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B13154 - NEUROBIOLOGY I

Why study neuroscience?
Something "fundamentally human"

→ Neuroscience is the study of -
- structure & fn of nervous system
- its role in behaviour
- normal & pathological states

Many disciplines are involved - genetics, biochem, psychiatry etc.

Human brain - extremely complex - 100 billion neurons with an avg of 10,000 synapses.

→ How do we study brain functions?
Depends on the level of complexity of the brain -

Molecule - Synapse - Neuron - Network - Brain in health & disease - Neural circuit mechanism

Decreasing complexity ←

Circuit mechanisms
Genetic studies
Electro-physiological methods
EEG
Behaviour
Behavioural assay (Animal models)

The brain receives information from the sensory world. Other than 5 senses, there's also proprioception (knowing the 3Dimensionality) → of the body & vestibuloception.

Different senses are processed in different parts of the brain. Broca provided first evidence for localisation of perception.

Broca's patient could understand language but not speak. There was a lesion in his brain in a region now called Broca's area which controls speech formation.

Wernicke's area is responsible for language comprehension - the patient couldn't understand but he could speak

What makes neurons unique?
- They are excitable – can conduct action potential
- They're highly interconnected – creates a complex network
- They can't replicate

How do neurons talk to each other?

★ Sherrington (1897) – proposed the term synapse
The potential moves from one neuron to another through chemical of eletrical synapse.

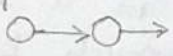
★ The action potential is carried through the axon by changes in ion channels & membrane potential

When neurotransmitters bind, they open ligand-gated Na^+ channels in the axon hillock. so Na^+ ions rush in and membrane is depolarised
The action potential propagates through voltage gated Na^+ channels. Then k^+ channels open & there's Pottasium efflux which repolarised the membrane
Finally, the Na-K pumps restore the original membrane conditions.

★ There are Excitatory and Inhibitory neurons. Excitatory cause Na influx & depolarise the membrane whereas Inhibitory ones cause Cl^- influx & hyperpolarise the membrane.
A neuron gets signals from ~~hubs~~ hundreds of neurons and the summation of the input signals decides the sign & magnitude of post-synaptic potential (PSP).

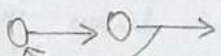
★ Micronetwork motifs – some common patterns of connection that occur frequently in neural circuits

Feedforward excitation

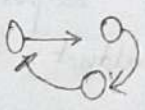


Feedforward inhibition

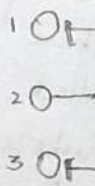
Feedback excitation



Feedback Inhibition



Lateral inhibition

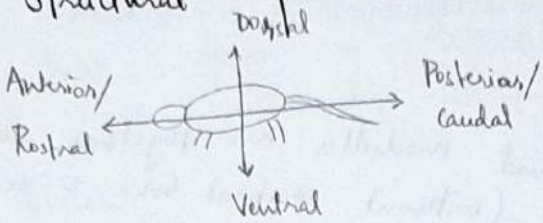


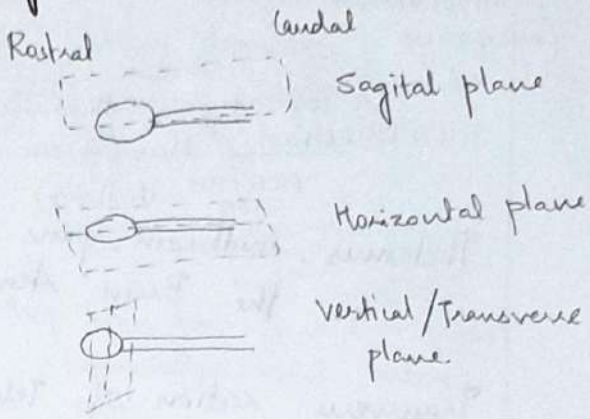
optical illusion image

② inhibits ① and ③

Lecture 02
Introduction to Neuroanatomy

Structural references





Parts of vertebrate brain

Spinal cord → Rhombencephalon (hind brain) → Mesencephalon (midbrain) → Diencephalon → Telencephalon

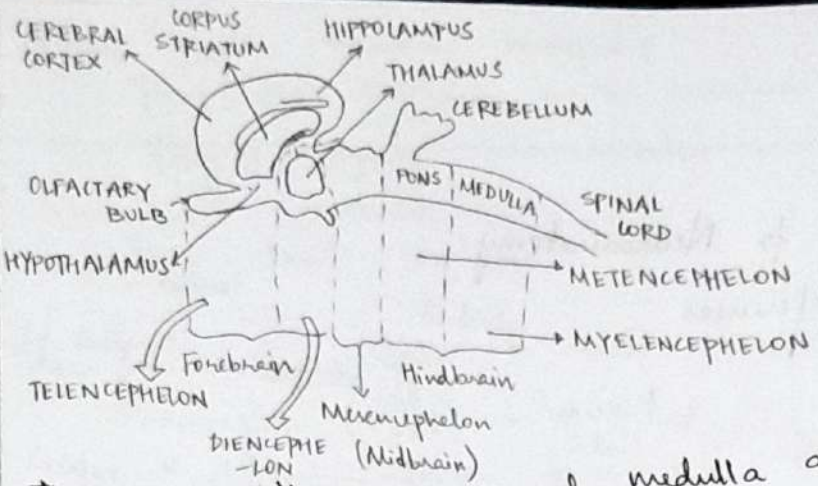
- Rhombencephalon
  - Medulla
  - Pons
  - Cerebellum
- Mesencephalon
  - Tectum (dorsal)
  - Tegmentum (ventral)
- Diencephalon
  - Thalamus
  - Hypothalamus
- Telencephalon
  - Olfactory bulb
  - Basal telencephalon
  - Cortex

- There are pockets / cavities in the brain which are connected by channels. They are called ventricles.
- All vertebrates have these different parts of the brain. But the relative sizes can vary vastly.
  # Lamprey has a v. small, inconspicuous cerebellum
  Larger lobe ⇒ more neurons ⇒ greater processing capacity

Comparing Rat and human brain

- The cerebral cortex has grown so much in humans that it covers midbrain and hindbrain in the top view.
- human brain is also very convoluted (sulci & gyri) which increases surface area and thus increases processing power.

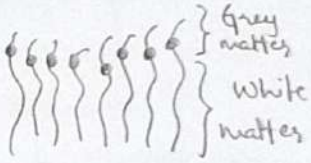
dorsal



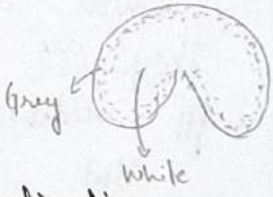
Thalamus, midbrain, pons and medulla are together called as the Brain stem (without cerebral lobes & cerebellum)

Transverse section of Teleost telencephelon

- The dull background is actually the interconnection of neurons and the spots represent the cell body of neurons.
- In fish and frog, the distribution of neuronal cell bodies is uniform.
- In reptiles and above, the neuronal cell bodies come together and their axonal fibres are bundled together. The top part is called grey matter and the axonal fibre is called white matter.



- This creates a cortex in the brain — brains of higher vertebrates are said to be Corticalized through corticalization
- We can find 6 distinct layers in the cortex of mamals, so they're said to have a neocortex.



In the cerebellum, white matter is inside & grey outside. Because the brain evolved to maximise the number of neurons which would be possible if they're on the outside (more SA). But in the position is inverted in the spinal cord because its an evolutionary inheritance — even fish spine has grey matter inside and white outside.

HODOLOGY - Study of interconnections in the brain or the organisation of pathways

→ **Neuropil**

It's a broad term defined as any area in the nervous system composed mostly of unmyelinated axons, dendrites and glial cells (hing density of synapses) that form a synaptically dense region containing a relatively low no. of cell bodies.

→ **Nucleus**

A distinguishable collection of neurons deep in the brain (CNS) that receive the same information and relay information to a common spot.

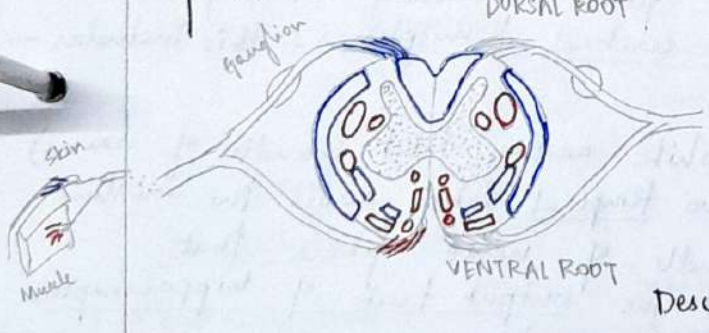
Eg: Lateral geniculate nucleus relays info from eye to cerebral cortex.

→ **Ganglia** (Greek: knot)

A collection of neurons (closely associated) in the PNS

Eg: Dorsal root ganglia contains cell bodies of sensory axons entering the spine via dorsal roots.

Spinal cord and Spinal Nerves



- Descending tracts
- Ascending tracts

Tracts contain bundles of nerves that carry information to and from sensory & brain systems

Descending - from the brain
Ascending - to the brain

Corpus callosum - its continuous with cortical white matter and forms an axonal bridge that links cortical neurons of two cerebral hemispheres.

Splitting Cc in monkeys & cats caused split brain where each hemisphere functioned independently. Cutting Cc in humans was not the same cuz hemispheres have different functions - only left interprets language.

★ Fornix & Anterior commisure - other structures that link the brain.

Spinal nerves (31 pairs)

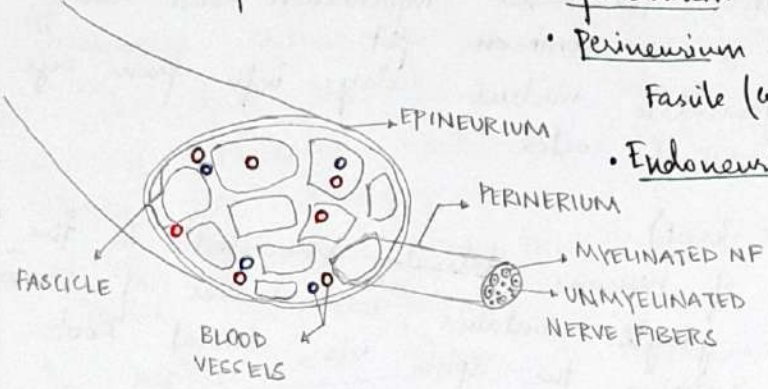
They're part of PNS. Each spinal nerve is connected to the spine by ventral (carries info from the brain ie. motor neurons) and dorsal (carries info to the brain ie. sensory neurons) roots. Both neurons are bundled together in the nerve

PNS can also be classified as —

- Somatic : All spinal nerves that innervate parts under voluntary control (muscles, skin)
- Visceral : Neurons that innervate internal organs, blood vessels and glands ie involuntary / autonomous

Structure of a nerve

- Epineurium - covering of the nerve
- Perineurium - covering of the fascicle (collection of nerve fibers)
- Endoneurium - covering around the myelin sheath.



Going back to Hodology

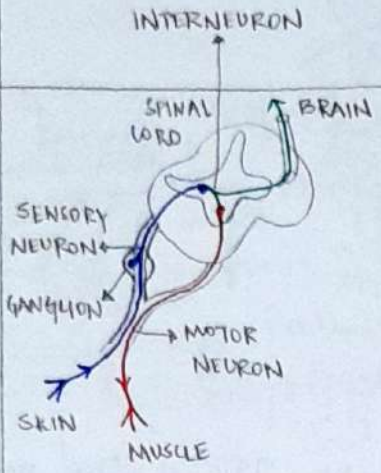
Commissural pathways are fibers connecting broadly similar regions of two cerebral hemispheres. This includes —

1. Corpus callosum
2. Anterior commissure - white matter tract (bundle of axons) connecting the two temporal lobes across the midline
3. Fornix - C-shaped bundle of nerve fibers that acts as the major output tract of hippocampus. It connects two halves of hippocampus.

VENTRAL - MOTOR - FROM
DORSAL - SENSORY - ~~FROM~~ TO

Bell-Magendie law

It's the finding that anterior spinal nerve roots (ventral) contain only motor nerves and posterior roots (dorsal) only sensory nerves; and that nerve impulses are conducted in only one direction in each case



Reflex Arc

It controls a reflex. Most sensory neurons pass through the spinal cord & form a synapse there. It allows for faster reflex action to occur by activating spinal motor nerves without the delay of routing it through the brain. The integrating centre in the spinal cord (where nerves synapse ie grey matter) processes the information and relays to motor neurons. Cognition of the action comes later.

→ Pain perception, Pressure perception & Propioception

* There are several types of corpuscles (Painian, Meissner's) and free nerve endings in the skin. Any deformation in the corpuscle causes action potential to be generated by opening pressure sensitive Sodium-ion channels in the axon membrane. This allows Na ion influx, creating a receptor potential

* Nociceptors terminals: Heat, acidosis or endogenous/exogenous agonists trigger pain receptors which cause influx of Na^+ and Ca^{2+} ions. Action potential is initiated and the sensation of pain is perceived by the brain

Intracellular Ca^{2+} signal causes local desensitisation.

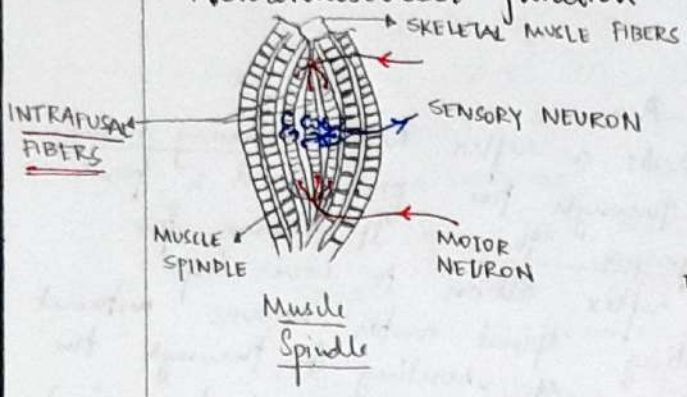
* Proprioception: Sense through which we ~~sense~~ perceive the position & movement of our body, including sense of equilibrium and balance, senses that depend on notions of force.

Proprioceptors sense changes in tension, sensations of stretch and muscles responses.

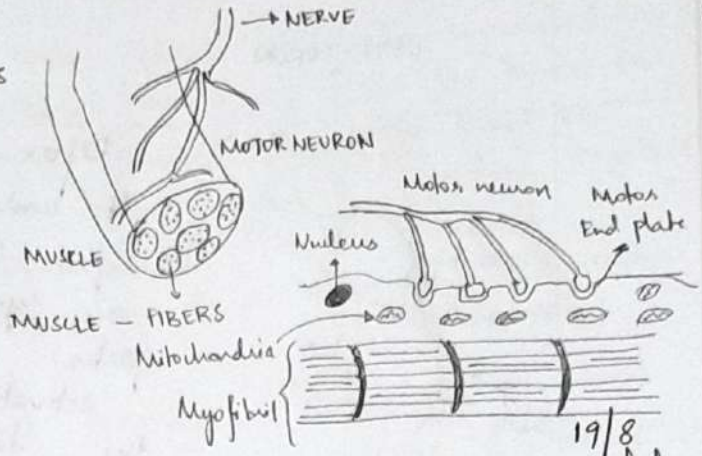
Proprioceptors are found in — (i) Synovial joints
(ii) Skeletal muscle (iii) Between muscles and tendons
(iv) Inner ear

(Charles Sherrington "proprio" → one's own)

Neuromuscular junction



Muscle Spindle



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Extrafusal muscle filaments – make up 99% of the musculature They're very rich in actin and myosin.

1% of muscle is intrafusal muscle which has axonal bundle and dendrites around them. when extrafusal muscles stretch, the intrafusal muscles also moves with it. This indicates to the brain the tension/stretch in the muscle

So, in a way, these intrafusal muscles acts as a sensory organ, turning mechanical perception into something the brain can interpret. Also a part of proprioception.

