

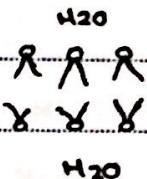
Cellular Mechanism of Brain Lecture 1 Part 1

■ The cell membrane is made of Phospholipids

- Phosphate head (Polar, Hydrophilic) → 
- Hydrocarbon tails (Non-polar, Lipophilic) → 

■ Phospholipid bilayers

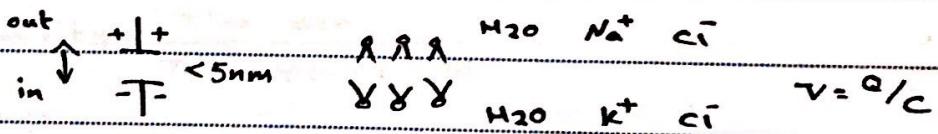
→ Forms Membrane



■ Phospholipid bilayer permeability:

- Highly permeable to Gases and small uncharged molecules
- Limited permeability to water
- Impermeable to ions and charged molecules
- Different concentrations of ions on two sides

■ Cells contain



■ Potential difference across the membrane is membrane potential.

■ Lipid membrane electrical properties

→ Specific capacitance of $\sim 1 \text{ pF/cm}^2$

→ Capacitance of typical cell: $10 \times 10^{-12} \text{ F}$ 

$$\rightarrow Q = CV \rightarrow Q = 10 \text{ pF} \times 0.1 = 1 \text{ pC} = 10 \text{ pF}$$

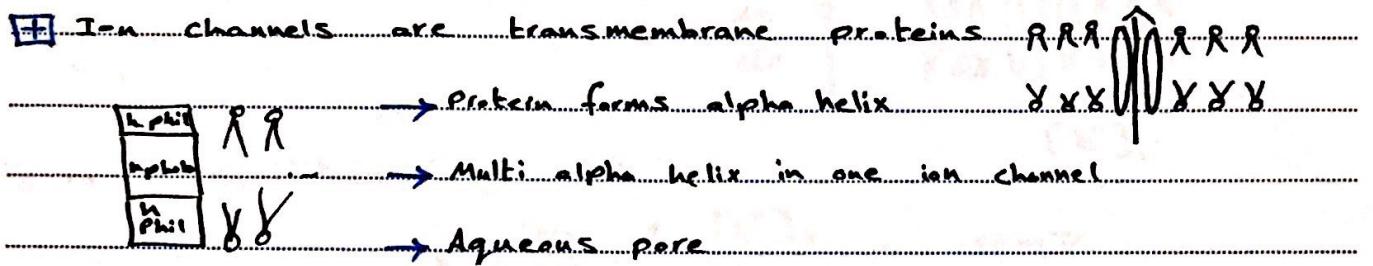
$$\rightarrow \text{ions} = \frac{1 \text{ pC}}{1.6 \times 10^{-19} \text{ C/ion}(K^+)} \approx 6 \times 10^{17} \text{ ions}$$

■ About 10^{17} K^+ ions are available in neurons.

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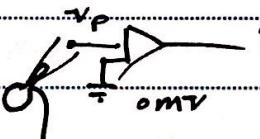
Cellular Mechanism of Brain Function Lecture 1 part 2

- ④ There are more than 1000 times available K^+ ions than ions needed for changing potentials
→ Can change potentials without significant effect on intracellular concentrations



Patch-clamp recordings

→ ion channels are either open



or closed.

$$\rightarrow \text{open probability} = \frac{\text{Time open}}{\text{Time open + closed}}$$

④ Ion channels are often highly selective

④ Single channel conductance is highly dependent on electrical field across membrane

④ Whole ion channel current can be computed as:

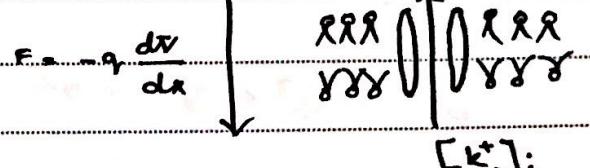
$$I = N \times i \times P \rightarrow \text{open prob}$$

Number of ion channels $\xleftarrow[\text{cell}]{}$ Current of each ion channel

Cellular Mechanism of Brain Lecture 1 part 3

- III Transporters → Much slower than ion channels
- can transport ions against electrochemical gradients
- uses ATP (energy)

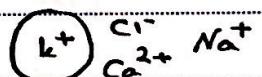
Electrochemical diffusion



Nernst equilibrium for K^+

$$E_{K^+} = \frac{RT}{2F} \ln \frac{[K^+]_o}{[K^+]_i} \approx -90 \text{ mV}$$

Ion concentrations



	intra	extra	F _{ion}
K^+	15 mM	4 mM	-97 mV
Na^+	12 mM	145 mM	+67 mV
Cl^-	5 mM	120 mM	-85 mV
Ca^{2+}	100 nM	1 mM	+123 mV

* Although an ion channel is permeable to selective ions, it has some degree of permeability to other ions.

Goldman-Hodgkin-Katz (GHK) equation

$$V_m = \frac{RT}{F} \ln \frac{P_{K^+}[K^+]_o + P_{Na^+}[Na^+]_o + P_{Cl^-}[Cl^-]_o}{P_{K^+}[K^+]_i + P_{Na^+}[Na^+]_i + P_{Cl^-}[Cl^-]_i} \approx -87.6 \text{ mV}$$

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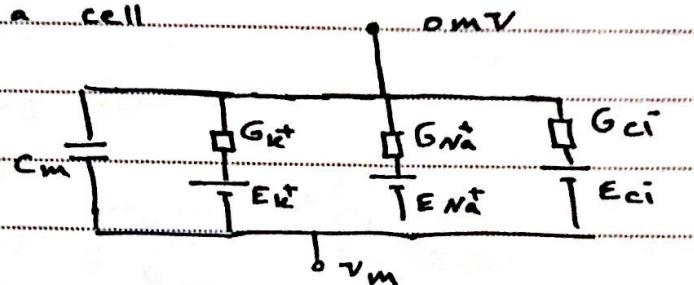
Cellular Mechanism of Brain Lecture 1 Part 4

Electrical equivalent of a cell

$$\rightarrow I_{K^+} = (V_m - E_K) G_{K^+}$$

$$\rightarrow I_{Na^+} = (V_m - E_{Na^+}) G_{Na^+}$$

$$\rightarrow I_{Cl^-} = (V_m - E_{Cl^-}) G_{Cl^-}$$



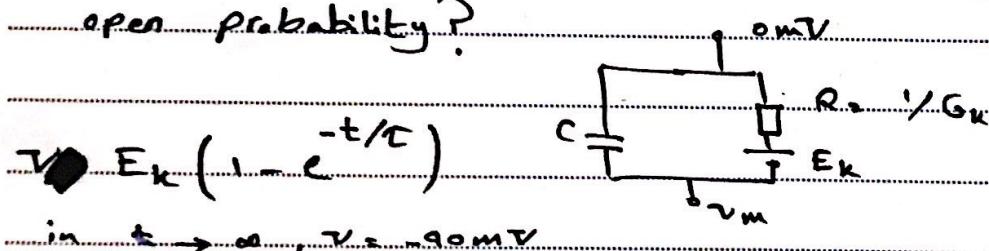
$$I_C = C_m \frac{dV_m}{dt}, \quad I_m = I_C + I_{K^+} + I_{Cl^-} + I_{Na^+}$$

Solving for steady state V_m ($I_m = 0$ and $\frac{dV_m}{dt} = 0$)

$$\rightarrow G_{\text{total}} = G_{K^+} + G_{Na^+} + G_{Cl^-}$$

$$\rightarrow V_m = \frac{G_K}{G_{\text{total}}} E_K + \frac{G_{Na^+}}{G_{\text{total}}} E_{Na^+} + \frac{G_{Cl^-}}{G_{\text{total}}} E_{Cl^-} = \sum_{i \in \text{ion}} \frac{G_i}{G_{\text{total}}} E_i$$

Q What happens to V_m if K^+ channels increase their open probability?

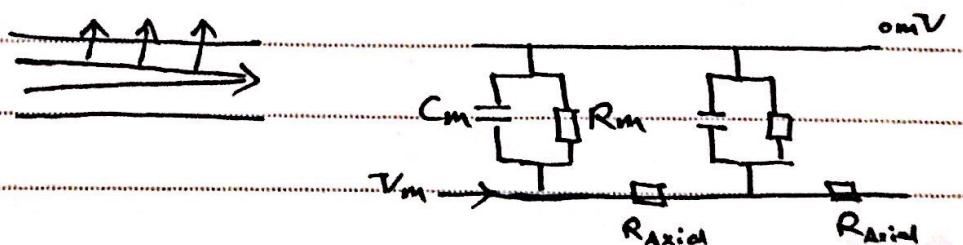


Depolarization ↑ vs. Hyperpolarization ↓

Q Neurons are not isopotential.

