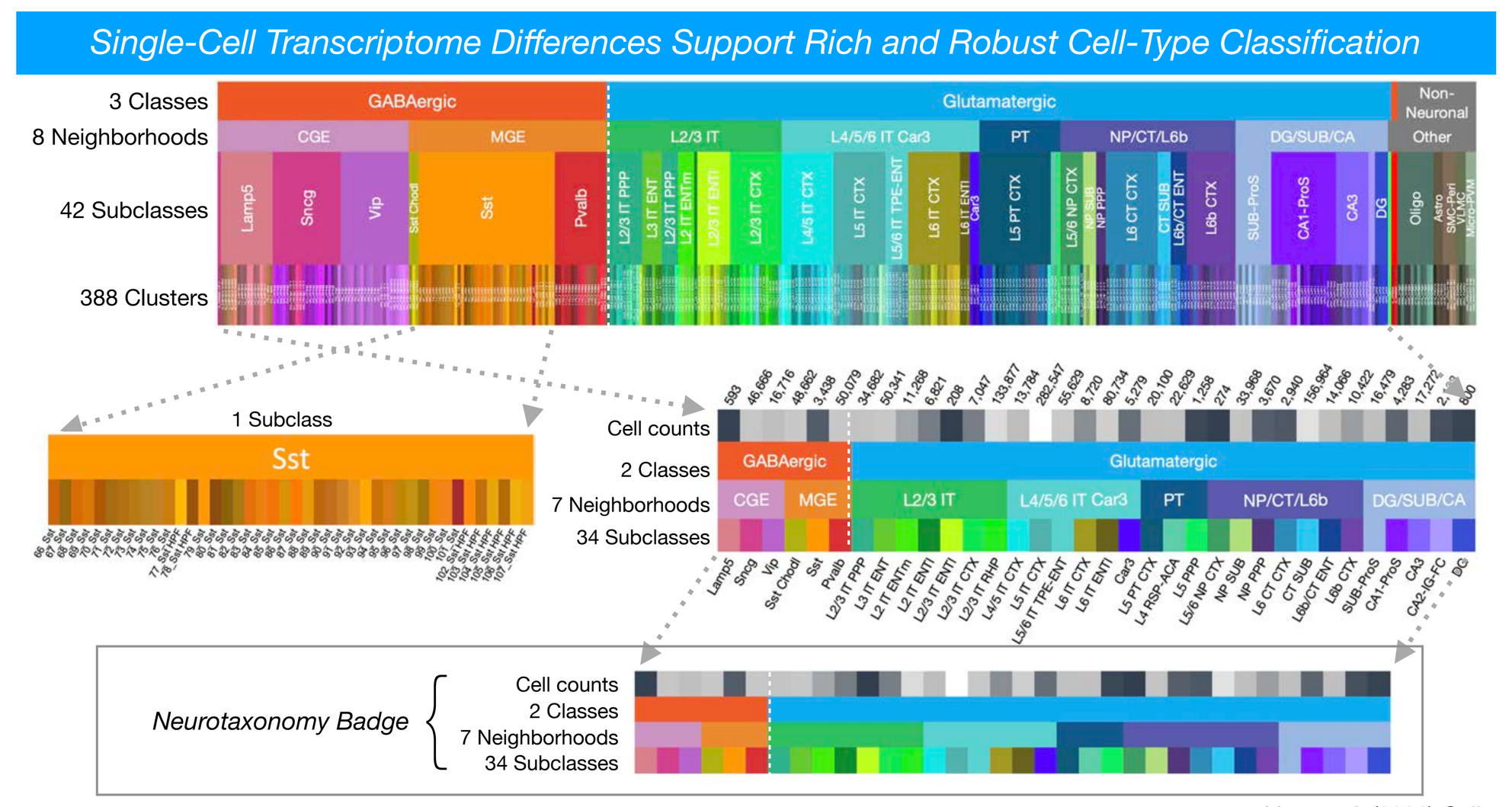
Almost All Neocortical Glutamatergic Neurons Express Cck and a CCK Receptor



Neuropeptide Precursor and Receptor Transcripts by Cell Type

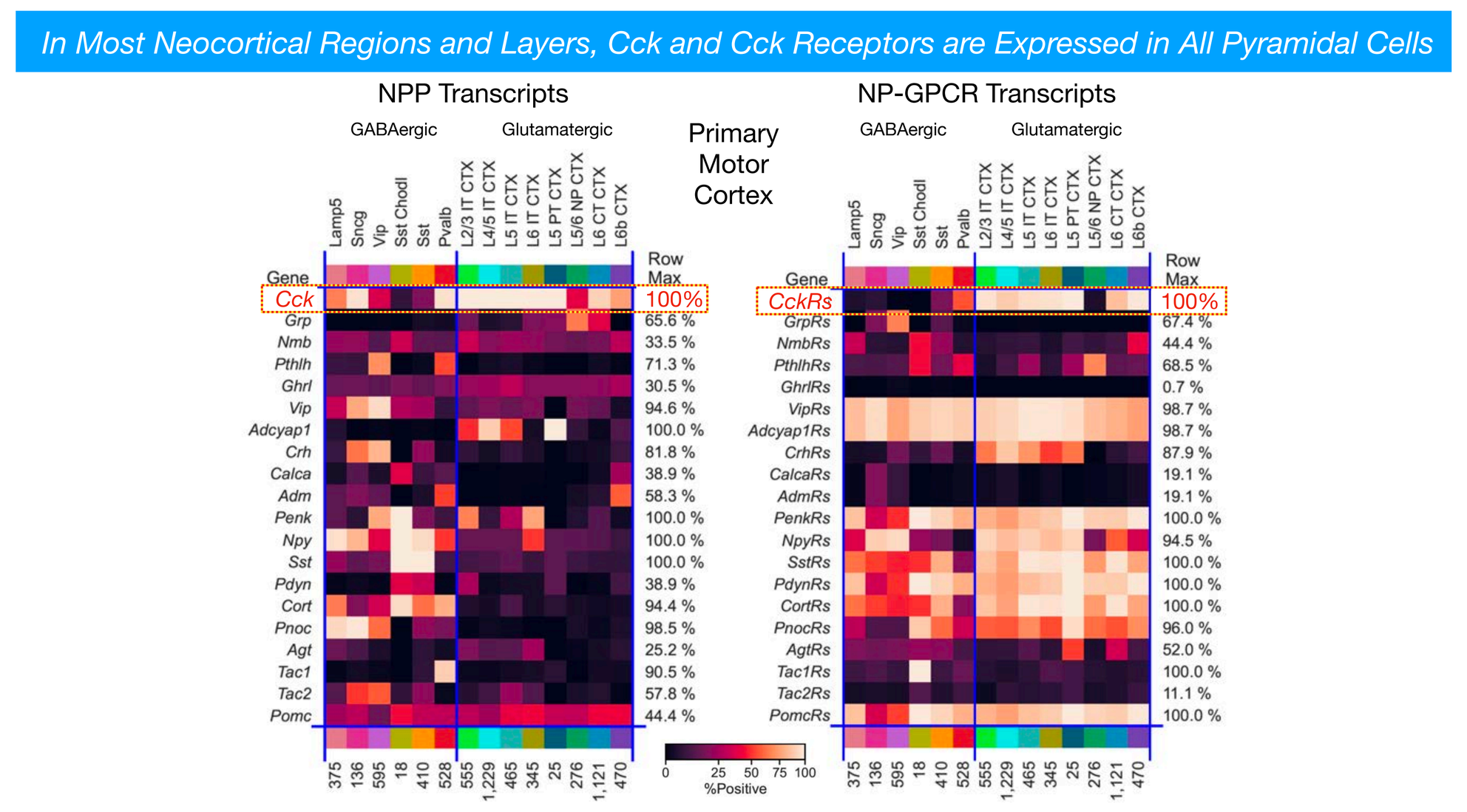
Here we focus on new evidence that certain individual neuron types and even many individual neurons of those types express genes for both precursor and receptor for one and the same peptide. In mouse cortex, the prime example is co-expression of *Cck* and *Cckbr* in the predominant glutamatergic pyramidal cell population. Transcriptomes furthermore show that such expression patterning is highly type-specific and that such specificity is tightly conserved amongst cortical regions. Such conservation speaks to developmental and functional importance that has been sustained across evolutionary time scales.