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Lecture 03

The Brain Stem

There are 12 pairs of cranial nerves – they enter the brain from the ventral side through the brain stem (except for Olfactory nerve).

Thalamus, midbrain, pons and medulla – brain stem

There are important nuclei (neuronal groups) in the brain stem

★ Nucleus gracilis & nucleus cuneatus – neuronal groups that act as junction box through whill all sensory info (ascending tract) paves through this to the thalamus.

★ Respiratory rhythmicity center – nuclei that control respiration – the movement of thorax

★ Cardiovascular center – controls the rate of heartbeat

So the brain stem controls very important involuntary / autonomic / vegetative functions – respiration, digestion, heart and blood vessel function, swallowing & sneezing.

REGULATION OF HEART

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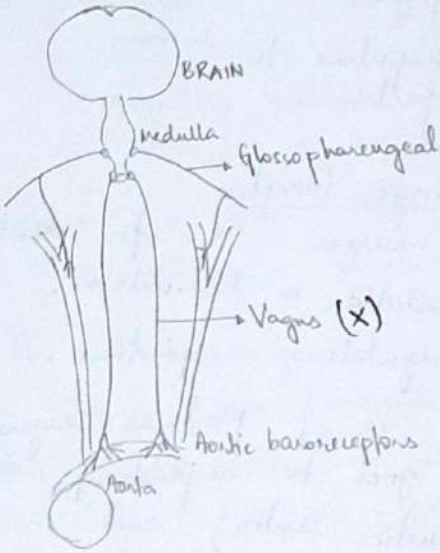
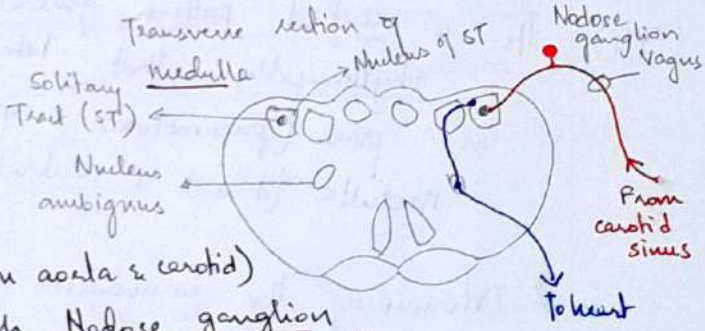


SENSORY PART

- The baroreceptors in aortic arch send info through the Vagus nerve (Aortic baroreceptors)
- Baroreceptors on carotid artery send info to brain stem through Glossopharengeal
- ↑ Firing of sensory neurons increases as BP increases



★ Information about BP (from aorta & carotid) is coming in through Nodose ganglion & into the Nucleus of ST (in the medulla)

Solitary tract – axons going further in the brain.

★ This info goes into Nucleus ambiguus, a from which an axon goes towards the heart.

The BP info is processed in the medulla & it decides whether to increase or decrease BP.

★ The motor fibres from Nu. amb. goes through the Vagus and the nerve ends at AV node & SA node.

They release acetylcholine which slows down heart (Parasymp)

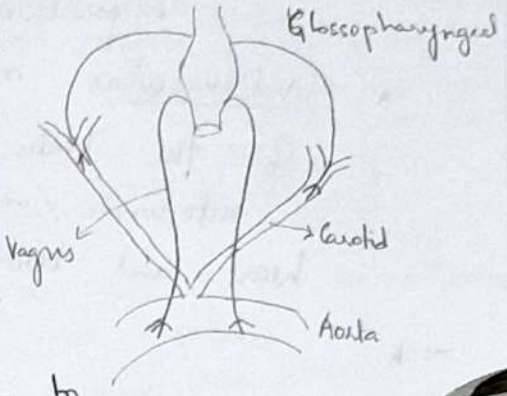
If heart has slowed too much, another group of neurons which go down the spinal cord, through a Sympathetic ganglia

post-ganglion nerve fibre → (10)

go to the heart as sympathetic nerve fibre, release (10) norepinephrine / noradrenaline which stimulates the heart to increase BP.

→ Amount of O_2 & CO_2 in blood.

* Medulla monitors the conc of O_2 & CO_2 in the blood through chemoreceptors - ~~chemo~~ peripheral arterial receptors on aorta & carotid which pass info through vagus & glossopharyngeal



* If CO_2 ↑ medulla sends impulses to thorax so we breathe faster.

Regulating Respiration - Pre Botzinger Complex

PreBotC is a cluster of neurons in the ventral respiratory group of medulla in brainstem.

They are important for regulating respiration

(CPG) Its a central pattern generator ie it produces signals rhythmically that later goes to respiratory centers in pons (pneumotaxic & apneustic centers) and medulla (dorsal & ventral respiratory groups).

Determining the boundaries of nucleus
- neurons that have connection to & from the same place
- group of neurons that produce the same neurotransmitter
- size of neurons

→ Emotional behaviour

Some neurons in the median of pons and medulla, called Nucleus of Raphe magnus / Raphe nuclei, produce serotonin (5-hydroxytryptamine). From this nuclei, some cell bodies project into forebrain limbic area that control emotional behavio

Raphe = Serotonin

Raphe nuclei - Seratonin
Locus ceruleus - norepinephrine
Substantia nigra - dopamine

Locus coeruleus

Its a nucleus in pons. Its the principal site of synthesis of norepinephine in the brain.
The cell bodies project into thalamus, amygdala, cortex, cerebellum etc. and release norepinephine there.
This hormone is involved in -

- Arousal & sleep-wake cycle
- Attention & memory
- Behavioural flexibility, inhibition, stress, emotions
- Cognitive control
- Posture & balance

There is upto 80% loss of locus ceruleus neurons in alzheimer's disease

The Midbrain

Mesencephalic aqueduct passes through the midbrain
On dorsal side, 4 lobes are visible - Superior and inferior colliculi (corpora quadrigemina) - on tectum side
In frog brain, there are only 2 lobes called Optic lobes. colliculi are homologous to them.

Transverse section of midbrain
Take the section through sup. colliculi. Cerebral aqueduct is also visible

- Oculomotor nucleus : The axons from this nucleus give rise to Oculomotor nucleus (III cranial nerve). They control the muscles which help move the eyeball
- Substantia nigra : they're a region of midbrain that look blackish. The neurons of Sub. nigra produce dopamine if ~70% of these neurons die and degenerate, it leads to Parkinson's disease
- Red nucleus - involved in motor coordination. Redness is caused by iron in form of haemoglobin & ferretin

Ventral Tegmental area

In midbrain, Substantia nigra & ventral tegmental area are the two groups of neurons that produce dopamine

- Sub. nig. innervates dorsal striatum. there, dopamine is important for motor control.
- VTA innervates ventral striatum, cerebral cortex and hypothalamus there dopamine is important fore generating pleasure, reward & happiness.

→ Thalamus

* Large mass of gray matter in dorsal part of diencephelon.

Diencephelon – epithalamus, thalamus, hypothalamus
Also encompares the 3rd ventricle
It's the portion of brain on which cortex is mounted

* Thalamus : relays sensory & motor signals to cerebral cortex and regulates consciousness, sleep, alertness

* Epithalamus = pineal gland + habenulas commissure + posterior comm

* Thalamus – made up of a series of nuclei responible for relaying info. The neurons are of excitatory & inhibitory nature. There thalamocortical neurons present selected info via nerve fibres to cerebral cortex

→ Basal ganglia

Dorsal & ventral striatum : rat terminology. In humans, its better defined into caudate & putamen.

Basal ganglia (made up of 6 structures – caudate, putamen, sub.nig globus pallidus, nucleus accumbens & subthalamic nucleus), control voluntary movements, habitual behaviours & emotions, procedural learning, cognition & eye movements.

→ Hypothalamus : endocrine & homeostatic control

It has distinctly arranged neurons that form nucleus.

Its involved in : thunger, thirst, temp. regulation
Fight-flight response
control of biological rhythm
Endocrine & ANS synchrony

* Suprachiasmatic nucleus – fires at different rates during day and night. Its a biological clock – sets our circadian rhythm

Present above optic chiasma – it gets info about day time through eyes & it relays to rest of brain which controls rest of physiological processes.

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Lecture 4

Cerebral cortex, Hippocampus & Olfactory system

Cortex has 4 pairs of lobes - Frontal, Parietal, Temporal and Occipital lobe. Frontal lobe is differentiated by Central Sulcus (crevice from one temporal lobe to other side). The gyri on either side are called Pre-central & post-central gyrus.

When we look at the transverse section, we can see the grey matter (cortex) and white matter.

Higher vertebrate neocortex has 6 layers.

- In first layer, there are no cell bodies - only the dendrites end in 1st layer. So has lots of synapses.
- In layers 2-5 there are different types of neurons with projects into other cortical areas
  - Layer two - neurons go to other lobes but same hemisphere
  - Layer three - goes to other hemisphere
  - Layer 5 - large neurons that project into subcortical structures (striatum, superior caliculus)
  - layer 6 - goes to thalamus - fusiform cells forming cortical columns
- The neocortex also receives information from different parts - thalamus transfers to layer 4
  - Other cortical parts transfer to 1, 2, 3, 5
- Cortex has 20 billion neurons and can be 1.5 - 5mm thick
- Level 2 - Stellate cells using GABA (γ amino butyric acid)
  Level 5 - Pyramidal cell which uses Glutamate (excitatory amino aid).
- The cortex is the highest region of processing

Level 2 - stellate cells - GABA
Level 5 - pyramidal - Glutamate

Tactile, pain and temperature pathways

* The sensory nerves attached to mechanoreceptor and temp receptors in the finger go to the dorsal root ganglion (1st order neuron), ascends up the spinal cord to the medulla (cuneate nucleus - 2nd order neuron) and then the 3rd order neuron goes through the midbrain to the thalamus which later projects into the cortex (post central gyrus) where all info from the skin (below neck) is interpreted

* Similarly info from other receptors is also transferred to the cortex through the thalamus (only olfactory & auditory doesn't go through thalamus) and only 3rd/4th order neurons project into the cortex.

* Post central gyrus — receives most of somato-sensory information
  Pre-central gyrus - processes motor information

Variation in structure of neocortex.

The layers of cortex vary based on their function

Primary sensory - Layer 4 is thick, similar to layers 5, 6
Association - Layer 4 is thinner & 5 & 6 are thick
Primary motor - Layer 4 is v. thin. layers 5, 6 are thick

Thalamus projects into layer 4

Korbinian Brodmann (1868 - 1918)

- He was a German neurobiologist. He created a cytoarchitectural map of human cerebral cortex.
- He divided the cortex into 52 distinct areas based on histological structure uniformity. He did this by sectioning the brain into small peices & studying them under microscope.
- This purely anatomical map has a functional purpose.

Area 17 - primary visual cortex
Area 3b - primary somatosensory cortex. (Post-central gyrus)

Broca's area

- Pierre Paul Broca (1824 - 1880) — a French doctor the studied 2 patients both of whom had lost the ability to speak after injury to posterior inferior frontal gyrus of brain (Broca's area).
- This region controls the respiratory system and mouth & lips to form legitimate words
- fMRI studies have shown that Broca's area is associated with language tasks.

Brodmann's area 44 & 45.

- This was a landmark find in neurobiology because it related to the question of function localisation in the brain — whether each function is controlled by a certain region. or brain on the whole

Cortical Homunculus

* Wilder Penfield (1891 - 1976) was a American - Canadian neurosurgeon who mapped the function of various regions of the brain.
* He did it while conducting surgery for epilepsy. He would give a slight shock to the brain and make the patient articulate what they felt

* Post-central gyrus - primary somatosensory cortex (Areas 3a, 3b, 1, 2)
The sensations from the body is received by this part of the cortex But the projections are not proportionate - the face and thumb are overrepresented whereas limbs and trunk are small.
* Pre-central gyrus - Primary motor area
When stimulated, the region of cortex makes that part of the body move. Again, palm & face are overrepresented This brain structure is involved in acquisition & performance of skilled movement

Contralateral nature of the brain

Simplified motor nerve tracts

The upper motor neurons that start at primary motor cortex, descend and cross-over to the other side at the medulla. Then it descends down the spinal cord, exits through ventral root and it stimulates the muscle by releasing acetylcholine at the neuromuscular junction.

Supplementary motor area (SMA) & premotor area (PMA) are other regions of cortex involved in motor regulation. Similarly, there are a few other sensory areas (auditory, visual & olfactory) but most of the cortex in humans is a part of Association region.

Evolutionarily, this area has expanded and reflects the analysis of sensory information. Especially in frontal & temporal lobe, the association areas are recent development and noteworthy of primate brain.

The emergence of 'mind' - ability to interpret and understand the sensory information - correlates best with expansion of association cortex.

The function of these cortices are loosely referred to as cognition.

Wernicke's area (Area 22) - Left temporal lobe

Its involved in comprehension of written & spoken language.

Wernicke's aphasia - person being able to speak in phrases that sound fluent yet lack meaning.

So, different parts of cortex are involved in different actions.

→ Hippocampus (Greek - seahorse)

It is a part of the cortex that's folded onto itself.

Its a part of the limbic system & its important for long-term memory, and learning.

there, the cortex has 3/4 layers (not 6) and its called the archicortex.

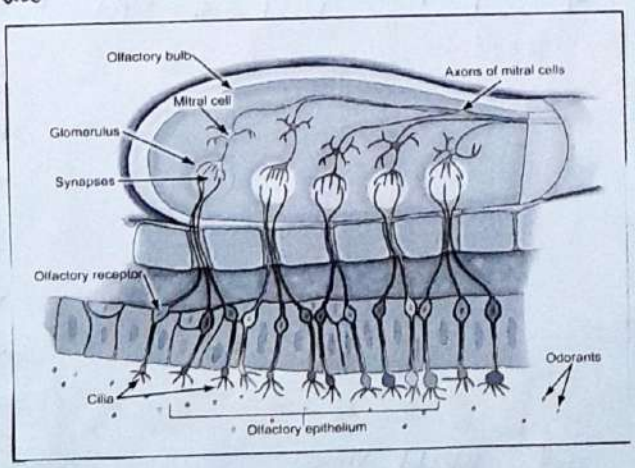
Hippocampal anatomy

The transverse section of hippocampus is mainly divided into Dentate gyrus and Cornu Ammonis (CA). They're further divided into specific regions.

Rafael Lorente de Nó - Spanish neuroscientist. He coined CA

→ Olfactory Bulb

- Hippocampus & olfactory bulb are present in the cortex of all vertebrate brain. Neocortex is only found in mamals
- Olfactory bulb has paleocortex - ~~4 or 5~~ 3 layers, not 6.
- It is a part of vertebrate forebrain that's involved in olfaction.
- Olfactory epithelium - sheet of cells in nasal cavity. It has olfactory receptors that are neurons with axons connecting to the CNS. But these receptors are recycle 4-8 weeks.
- Epithelium is covered with mucus layer. Odorants need to dissolve in the mucus before reaching the receptors
- Olfactory nerves have a single, thin dendrite in the epithelium from which several long cilia emanate in the mucus layer. Odorants bind to the surface of the cilia and activate the transduction proces.



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Lecture 05

Neuronal Circuits in the brain

Visual circuit

* Optic nerves meet and cross at the optic chiasma (some fibres) and continue onto the thalamus.
* In the eye, retinal ganglion cells give rise to axons that travel through the optic nerve. At the optic chiasma, some nerve fibers continue to the same side while others go to the other hemisphere.
  * Same side : ipsilateral
  * Opposite side : contralateral
* The fibers that go into opposite side can be divided into 4 groups —
  - One goes into suprachiasmatic nucleus (biological clock) (Melanopsin →)
  - Another group goes to Lateral Geniculate nucleus in thalamus
    OPTIC RADIATION
    LGN - receives lot of info from the eye
    give rise to thousands of fibers that project into occipital lobe / primary visual cortex
  - Another group goes to Pretectum (midbrain) which controls reflex of pupil & lens. i.e controlling the amt of light & focal length
  - The last contralateral group goes to superior colliculus It plays an important role in orienting movement of head & eyes to focus on a moving object
* Eye can be vertically sectioned into Temporal & Nasal half. All temporal neuronal fibers (in both eye) project ipsilaterally whereas nasal fibers project contralaterally.