→ Neuronal arbors can be considered leaky

electrical cables with capacitance



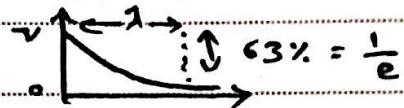
NIHL

Cellular Mechanism of Brain Lecture 1 Part 5

EE) Cable equation at steady state

□ Remove capacitance

$$\rightarrow \lambda (\text{length const.}) = \sqrt{\frac{R_m}{R_{\text{Axial}}}}$$



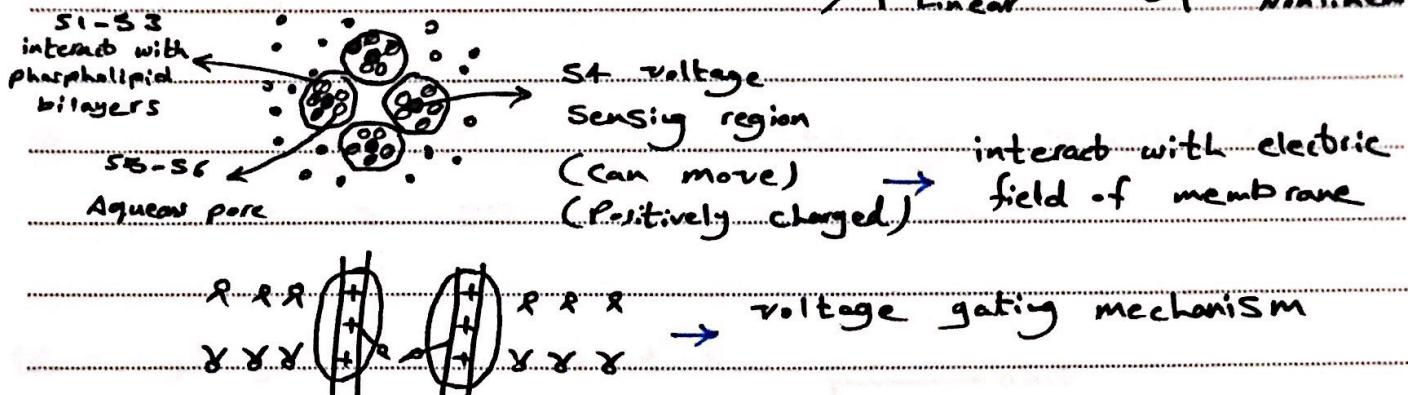
E) Spatio-temporal dynamics of V_m

$$\frac{R_m}{R_{\text{Axial}}} \frac{\partial^2 V(x,t)}{\partial x^2} - R_m C_m \frac{\partial V(x,t)}{\partial t} - V(x,t) = 0$$

⊕ Numerical computer simulations are available for
Neuronal potentials

Cellular Mechanism of Brain Lecture 2 Part 1

■ Voltage gated ion channels



- ④ Open probability is a function of membrane potential.
→ Voltage dependent conductance



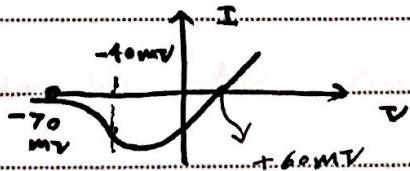
■ Voltage dependent Na^+ and K^+ channels



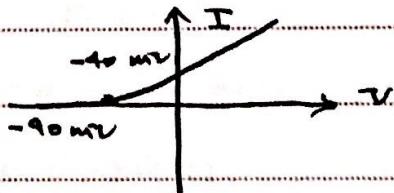
■ Explosive Na^+ conductance

→ Enters positive feedback

if brought to -40mV



■ Stabilizing K^+ conductance

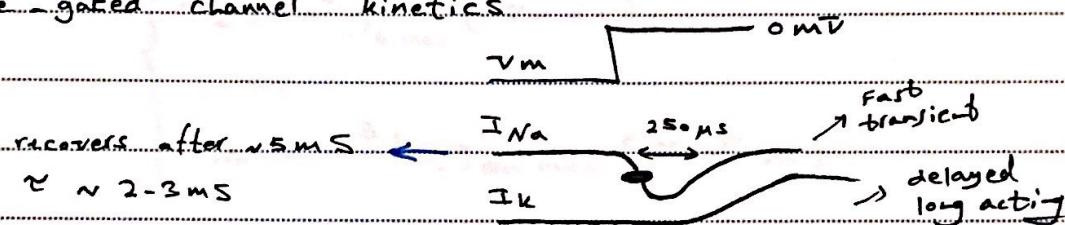


Cellular Mechanism of Brain Lecture 2 Part 2

② Opening and closing of ion channels occur in microsecond time scale.

③ Voltage gating of ion channels take longer, because it's dependent to 3D confirmation of channel.

■ Voltage-gated channel kinetics



■ Na₊ channel diversity

→ encoded by 9 genes $Na_v 1.1 \dots Na_v 1.9$



β subunit 9 proteins and 4 γ beta subunits

→ Provides diversity

④ Also cell type and subcellular localization provide diversity

■ Potassium channel diversity

□ 80 genes → Gives great diversity

□ Ca^{2+} activated K channels (BK and SK)

□ G-protein coupled K channels (GIRK)

□ Tandem pore potassium channels (setting the resting potential)

Cellular Mechanism of Brain Lecture 2 Part 3

④ Action potentials last about 1 ms

■ First record of action potential: Hodgkin and Huxley

→ Intracellular recording from the giant axon of a squid

■ Hodgkin-Huxley model for K^+ conductance

$$\left\{ \begin{array}{l} I_K = n^+ g_{K\max} (V - E_K) \\ I_{Na} = m^3 h g_{Na\max} (V - E_{Na}) \end{array} \right.$$

④ Action potential is initiated at -55mV where inward current of Na^+ triggers a positive feedback loop.

④ There exist diversity in action potential in different cell types. In inhibitory cells, repolarization happens faster because of different potassium channels.

④ Action potential allows reliable information transfer in long distances.

■ Action potential threshold depends on

→ densities

→ activation V_m and dynamics

→ inactivation V_m and dynamics

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Cellular Mechanism of Brain Lecture 2 Part 4

- ④ Action potential is almost always initiated at axon initial segment
 - 5 μm under the cell body
 - High density of voltage-gated Sodium channels
 - Voltage-gated Sodium channels bind to Ankyrin-G protein
 - Lowest activation threshold for AP

~~Action Potential Propagation~~

- Action potential propagation speed depends on (increases)

- Higher membrane resistance

- Lower axial resistance

- Lower membrane capacitance

$$\left\{ \begin{array}{l} \lambda = \sqrt{R_m/R_{axial}} \\ T = R_m C_m \\ \lambda^2 \frac{\partial^2 V}{\partial x^2} - T \frac{\partial V}{\partial x} = 0 \end{array} \right.$$

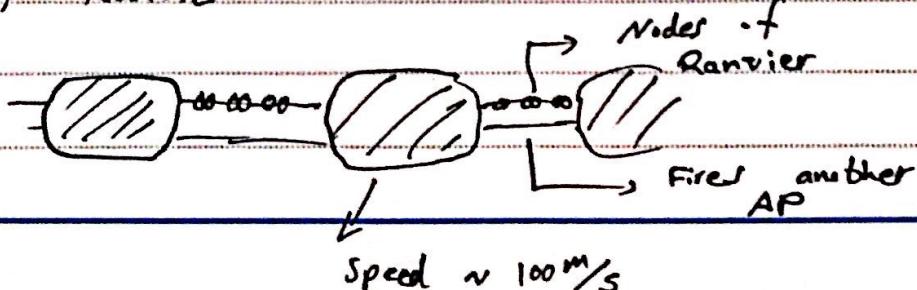
- ④ Typical AP Speed = ~ 1 m/s

- Myelination: Specialized glial cells wrap thin process

around selected axons. They contain 80% lipid and so they're great electrical insulators.

They increase axonal membrane resistance by a factor of ~ 5000 and decrease axonal capacitance by a factor of ~ 50 (similar to axial ~~and~~ coating)

- Nodes of Ranvier



Cellular Mechanism of Brain Lecture 2 Part 5

Whole cell recording steps:

① Prepare the brain slices

② Image the neurons → High contrast infrared video microscopy

③ Prepare recording electrodes

④ Whole cell patch-clamp recording

* The mammalian brain can be cut into thin ($300\text{ }\mu\text{m}$) slices, and the neurons remain viable.

* Break the membrane for whole-cell patch clamp configuration.

* In voltage-clamp, voltage is fixed and current measured.

In current-clamp, current is fixed and voltage is measured

Cellular Mechanism of Brain Lecture 3 Part 1

■ Neurotransmitters diversity

- Glutamate (80% of ^{NTS}) □ γ -aminobutyric acid (GABA)
 - Acetylcholine (heart)
new muscular □ Dopamine (Reward based learning)
 - Met-enkephalin □ Oxytocin
- neuropeptides

④ Presynaptic specializations where neurotransmitters reside are called bouton.

- They have voltage-gated Ca^{2+} channels
 - Causing Ca^{2+} influx
 - Causes release of neurotransmitters into synaptic cleft
 - Activates ligand-gated ion channels
 - P.s. - synaptic Potential

⑤ Post-synaptic potential occurs with latency of 1ms after AP in pre-synaptic neuron.