Single-Cell Transcriptome Differences Support Rich and Robust Cell-Type Classification

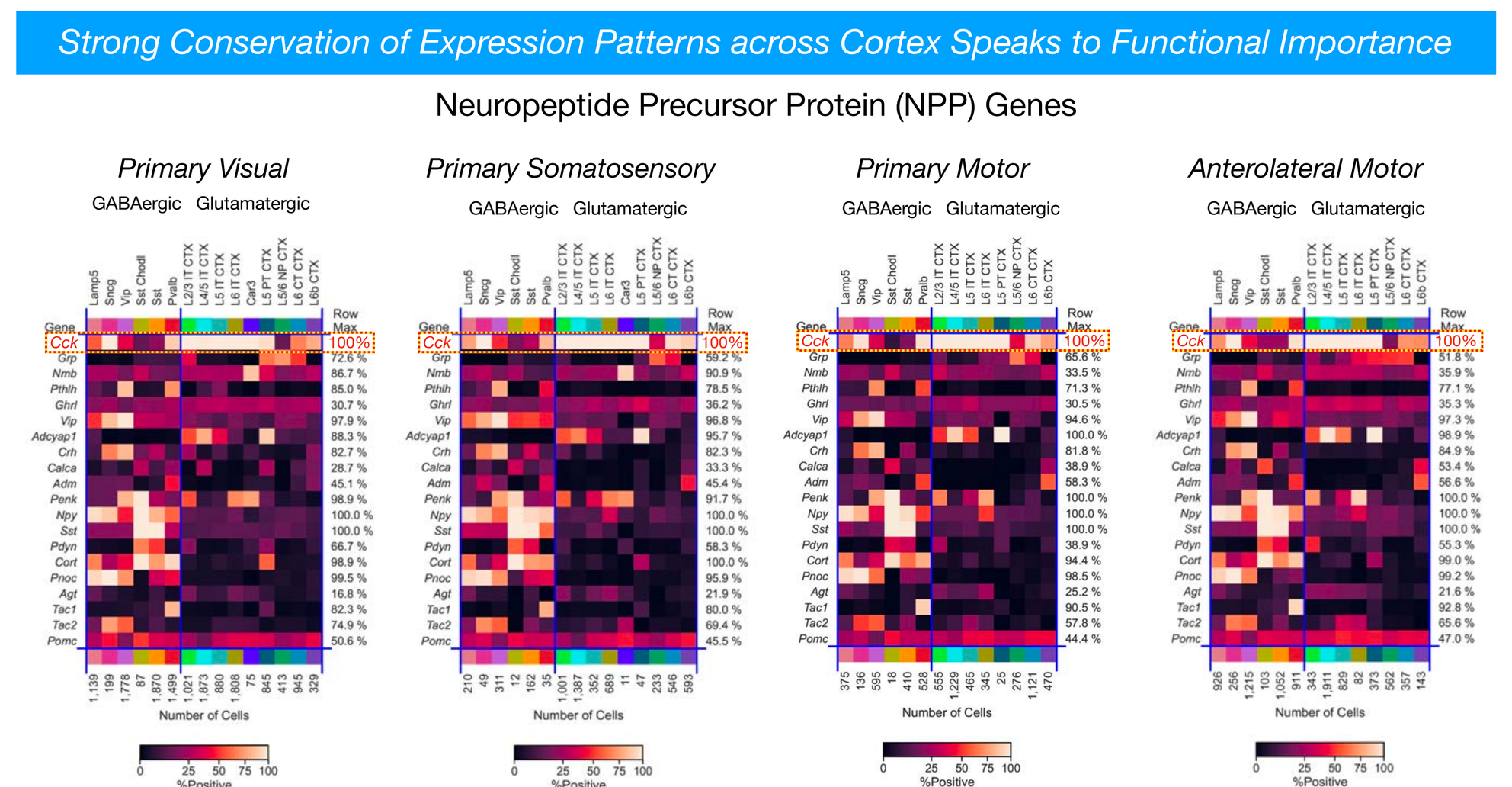


Yao, et al. (2021) Cell

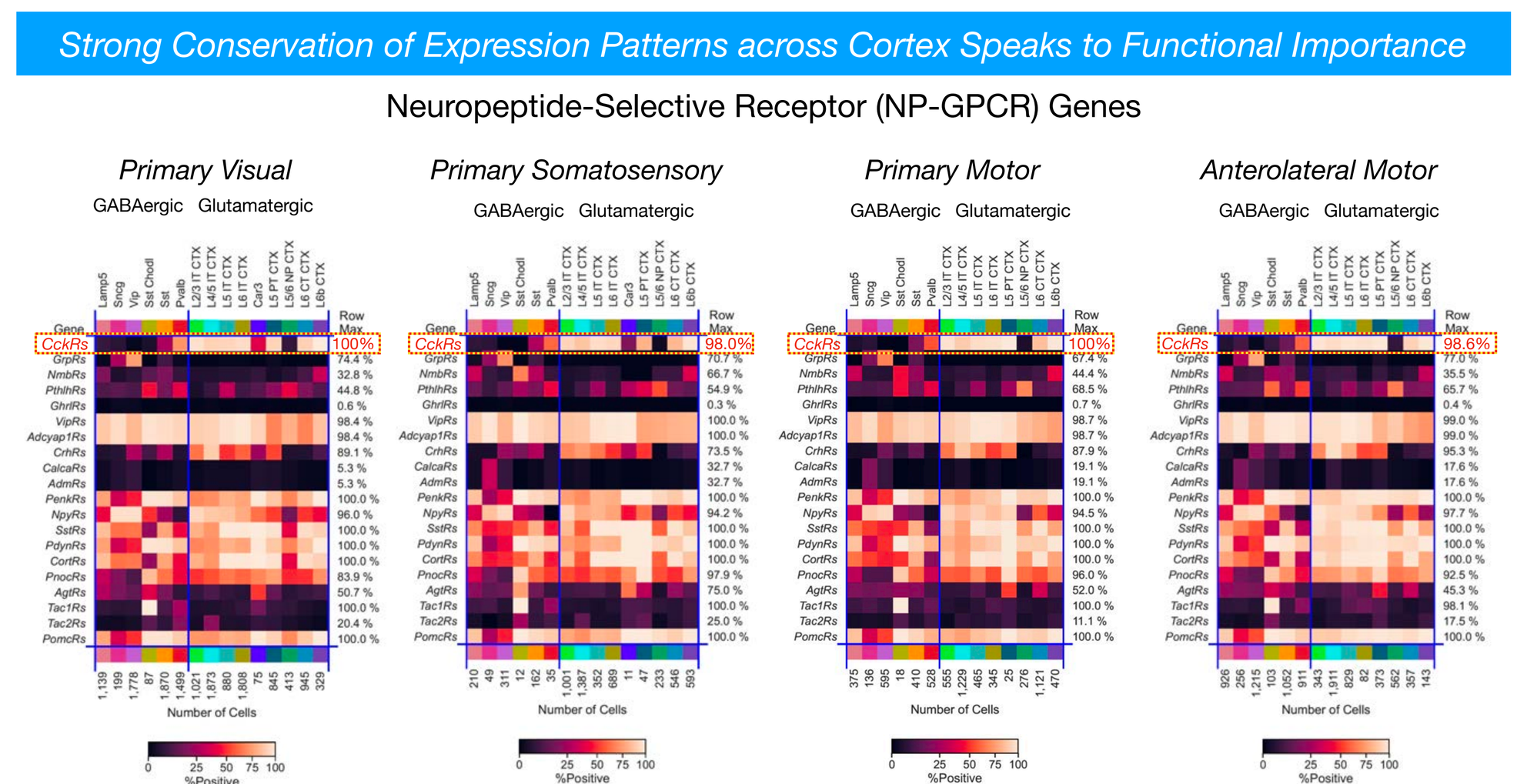