LGN has alternating layers (6?) of temporal & nasal nerve fibers which project correspondingly. So each hemisphere gets information from one side of view. Integration of this information gives rise to a 3D world view.

If one eye is removed, then the axons which are supposed to receive information degenerate. This gives rise to alternate layers of functioning & non-functioning layers. Hence, Area 17 is also called Striate cortex

→ Pupillary light reflex pathway (ANS)

The projections of optic nerve into the Pretectal nucleus in midbrain are carried to Edinger-Westphal nucleus in nuclear complex of oculomotor nerve

Oculomotor nerve goes on to innervate the ciliary ganglion, from which short posterior ciliary nerves arise and enter the eye to innervate pupillary sphincter. This controls the amount of light entering the eye.

→ Retino-hypothalamic tract innervates suprachiasmatic nucleus. (SCN)

Melanopsin containing retinal ganglion cells (RGC) are weakly sensitive to light — only transfers info about day and night. These nerves project into SCN whose function regulates the circadian rhythm.

Auditory pathway

Cochlea (inner ear) has receptors that sense the sound
Vestibular apparatus - they sense balance & the movement of your head wrt body.

* Organ of Corti in cochlea has sensors that convert mechanical disturbances into nerve impulses. It is first carried to spiral ganglion.

* Fibers from spiral ganglion travel over the Auditory nerve

* Spiral Ganglion —(Auditory nerve)→ Dorsal & ventral cochlear nucleus (Medulla) → Superior olive (any) (nucleus) —(Lateral lemniscus)→ Inferior colliculus (Midbrain) → Medial Geniculate Nucleus (MGN) (Thalamus) → Auditory cortex (Neocortex)

* From auditory cortex, the signals are transduced to Wernicke's area which helps comprehend the meaning.

* Information from each cochlear nucleus is transmitted bilaterally (ig it means that info from both each ear goes to both hemispheres)

* Auditory cortex
  There's a primary auditory cortex, in which there are demarcations where some neurons perceive some range of frequencies. Also, there's a secondary and cortex.

Barrel cortex of Rodents
Rodents and cats have whiskers – they are mechanosensory
- They are similar to our fingers, very sensitive
  Barrel cortex is the region where this sensory information is carried.
- The base of each whisker has a nerve terminal whose cell body is located in the trigeminal ganglion. deep in the brain. This nerve is a branch of Trigeminal nerve (V) called Infraorbital nerve
- It further goes into trigeminal nuclei in brainstem, then switches / crosses into other hemisphere through thalamic relay nuclei & projects into Barrel cortex.
- This region is a representation of the whisker pad and there's a one-to-one correspondence. Each nerve carrying info to Barrel cortex looks like a cylinder or barrel

Nigrostriatal pathway.
There are projections from Substantia nigra to the dorsal striatum (caudate & putamen) in forebrain which lies below the corpus callosum.
This pathway is critical in the production of movement as a part of Basal ganglia motor loop.
If dopamine producing SN cells degenerate ⇒ Parkinson's.
Dopamine plays an inhibitory role here?
Symptoms – hypokinesia, tremors, rigidity & postural imbalance

Reward center in the brain

James Olds & Peter Milner co-discovered the pleasure center. In 1953, they were looking for areas that help animals in maze solving. They observed that rats preferred to return to the region where they received the last pulse of electrical stimulation.

If the electrode is inserted into pleasure center, and the rat can press a lever to activate it & feel happy Animal learns to do this readily — operant learning

Pathway

Ventral tegmental area (VTA) in midbrain projects into ventral striatum and the cortex. When this pathway is excited, the brain derives pleasure from it.

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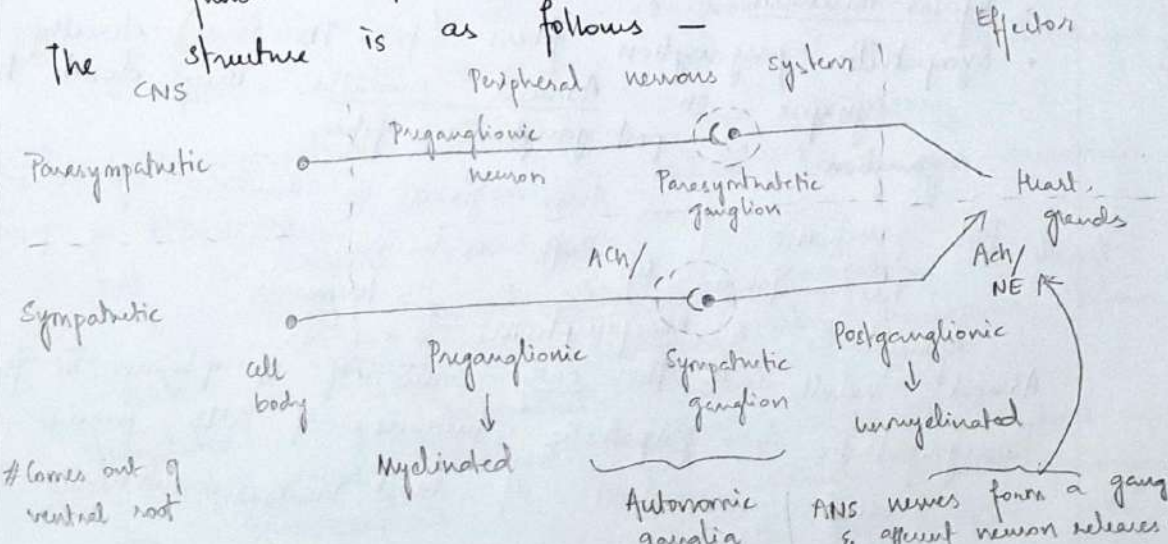
Lecture 5

ANS, Ventricular system, Blood-Brain barrier, Stereotaxy etc

The Autonomous Nervous System.

This part of the nervous system which control involuntary actions — motions of smooth muscles and function of glands.

The structure is as follows —



Structure of autonomous NS

* In Somatic NS, there's a single motor neuron which releases ACh

If components of both sympathetic & parasympathetic NS reach the organ, then they're said to have Dual Nerve supply.

Preganglionic fibre < Postganglionic fibre in Sympathetic system.

All preganglionic cell bodies of sympathetic system are in the thorax & lumbar (T_1 to L_2) of spinal cord. There ganglia are close to spinal cord. → Thoracolumbar outflow

In Parasympathetic system: postganglion > preganglion.

Its cell bodies are mainly located in the brain stem (GVE nuclei) called Cranial outflow, and in the sacrum, called Sacral outflow. (uterus, urinary bladder, ejaculatory duct etc)

Thoracolumbar ANS - Sympathetic

Craniosacral ANS - parasympathetic

Sympathetic ANS

The fibres arise from thoracolumbar area and terminate at the chain of sympathetic ganglia which are on either side of spinal cord (outside CNS)

The effects of ANS are listed in the image.

Some fibres don't synapse at the ganglia chain they extend and form a ganglia later called

- Coeliac ganglia - stomach, pancreas, spleen, liver
- Inferior mesenteric ganglia - urinary bladder, stimulates orgasm
- Sympathetic preganglion fibers (from T_{12} level) directly synapse on Adrenal medulla. They don't have ganglion or post ganglionic fibre

Post ganglionic fibres have given up neuronal fn here & have taken up the endocrine function of producing epinephrine & norepinephrine → hormones → dual role

Adrenal medulla is the only source of epinephrine in the body

Parasympathetic & Sympathetic divisions of ANS provide dual innervation of most visceral autonomic effectors.

Sympathetic ganglia are connected & talk to each other. Not same for parasympathetic.

Exceptions

Parasympathetic nervous system.

It acts antagonistically to the Sympathetic NS. in most cases, but in some cases, there's no antagonism. (figure out which ones!)

Parasympathetic innervation of head & neck.

III cranial nerve → Ciliary ganglion → focal length of lens
VII → Spinopalatine → lacrimal gland
VIII → Submaxillary → Salivary (submaxilary & sublingual)
IX → Otic ganglion → Parotid salivary, mucous mem. of mouth
X (vagus) → Rest of the body

Antagonistic nerve supply to the heart

Sympathetic nerves (which synapse at ganglia chain) supply nerves to the heart (SA & VA node & other places)

Vagus nerve — parasympathetic also sends signals to the heart, which are antagonistic.

The preganglionic & post ganglionic of parasympathetic nervous system are cholinergic — they release Acetyl choline

In sympathetic NS, all preganglionic fibres are cholinergic whereas post ganglionic fibres are noradrenergic

The parasympathetic stimulations are excitatory — it stimulates secretion of saliva, tears, gut secretion

but if slows heartbeat

For the heart, noradrenaline is excitatory — heart rate & strength of pumping also increases when stimulated by sympathetic nerves.

This info about increased heart beat will be picked up by baroreceptors & medulla which directs parasympathetic nerves to release ACh and inhibit the heart muscles. So the effect of neurotransmitter is contextual based on effector organ

Heart has its own system to maintain rhythm of heartbeat - neurogenic

There is more complexity to the ANS. For instance the sympathetic NS that goes to the skin is entirely cholinergic.

Meninges

The brain is covered by a covering called meninges which are 3 layers (from in to out) —

- Piamater - fits the brain v. closely like a glove
- Arachnoid mater - fits loosely
- Dura mater

There is some space between piamater & arachnoid mater that's filled with fluid.

- The vertebrate brain has certain hollow pockets in the brain which contains fluids. (CSF)
- They're called ventricles and they're lined by special glial tissue called Ependyma
- This prevents the brain from directly coming into contact with CSF
- There are 4 ventricles —

Forebrain { 2 lateral ventricles
Third ventricle — separates 2 parts of Thalamus

↓ Cerebral aqueduct } Midbrain

Fourth ventricle } Hindbrain

↓

Canal that runs down the spinal cord

Cerebrospinal fluid system

Cerebral cavity has a volume of 1600 - 1700 ml & 150 ml of this is occupied by CSF.

It is kept in circulation through the ventricles & cisternae outside the brain.

About 500 ml of CSF is produced each day by Choroid plexus (Ependymal cells of lateral & third ventricles & 4th ventricle) which flows down the ventricles. Then it diffuses to the space outside the brain & spinal cord.

Then it is absorbed back into the blood

It flows out through Foramen of Magendie (1) and lateral Foramina of Luschka (2) into the outside of the brain.

* Muscarine – alkaloid compound found in some mushrooms
* Sweat glands don't have dual innervation – no PNS

Both nicotine & muscarin are excitatory – similar, but not same effect as ACh

Lecture 6

Types of receptors

Cholinergic receptors – they get stimulated by ACh. 2 types –

1. Nicotinic (N_2) receptors: there's a site which combines with nicotine

4 subunits → 2 types – Muscular, Neuron

They're found in sympathetic (chain) ganglia, adrenal medulla & parasympathetic ganglia.

2. Muscarinic (M) receptors: they're found at the terminal of * effector organ nerves which release ACh. These receptors combine muscarine

GPCR with 7 TM domains; 5 types

Found in: sweat glands (in skin) – exception of sympathetic NS where even post ganglionic nerve is cholinergic

Brain, heart, smooth muscles, glands

Adrenergic receptors – they're found at the effector targets of post-ganglionic terminal of sympathetic nerves

These receptors (cholinergic) are ligand-gated Na^+ ion channels.

The antagonists for these receptors are –

Metabotropic → Muscarinic – Atropine, Hyoscine

Tonotropic → Nicotinic – Tubocurarine, hexamethonium

} Competitive inhibition of ACh.

Adrenergic receptors

They are G-protein coupled receptors that recognize and selectively bind to epinephrine, norepinephrine and catecholamines, which are released by sympathetic Ns & adrenal medulla

This also has 7 TM domains. There are mainly 2 types –

Alpha	Beta
+++ Norepinephrine	+++ Epinephrine
+ Epinephrine	+ Norepinephrine
Smooth muscle contraction	Smooth muscle relaxation.

Different smooth muscles have greater abundance of different kinds of receptors. This is v. important in regulating the blood flow in the body.

(26) Q: Discuss the effect of adrenergic receptors on heart.

CSF again

One if goes out of Foramina in hindbrain, it gets pushed to the top of the brain - superior sagittal sinus - where it returns to the blood.

Choroid plexus - the cells secrete CSF & release it into the ventricles

Blood brain barrier

Blood is mainly supplied through Posterior, Middle and Anterior cerebral artesies which further divide into arterioles and capillaries.

The smooth muscles decrease from artery to arteriole & they're not present in arteries.

Paul Ehrlich - intravenous dyes (like trypan blue) didn't stain the brain but stained other organs

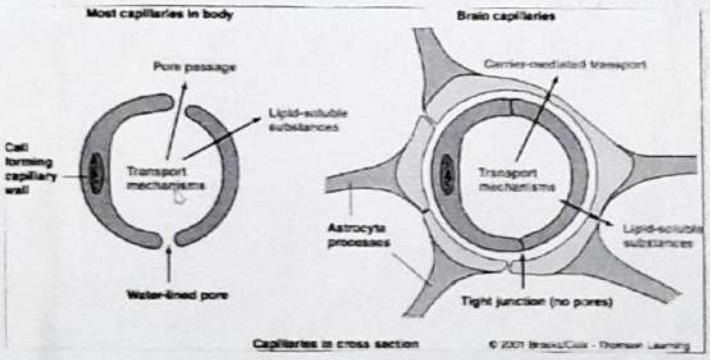
There are 3 morphological features that distinguish brain capillaries from those in other regions -

No diapedesis of WBCs →

1. There are tight junctions b/w endothelial cells
2. Capillaries are surrounded by astrocyte foot processes
   So, there's a need for carrier mediated transport
3. Mitochondria are more numerous in endothelial cells of brain capillaries.

Many molecules cannot cross the BBB -

* Amino acids & glucose is transported by carriers
* Alcohol (hydrophilic) easily crosses the membrane
* Dopamine can't cross but levoDOPA can - used for treatment of Parkinson's
* Morphine can't cross, fast heroin can - has quick onset of action
  ↳ slowly



Lidocaine injection – its a local anaesthetic that also suppresses the function of neurons

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Lecture 7

The Cerebellum : Anatomy & microcircuitry

Stimulating cerebellum doesn't produce any twitch but it helps in coordination of voluntary movement.

It coordinates the timing & force of these different muscle groups involved to produce fluid movement

Dorsal view

Cerebellum is divided into anterior and posterior lobes

It has many lines transverse to the length of brain which is because of the folds in cerebellar cortex

These shallow ridges are called folia. (same as gyri)

Sagital section

- There are 3 cerebellar penduncles (superior, middle, inferior) in the pons which mechanically support cerebellum and the axons (myelinated white matter) that take info back and forth from Cerebellum and rest of brain.
- Cerebellum also has grey matter i.e neuronal cell bodies.
- Buried deep in the cerebellum, there is the cerebellar nucleus. (4 pairs)
- The folia & lobes increase the S.A. of cerebellar cortex.

Deep Cerebellar nuclei

All outputs from cerebellum originate from these nuclei

So lesioning this nuclei is like lesioning the cerebellum

1. Dentate nucleus
2. Emboliform nucleus
3. Globose nuclei (2 on each side)
4. Fastigial nucleus

Grey matter in cerebellum is also cerebellar cortex and is split into 3 layers -

1. Molecular layer - a region of a lot of synapses
2. Purkinje cell layer - very large neurons that form middle layer
3. Granule cell layer - innermost layer made of tiny

Purkinje cells

Discovered by Johannes Purkinje. It has a large cell body (18-20 μm). There is great dendritic arborisation from pure cell bodies.

Staining by Golgi technique shows entire neuron.

Each Purkinje cell has one ~~neuro~~ axon that goes down and ends at one of the deep nuclei

Each cell is capable of forming 50k synapses

The dendritic tree of the cell is almost 2 dimensional.