■ Excitatory synapses: Glutamate activates post-synaptic ionotropic glutamate receptors permeable to Na^+ and K^+ with reversal potential $\sim 0\text{mV}$ causing EPSP

■ GABA activates post-synaptic ionotropic GABA receptors permeable to Cl^- with reversal potential $\sim -70\text{mV}$ causing IPSP

⑥ Distance between pre-synaptic & post-synaptic membrane NILAI is about ~~50~~ 50 nm

Cellular Mechanism of Brain Lecture 3 Part 2

- Synaptic vesicles filled with high concentration of neurotransmitters (40nm in diameter)
 - Causes quantal release of neurotransmitters
 - Made of Lipids

- Exocytosis: Release of contents of synaptic vesicles and their diffusion into synaptic cleft

- Neuron may have multiple synapses with another neuron for more stable communication

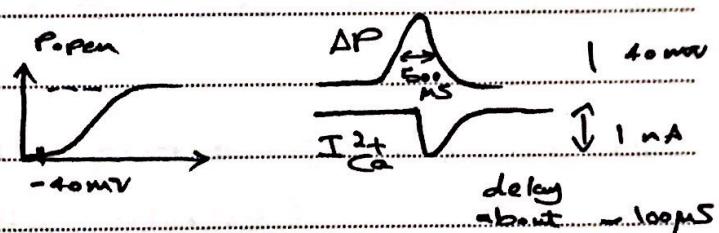
- Variations of neuron to neuron communication
 - ① Electrical Synapses ~~(electrically coupled)~~ (electrically coupled)
 - Gap junctions ~~(Holes)~~
 - ② Volume transmission → Leak into extracellular space
 - Important rules
 - ③ Some types of neurotransmitters only operate with volume transmission (not one to one)
 - ③ Retrograde signaling → PS dendrites release NTs
 - Synapse is bidirectional communication channel
 - Dendrodendritic release is also possible
 - (D1 triggers D2 and D2 triggers D1)

Cellular Mechanism of Brain Lecture 3 Part 3

■ Neurotransmitter release steps

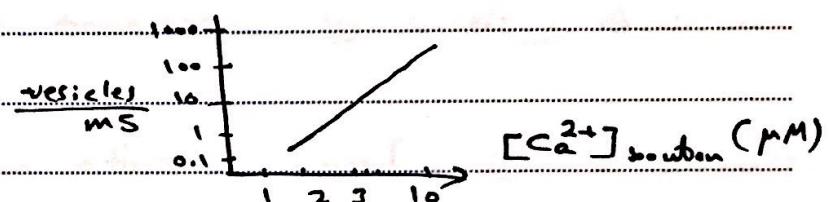
- ① synaptic vesicles need to be ready
- ② vesicle waits for increase of calcium concentration
- ③ increase in calcium drives fusion of vesicle to membrane

④ For Calcium channels:



⑤ Release rate of neurotransmitters $\propto [Ca^{2+}]^4$

$$\rightarrow \text{Slope} \approx 4$$



■ Molecular mechanism of vesicle fusion is caused by

SNARE complex {

- Synaptobrevin →
- SNAP-25 → Pull plasma membrane
- Syntaxin ↑ and vesicle membrane

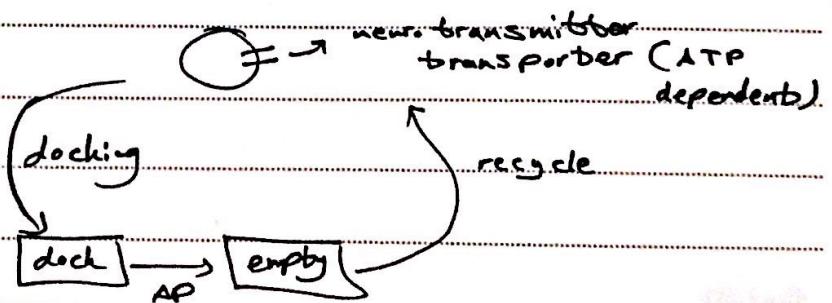
near each other

⑥ Calcium binds to Synaptobrevin protein and when that happens, it interacts with SNARE and vesicle and opens the fusion pore

■ Synaptic vesicle cycle

V-Type ATPase Protein Pump, becomes acidic and positively charged

acid + potential



⇒ gives energy
to ~~AT~~ NT transporters

Cellular Mechanism of Brain Lecture 3 Part A

III Synaptic vesicle recycle scenarios:

- kiss and stay (stays in same spot and refills)
 - kiss and run (~~is~~ goes away, refills and docks again)
 - vesicle fuses completely with membrane
- * Kiss and run and kiss and stay occur in 50 to 100 ms.

* Exocytosis is an stochastic process (docked vesicle has ~ 10% chance of being released)

→ process can be highly regulated

- increase synaptic transmission by increasing calcium influx
- by action potential waveform (by K^+ channels)
- Acting on calcium channels themselves
- Vesicle filling
- Vesicle pH and electric potential
- Vesicular neurotransmitter transporters and pumping
- Size of vesicles and ~~their~~ amount
- Vesicle release
 - Docking and priming
 - Ca^{2+} sensitivity

Cellular Mechanism of Brain Lecture 3 Part 5

Pre-synaptic dynamics

- Short-term (ms) ↑ Facilitation, Depression → depends immediately on the calcium and vesicles
- Post-tetanic potentiation (minutes)
- Long-term presynaptic plasticity (hours) (memories)

Facilitation: Caused by accumulation of Calcium in pre-synaptic terminal

* Calcium pumps suck out the calcium and reduce its concentrations

* Other proteins binding Calcium act as Calcium buffers

* $(1.2)^4 \approx + 20\%$ increase in Calcium concentration doubles the neurotransmitter release

Depression: After an AP, vesicles are still not ready for another release

Post-tetanic potentiation: only happens in certain synapses

- AP after high freq. tetanic stimulation causes more NT release and greater PTP
- High-freq stimulation causes increase in Calcium which activates enzyme called protein kinase C (PKC)
→ PKC acts on neurotransmitter release machinery
→ release rate at any given Calcium concentration increases
- ② Enhances NT release for about a minute (short term memory)

Cellular Mechanism of Brain Function Lecture 3 Part 6

■ Presynaptic long-term potentiation

□ Occurs in same ~~two~~ types of synapses

□ After high Freq. stimulation:

□ enzyme called Adenylate cyclase activated

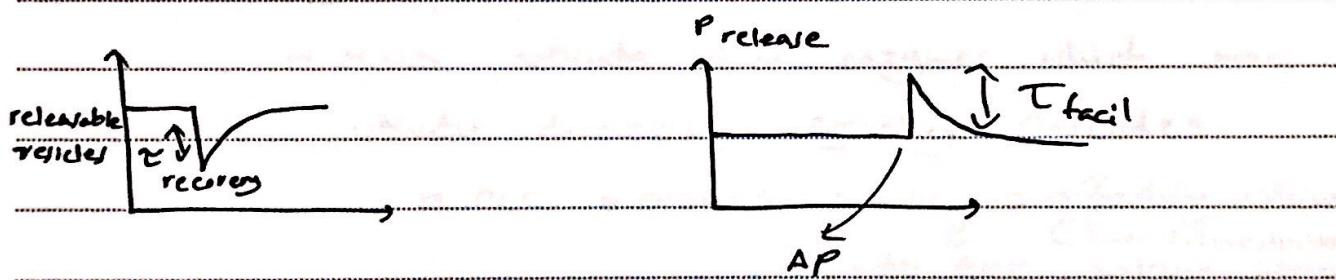
→ enzyme turns ATP to cAMP

→ cAMP activates protein kinase A (PKA)

→ PKA acts on Syt B (Synaptobrevin B)

Causes long lasting effect on NT release

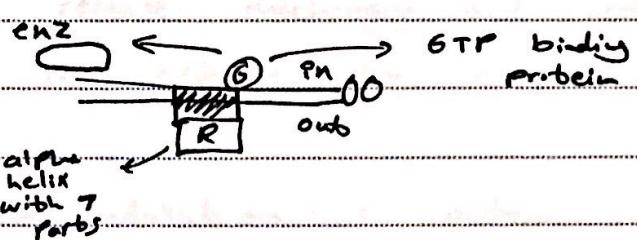
■ Modeling presynaptic Dynamics



Cellular Mechanism of Brain Lecture I Part 47

■ Pre-synaptic neuromodulation: Neurotransmitters can bind to receptors on boutons, ~~affecting~~ the release of neurotransmitters.

□ Pre-synaptic metabotropic receptors



□ GTP after activation splits to two parts,

alpha and beta/gamma

→ ~~will~~ diffuse inside the cell act on ion channels

□ Might activate other enzymes which may activate downscaling signaling cascades

□ PLC — enzyme, splits → affecting calcium
 ↓
 (from intracellular activator PKC Calcium stores)

□ AC — enzyme → increase cAMP

④ Process which triggered by pre-synaptic metabotropic receptors may take > 100ms to occur and may last for some seconds to hours.

④ G protein might inactivate K^+ channels, changing the AP waveform and enhancing NT release

④ G protein might affect Calcium channels or release NIAI machinery, or recycling and docking or etc

Cellular Mechanism of Brain Lecture 3 part 8

■ One major form of presynaptic modulation is called presynaptic inhibition.

□ It affects Ca^{2+} channels and inhibits calcium influx.

□ ~~It~~ acts on release machinery and reduces release rates in response to calcium.

■ Post-synaptic neuromodulation: G protein in post-synaptic dendrites ~~can~~ may affect GIRK (K^+) channels or K_{ir} channels, causing slow post-synaptic potential.

④ Presynaptic inhibition typically reduces depression and thus increases facilitation.