In Most Neocortical Regions and Layers, Cck and Cck Receptors are Expressed in All Pyramidal Cells



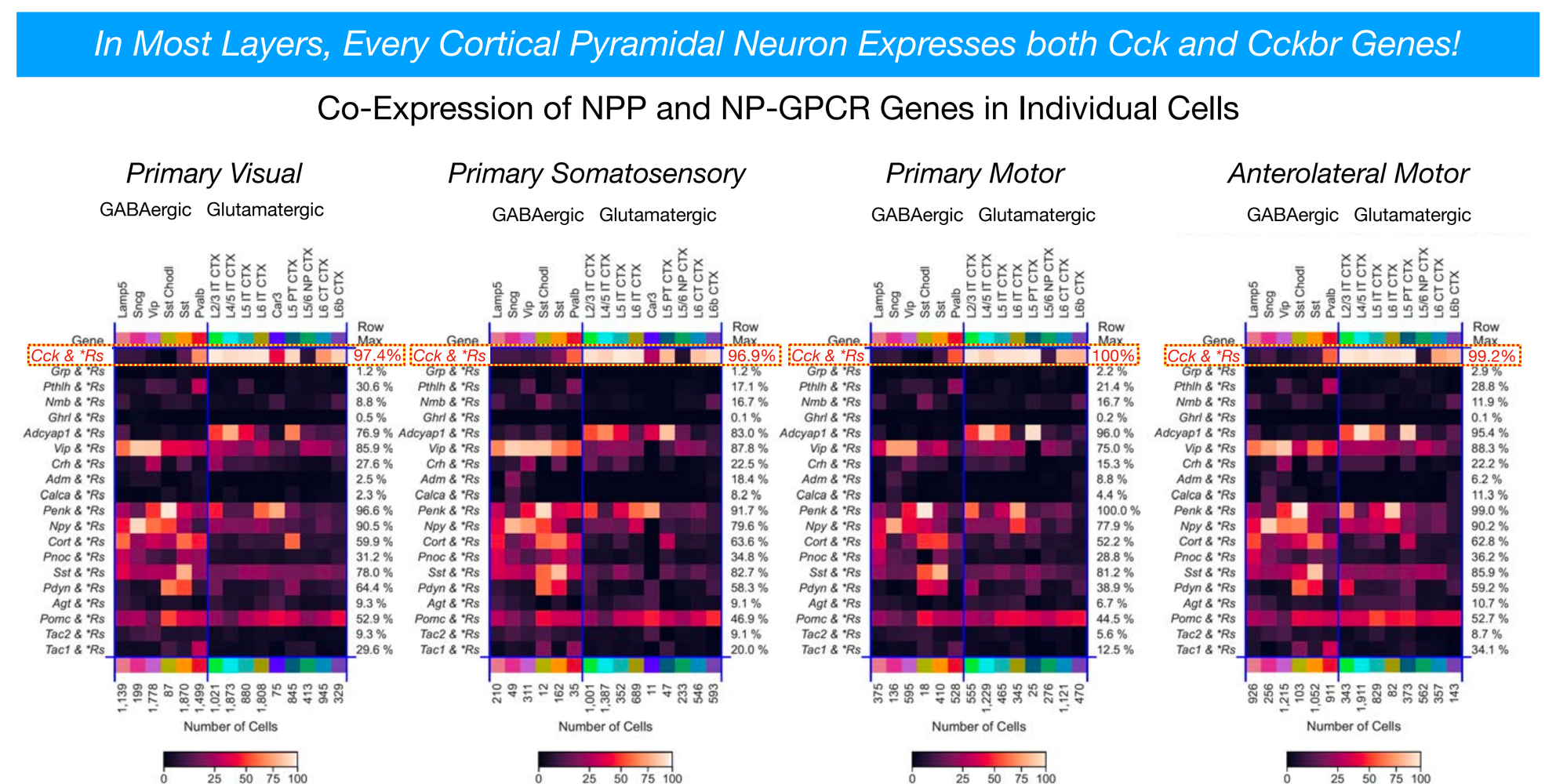
Strong Conservation of Expression Patterns across Cortex Speaks to Functional Importance