All purkinje cells are oriented parallely

From the side, this looks almost flat and its oriented perpendicular to the folia (i.e. each ~~folia~~ dendritic tree project into a range of folia)

Neurons from the Inferior Olivary nucleus (Medulla) give rise to 2 branches of nerve fibers. One branch projects into 1. deep nuclei and the other branch projects into the molecular layer and forms close excitatory association with 2. Purkinje dendrites. These neurons are called Climbing ~~neuron~~ fibers → One fiber ↔ one P. cell

They climb and wrap around the dendrites

The axons for Purkinje cells end at Deep nuclei and there are nerve fibers from deep nuclei to the Red nucleus in the mid brain

Fibers from Red nucleus come down as two main branches - one ends at Inferior olive whereas the other goes down the spine as Rubrospinal tract.

So, Inferior Olive funtions as relay station b/w spinal cord and cerebellum, integrating motor and sensory info to provide feedback & training to cerebellar neurons.

B Mossy fibers originate from pontine nuclei, spinal cord, brain stem reticular formation & vestibular nuclei.

The cerebellum also receives neural inputs from other parts of the brain (pons & other afferents) and these are called Mossy fibers.

Mossy fibers also innervate deep nuclei and into the cerebellar cortex where they end in the granule cell layer, synapsing with granule cells.

As a part of this circuit, fibers from deep nuclei go to the cerebral cortex (primary motor area) through the ventrolateral nucleus in the thalamus.

Fibers from cerebral cortex that to neurons in the pons from where some mossy fibers arise.

Thus, cerebellum is critical for proper execution of planned, voluntary, multijoint movements. The decision is taken in Prim. Motor. area but cerebellum instructs it wrt direction, timing & force. Cerebellum is also the place for motor learning.

Purkinje cells talk to various folia whereas the Granule cells (T shaped) run their fibers along the folia and talk to several Purkinje cells. The organisation of these cells is quite geometric.

Other types of cells in the cerebellum (stellate, Golgi, basket etc) help in processing information but its Purkinje axons that carry output from cortex to deep nuclei.

So the deep nuclei receives input from several sources

Mossy & climbing fibers have excitatory connections with deep nuclei, granule cells and purkinje cells.

The axons of Purkinje cells are gabaergic (GABA producing) and inhibit the neurons of deep nuclei.

So deep nuclei computes all the inputs and then sends info to other parts of brain.

Lecture 8 - Subitha (1) 8/9/21

Hippocrates was one of the few who believed that the brain was the seat of intelligence.

Goal of neuroscience : understand how the brain works

About 85 billion neurons in humans & 16 billion in the cortex
In rodents, the size of neurons increases with size of the brain, but that's not true in primates - the neurons are smaller which allows the brain to be packed with great no. of neurons.

In chimps and other primates, the brain size is small.
The brain is energetically costly - there's a linear relationship b/w no. of neurons and cost of energy
We can afford as many neurons as we do, and not spend 8-10 hours eating, because of cooking

10^{11} neurons each of which can make 10^3 - 10^4 connections

Origins of Neuroscience
7000 years ago : Trepanation - boring holes in skull to cure madness
5000 yrs ago : Egyptians thought of heart as seat of soul
460 BC : Hippocrates
350 BC : Aristotle - thought brain was a radiator that cooled the heart
200 AD : Galen - ventricles and CSF confirmed his theory of 4 humours. this theory prevailed for 1500 yrs
17th - 18th century - Shift from Galen's theory

Cajal and Golgi - Nobel 1906
- Golgi developed a stain that launched modern neuroscience
he was a staunch defender of Reticular theory - that nerves were like a network of pipes.
- Ramon y Cajal - the neuron doctrine that each neuron is a discrete unit.
Beautiful drawings

lots of projects (private & by different countries) have invested a lot to understand the brain. But these projects have been criticised about its reverse engineering methods. The goal is to understand the brain to find cure for disorders.

Levels of investigation

* Molecules → Synapses → Neurons → Networks → Maps → Systems → CNS

This hierarchy of organisation is convenient but oversimplific
Studying phenomena at isolated levels is an impediment to better understanding the brain.
Also, most of the molecular processes are incompletely understood.

Other criticism: Neuroscience needs behaviour - correcting a reductionist approach Krakauer 2017

Can a neuroscientist understand a microprocessor? Kording et al

Neuroscience is replacing psychology in understanding human life but it is unwilling to address psychosocial concerns.

What are brains for?

- A sea squirt digests its own brain when it becomes an adult to lead a sessile life. So brain is required for fast, coordinated movement → deliberate, directed, internally generated
- It allows creatures to perceive the world & respond to it. But its not just an ~~ipo~~ input-output device
- Skinner - Behaviourism - Nurture: Brain has the ability to learn and remember - make associations
  - ↳ Blank slate argument
- Galton & Davenport - Nature ie deterministic view that nature and behaviour are predetermined.

Brain is the most complex organ – capable of learning, perception, memory, anticipation, arousal, context dependent reaction and much more – produces feelings, consciousness & ideas in humans

"Kidneys make piss but brains make epistemology"

Understanding the brain stretches our capabilities, scientific and linguistic

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Lecture 9

No 2 brains are identical but all brains have things in common at different levels.

The molecular mechanism, even molecules used, are very similar. The chemical sensing is one of the oldest.

So we can use models and model systems. With the underlying idea that there are common properties, we can study a tractable, representative animal and generalise our findings.

Also the number of neurons and size of neurons are convenient. Simpler networks

There is a lot more variation in invertebrates than mammals

All organisms are limited in what they do & they have evolved some skills, say spatial navigation in honey bees, which is reflected in their brains

So we can explore these boundaries.

Complexity of brain is also associated with a repertoire of molecules in coordination with number, size and connection of neurons.

Paramecium : ionic currents

They swim in stagnant pools – mate, feed on bacteria, run away etc

It is considered as the first version of the neuron.

It shows complex behaviours & senses a variety of stimuli

Posterior bump : it speeds up

Anterior bump : it slows down

The signalling molecules to process information is
K^+, Na^+, Ca^{2+} & Cl^-

Neural strategy
1) Converts mechanical stimulus to a local electric signal created by opening of ionic channels
2) This local signalling triggers a cell wide response that alters the beating of cilia
3) Swim returns to normal as electrical signal subsides

This is a useful level to study because there are direct links between behaviours & subcellular signaling

Physics of neural signalling
All biological membranes are negatively charged & maintained ~ -30 mV (pond water). There's an excess of K^+ inside the cell, and Ca^+ & Na^+ outside the cell.
This arises because of -
- Negatively charged proteins inside
- Membrane proteins (transposters & pumps) that actively pump ions out of cytoplasm
- This is maintained by the hydrophobic membrane

Ion channels & electrical signalling
↳ membrane proteins that allow ions to cross. Some are selective — specific to Na or Ca

When Na+ ions come in (Depolarised) - cell becomes (membrane potential) +ve ($\because V_m = V_{in} - V_{out}$)
K+ ions go out - membrane becomes more -ve
↳ in the same restoring the equilibrium

The local signal (Na-in, K-out) doesn't die out immediately because membrane is insulating & ion channels are slow
So this local signal can spread & persist.
Eventually the cell goes back to its original state when excess ions are pumped
A membrane with varying potential in rapid response to stimuli is considered (by some) as the most basic unit of cognition

↑ MEMBRANE EXCITABILITY
Entirely unique to living cells
So, this is important to understand cognition at higher levels

Signalling in Paramecium - Posterior bump

- Bumping leads to a mechanical deformation
- This opens the mechanosensitive K^+ ion channels - rapid outward flux of K^+ ions leading to cell being even more negatively charged
- This makes the cilia beat faster & cell shoots forward
- These mechanosensitive channels are in the posterior. The size of change in membrane potential is proportional to the strength of the bump.

Anterior bump action potential

- It opens Ca^{2+} ion channels ⇒ cell becomes more +ve
- for a depolarization above a certain threshold, second-stage larger response is triggered that spreads across cell
- This 2nd response is not graded - it results in an electrical signal once the stimulus is above certain threshold : called an Action Potential (AP)
- This spreads through the cell spatially due to voltage sensitive channels Ca^{2+} channels

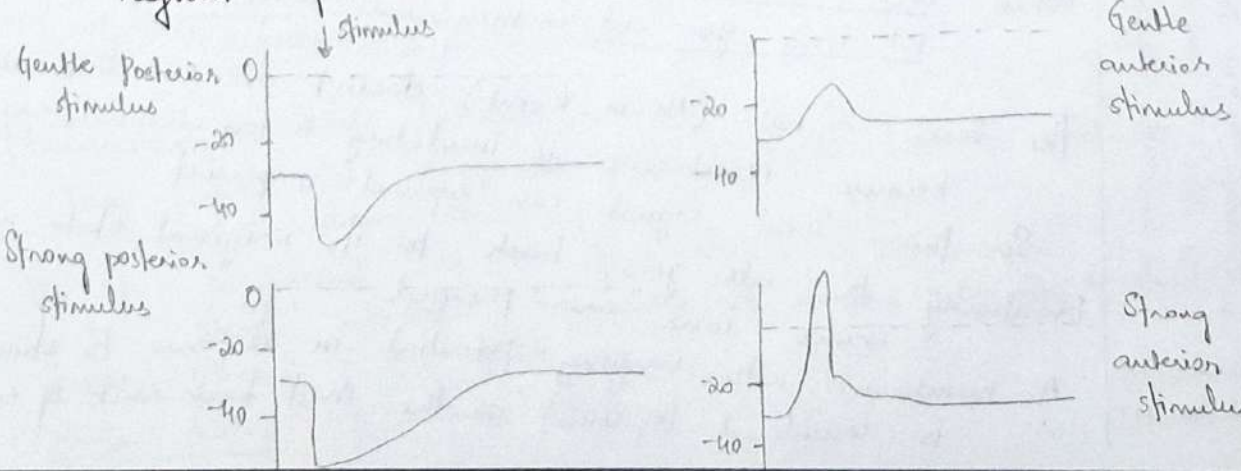
Global signaling

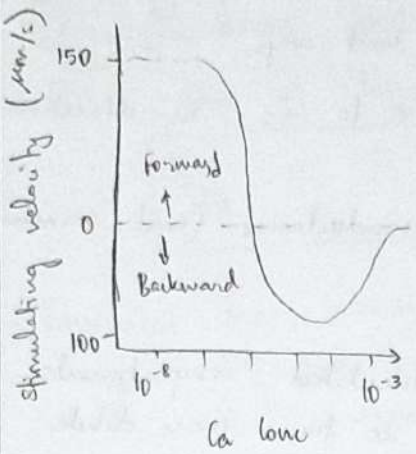
Depolarisation of a patch triggers the opening of voltage sensitive channels in the neighborhood.

Calcium accumulation leads to reversal of the cilia.

So paramecium slows down, swims backward briefly and heads off in a new direction.

Driver of ciliary reversal is Ca^{2+}. After its pumped out, regular forward motion is resumed





As Ca^{++} ions increases, the cilia slow down, then move back briefly, then turn around & accelerate

Recovery mechanism

- Very important. It happens through a combination of fast and slow acting pumps.
- Ca^{+} dependent k^{+} ion channels open in response to calcium influx — so k^{+} goes out momentarily to repolarise the membrane
- Membrane transporter protein use ATP to eliminate extra Ca^{+}

Concept of Action Potential

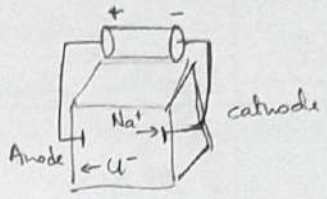
* They are not graded. Its an on/off response that doesn't degrade. It has fixed size & duration
* Advantage: by setting a threshold, this eliminates noise ie becomes robust since they've to go long distances, it helps that they don't degrade
* Qualitative description: electrical & chemical signalling across cellular barriers over ion channels & synaptic transmission
* Cells capable of carrying out AP are called excitable
  All the action happens at the membrane.

Diffusion

Random T-dependent movement to gain uniform distribution
There's a net flux from region of high conc → low conc
Essential conditions:
1) Permeable membrane
2) Concentration gradient

Electricity

Net charge movement of Na^+ toward cathode (-ve) & Cl^- towards anode (+ve)



Movement of charge : current (I) unit : amp

Convention: current flows from +ve to -ve : in direction of Na^+

Ohms law : $V = I \times R$

$R = \frac{1}{g}$ g: conductance (unit: seimens)

Equilibrium / Reverse potential

Say there are 2 electrically insulated compartments ~~what~~ with 'outside' being 20 times more dilute

Now insert potassium channels: –

1. K^+ would go from inside to outside down the conc gradient.
2. Inside becomes more negative wrt outside and impedes continued flow of K^+
3. Thus potential difference is established
4. At some potential diff (E_K). the <u>electrical force pulling K^+ in</u> = <u>diffusion pushing K^+ out</u>

This electric potential difference that exactly balances the ionic conc gradient is called Equilibrium potential.

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Lecture 10

Ion movement in excitable cells

Ions in neurons : Na^+, K^+, Ca^+, Cl^-

Excitable : In neurons, it means the ability to fire an action poten

These ions are distinctly inhomogenously distributed in the cell

$[Na^+]_i << [Na^+]_o$

$[Ca^{2+}]_i << [Ca^{2+}]_o$ *

* $[K^+]_i >> [K^+]_o$

$[Cl^-]_i << [Cl^-]_o$

This situation sets up a gradient – there's a flow of these ions across the conc gradient.

But these particles ~~are~~ also ~~are~~ have an electric field associated with it.

Physical Laws that dictate ion movement

1. Fick's law of Diffusion

$$J_{diff} = -D \frac{\partial [c]}{\partial x}$$

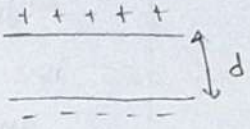
J : diffusion flux (molecules/sec-cm^2)

D : diffusion constant (cm^2/sec)

$[c]$: # molecules/cm^3

Empirical law which states that diffusion takes place down the conc. gradient & its proportional to the magnitude of the concentration gradient

2. Ohm's law of drift



Electric field : $E = V/d$

↳ Force / unit charge

$$\Rightarrow E \cdot d = \frac{F}{q} \cdot d = \frac{W}{q} \Rightarrow W = d \cdot Eq$$

Also $V_f - V_i = \frac{Fd}{q} = -Ed$

$$\frac{W}{q} = \Delta V \Rightarrow W = \Delta V \cdot q$$

~~Statement~~ Definition : Change in voltage is defined as work done per unit charge against an electric field.

The flow of charged particles in an electric field is described by -

$$J_{drift} = \partial_{el} \bar{E}$$

$$J_{drift} = -\mu z [c] \frac{\partial V}{\partial x}$$

∂_{el} : electrical conductivity (mol/vol-sec-cm)

J : drift flux (mol/sec-cm^2)

E : electric field $-\frac{\partial V}{\partial x} = \frac{F}{q}$

μ : mobility (cm^2/vol-sec)

z : valence of ion species

$[c]$ conc. (mol/cm^3)

Statement :

Drift of positively charged particles takes place down the electrical potential gradient and is directly proportional to the magnitude of that gradient

Proportionality is : $\mu z [c]$

3. Einstein's relation between diffusion & mobility

(28) Is demonstrated that frictional force exerted by fluid/medium is the same for drift as it is for diffusion at thermal equilibrium

$$D = \frac{KT}{q}\mu$$

D: diffusion const.
K: Boltzmann constant
T: Absolute temperature
μ: mobility
q: charge

Statement:
Diffusion & drift processes/forces are additive as the resistance presented by the medium is the same in both cases.

4. Space charge neutrality

In a given volume, the total charge of cations is approximately equal to the total charge of anions: -

$$\sum_i z_i^c e [c_i] = \sum_j z_j^a e [c_j]$$

z_i, z_j are the valence of cations & anions
e: monovalent charge

The only exception to this rule is the cell membrane due to charge separation

Together these 4 laws help us understand AP.

by how much?