Cellular Mechanism of Brain Lecture 3 Part 9

■ Considerations for EM of biological structures

- Samples contain atoms of low molecular weight
- Most biological samples contain water
- high vacuum
- intense heat of the beam
- size of specimen

④ Heavy atoms interact with beams

■ Preparing samples

- ① □ Fixation chemical fixation or cryofixation
- ② □ Embedding resins
- ③ □ Sectioning section thinly (40 to 200 nm)
- ④ □ EM imaging image in ambient temperature (TEM)

- ⑤ Chemical fixation
 - Coagulative fixation (making proteins immobile)
 - Aldehydes (non coagulative) (Polymerize proteins)

Heavy metals are

- used for stain (Heavy metal for EM) → stains membrane and proteins

- ⑥ By backscattered electrons, reflected from samples, we are able to see EM (and see through sample structure)
- ⑦ electron beam can be used together with ion beam
Can polish away few nm thick layers of sample

Cellular Mechanism of Brain Lecture + Part 2

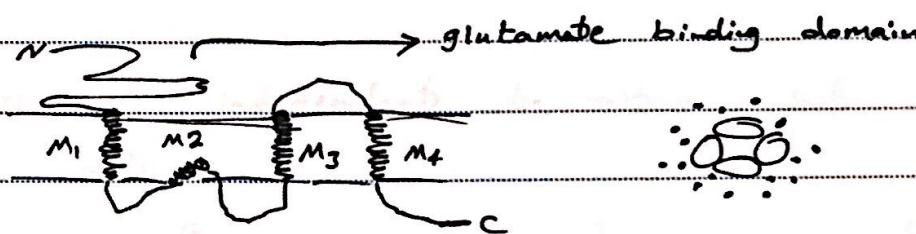
I. Iontropic glutamate receptors

□ 100 mM glutamate in each vesicle

□ Fusion causes 1mM for 1ms of glutamate in synaptic cleft

□ AMPA receptor and NMDA receptors

□ Iontropic glutamate receptors structure



⊕ Permeable to Na^+ , K^+ and in some cases, Ca^{2+}

II. Subtypes

□ AMPA receptors

□ NMDA receptors

□ Na^+ and K^+

□ Na^+ , K^+ and Ca^{2+}

□ reversal potential $\approx \text{amV}$

□ reversal potential $\sim \text{amV}$

□ Single channel $G \approx 5 \mu\text{S}$

□ Single channel $G \approx 50 \mu\text{S}$

□ 4 genes (GRIA1-4)

□ 7 genes

GluR1-4

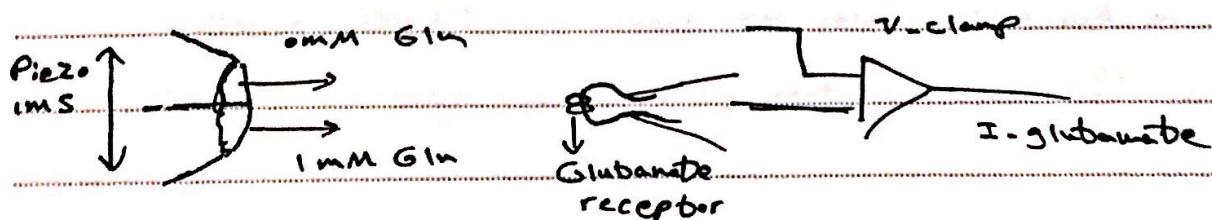
or

GluR A-D

⊕ Glycine or D-Serine

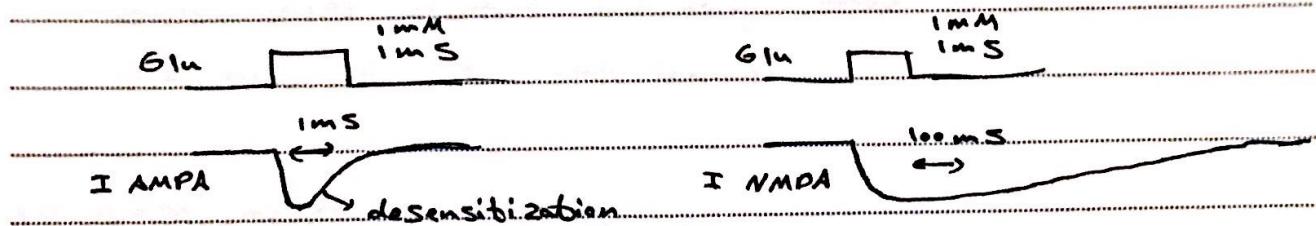
are needed to be
presented (almost always
is)

III. Measuring kinetics of Ligand-gated ion channels

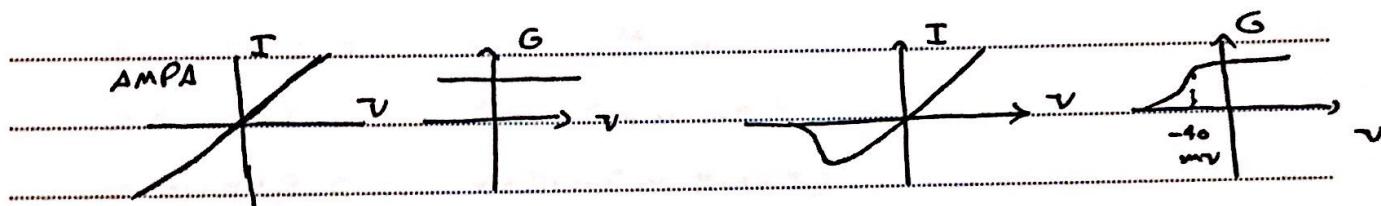


Cellular Mechanism of Brain Lecture 4 Part 2

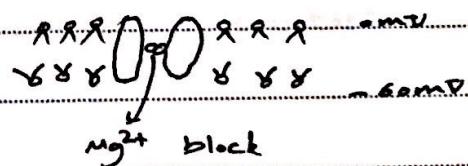
AMPA vs NMDA (Piczo experiment)



* AMPA receptors are independent to voltage, but NMDAs are not.



* NMDA receptors are voltage dependent because of Magnesium block (Mg^{2+}) which is always present in extracellular space. So they are less effective on resting potential.



AMPA receptors diversity

- GluA 1-4 genes
- Most AMPA receptors have GluA 2 gene
- Having GluA 2 → Linear IV relationship and no Ca^{2+} permeable
- Lacking GluA 2 → Inwardly rectifying and Ca^{2+} permeable

Cellular Mechanism of Brain Lecture + Part 3

III Kainate receptors (grkl-5 genes)

- Like AMPA receptors but having ^{lesser} currents and slower
- Function is not well known.

IV NMDA receptors diversity

□ GluN1, GluN2A-D and GluN3A-B genes

↳ required (at least one)

□ GluN2A, B → strong Mg^{2+} block

□ GluN2C, D → weak Mg^{2+} block

□ GluN2A → fast ($\sim 10ms$)

□ GluN2B, C → medium ($\sim 20ms$)

□ GluN2D → slow ($\sim 1s$)

V Metabotropic glutamate receptors

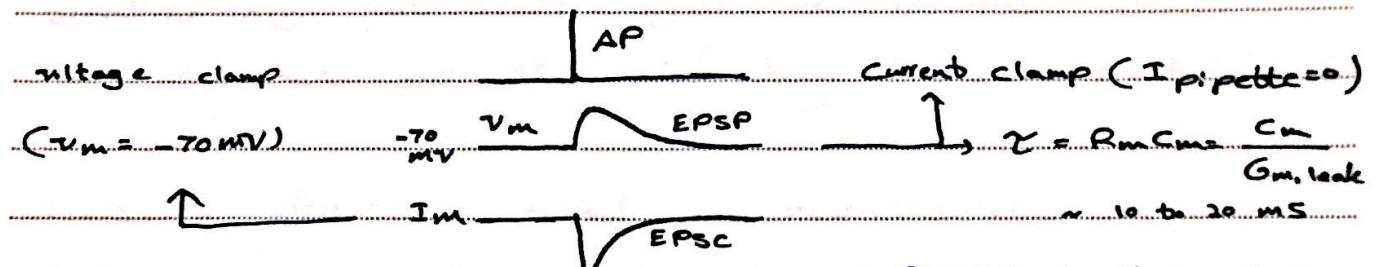
- 7 Transmembrane, G-protein coupled receptors

□ grm1-m8 genes

④ All mammalian glutamate receptors are chloride channels.

Cellular Mechanism of Brain Lecture 4 Part 4

EPSPs VS EPSCs



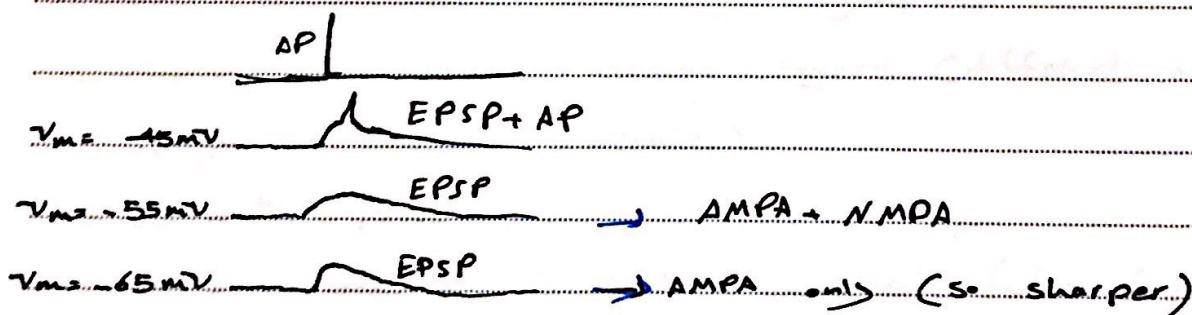
* EPSP is slower because membrane potential needs to be discharged

Calyx of Held synapse



> 200 synaptic connections

Part 2: Synaptic voltage dependence of EPSPs



* Situation is more complex because synapses are distributed on dendrites (far from soma) and filtering by cable properties happen.