Strong Conservation of Expression Patterns across Cortex Speaks to Functional Importance

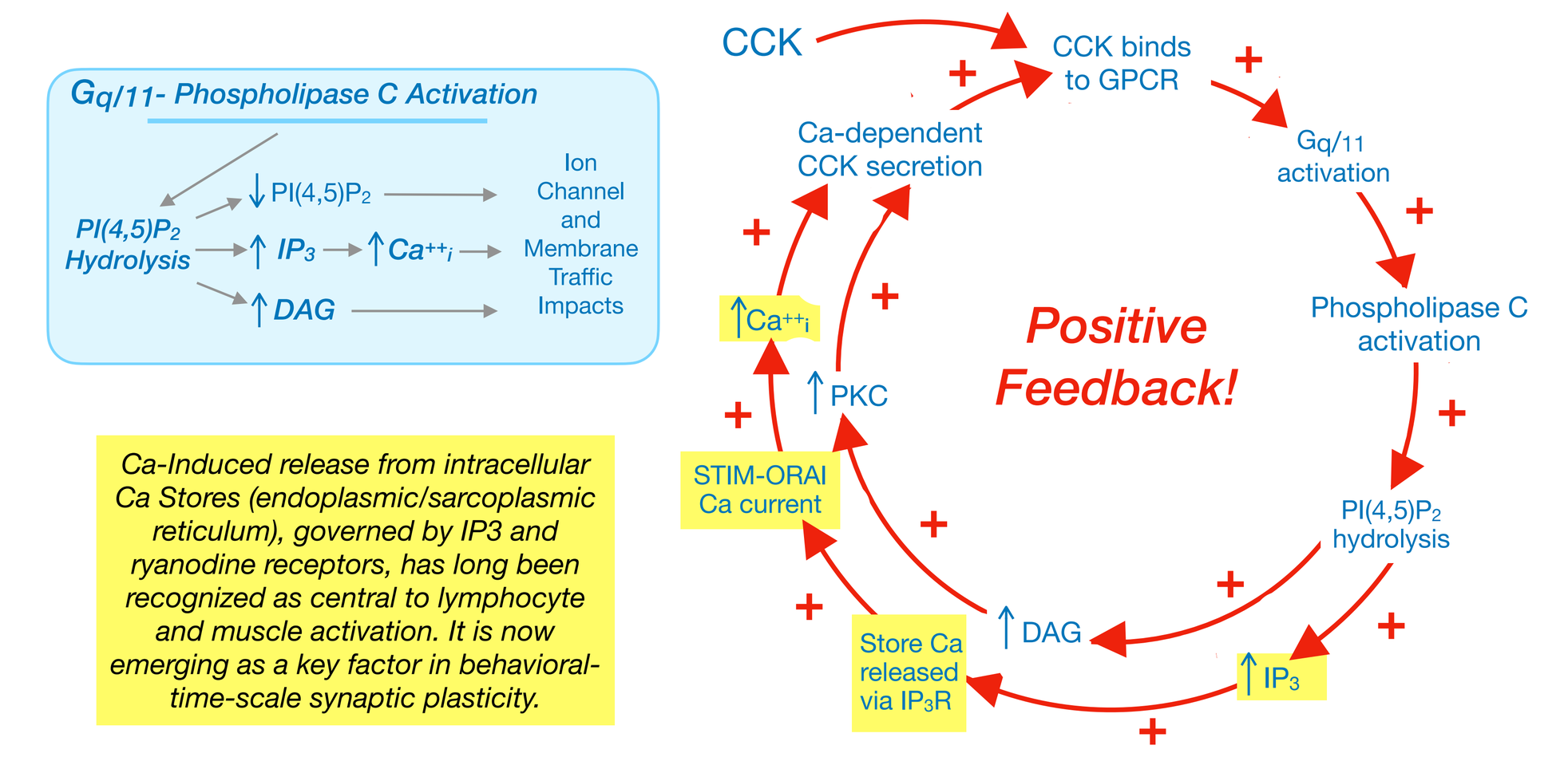


In Most Layers, Every Cortical Pyramidal Neuron Expresses both Cck and Cckbr Genes!



CCK firecracker?... (Regeneration, Propagation, Thresholds?)

Co-expression of *Cck* and *Cckbr* in neocortical pyramidal cells entails the intriguing prospect of regenerative positive feedback, with activation of the receptor by CCK leading via a Gq/11 transduction pathway to phospholipase C activation, to intracellular Ca elevation, and then in turn further secretion of CCK. Such regeneration could support signal amplification, non-decremental propagation, threshold non-linearities and other computational processes.