→ Charge separation & unaccounted for charges

membrane capacitance = 1 $\mu F/cm^2$

means that you need 10^{-6} uncompensated coloumbs of charge / cm^2 to generate 1 volt across the membrane.

$Q = cV$ $\quad A = 4\pi R^2$ $\quad V = \frac{4}{3}\pi R^3$ (spherical cell)

Calculate the fraction of uncompensated ions on each side of the membrane, that is needed to produce 100 mV is a spherical shell of $R = 25\ \mu m$
Conc of ions: [c] = 0.5 moles

Lecture 11

How many uncompensated ions do we need to create an action potential

$$\boxed{Q = C \cdot V}$$

$$ne = CV \Rightarrow n = \frac{10^{-6} \times 10^{-1}}{1.6 \times 10^{-19}} = 6 \times 10^{11} \text{ /cm}^2$$

$$SA = 4\pi r^2 = 4\pi (25 \times 10^{-4} \text{ cm})^2$$

$$\Rightarrow N = n \times SA = 4.7 \times 10^{7} \text{ ions} \rightarrow \text{uncompensated}$$

Total no. of particles = 0.5 molar × volume = $\frac{0.5 \times 6.02 \times 10^{23}}{1000 \text{ cm}^3} \times \frac{4}{3}\pi (0.0025)^3$

Total no. of particles = ~~2.5 × 10^11~~ 7.8×10^{11} → diff from S's calc

$$\text{Fraction} = \frac{4.7 \times 10^{7}}{7.8 \times 10^{11}} = 0.6 \times 10^{-4} = 0.00006\ \%$$

Nernst Plank Equation (NPE)

Movement of ions is influenced by electric feild and concentration gradient which originate from heterogeneity of ion distribution and separation of charge across membrane

Concentration differences are maintained

1. Active transport (ion pumps that transport ions)
2. Selective permeability of membrane to ions

Charge separation

1. There are negatively charged tails of proteins on the inside of the cell
2. Selective permeability of the membrane

If the membrane wasn't permeable, there'd be a lot more K^+ on the inside

Ion flux under the influence of both conc. gradient and electric field —

$$J = J_{drift} + J_{diff}$$

$$J = -\mu z [c] \frac{\partial v}{\partial x} - D \frac{\partial c}{\partial x}$$

Invoking Einstein's relationship —

$$\boxed{J = -\left(\mu z [c] \frac{\partial v}{\partial x} + \frac{\mu kT}{q} \frac{\partial c}{\partial x}\right)} \quad -(1)$$

NPE in ion flux form.

Divide the equation by N_A —

$$\frac{J}{N_A} = j = -\frac{\mu z [c]}{N_A} \frac{\partial v}{\partial x} - \frac{\mu kT}{N_A q} \frac{\partial c}{\partial x}$$

Define: $\frac{\mu}{N_A} = \aleph$ (Aleph) — molar mobility

$qN_A = F$ (total charge in a mole)

$R = N_A k$ (gas constant)

We get —

$$\boxed{j = -\left(\aleph z [c] \frac{\partial v}{\partial x} + \frac{\aleph RT}{F} \frac{\partial c}{\partial x}\right)} \quad -(2)$$

Molar form

$I = j \times$ charge : current density.

Multiply (2) by total charge —

$$\boxed{I = -\left(\aleph z^2 F [c] \frac{\partial v}{\partial x} + \aleph z RT \frac{\partial c}{\partial x}\right)} \quad -(3)$$

Current density form

j : molar flux mol/sec-cm²

N_A : Anagadro no.

R : Gas constant (1.98 cal/k mol)

F : Faraday constant (96480 C/mol)

$\aleph$: $\frac{\mu}{N_A}$ (cm²/V-sec-mol

$I = \frac{\text{Ampere}}{cm^2}$

Statement of NPE

It describes ionic current flow driven by the electrochemical potentials.

Negative sign : Current flows in opp. directn of $\frac{\partial v}{\partial x}$ and $\frac{\partial c}{\partial x}$ ✱

Under what conditions is the current 0?

The Nernst Equation

NPE : $I = -\left(\nu Z^2 F [c] \frac{\partial V}{\partial x} + \nu Z R T \frac{\partial c}{\partial x}\right)$

when $I = 0$:-

$$\frac{\partial V}{\partial x} = -\frac{RT}{ZF} \frac{1}{[c]} \frac{\partial [c]}{\partial x}$$

Integrate on both sides

$$\int_{x_1}^{x_2} \frac{\partial V}{\partial x} dx = -\frac{RT}{ZF} \int_{x_1}^{x_2} \frac{\partial [c]}{[c] \cdot \partial x} \cdot dx$$

$$\int_{V_1}^{V_2} dV = -\frac{RT}{ZF} \int_{c_1}^{c_2} \frac{d[c]}{[c]}$$

$$V_2 - V_1 = -\frac{RT}{ZF} \ln\left(\frac{c_2}{c_1}\right)$$

Define membrane potential

$V_m = V_{in} - V_{out}$

$V_2 - V_1$

$$\Rightarrow V_{in} = -\frac{RT}{ZF} \ln\left(\frac{[c_{in}]}{[c_{out}]}\right)$$

$V_{out} = 0$ when membrane is at rest i.e. equilibrium potential

$$\therefore \boxed{V_{in} = \frac{RT}{ZF} \ln\left(\frac{c_o}{c_{in}}\right)}$$

For potassium : $c_i \gg c_o$ $\Rightarrow$ Potential is negative

Problems —

1. $T = 20^\circ C$ $Z = +1$ $E_i = 58\ mV$ $\log_{10}\left(\frac{c_o}{c_i}\right) = ?$

$T = 37^\circ C$ $Z = +1$, $E_i = 62\ mV$ $\log_{10}\left(\frac{c_o}{c_i}\right) = ?$

$\log_b x = \frac{\log_a x}{\log_b a}$: changing base of the log

(12)

2. Calculate E_i for squid giant axon

ion species	$[C_i]$ mM	$[C_o]$ mM
K	400	20
Na	40	440
Cl^-	40	560
Ca^{2+}	0.4×10^{-4}	10

15/9/21

Lecture 4 Assignment

01. $$V_{in} = \frac{RT}{ZF} \ln\left(\frac{C_o}{C_i}\right) = 2.3 \frac{RT}{ZF} \log_{10}\left(\frac{C_o}{C_i}\right)$$

a) $58 \times 10^{-3} = 2.3 \times \frac{8.31 \times 293}{1 \times 96480} \times \log_{10}\left(\frac{C_o}{C_i}\right) \Rightarrow \log_{10}\left(\frac{C_o}{C_i}\right) = 0.99923$

b) $62 \times 10^{-3} = 2.3 \times \frac{8.31 \times 310}{1 \times 96480} \times \log_{10}\left(\frac{C_o}{C_i}\right) \Rightarrow \log_{10}\left(\frac{C_o}{C_i}\right) = 1.009$

02.

Ion species	$[C_o]$	$[C_i]$	Z	$\log_{10}\left(\frac{C_o}{C_i}\right)$	E_i
K^+	20	400	+1	-1.3	-75.4 mV
Na^+	440	40	+1	1.04	+60.3 mV
Cl^-	560	40	-1	1.14	-66.2 mV
Ca^{2+}	10	0.4×10^{-4}	+2	5.4	156.7 mV

T = 293 K
R = 8.31
F = 96480

16/9

Lecture 5

Ion distribution

The inhomogenous distribution results from active & passive transport

1. Na-K pump
   - uses ATP
   - seen in most cell types
   - primary reason for difference in conc. of Na & K

$3Na^+ \rightarrow$ pumped out
$2K^+ \rightarrow$ pumped in

$\Rightarrow [Na^+]_o >> [Na^+]_i$ and $[K^+]_o << [K^+]_i$

2. Na - Ca exchangers - it doesn't use ATP
- it takes advantage of conc gradient of Na.

Na^+ → in
Ca^{2+} → out

3. Plasma membrane calcium ATPAse (PMCA) and SERCA
PMCA pumps excess calcium in cytosol to the outside,
but & SERCA pumps excess Ca^{2+} inside the ER.
Ca^{2+} is both chemically & electrically active so Ca levels in the cell are closely regulated.

Passive properties
* Cell membrane is selectively permeable to ions.
At resting membrane potential, cell is most permeable to K^+ & very little permeable to Na^+, Ca^{2+} & kinase proteins, & Cl^- (-vely charged) and anions like SO_4^{2-}.
Anions & -ve proteins are present inside the cell

Donan equilibrium
If there are no active transporters and the membrane is sufficiently permeable to several ions, then these ions are said to be passively distributed. The reversal of Nernst potential is the action potential.
Membrane potential of the cell is equal to Nernst potential of each of these ions

$$V_{in} = \frac{RT}{zF} \ln\left(\frac{C_o}{C_i}\right)$$

Donan Rule says: If C^{+m} (cations) and A^{-n} is anions,

$$V_{in} = V_C = V_A$$

$$\boxed{\frac{1}{m} \ln\left(\frac{C_o}{C_i}\right) = -\frac{1}{n} \ln\left(\frac{A_o}{A_i}\right)}$$

$$\Rightarrow \left(\frac{C_o}{C_i}\right)^{1/m} = \left(\frac{A_i}{A_o}\right)^{1/n} \quad - (*)$$

If membrane is permeable to K and Cl — $\frac{[K_o]}{[K_i]} = \frac{[Cl_i]}{[Cl_o]}$ — (1)

Using space charge neutrality (ignore Na^+) –

$$[K_i] = [Cl_i] + [A_i] \quad -(2)$$

$$[K_o] = [Cl_o]$$

Using (1) from prev. page.

$$[Cl_i] = [Cl_o]\frac{[K_o]}{[K_i]} = \frac{[K_o]^2}{[K_i]}$$

Using (2) in this,

$$[K_i] = \frac{[K_o]^2}{[K_i]} + [A_i]$$

$$\Rightarrow [K_i]^2 = [K_o]^2 + [A_i][K_i]$$

$$\Rightarrow [K_i] \gg [K_o]$$

$$[Cl_i] \quad [Cl_o]$$

Just from permeabilities, we can say that K ~~and Cl~~ inside the cell is much greater than outside. Cl is just the opposite.

Example

Consider we have 2 compartments, separated by a semipermeable membrane – not permeable to A^-

	I (i)	II (o)
A^-	100	0
K^+	150	150
Cl^-	50	150

At what conc. will the compartments reach electrochemical equilibrium.

$$\frac{[Cl_i]}{[Cl_o]} = \frac{[K_o]}{[K_i]} \Rightarrow \frac{K_i}{K_o} = \frac{Cl_o}{Cl_i}$$

$$\Rightarrow \frac{[150+x]}{[150-x]} = \frac{[150-x]}{[50+x]} \Rightarrow (150-x)^2 = (50+x)(150+x)$$

$$x^2 - 300x + 22500 = x^2 + 200x + 7500$$

$$x = 30$$

New distribution:

A	=	100 0
K	=	180 120
Cl	=	80 120

J

Lecture 6 - Subitha

In NPE, we didn't really account for selective permeability of membranes.

Membrane permeability P

$$J = -P\,\Delta[c] \quad -(1) \quad \text{(empirically)}$$

J : mol/cm² sec

P : cm/sec

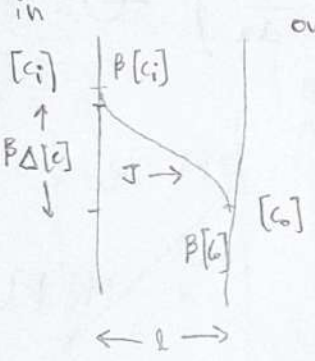
$$J = -D\,\frac{d[c]}{dx} \quad -(2)$$

Lets assume that [c] drops linearly within a membrane -

$$\frac{d[c]}{dx} = \beta\,\frac{\Delta[c]}{l} \quad -(3)$$

β : (constant) water-membrane partition coefficient (no unit)

l : width/thickness of membrane (cm)



Using (2) & (3)

$$J = -\beta\,\frac{\Delta[c]}{l}\cdot D \quad -(4)$$

Comparing with (1)

$$\boxed{P = \frac{D\beta}{l}} \quad -(5)$$

From Einstein's relation :

$$D = \frac{\mu kT}{q}$$

$$D = \frac{\nu RT}{F} \qquad \text{where } \nu = \frac{\mu}{N_A} \quad F = qN_A \quad R = N_A \cdot k$$

So, $$\boxed{P = \frac{\beta \nu RT}{lF}}$$

$P \propto \frac{\text{mobility, coefficient}}{\text{width}}$.

Goldman - Hodgkin - Katz Equation

Adding sophistication to NPE, GHK allows us to consider reversal potential at rest and selective permeability of multiple ion species while talking about reversal potential.

Assumptions –

1. Within the cell membrane, NPE is valid - through the thickness of cell membrane
2. Ions do not interact with each other
3. Electric field is constant within the membrane.

$$E = -\frac{dV}{dx} = -\frac{V}{l}$$

So. GHK is also called the const. field model

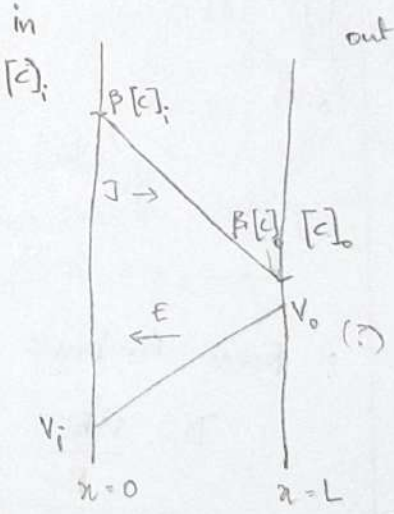
Based on NPE, we know –

$$I = -\left(\nu z^2 F [c] \frac{dV}{dx} + \nu z RT \frac{d[c]}{dx} \right) \quad -(2)$$

$$\frac{dV}{dx} = \frac{V}{l}$$

Voltage current Conc

$$I = \nu z^2 F [c] \frac{V}{l} - \nu z RT \frac{d[c]}{dx}$$



Define a new variable –

$$y = I - \frac{\nu z^2 F [c] V}{l} \quad -(4)$$

$$\frac{dy}{dx} = \frac{dI}{dx} - \frac{\nu z^2 F V}{l} \frac{d[c]}{dx}$$

At steady state – $\frac{dI}{dx} = 0$

$$\frac{dy}{dx} = -\frac{\nu z^2 F V}{l} \frac{d[c]}{dx} \quad -(5)$$

From (2) and (4) –

$$y = -\nu z RT \frac{d[c]}{dx} \Rightarrow \frac{d[c]}{dx} = \frac{-y}{\nu z RT} \quad -(6)$$

(?)

⇒ $\frac{dV}{dx}$: negative −ve

$\frac{d[c]}{dx}$: +ve

From (5) $\frac{d[c]}{dx} = \frac{-1}{\nu z^2 \beta F V} \frac{dy}{dx}$

So (6) becomes –

$$y = -\nu z RT \cdot \frac{-1}{\nu z^2 F V} \frac{dy}{dx}$$

$$\frac{dy}{dx} = \frac{zFV}{lRT} y$$

$$\frac{lRT}{zFV} \int_{y(x=0)}^{y(x=l)} \frac{1}{y} dy = \int_{x=0}^{l} dx$$

$$\frac{lRT}{zFV} \Big|_{y(x=0)}^{y(x=l)} \ln y = l$$

$y = I - \frac{\nu z^2 F [c] V}{l}$

At $x=0$, $[c]_{x=0} = \beta [c]_i$

$x = l$ $[c]_{x=l} = \beta [c]_0$

$$\frac{zFV}{RT} = \ln \left[\frac{I - \frac{\nu z^2 F [c]_0 V \beta}{l}}{I - \frac{\nu z^2 F [c]_i V \beta}{l}} \right]$$

Let's call $\boxed{\frac{zFV}{RT} = \Xi}$

$$e^{-\Xi} = \frac{Il - \nu z^2 F V \beta [c]_i}{Il - \nu F z^2 V \beta [c]_0}$$

This flips ∵ of taking negative exponent

Solving for I –

$$Il e^{-\Xi} - \nu z^2 F V \beta [c]_0 e^{-\Xi} = Il - \nu z^2 F V \beta [c]_i$$

$$I l \left(e^{-\Xi} - 1\right) = \nu z^2 F V \beta \left([c]_0 e^{-\Xi} - [c]_i\right)$$

$$I = \frac{\nu z^2 F V \beta}{l} \frac{\left([c]_i - [c]_0 e^{-\Xi}\right)}{\left(1 - e^{-\Xi}\right)}$$

$P = \frac{\beta \nu RT}{lF}$

$$I = \frac{\nu z \Xi RT \beta}{l} (\cdots) = PFz\Xi \left(\frac{[c]_i - [c]_0 e^{-\Xi}}{1 - e^{-\Xi}} \right) \quad \Xi = \frac{zFV}{RT}$$

$$I = PFZ\Sigma \left[\frac{[c]_i - [c]_o e^{-\xi}}{1 - e^{-\xi}} \right]$$

: Similar to Ohm's law - I-V relationship, but not linear.