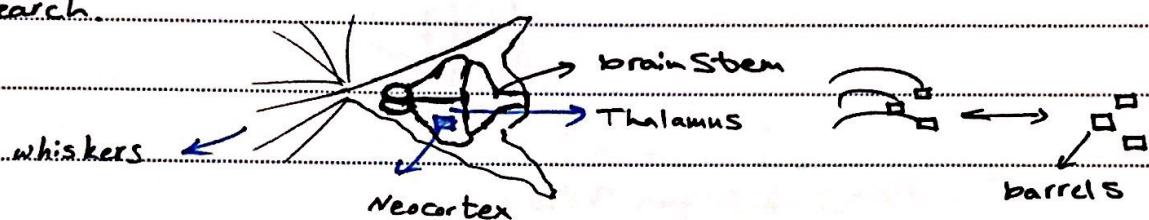
* Temporal and Spatial summations of PSPs

cause [REDACTED] IP (can happen at same time in soma or can happen locally, causing activation of NMDA receptors)

Cellular Mechanism of Brain Lecture 4 Part 5

■ Somatosensory part of thalamus → Feeling of touch
Somatosensory part sends signal to neocortex (where conscious perception of being touched resides)

④ Mouse is attractive animal in neurophysiology because of its extensive genetics and extensive use in biological research.



④ Neocortex can be divided into layers

□ L1 (superficial) with only synapses

□ Morphologies change across layers (different subtypes though all are glutameric)

□ Laminate positions

□ Thalamic innervation of L1 is a prominent feature of primary sensory cortices

④ We use optical imaging to see which barrels of mouse brain are activated (fNIRS)

④ We can use multiple pipettes and current injection to observe the connectivity in a microcircuit of synapses
→ we can then extract connection probability matrix between layers

L2 L3 L4 → Presynaptic

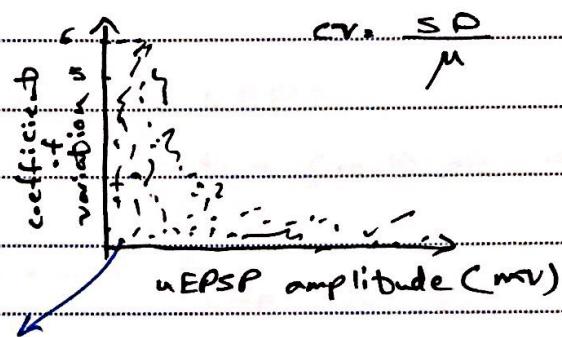
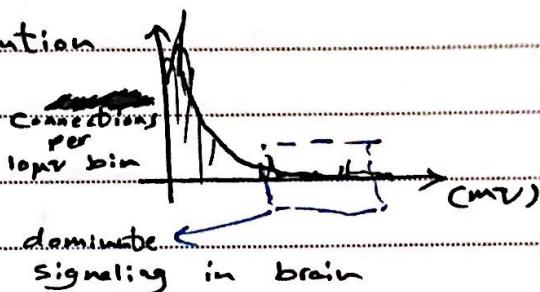
④ Deeper layers are integrators

Post Synaptic	L2	L3	L4	Presynaptic
	1	2	-	
	-	2	1	
	2	1	..	

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Cellular Mechanism of Brain Lecture + Part 6

■ uEPSP amplitude distribution



smaller connections

have much more

variety in responses

* Glutamate synapses are involved in signaling all sensory information from periphery to cortex.

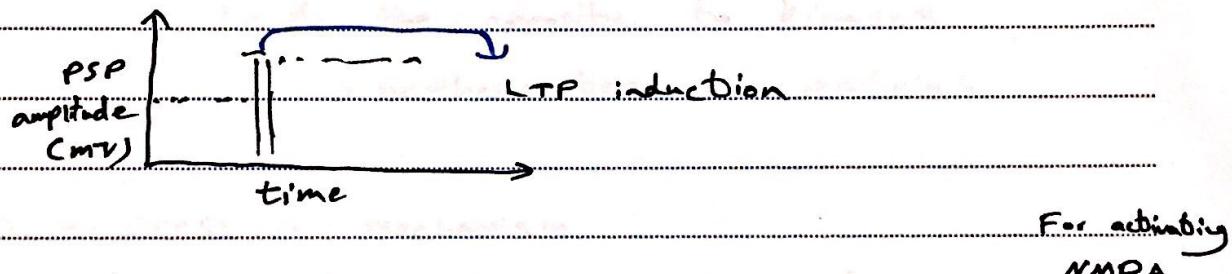
* Glutamatergic synapses in local neocortical microcircuits connect specific cell types with diverse synaptic properties.

Cellular Mechanism of Brain Lecture + Part 7

- Post-synaptic plasticity of glutamatergic synapses
 - by change in receptors composition
 - pre-synaptic plasticity is dominant in ms and s time scale
 - Long-term plasticity that forms memories happens by post-synaptic plasticity

■ Long-term Potentiation (LTP)

- 100 Hz for 1s stimulation (multiple times)



■ LTP induction methods

- ① High Freq (100 Hz) stimulation of many axons
- ② Injecting depol. current through pipette during synaptic stimulation
- ③ Pairing postsynaptic AP firing with EPSP input

For activating NMDA

all can activate NMDAs

■ LTP induction requires

- ① NMDA activation
- ② Post-synaptic cytosolic calcium increase
- ③ Activation of protein kinases (CaMKII)

↳ Adds phosphate group to AMPA receptor,

changing its conductance or may add

additional AMPA receptors (binding them at post-synaptic areas)

Cellular Mechanism of Brain Lecture 4 Part 8

+ Long-term depression (LTD) induction

① Repetitive 1Hz stimulation of many axons

② Injecting weak depolarizing current with pipette during synaptic stimulation

+ LTD induction requires

① NMDA receptor activation

② Activation of protein phosphatases (Calcineurin)

↳ do the opposite to kinases

→ unphosphates the proteins

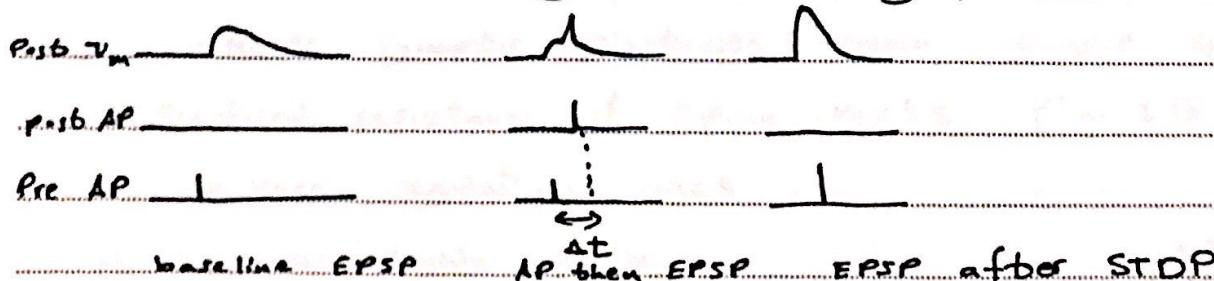
+ LTD expression mechanism

① Calcineurin-mediated dephosphorylation of AMPA receptors

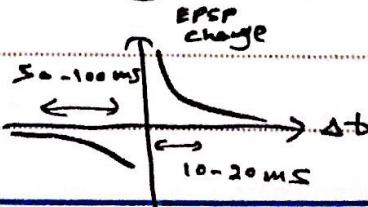
② AMPA receptors are removed from synapses

+ Spike timing-dependent plasticity (STDP)

□ Simultaneous presynaptic & postsynaptic AP



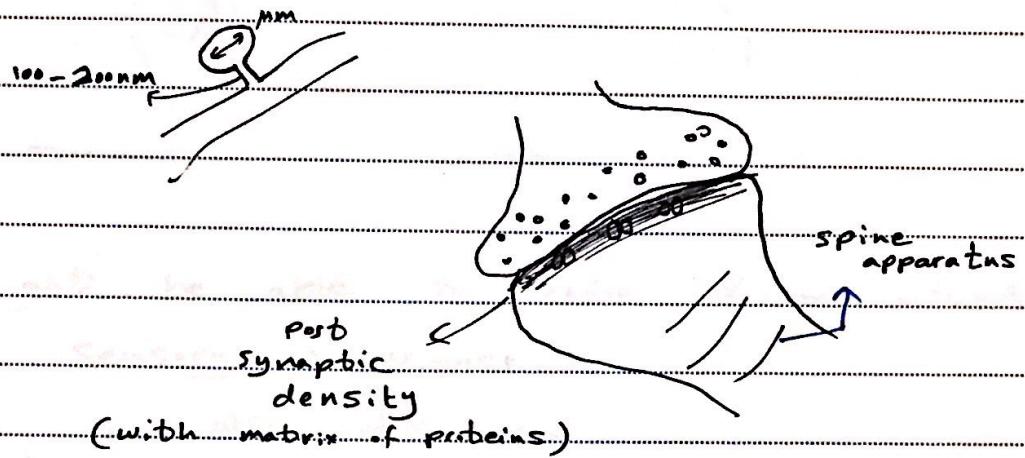
④ If $\Delta t < 0$, plasticity changes sign (smaller EPSP after STDP)



NIHL

Cellular Mechanism of Brain Lecture + Part 9

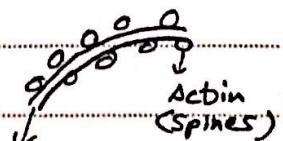
- Dendrites are often decorated with spines.
- Spines play important role in making synaptic connections.