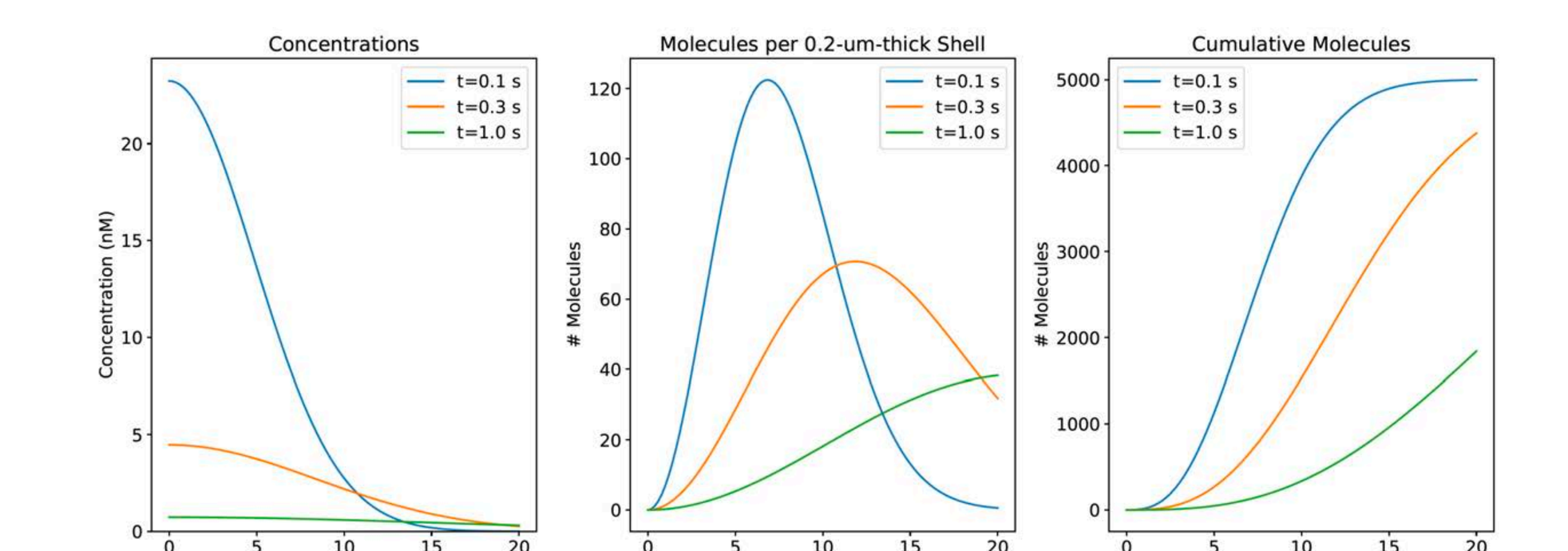


What can diffusion theory tell us about neuropeptide networks?

Functional peptidergic network graphs must be determined somehow by convolutions of (1) cell-type-specific differential expression of ligand and receptor genes with (2) cellular morphologies and (3) the action radius of peptide released from a single vesicle through the neuropil interstitium to a conjugate receptor. Below we offer some action radius predictions, based on a diffusion theory model that incorporates empirically determined included volume and tortuosity factors (Nicholson 2000).

Prediction of CCK Diffusion through Interstitial Space from Release of a Single CCK Vesicle

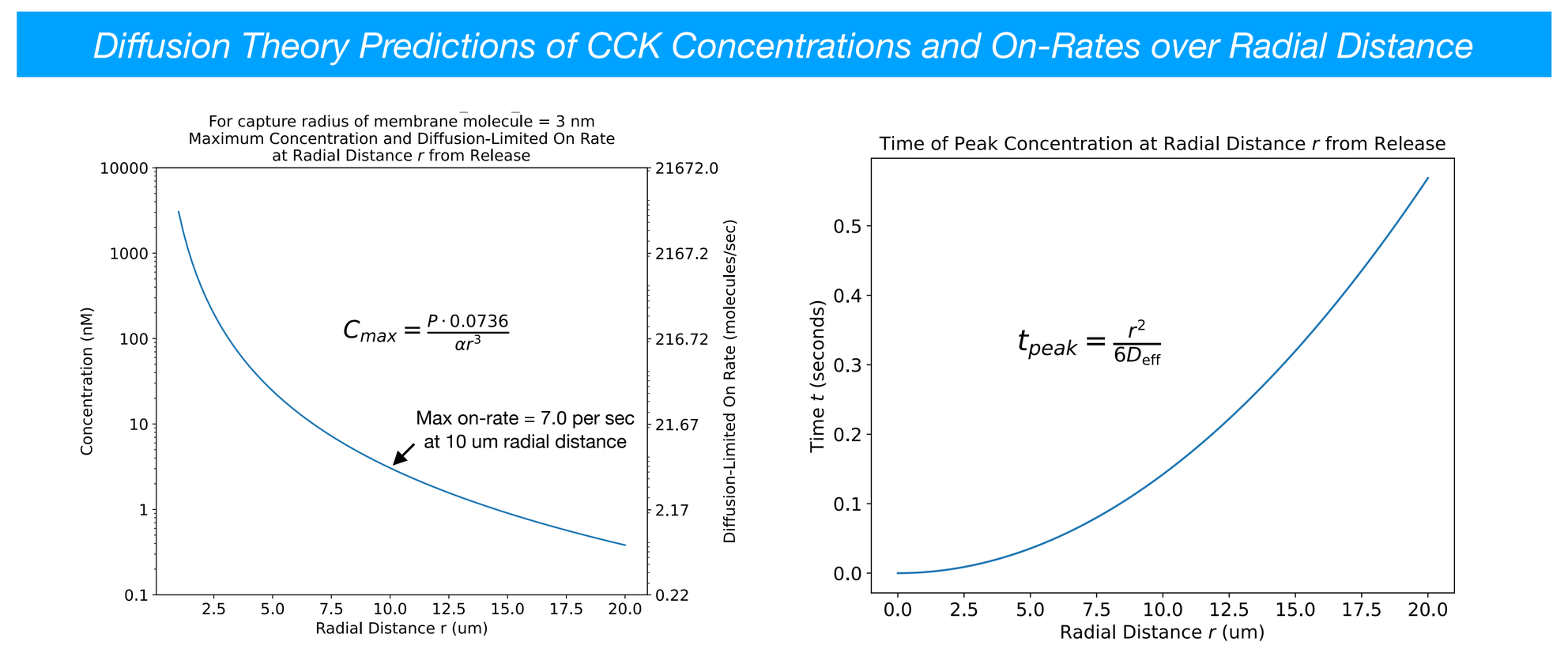
$$C = \frac{P}{\alpha(4D_{\mathrm{eff}}\pi t)^{3/2}}\exp\left(-\frac{r^2}{4D_{\mathrm{eff}}t}\right)$$



3D Radial Diffusion after Instantaneous Point Source Release of 5,000 molecules
D_eff = 1.17e-06 cm^2/sec, Alpha = 0.2, Lambda = 1.6

RMS displacements (um) = 8.39, 14.52, 26.52
Rough RMS-based guesses of concentrations (nM) = 16.86, 3.24, 0.53

Diffusion Theory Predictions of CCK Concentrations and On-Rates over Radial Distance



Peak Concentrations after Instantaneous Point Source Release of 5,000 molecules
Alpha = 0.2

Acknowledgements and Resources

Help, Collaboration and Inspiration
The late Paul G. Allen
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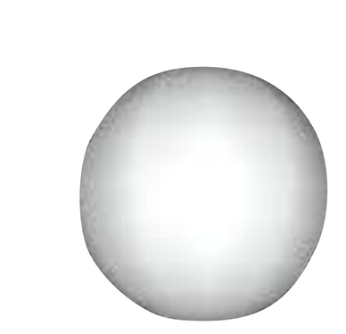
Data Resources
Single-Cell RNA-Seq and Taxonomy:
The Allen Institute for Brain Science
Volume Electron Microscopy:
The MICrONs Consortium
(Chiefs: Tolias, Reid, Seung)