Here, we've added electrochemical forces and the permeability of the membrane

This is the GHK current equation, which can be written as

$$I = I_{out} - I_{in}$$

$$I_o = FFZ\Sigma \frac{[c]_i}{1 - e^{-\xi}} \qquad I_{in} = - PFZ\Sigma \frac{[c]_o e^{-\xi}}{1 - e^{-\xi}}$$

★ Case I : $[c]_o = [c]_i$

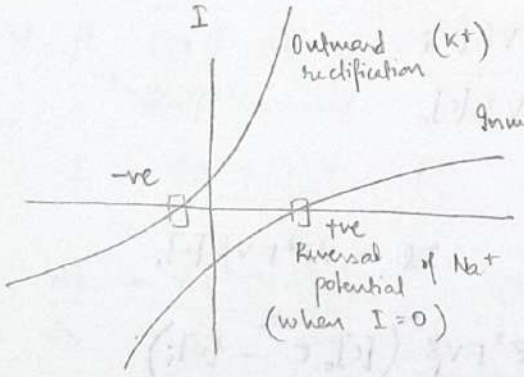
$\Rightarrow I = PFZ\Sigma [c]_o$ $\Rightarrow$ IV relation is linear

★ Case II : $[c]_i \gg [c_o]$ ~ k^+ ion : called Outward rectified

here IV is not linear - but how will slope change?

The slope becomes steeper ∴ its easy for k^+ to escape out as V increases.

★ Case III : $[c]_i \ll [c_o]$ ~ Na^+ ion : Inwardly rectified



Reversal potential -
+ve for Na^+
-ve for K^+

Lecture 7

GHK current equation

One of the assumptions : movement of ions is independent of each other.

$$I = I_K + I_{Na} + I_{Cl}$$

Remember : $I = PZF\Xi \dfrac{([ci] - [c]_o e^{-\Xi})}{(1 - e^{-\Xi})}$

So, $I = P_K Z_K \Xi_K \dfrac{([K]_i - [K]_o e^{-\Xi_K})}{(1 - e^{-\Xi_K})} + P_{Na} Z_{Na} \Xi_{Na} \dfrac{([Na]_i - [Na]_o e^{-\Xi_{Na}})}{(1 - e^{-\Xi_{Na}})}$

$+ P_{Cl} Z_{Cl} \Xi_{Cl} \dfrac{([Cl]_i - [Cl]_o e^{-\Xi_{Cl}})}{(1 - e^{-\Xi_{Cl}})}$

What happened to F ??

Z_{Cl} is -ve

WKT, $Z_{Na} = Z_K = -Z_{Cl} = Z$ $\quad \Xi = \dfrac{ZFV}{RT}$ $\Rightarrow \Xi_K = -\Xi_{Cl} = \Xi$

So, $I_{Cl} = P_{Cl} FZ\Xi \dfrac{([Cl]_o - [Cl]_{in} e^{-\Xi})}{(1 - e^{-\Xi})}$ $\Rightarrow \dfrac{[Cl]_i - [Cl]_o e^{\Xi}}{1 - e^{\Xi}}$ $\Rightarrow \dfrac{[Cl]_i - [Cl]_o / e^{-\Xi}}{1 - 1/e^{-\Xi}}$ $\rightarrow \left(\dfrac{[Cl]_i e^{-\Xi} - [Cl]_o}{e^{-\Xi} - 1}\right)$

We define : $y = [K]_i + \dfrac{P_{Na}}{P_K}[Na]_i + \dfrac{P_{Cl}}{P_K}[Cl]_o$

$w = [K]_o + \dfrac{P_{Na}}{P_K}[Na]_o + \dfrac{P_{Cl}}{P_K}[Cl]_i$

We can write total current as —

$I = P_K ZF\Xi \dfrac{(y - we^{-\Xi})}{(1 - e^{-\Xi})}$: GHK current equation.

Resting potential is when $I = 0 \Rightarrow y = we^{-\Xi}$

$\Rightarrow \Xi = \ln\left(\dfrac{w}{y}\right) = \dfrac{ZVF}{RT}$

$$V = \frac{RT}{F} \ln\left[\frac{P_K[K]_o + P_{Na}[Na]_o + P_{Cl}[Cl]_i}{P_K[K_o]_i + P_{Na}[Na]_i + P_{Cl}[Cl]_o}\right]$$

: GHK voltage equation

This is the resting potential based on the permeabilities and concentrations of different ions.

Squid Giant Axon

	inside	outside
K	400	10
Na	50	560
Cl	40	540

$T = 20^\circ C$

$P_K : P_{Na} : P_{Cl} = 1 : 0.03 : 0.1$

$$V = \frac{8.30 \times 293}{96840} \ln \left[\frac{10 + 0.03(560) + 0.1(40)}{400 + 0.03(50) + 0.1(540)} \right]$$

$$V = 0.058 \log_{10} \left(\frac{30.8}{455.5} \right) = 58\,mV \times \log_{10}(0.0676)$$

$V = 58\,mV \times (-1.17)$

$\therefore V = -67.8\,mV$ 　　S's axon : $-70\,mV$

The resting potential is v. close to potential of K.

During an AP, permeability is v. diffent —

$P_K : P_{Na} : P_{Cl} = 1 : 15 : 0.1$

$$V = 0.058 \times \log_{10} \left(\frac{10 + 15(560) + 0.1(40)}{400 + 15(50) + 0.1(540)} \right)$$

$$V = 0.058 \times \log_{10} \left(\frac{8450}{1204} \right)$$

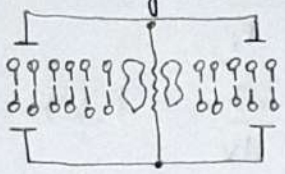
$\therefore V = +49\,mV$

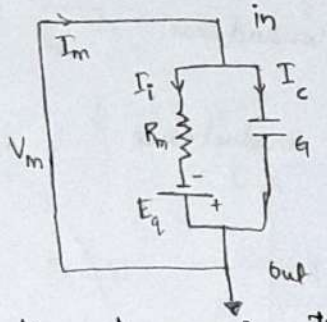
Summary —

1. Na, K, Cl, Ca — heterogeneous distribution & movement
2. 4 physical laws — Fick's, Ohm's, Einstein relat^n, Space-charge ment.
3. NPE and NE — reversal potential
4. Adding permeability we get : GHK : current & voltage

Lecture 8 (Sutirtha)

Electrically excitable membranes.





Now we're bringing in active transport. Think of changing permeability as voltage dependent ion channel

Kirchoff's laws – total current can be written as –

$$I_m = I_c + I_i \quad \rightarrow \text{Ionic current}$$

$$\boxed{I = C_m \frac{dV_m}{dt} + \frac{(V_m - E_r)}{R_m}}$$

E_r : reversal potential

$\frac{1}{R_m} = G_m$ Conductance

I : A/cm^2

G_m : S/cm^2

V_m : membrane potential.

Ionic current –

$$I_i = I_{Na} + I_k + I_{Cl} + I_{Ca}$$

Each ionic current is associated with a channel –

$$I_{Na} = g_{Na} \underbrace{(V_m - E_{Na})}_{\text{driving force.}}$$

Greater the difference b/w voltages, greater the current driving the ions

there, $E_{Na} = \frac{RT}{zF} \ln \frac{[c]_o}{[c]_i}$

Membrane conductances

They simulate ion channels

$G_m = \frac{1}{R_m}$

Conductance can be voltage dependent, ligand dependent & they can be time dependent (i.e. they can open instantaneously or sluggishly).

I_c : capacitor current

I

Linear conductance

$$I_i = G_m (V_m - E_i)$$

$$G_m = \frac{I_i}{V_m - E_i} \quad : \text{Chord conductance}$$

$$G_m = \frac{dI}{dV_m} \quad : \text{Slope conductance}$$

In linear membranes, chord = slope conductance

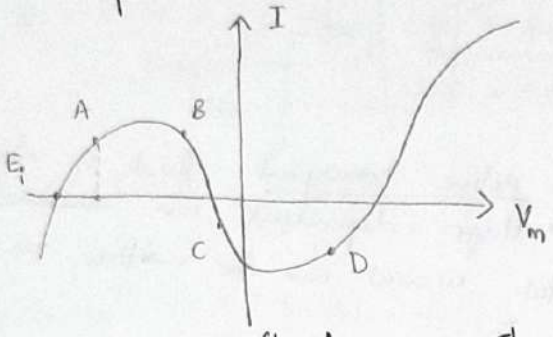
This means : $\frac{I_i}{V_m - E_i} = \frac{dI}{dV_m}$

II Non-linear membranes (I is a fn of voltage)

$I = F(V)$

Chord conductance : $G = \frac{F(V)}{V_m - E_i}$

Slope conductance : $G = \frac{dI}{dV} = F'(V)$



: Change in current in a channel with multiple ions – because of several E_is

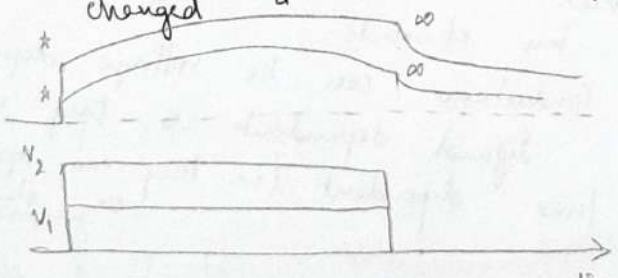
	Chord	Slope
A	+ve	+ve
B	+ve	-ve
C	-ve	-ve
D	-ve	+ve

Chord → amplitude of I at any given V

Slope → small variation in I wrt. V

III Time varying currents (I is a non-linear fn of V_m, t)

Two types of measurements – I just before V is changed & I in steady state.



* instantaneous value

∞ steady state

I^* & I^∞ are not functions of time

Instantaneous currents –

Chord $G^* = \frac{I^*}{V_m - E_i} = f(V, t^*)$

Slope $\frac{dI}{dV} = f'(V, t^*)$

Steady state

chord $\quad g^{\infty} = \dfrac{I^{\infty}}{V_m - E_i} = f(V, t^{\infty})$

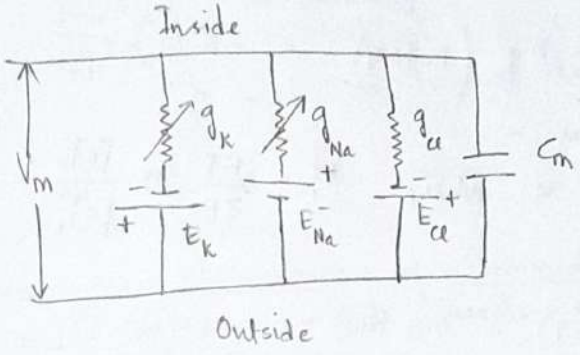
Slope $\quad \dfrac{dI^{\infty}}{dV} = f'(V, t^{\infty})$

??? Some observations –

1. $I_i = f(V, E_i, t) > 0 \quad E_i > 0$ – conductance is always positive when $E_i > 0$ (?)

2. $I_i = f(V, E_i, t) > 0$ when $E_i > 0$
   $I = f(V, E_i, t) < 0$ when $E_i < 0$
   } Single reversal potential and direction of current

Parallel Conductance Model



$$I = I_c + I_{Na} + I_K + I_{Cl}$$

$$I = C_m \frac{dV}{dt} + g_K (V_m - E_K) + g_{Na} (V_m - E_{Na}) + g_{Cl} (V_m - E_{Cl})$$

This is the equivalent electrical circuit description of a neuronal membrane permeable to several ions

At steady state, $I_{total} = 0 \quad \dfrac{dV}{dt} = 0$

$$V = \frac{g_K E_K + g_{Na} E_{Na} + g_{Cl} E_{Cl}}{g_K + g_{Na} + g_{Cl}}$$

Similar to GHK
V is a fn of conductances and reversal potentials

Example — Aplysia

	$[c]_i$	$[c]_o$	E_i	
K	168	6	-83.9	$P_K : P_{Na} : P_{Cl} = 1 : 0.019 : 0.381$
Na	50	337	48	$g_K = 0.5$
				$g_{Na} = 0.11$
Cl	41	340	(+)53.3	$g_{Cl} = 0.32$

Find V_{rest} according to GHK, parallel conductance:
What would be the effect of 10 fold increase in $[K]_o$?

★ GHK : $V = \frac{RT}{F} \ln \left(\frac{P_K [K]_o + P_{Na} [Na]_o + P_{Cl} [Cl]_o}{P_K [K]_i + P_{Na} [Na]_i + P_{Cl} [Cl]_i} \right)$ wrong

So $V = 0.058 \times \log_{10} \left[\frac{6 + 6.4 + 129.5}{168 + 0.95 + 15.6} \right] = 0.058 \times \log_{10} \left(\frac{141.9}{184.6} \right)$

$V = 0.058 \times \log_{10} (0.769) = -6.61 \text{ mV}$

★ Parallel conductance –

$V = \frac{\Sigma g_i E_i}{\Sigma g_i}$ × where $E_i = \frac{RT}{zF} \ln \frac{[c]_o}{[c]_i}$

$V = \frac{0.5(-83.9) + 0.11(48) + 0.32(+53.3)}{0.93}$ $\because z_{Cl} = -1$

$V = \frac{-41.95 + 5.28 + 17.056}{0.93} = -21.1 \text{ mV}$

★ 10 × increase in $[K]_o$

By GHK, $V = -1.5$ mV

In parallel conductance, $E_K = -25.9$

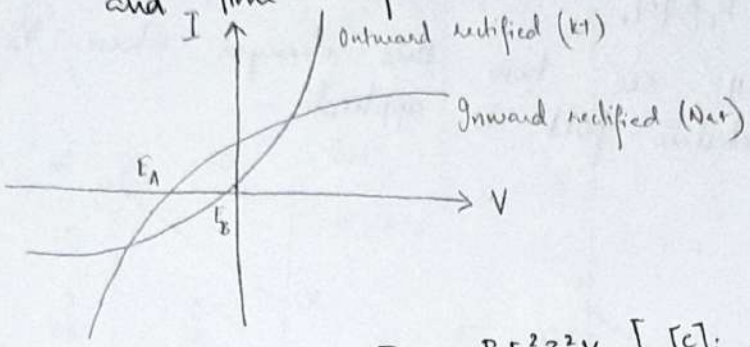
$\Rightarrow V = +10$ mV

These two are ~~same~~ descriptions of the same system through different approaches, adding more complexity at each turn.

Lecture 9

Non linear excitable membranes

Non linearity can come from 2 sources - voltage and time dependence



GHK current eqn : $I = \frac{PF^2Z^2V}{RT}\left[\frac{[c]_i - [c]_o e^{-\frac{ZVF}{RT}}}{1 - e^{-\frac{ZVF}{RT}}}\right]$

If $\frac{[c]_o}{[c]_i} < 1$ → outward rectified

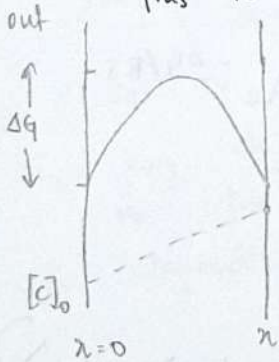
$\frac{[c]_o}{[c]_i} > 1$ → inward rectified

Here we assumed that voltage drop across membrane was linear.