- * Spine apparatus is a part of endoplasmic reticulum inside spines (Local protein synthesis).
They're also intracellular Ca^{2+} stores, for regulating synaptic plasticity.

+ Spine rules

- Bottleneck for diffusion (Localized signaling)
→ Helps synaptic plasticity remain synapse specific.
- Electrical resistance of spine necks ($\approx 2 \text{ G}\Omega$)
→ Have regulation rules.
- They are highly motile.
 - They move because of Actin.



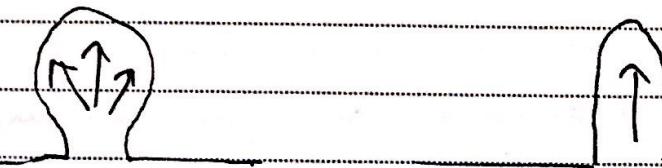
- * Actin is a protein that polymerize

and depolymerize and gives ~~motility~~ ^{cells} ability to move

MAP 2
microtubules

Cellular Mechanism of Brain Lecture 4 Part 10

- ④ New spines may grow where there weren't any



- One might be able to rewire Neural networks through sensory experience.

- Spines can also disappear

- ④ Spines have volume < 1 fL

Cellular Mechanism of Brain Lecture 5 Part 1

④ Inhibition is needed to balance the amount of excitation.

GABAergic synapses

- GABA is synthesized from Glutamate (with an enzyme) named GAD

- Vesicular GABA transporters (VGAT)

- Ionotropic GABA receptors conduct post-synaptic current

GABA_A Receptor

- Permeable to ions (Cl^-)

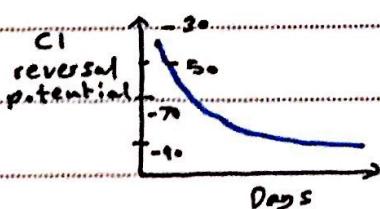
- $E_{\text{Cl}} = -85 \text{ mV}$ $E_{\text{Cl}} = \frac{RT}{2F} \ln \frac{[\text{Cl}^-]_i}{[\text{Cl}^-]_o}$

- Leaky chloride channels bring the Cl^- out at resting potential

- Chloride transporters (notably KCC2) take chloride to out of cell (takes both Cl^- and K^+ outside)

⑤ During early development, intracellular and extracellular density of Cl^- are different and GABA is not inhibitory

□ In rodents:



early development

Adult brain

- Low KCC2 expression

- High KCC2 expression

- High cytosolic Chloride

- Low cytosolic chloride

⑥ Chloride concentrations might differ in different neuronal compartments.

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Cellular Mechanism of Brain Lecture 5 Part 2

Glutamatergic VS GABAergic Synapses

Glutamate

GABA

TGluT

TGAT

AMPA / NMDA (PSP 95)

GABA_A (Gephyrin)

Spine

Shaft or Soma

(may be also on spines
act as regulators)

■ GABA_B (Metabotropic receptors)

□ 7 Transmembrane receptors

□ Activate G proteins → { Activate K^+ channels (slow - 200ms scale)
inhibits C^{2+} channels

□ GABA_D proteins in presynaptic specializations

→ inhibits neurotransmitter release

■ Spinal Cord

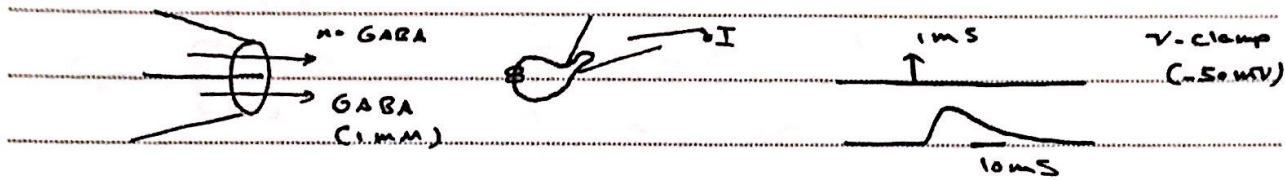
□ GABA is less prominent

□ Glycine acts as a major neurotransmitter

□ Very similar to GABA

Cellular Mechanism of Brain Lecture 5 Part 3

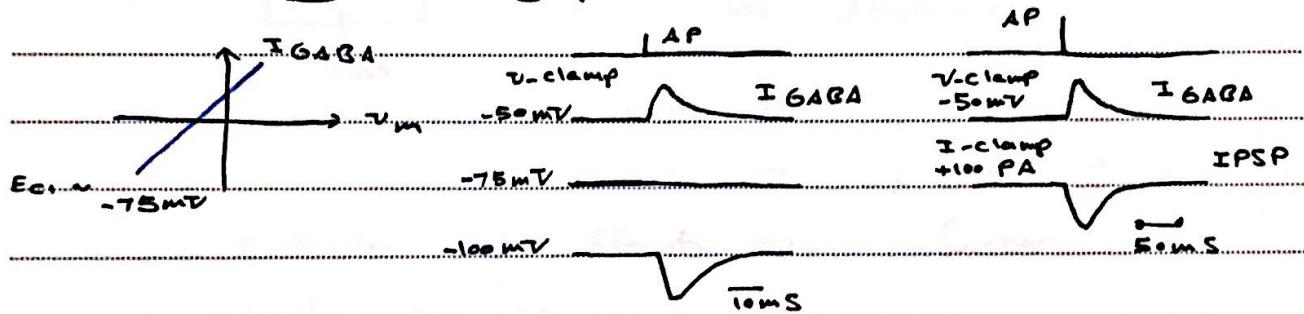
■ Piezo experiment for GABA



→ Fast conductance, but still slower than AMPA

(5 to 10 times slower)

■ Inhibitory post-synaptic potentials

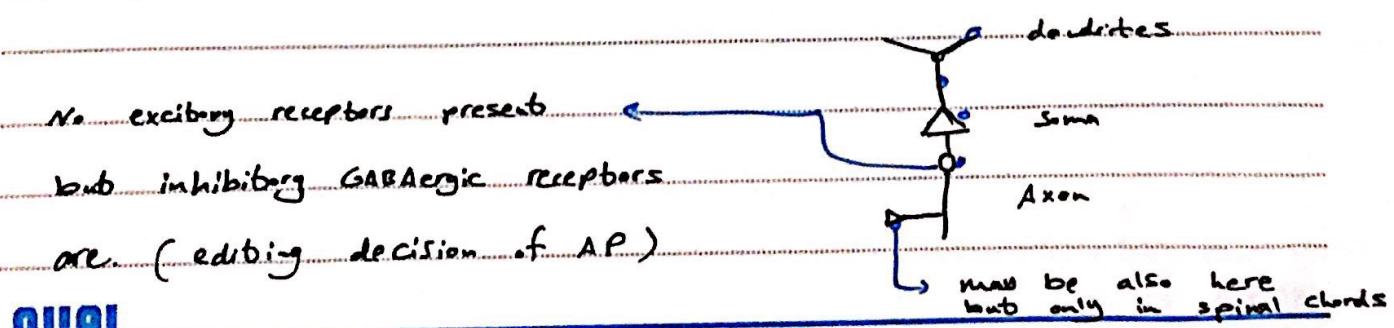


■ Shunting inhibition

The resting V_m is often close to the chloride reversal potential. If $V_m = E_Cl$, then there is no flow of current and therefore no IPSP, but there is nonetheless a conductance, which changes the input resistance. (decrease)

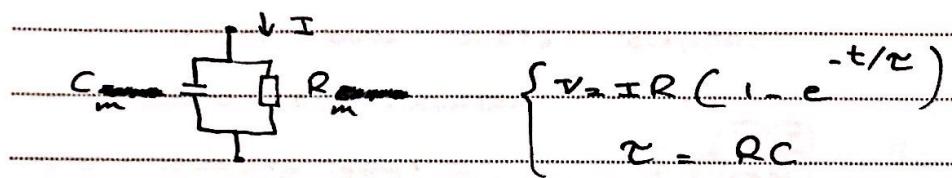


■ Target structures of GABAergic inhibition

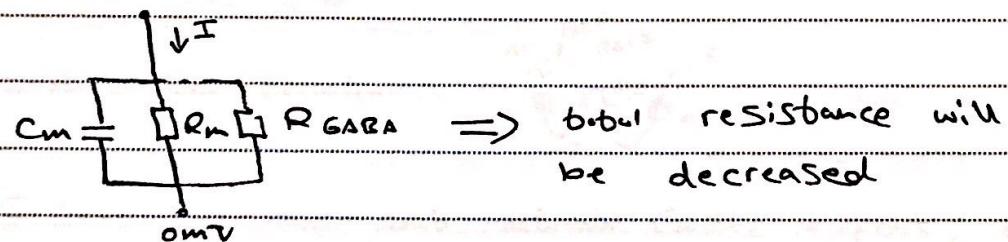


Cellular Mechanism of Brain Lecture 5 Part 4

Shunting Inhibition Circuit modeling:



Adding GABA conductance:



\Rightarrow without GABA cond

\Rightarrow with GABA cond

\rightarrow EPSPs will affect V_m faster
but smaller

Cellular Mechanism of Brain Lecture 5 Part 5

+ Benzodiazepines

□ Act upon GABA_A receptors

□ GABA_A receptors

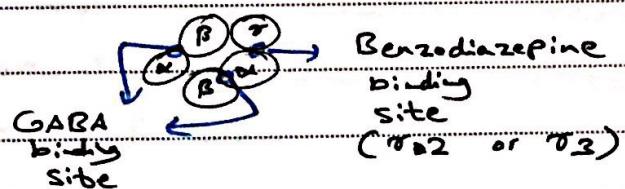
□ GABA_A genes

□ $\alpha 1-6$

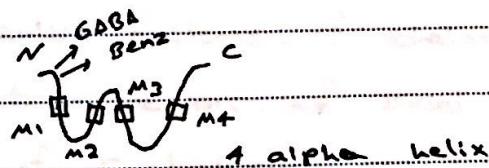
□ $\beta 1-4$

□ $\gamma 1-3$

□ $\delta, \pi, \epsilon, \theta$



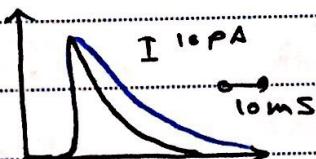
□ Amino Acid Structure



⊕ Benzodiazepines don't activate GABA_A receptors, but

increase the affinity of GABA for binding to GABA_A receptor. They also prolong the duration of

IPSCs.



⊕ Benzodiazepines has no effect on $\alpha 4/\delta 6$

+ Effects of Benzodiazepines

① Sedation

② Anxiolysis

Cellular Mechanism of Brain Lecture 5 Part 6

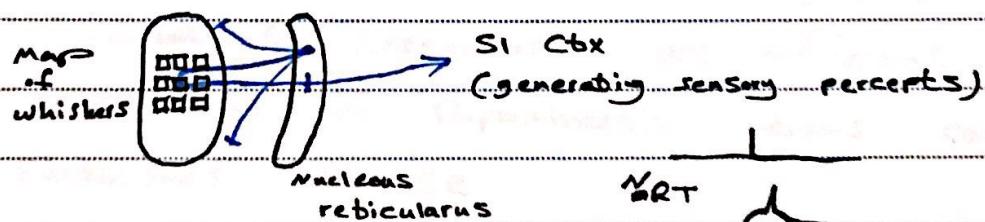
II GABAergic projections

- Neocortex only have local GABAergic neurons
- Some parts have long-range GABAergic projections to other parts of brain

□ Three examples

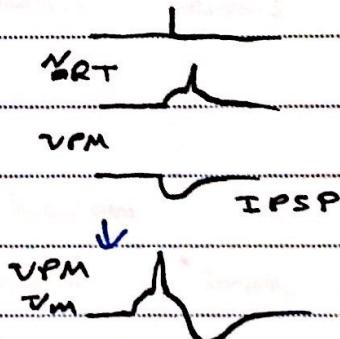
- ① Neurons in reticular nucleus of thalamus
- ② Striatal projection neurons of basal ganglia
- ③ Purkinje cells of cerebellum

□ Ventral posterior nucleus



④ Also sends inhibitory

signals to other parts.



□ Striatum and substantia nigra

- Gets input from ① Cortex (SI) and ② Higher thalamic areas

→ inhibits SNr (which have ~~GABAergic~~ GABAergic neurons to ~~inhibit~~ inhibit brainstem motor area and prevent movement)

→ [Disinhibition]

- ④ Striatum also gets input from Dopaminergic projections from basal ganglia

→ Basal ganglia controls goal-directed behaviour

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Cellular Mechanism of Brain Lecture 5 Part 7

■ Basal ganglia, dopamine and Parkinson's disease

- Dopamine activates D₁R and D₂R receptors with volume transmission (in striatum)

□ D₁R → Direct path to Substantia nigra

→ inhibits SNr → Go pathway

□ D₂R → indirect path to inhibit globus pallidus

→ inhibits SNr → No Go pathway

⊕ Plasticity that happens in synapses with D₁ and D₂ receptors are different

- Degeneration of Dopaminergic neurons causes Parkinson's disease

■ Purkinje cells of the cerebellum

□ Cerebellum (in Latin) means "small brain"

□ Purkinje cells are in cerebellar cortex, which are GABAergic neurons inhibit the deep cerebellar nuclei → Helping fine sensorimotor skills

■ Feedback inhibition uses

- ① Prevents run-away excitation
- ② Help select the most strong excitatory inputs
- ③ Help sharpen spatiotemporal receptive fields

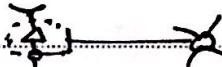
■ Dopamine is released as a reward signal and when animal is expecting reward, it's released in striatum and [redacted] acts as reinforce signal useful for learning and synaptic plasticity (encourage to do what it's doing)

Cellular Mechanism of Brain Lecture 5 Part B

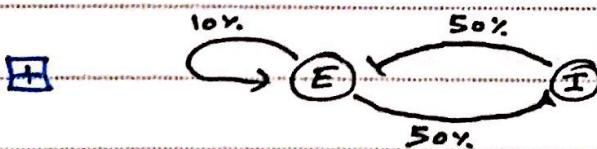
+ Inhibitory GABAergic neurons of the neocortex

- Don't have long range projections
- Small fraction (15%) of cells
- Morphology and electrophysiologies are different
- Four different types
 - Parvalbumin-expressing (Fast spiking) (100 Hz)
 - Somatostatin-expressing
 - Vasactive intestinal peptide expressing
 - Neurogliaform cells

■ Parvalbumin expressing cells

- Two types ◦ Basket (^{Covers Soma}) 
- Largest family ◦ (Fast hyperpolarizing IPSP)
 - Make synapses directly to axon initial segment.

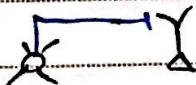
⊗ Excitory neurons are connected with each other with 10% probability, whereas excitatory and inhibitory neurons are connected with 50% prob. (Parvalbumin)



Strong inhibitory loop

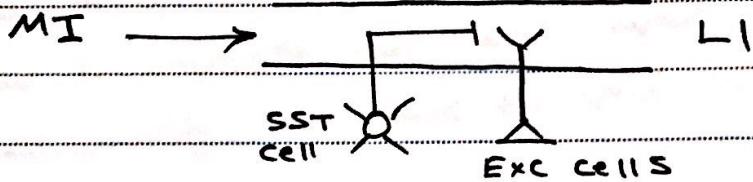
Cellular Mechanism of Brain Lecture 5 Part 9

■ Somatostatin-expressing GABAergic neurons



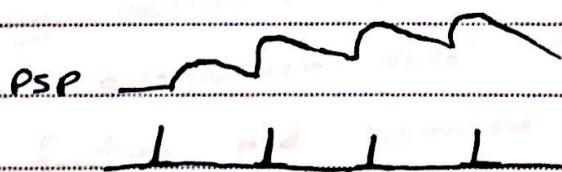
④ They strongly innervate distal dendrites of excitatory pyramidal neurons.

→ Distal dendritic inhibition



⑤ Seems to gating function for sensory inputs

□ They also receive facilitating excitatory input from nearby cells.



⑥ This feature is unique.

■ VIP cells (inhibit other inhibitory neurons)

→ Disinhibition

■ Neurogliaform neurons (volume transmission of GABA to activate GABA_B receptors and drive slow IPSPs)

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Cellular Mechanisms of Brain Lecture 6 Part 2

Measure neuronal activity methods

- Electrophysiology →
 - Whole-cell patch-clamp recording
 - Extracellular recordings of action potentials
- Optical imaging →
 - Fluorescence microscopy * using extracellular potentials
 - High resolution structural and functional measurements

④ Correlations of neuronal activity is essential to measure, but correlations do not necessarily imply causality.
→ in order to investigate the causal impact of a specific neuronal activity, we need to specifically perturb that activity

□ Optogenetics: controlling neuronal activity by light

① Optical control

② Genetics

■ After measuring correlations and applying perturbations, we need to quantitatively model the causal interactions driving brain function and behaviour.

④ The closest common ancestor between man and mouse is thought to have lived ~80 million years ago.

④ 99% of genes have homologs in man and mouse.

Mammalian brain size

① Mouse (20g)

$\sim 10^9$
 $\sim 10^8$ neurons

② Macaque

~ 100 g

$\sim 5 \times 10^9$ neurons

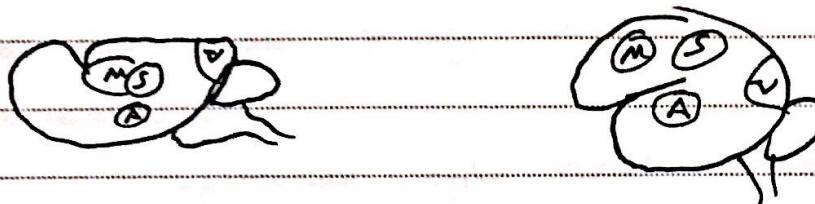
③ Human (60kg)

~ 1500 g
 $\sim 10^{11}$ neurons

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Cellular Mechanisms of Brain Lecture 6 Part 2

Human-mouse similarities



- * structures, locations and layers are very similar
- * Highly specific genetic manipulations in well-defined cell types is essential for causal and mechanistic understanding of Brain function. → Cre-LoxP system
- * Projection maps of the mouse brain is provided by Allan institute
- * Morris water maze is an experiment to study mouse behaviour

Voltage sensitive Fluorescent dyes (VSD)

□ Lipophilic compound

□ Noisy

□ Not possible for recording voltage in living animals so far
→ However, it can be used in population of neurons

* 500 microns top the neocortex - 100 μm scale

* The brain scatters light strongly, with less scattering at long wavelength → 900 nm infrared works good.