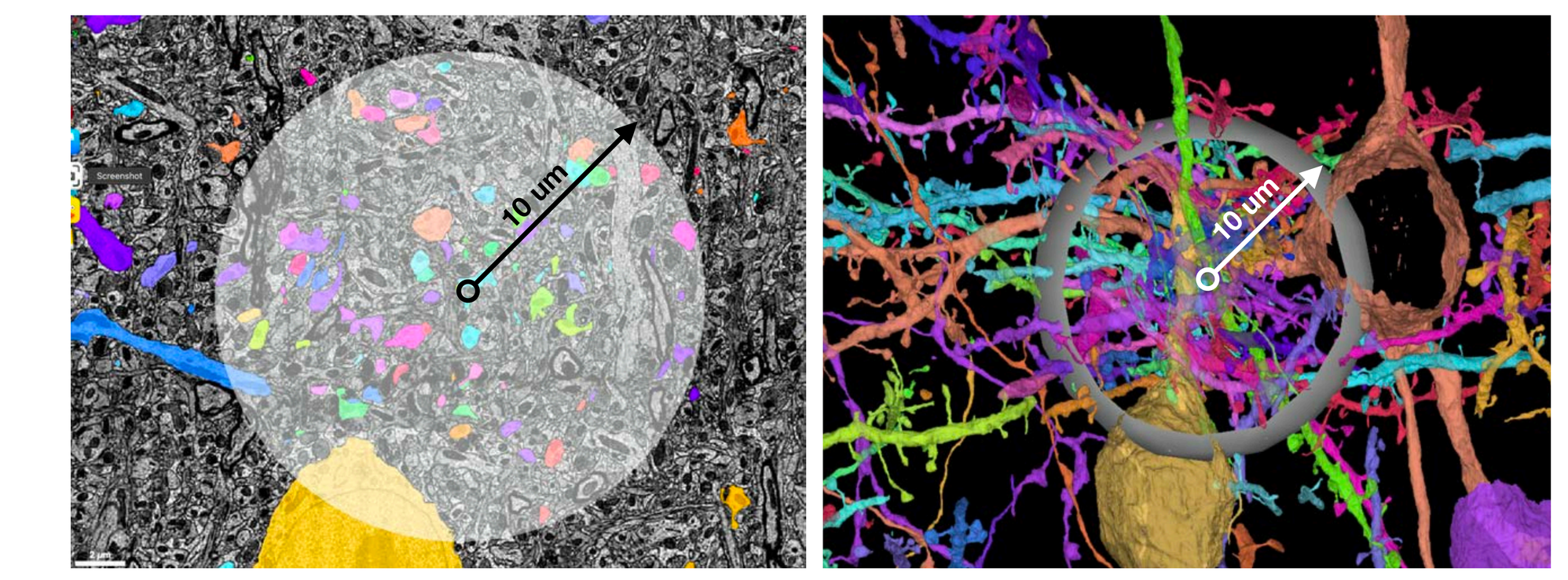


A GitHub Repo
contains this slide deck,
a supporting bibliography,
pointers to all data resource files,
and all data graphics scripts

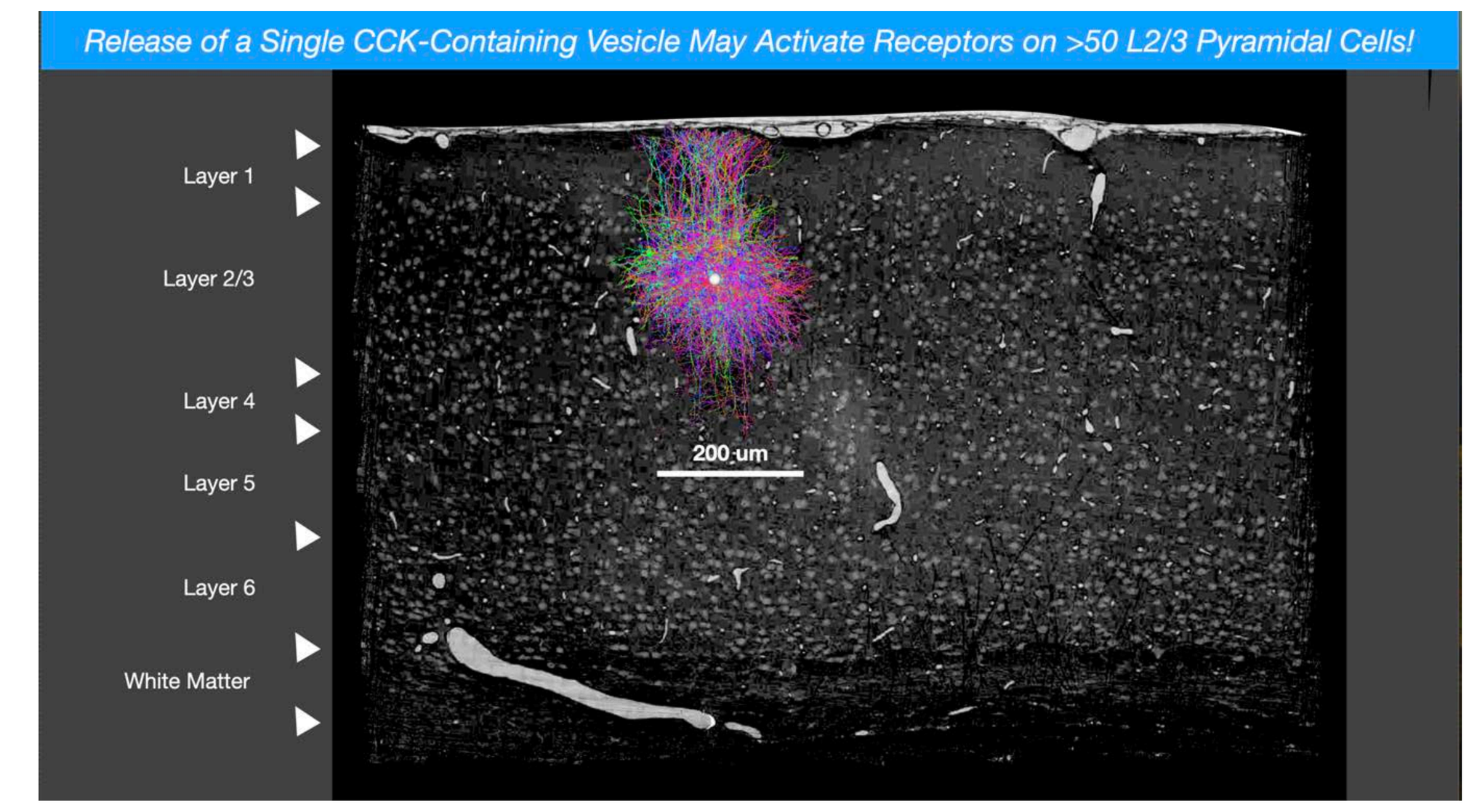
What Can Volume EM Tell Us about Peptidergic Networks?