But there are observations of inward rect. when $\frac{[c]_o}{[c]_i} < 1$ and converse which needs extra explanation

Energy Barrier model

This is a thermodynamics approach - mesoscopic physics



$$\beta[c]_o \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \beta[c]_i$$

Arrhenius equation :

$$k = Ae^{-E_A/RT}$$

Here, the ions have to cross the energy barrier to jump back and forth between the membrane at rate of k_1 and k_2

At thermodynamic equilibrium – rate coefficients k_1, k_2 can be related to free energy by –

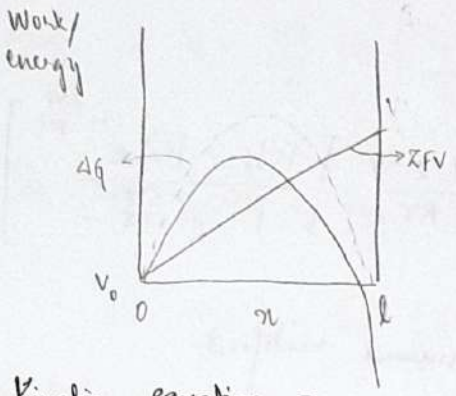
$$k_1 = A e^{\Delta G/RT} \qquad k_2 = A e^{\Delta G/RT}$$

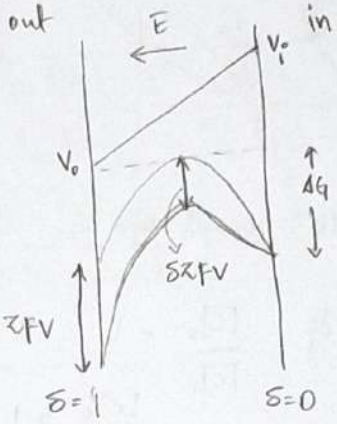
$k_1 = k_2$, assuming that ΔG is the same.

Using mass action law,

$$J_1 = k_1 \beta [c]_0 \qquad J_2 = k_2 \beta [c]_i$$

Next, we'll see how this changes when V_m changes ie electric field is applied –





Kinetic equation –

$$\beta [c]_0 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \beta [c]_i$$

$$k_1 = A e^{-(\Delta G + zFV - \delta zFV)/RT}$$

$$k_2 = A e^{-(\Delta G - \delta zFV)/RT}$$

So –

$$k_1 = k_0 e^{-(1-\delta) zFV/RT}$$

$$k_2 = k_0 e^{+\delta zFV/RT}$$

where $k_0 = A e^{-\Delta G/RT}$

δ : fractional influence of V on ΔG.

It accounts for non-uniform influence of the membrane on ΔG.

If $\delta = 1$, the peak of energy barrier is on the left boundary

If $\delta = 0$, the energy barrier peak is on the opp. side

Using this, we write the fluxes –

$$J_1 = k_1 \beta [c]_0$$

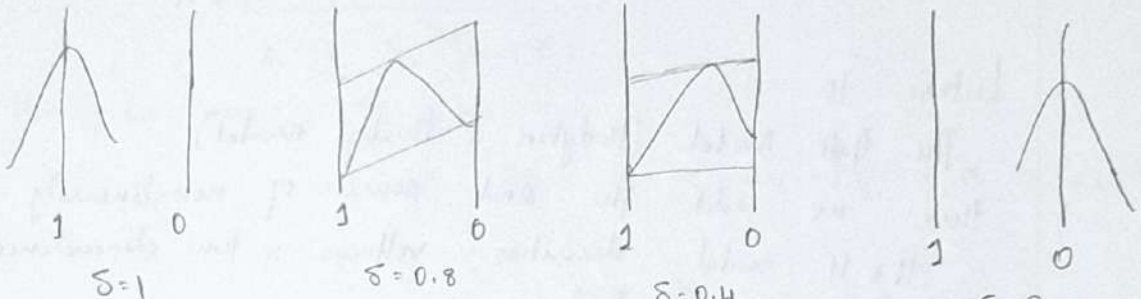
$$J_2 = k_2 \beta [c]_i$$

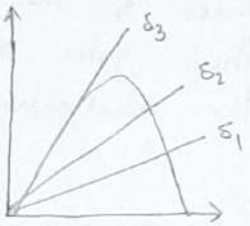
$$I = zF (J_{out} - J_{in})$$

$$* \quad I = zF\beta k_0 \left([c]_i e^{\delta zFV/RT} - [c]_0 e^{-(1-\delta) zFV/RT} \right) \quad *$$

Adding ef line to Gaussian? Is that what we did?

δ : a factor of asymmetry that gives fractional influence of V on ΔG





$W = \Delta G + \delta$

Adding gaussian to st lines with different slopes

$y = mx + c \qquad c = 0$

$\boxed{y = \delta . ZFV}$

★ When $\delta = 0$ ⇒ barrier inside (δ said outside!)

$$I = ZF\beta k_0 \left([c]_i - [c]_0 e^{-ZFV/RT} \right)$$

So IV relation is inward rectified

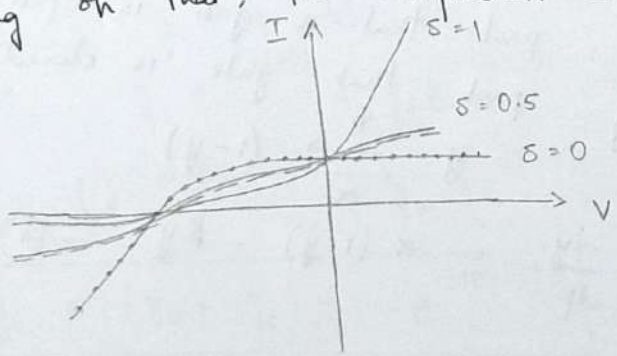
★ $\delta = 1$ ⇒ barrier is outside the membrane

$$I = ZF\beta k_0 \left([c]_i e^{\delta ZFV/RT} - [c]_0 \right)$$

So IV relation is outward rectified

★ For any other value of δ, IV rectification depends on the ratio of $[c]_i : [c]_0$

So depending on that, the rectification changes.



For $\frac{[c]_0}{[c]_i} = \frac{1}{53}$

(08)

At reversal potential, $I = 0$

So, we get —

$$[c]_i e^{5zFV/RT} = [c]_o e^{-(1-5)zFV/RT}$$

$$\Rightarrow \text{we a} \quad V = \frac{RT}{zF} \ln \frac{[c]_o}{[c]_i}$$

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Lecture 10

The Gate Model (Hodgkin & Huxley model)

Here, we add the 2nd source of non-linearity : time

H & H model describes voltage & time dependence of ion conductance

Each gate has single energy barrier & they open/close in response to voltage. These gates are channels i.e. ions can only traverse the membrane when these gates are open

Assumptions

1. Ions always flow down conc. gradient
2. Ion channel pore have gates that are controlled by voltage dependent
3. Movement of each gate is described by a single barrier.
4. Each ion channel responds to changes in electric field by opening / closing —

$$\text{Open} \underset{\alpha(V)}{\overset{\beta(V)}{\rightleftharpoons}} \text{Close} \qquad \alpha, \beta$$

5. Reaction between open & close follow 1st order kinetics.

Model

y : prob. that a gate is open

$1-y$: prob. that gate is closed

$$y \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} (1-y)$$

$$\frac{dy}{dt} = \alpha(1-y) - \beta y \quad - (1)$$

At steady state, $y = y_\infty$ and $\frac{dy}{dt} = 0$

So, $\boxed{y_\infty = \frac{\alpha}{\alpha + \beta}}$ where α & β are rates

$$\alpha = y_\infty (\alpha + \beta)$$

We can write $\frac{dy}{dt}$ as —

$$\frac{dy}{dt} = \alpha(1-y) - \beta y = \alpha - \alpha y - \beta y = \alpha - y(\alpha+\beta)$$

$$\frac{dy}{dt} = y_\infty(\alpha+\beta) - y(\alpha+\beta)$$ Integrating —

$$\int \frac{dy}{y_\infty - y} = (\alpha+\beta) \int dt$$

$$-\ln(y_\infty - y) = (\alpha+\beta)t + c$$

$$\Rightarrow y_\infty - y = e^{-(\alpha+\beta)t + c}$$

$$y = y_\infty - Ae^{-(\alpha+\beta)t}$$

* Say, at $t=0$, $y(t=0) = y_0$ (initial prob. of open channel)

So, $A = y_\infty - y_0$

We get —

$$\boxed{y(t) = y_\infty - (y_\infty - y_0)\, e^{-(\alpha+\beta)t}} \quad -(1)$$

This gives us the time-varying prob. of openness of the gate.

Add & subtract y_0 to (1)

$$y(t) = y_\infty - y_\infty e^{-(\alpha+\beta)t} + y_0 e^{-(\alpha+\beta)t} - y_0 + y_0$$

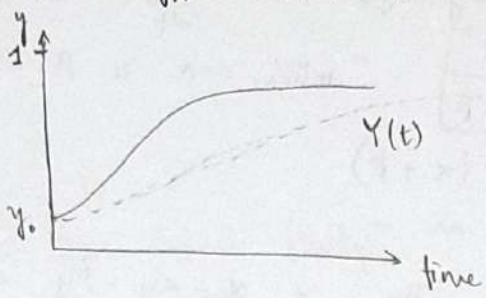
$$= y_0\left(e^{-(\alpha+\beta)t} - 1\right) - y_\infty\left(e^{-(\alpha+\beta)t} - 1\right) + y_0$$

$$\boxed{y(t) = y_0 + (y_\infty - y_0)\left(1 - e^{-(\alpha+\beta)t}\right)}$$

If there are p independent gates —

$$Y(t) = [y(t)]^p$$

$$y(t) = y_\infty - (y_\infty - y_0)\, e^{-(\alpha+\beta)t}$$

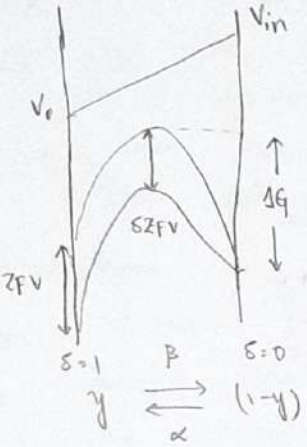


$y_\infty = \frac{\alpha}{\alpha+\beta}$ where α, β are voltage dependent rates

so for different V, y_∞ is different

Voltage Dependence

But we assumed that each gate has a single energy barrier



From energy barrier model, WKT,

$$\alpha = A e^{-(\Delta G - \delta z F V)/RT}$$

$$\alpha = \alpha_0 e^{\delta z F V/RT}$$

$$\beta = \beta_0 e^{-(1-\delta) z F V/RT}$$

is it $e^{-\delta}$??

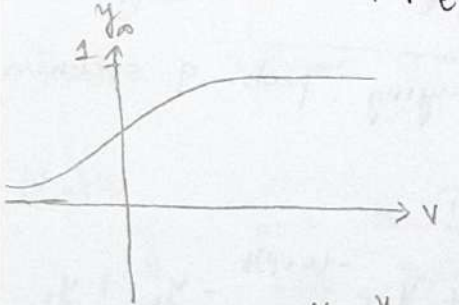
The prob. of this ~~getting~~ being open is time dependent.

$$y_\infty = \frac{\alpha}{\alpha+\beta} = \frac{\alpha_0 e^{\delta z F V/RT}}{\alpha_0 e^{\delta z F V/RT} + \beta_0 e^{-(1-\delta) z F V/RT}}$$

Simplify: if $\alpha_0 = \beta_0$ —

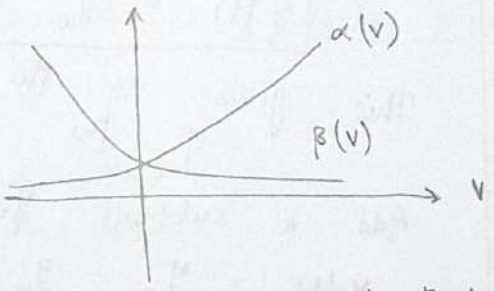
$$y_\infty(V) = \frac{1}{1 + e^{-zFV/RT}}$$

— makes sense if ⊖ are not there



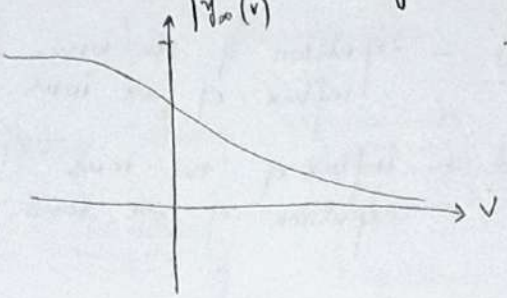
For a value of V, y_∞ settles at a different value

This is a depolarization activated gate

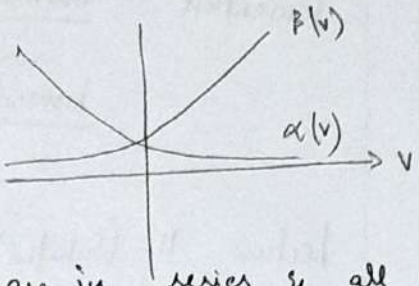


As V increases, p that a gate is open increases

What if some gates close as membrane depolarises —



hyperpolarisation activated gate



We consider that all the gates are in series & all need to be open for ions to cross from one side of the membrane to another.

Hodgkin & Huxley Analysis of Squid Giant Axon

- Electrical signals are transmitted b/w cells
- Passive spread of graded potentials (over short distances)
- Propagation of all or none (over long distances)
- Ions diffuse down the electrochemical gradient
- AP is generated by voltage & time dependent conductances

Best studied preparation : Squid giant axon

Voltage clamp experiments

2 electrodes are inserted into the axon —

1. For recording transmembrane voltage
2. For passing current to the axon to keep the membrane potential constant

Reasons —

Capacitance current = 0 $\quad \because \frac{dV}{dt} = 0 \Rightarrow I_c = C\frac{dV}{dt} = 0$

By clamping, it measures the time dependent characteristics of membrane for a particular ~~current~~ voltage, how does current change

(61)

Principle: Clamp current required to keep the membrane potential constant is equal & opposite of the ionic current flowing ~~over~~ across the membrane
So this gives v. accurate measurements

Convention: outward current - expulsion of +ve ions (K^+ ions)
influx of -ve ions

inward current - influx of +ve ions (Na^+)
expulsion of -ve ions

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Lecture 11 (Sukitha)

The process of membrane excitability - the way in which transport of ions is regulated - provides a foundation for a theory to understand consciousness
The opening & closing of channels is not just a response to stimuli - they constitute a basis for perception, cognition & movement in animals.