* Two photon excitation [red] also helps focusing the excitation point ($F \propto P^2$ anumb. of excitation depends on ~~2nd square~~ 2nd power of photon density) * can be used *in vivo*
* scale of $\sim 1 \mu\text{m}$

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Cellular Mechanisms of Brain Lecture 6 Part 3

Calcium imaging

- It's easy to make calcium-binding molecules that change their fluorescence properties.

- F → Provide higher resolution ways for brain imaging (at level of synapses).

* can be done at real-time

* Low temporal resolution ($\sim 10\text{ms}$)

Extracellular electrode recording

- Two types of potential.

Local Field Potentials
($5\mu\text{m}$) ↗

- ① Synaptic currents (with activating AMPA receptors) (L.F.P.)

- ② Firing action potential

- ③ We can use Electrode array.

→ Utah array (10×10 , $400\mu\text{m}$ apart.)

→ Silicon probes ($5\mu\text{m}$ apart.)

- ④ In-vivo whole cell recording is also possible for animals.

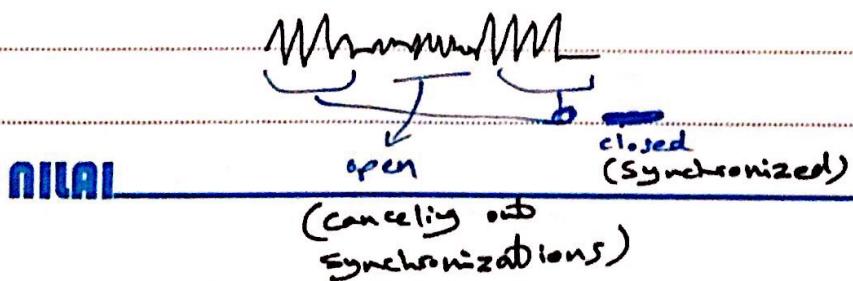
- ⑤ APs are being fired with mean = 1Hz and Median = 0.1Hz

- ⑥ Dual-cell recordings can be used for assessing fluctuations in two neurons together.

- Mouse whisker example - measuring correlation of fluctuations in neighbouring cells

* quiet whiskers v.s moving whiskers

* Also can use EEG (open v.s closed eyes potentials)



Cellular Mechanisms of Brain Lecture & Part 4

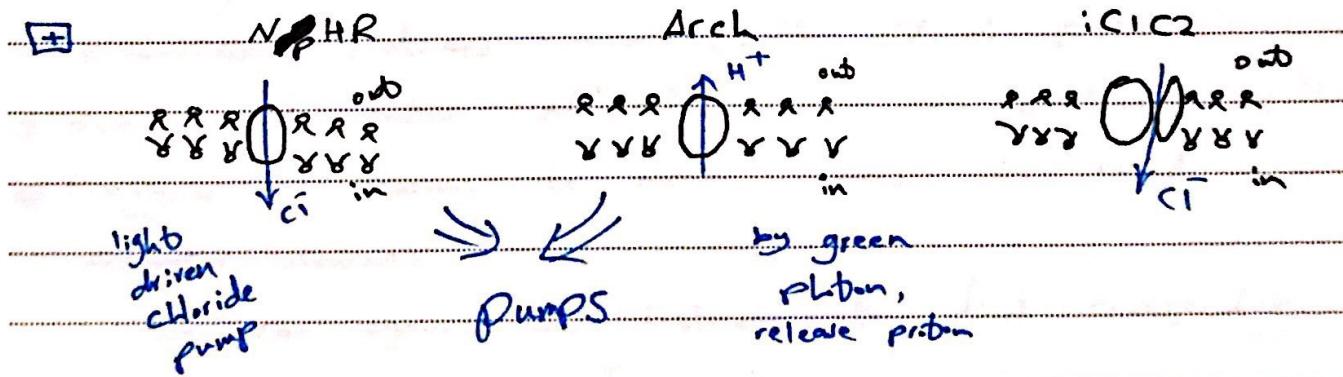
- * There is variety of AP firings in different types of cells.

Controlling neurons

channelrhodopsin-2

- [+] optogenetic revolution started with ~~other~~ light-activated cation channel ChR2 (with inward Na^+ and Ca^{2+} leading to curr)
- Activated by Retinal molecule bind to it, which is sensitive to light
- * Drawback is that we don't know exactly how many cells or which cell-types were stimulating
→ Use two-photon excitation of ChR2 for controlling at single-cell level (instead of blue light)

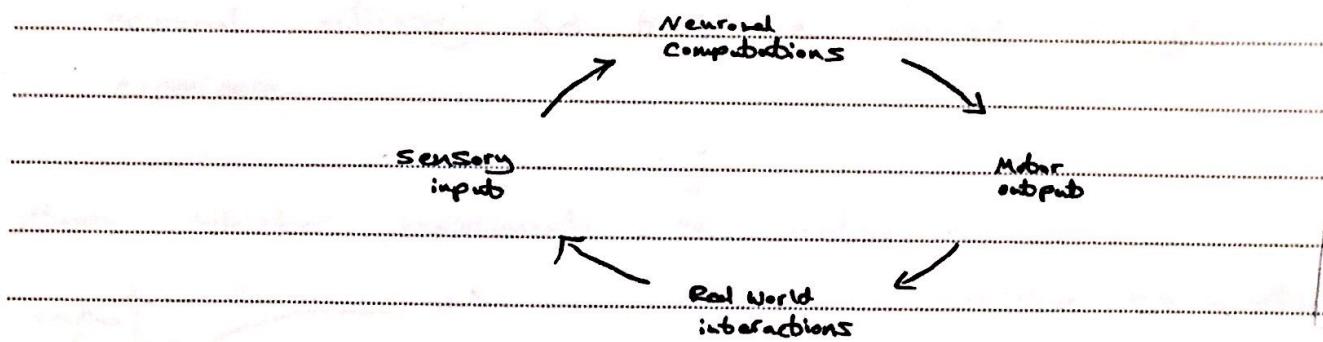
optogenetic inhibition



- * Pumps are slower than ion channels
- * optogenetic actuators are being developed to control many other processes.

Cellular Mechanisms of Brain Lecture 7 Part 2

Sensorimotor loops

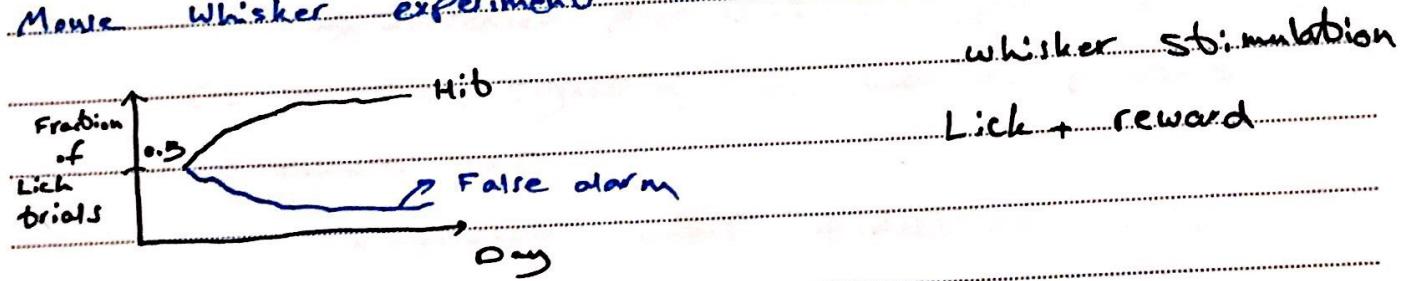


- ① At neuromuscular junctions, neurotransmitter acetylcholine (ACh) is released.
 - Muscle cell also fires AP like neurons, which increases cytosolic Calcium concentration, causing muscle contraction.
- ② We can localize location of neurons, projecting and controlling specific muscles by injecting genetically engineered Rabies virus (with colored proteins)
- ③ Neurons that control different muscles are clustered together
- ④ Presynaptic neurons to motor neurons are called premotor neurons.
 - We can again use Rabies to find corresponding premotor neurons of motor neurons.
- ⑤ Sensory information is often actively and selectively acquired.

Cellular Mechanisms of Brain Lecture 7 Part 2

- Sensory percepts are internal constructs, created by neuronal activity. And they are learned through experience.

Monie Whisker experiment for behaviour



whisker stimulation

Lick + reward

- * Considering C2 barrel in neocortex, we can experiment if it involves in licking process
→ use TTX toxic injection (blocker of Na^+ channels, preventing APs)
* we can also inject CNQX, APV (blocking AMPA receptors)
* we can also use optogenetics for direct stimulation of C2 barrel

Cellular Mechanisms of Brain Lecture 7 Part 3

Dopaminergic innervation of striatum

Two types of dopamine receptors:

- D1 receptor (G_s and G_{off} proteins)

- Stimulation of adenylate cyclase

- Increased production of cyclic AMP (LTP)

- D2 receptors, signalling G_i and G_o proteins

- Inhibit AC

- Decrease cAMP production (LTD)

whisker detection task learning.