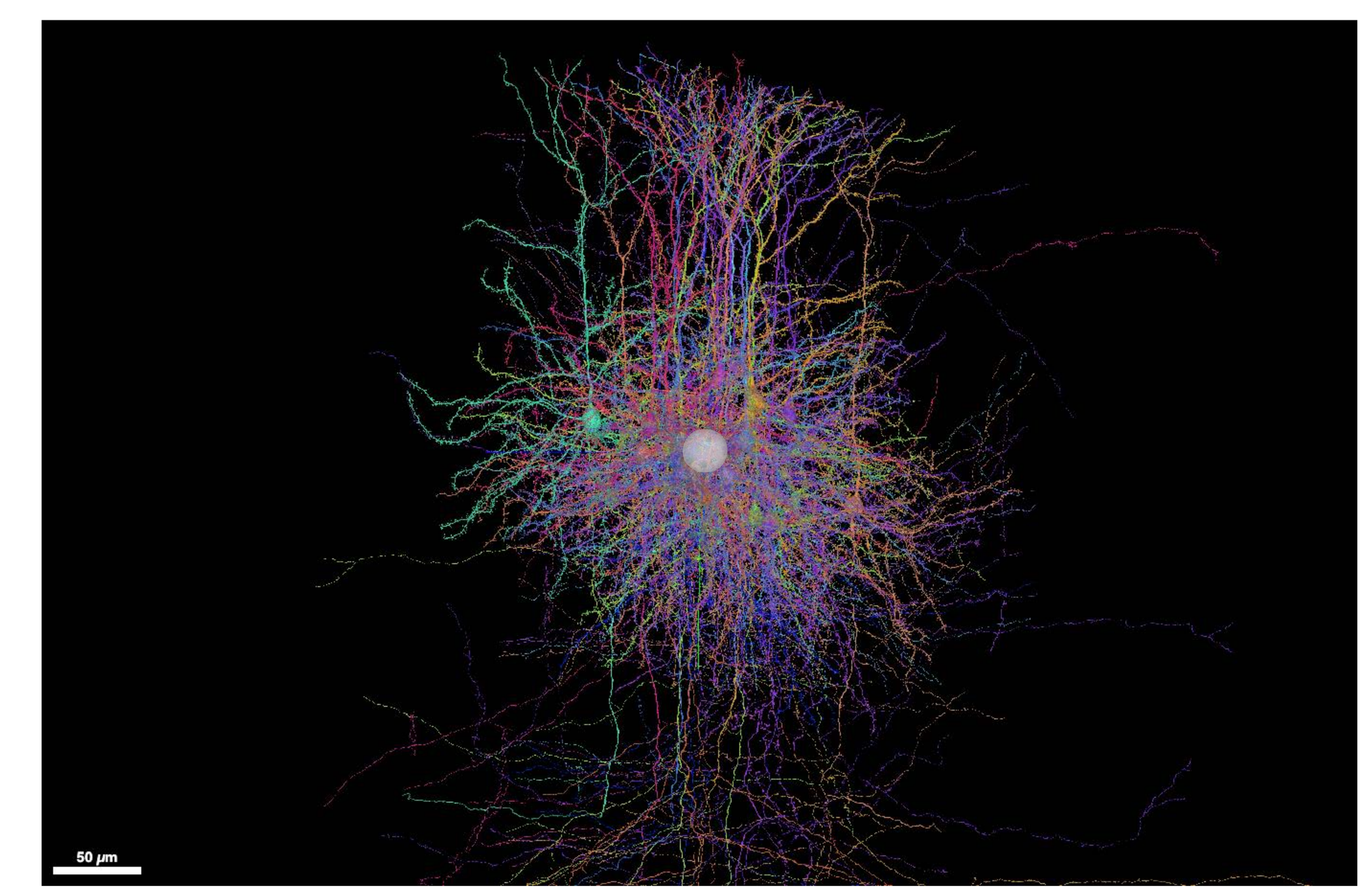
The diffusion theory treatment we have sketched suggests that a distance of 10 um can be taken as a fairly conservative estimate of single vesicle action radius. To explore possible implications of such a distance value, we used *Neuroglancer* (Maitin-Shepard, 2016) to visualize MICrONS project volume EM data (MICrONS Consortium, 2023). Our aim here has been to build intuition about what target cell populations might respond to a single dendritic neuropeptide release event. The renderings below suggest that release of a single CCK vesicle from one layer 2/3 basal dendrite, with an action radius limited to 10 um, might activate a *Cckbr*-encoded receptor on 50 or more other layer 2/3 pyramidal cells. These renderings zoom in on a single MICrONSvolume rendering to illustrate.

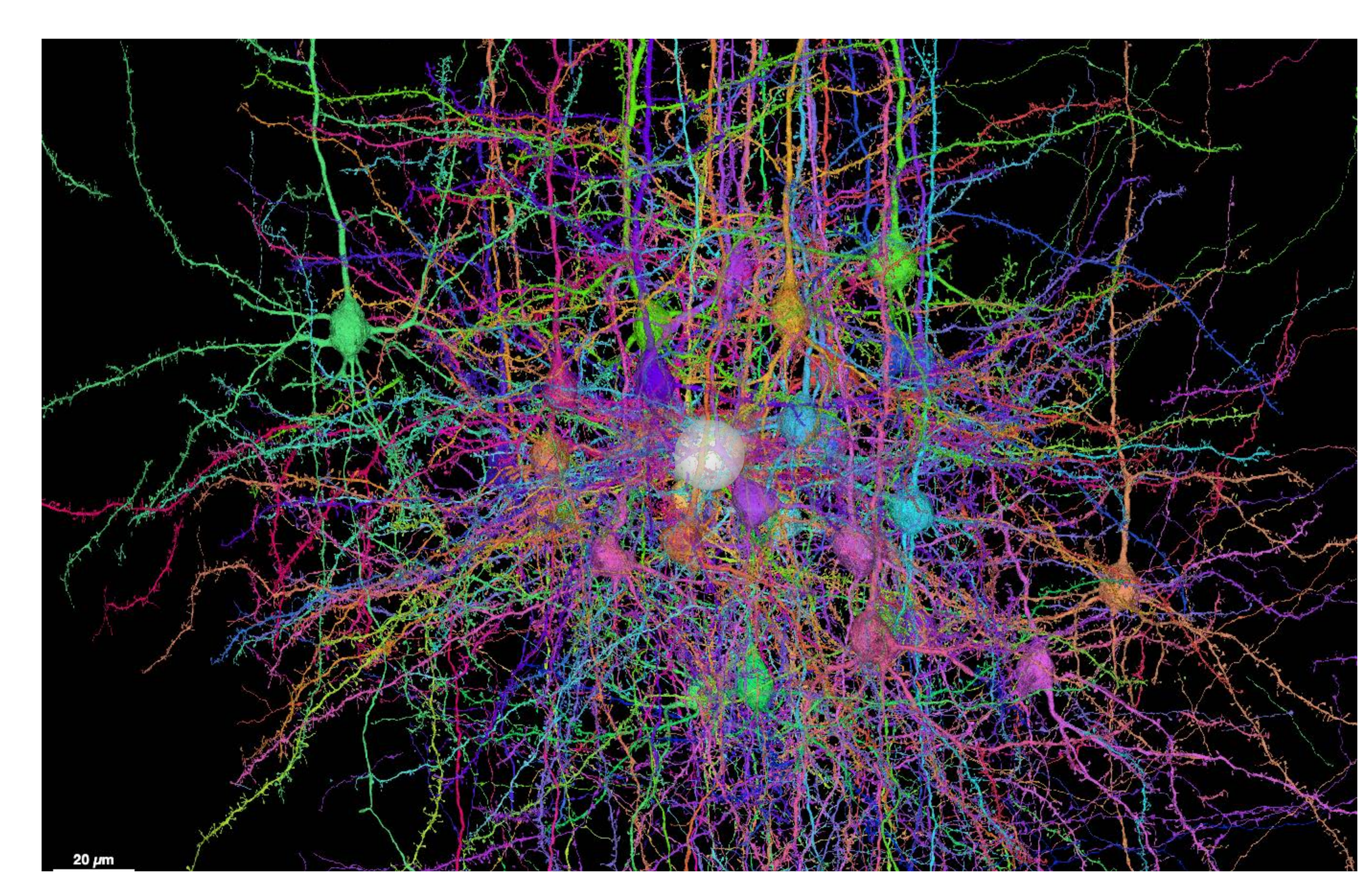
How Many Layer 2/3 Pyramidal Cells Send Dendrites through a 10-um Radius Sphere?

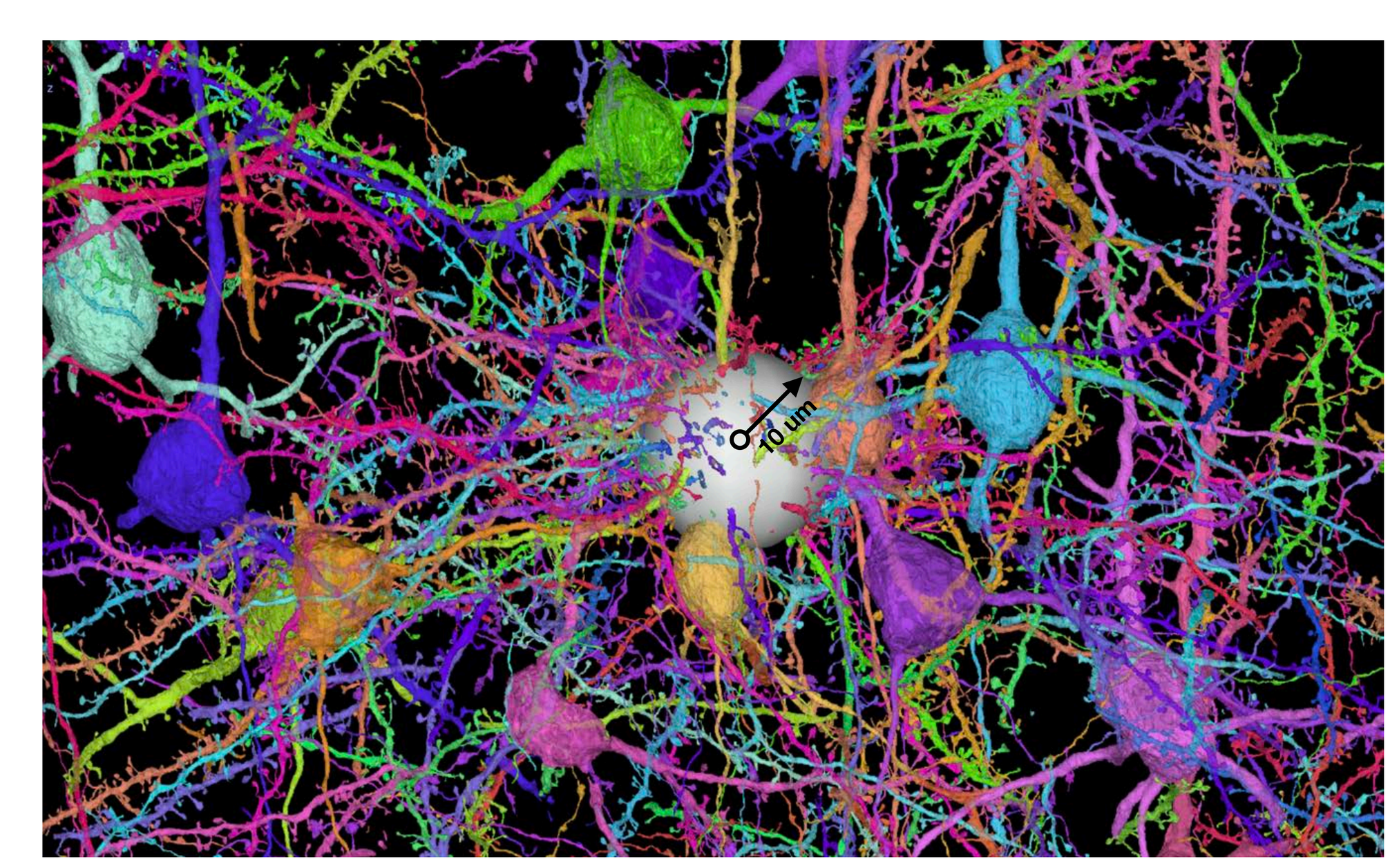


Release of a Single CCK-Containing Vesicle May Activate Receptors on >50 L2/3 Pyramidal Cells!









We wish to thank Allen Institute founder, the late Paul G. Allen, for his vision, encouragement and support.