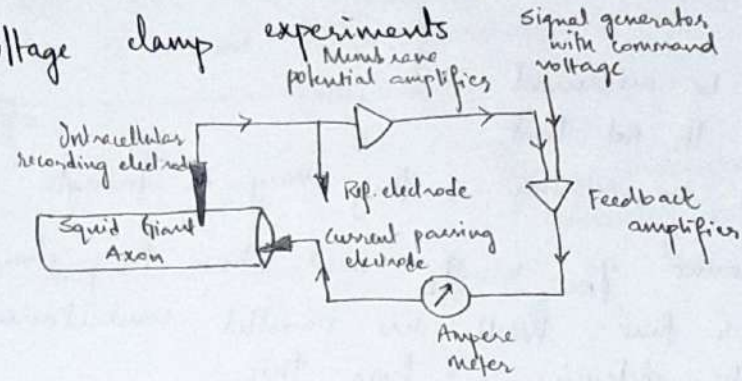
Architeuthis - the squid giant axon
Giant squid - can grow to very large size - largst invertebrate
Hard to study

Concepts so far -

- Electric property of membranes can be characterised by parameters of electrical circuits.
  Dielectric property of membrane - electric capacitance
  Ion channels - conductance
- Linear membrane - constant membrane conductance
  Non-linear membrane - conductances that vary wrt voltage & time
- Membrane voltage is determined by equilibrium potentials and relative conductances of permeant ions
- NPE, GHK model, Barries model, H & H model (Gate model)

With this, we'll describe the AP quantitatively, including its characteristics – all or none

Voltage clamp experiments

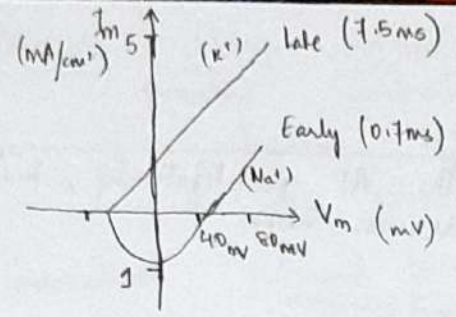


This operates by negative feedback – V_m is measured by amplifier & sends output to Feedback amplifier, which subtracts V_m from command voltage (signal generator). The voltage is maintained by sending current (equal & opp to ion current) via another electrode. This eliminates capacitance current. Also voltage & time dependence of channels can be measured.

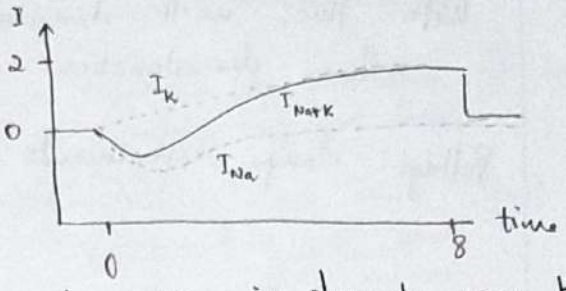
Early onset inward current – Na^+
Late onset outward current – K^+

- Around ~ -30 mV, there's a negative current ⇒ there's an influx of positive ions (Na^+) (∵ eq & opp measurement)
- At 60 mV, there's a positive current ⇒ outflux of positive ions (K^+)

→ Early onset of inward current (Na^+) & late onset of outward current (K^+)
They can be blocked by using pharmacological agents TTX and TEA



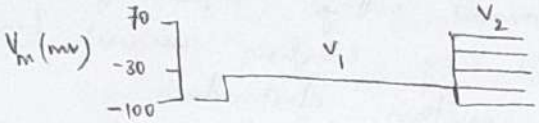
Peaks of early & late current with varying V_m



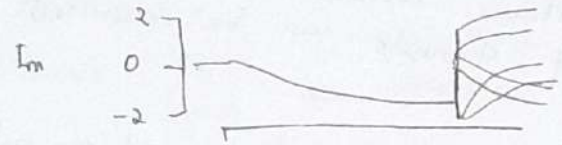
I_{Na} & I_K can be measured independently. Its not that either currents are missing — they vary in strength over time

Aim: to determine g_{Na} & g_K and how they change with voltage & time. We'll use parallel conductance formulae to determine V from this.

→ Instantaneous expts with early onset current



2 voltages applied — V_1 and then suddenly V_2



V_1 : $I_{Na_1}(V_1, t_1) = g_{Na}(V_1, t_1) \times (V_1 - E_{Na})$

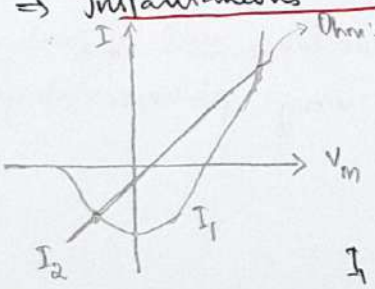
V_2 : $I_{Na_2}(V_2, t_1^*) = g_{Na}(V_2, t_1^*) \times (V_2 - E_{Na})$

Assumptn; $g_{Na}(V_1, t_1) = g_{Na}(V_2, t_1^*)$ as g_{Na} doesn't have enough time to change

We get —

$I_{Na_2} - I_{Na_1} = g_{Na}(V_1, t_1) \times (V_2 - V_1)$ — true for any V_2

⇒ Instantaneous conductances (IV curve) follow Ohm's law



Membrane exhibits instantaneous IV relations for Na+ & K+

So by measuring I_{Na} & I_K, H & H were able to measure g_{Na} & g_K

I_1 & I_2 intersect at 2 points ... (contd)

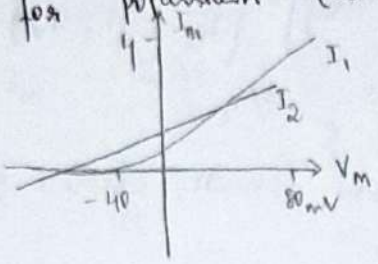
pg 69

Points of intersection

$I_1 = I_2 = 0$ when $V_1 = V_2 = E_{Na}$

But this is not true – I is slightly +ve because of some other currents.

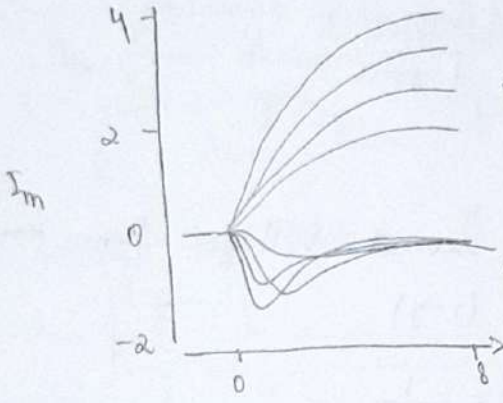
For potassium (late onset current) –



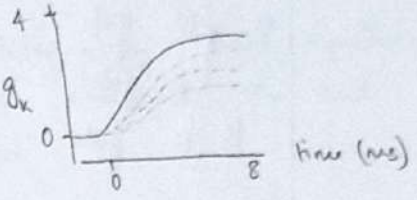
Thus, instantaneous I_{Na} & I_K currents turn out to be linear,

Each st. line will give a value of g_{Na} & g_K

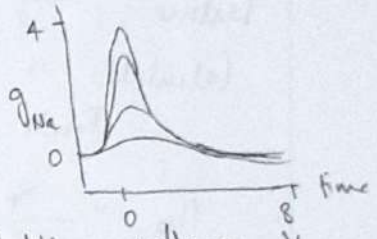
Calculating g_{Na} & g_K for every instant: response to single curve



$$g_K(V,t) = \frac{I_K(V,t)}{V - E_K}$$



$$g_{Na}(V,t) = \frac{I_{Na}(V,t)}{V - E_{Na}}$$



Each family of lines describes various holding voltages V

We use $g = \frac{I}{V-E}$ because NOT instantaneous conductance is linear

We've seen that instantaneous currents are linear –

$$I_m = C_m \frac{dV}{dt} + g_K(V,t)(V - E_K) + g_{Na}(V,t)(V - E_{Na}) + g_L(E - V_L)$$

Based on parallel conductance circuit

Also: $g_K(V,t) = \gamma_K(V,t)\,\bar{g}_K = \underline{n^4 \bar{g}_K}$

$g_{Na}(V,t) = \gamma_{Na}(V,t)\cdot\bar{g}_{Na} = \underline{m^3 h\,\bar{g}_{Na}}$ } *

$\bar{g}$: max conductance over all values

$$g_K(t) = \bar{g}_K n^4 = \bar{g}_K \left[n_0 - (n_0 - n_\infty)\left(1 - e^{-t/\tau_n}\right) \right]^4$$

4 gates

n^4 : prob of being open

4 single type of gate

For sodium, the model needed 2 types of gates — one that would open with depolarisation (m) & another that would close with depolarisation (h) —

$\bar{g}_{Na}$: Maximum conductance over all values

$$g_{Na}(t) = \bar{g}_{Na} m^3 h$$

$$= \bar{g}_{Na} \left[m_0 - (m_0 - m_\infty)\left(1 - e^{-t/\tau_m}\right) \right]^3 \left[h_\infty + (h_0 - h_\infty) e^{-t/\tau_h} \right]$$

$$= \bar{g}_{Na} m_\infty^3 h_0 \left(1 - e^{-t/\tau_m}\right)^3 e^{-t/\tau_h}$$

Since m_0 & h_∞ are negligibly small, there's good agreement with data

Na { m gate — very fast (opens when depolarised)
Na { h gate — very sluggish (closes when depolarised)
K { n gate — very sluggish (opens ⟶ ⟶)

3/10

Lecture 12

Calculate α and β for several voltages from expt. data

Recall : $y \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} (1-y)$

$$n_\infty = \frac{\alpha_n}{\alpha_n + \beta_n} \qquad \tau_n = \frac{1}{\alpha_n + \beta_n}$$

$$\Rightarrow \alpha_n = \frac{n_\infty}{\tau_n} \qquad \beta_n = \frac{1 - n_\infty}{\tau_n}$$

Calculate α & β for different values of V_c

At t_∞ i.e $t \to \infty$

$$\Rightarrow \boxed{g_K(t_\infty) = \bar{g}_K (n_\infty)^4}$$

At $t = \tau_n \Rightarrow 1 - \frac{1}{e} = 0.63$

Assume $n_0 = 0$

$$g_K(t = \tau_n) = \bar{g}_K \left(0.37 n_0 + 0.63 n_\infty\right)^4$$

Since we know value of g_K at τ_n, we can find out τ_n and use τ_n & n_∞ to find α_n

Using this, we can find α and β for given V_m.

When we plot values of α and β, it follows the gate model prediction – Refer pg 60 & 61 & 70

They used different models to fit functions to the obtained data

Hodgkin and Huxley Equations –

$$I_m = C_m \frac{dV}{dt} + \bar{g}_k n^4 (V - E_k) + \bar{g}_{Na} m^3 h (V - E_{Na}) + g_l (V - E_l)$$

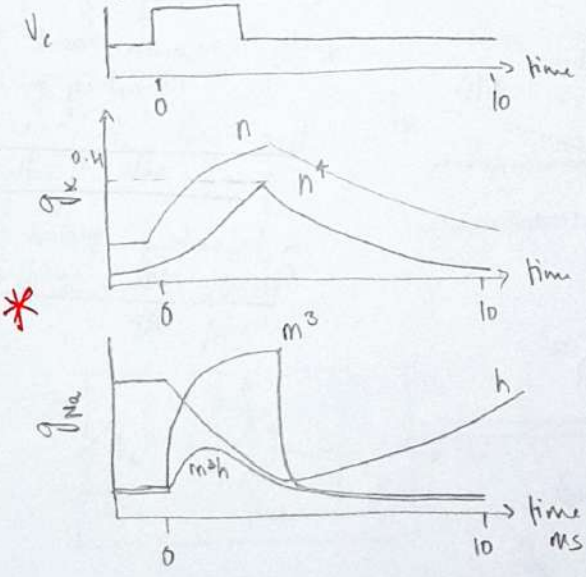
$$\frac{dn}{dt} = \alpha_n (1-n) - \beta_n n$$

$$\frac{dm}{dt} = \alpha_m (1-m) - \beta_m m$$

$$\frac{dh}{dt} = \alpha_h (1-h) - \beta_h h$$

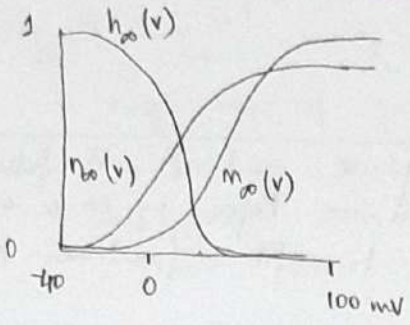
It is a 4 dimensional model. Integrating these equations will give AP. Everytime V is changed, α_n and β_n also have to be changed

Plotting the solution of m, n & h gates

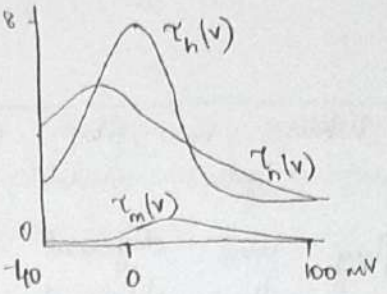


Na channels have a short time slot when they can rush in. After that, channels close

But entering phase of Na is a positive feedback loop because it depolarises the membrane



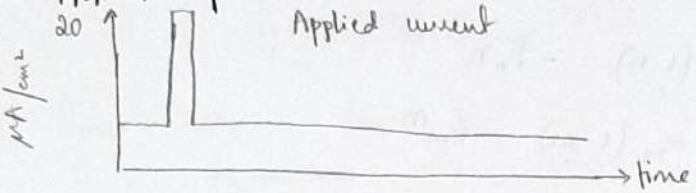
Voltage dependence

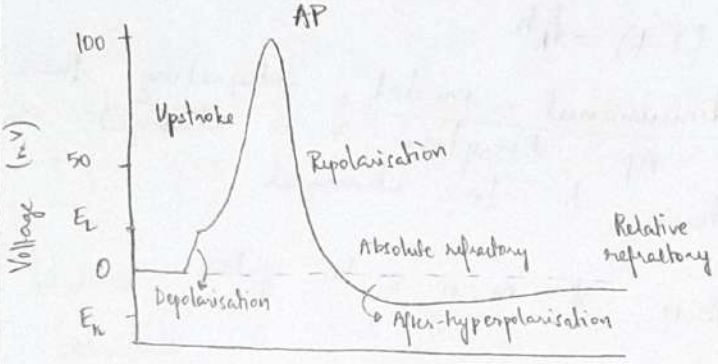


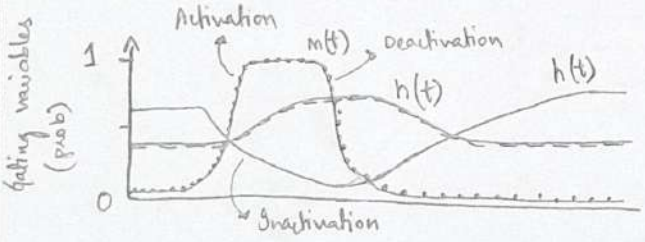
Time dependence

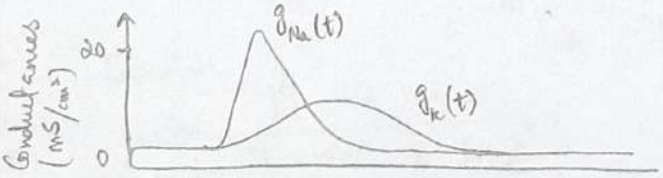
$\uparrow\tau$ => slower opening

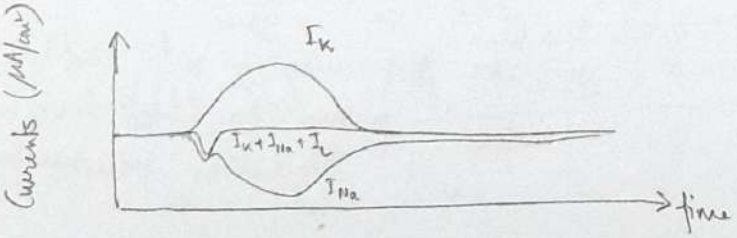
Action potential



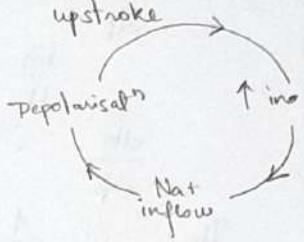








* When depolarised, g_{Na} goes up quickly, causing an upstroke



* Refractory period: this is (a) because of sluggishness of K^+ channel, which closes slowly, allowing K^+ (b) to keep going out

* The neuron can't fire in the refractory period — this sets the max. firing rate of the neuron

* Refractory period also ensures the unidirectionality of AP

Local Anesthesia

Cocaine was isolated from coca leaves in 1860 by Dr. Niemann

Lidocaine - synthetic substitute that blocks Na channels

Prevents AP by binding to VDSCs (S6 α helix domain of IV of the protein)

Recap of Lect 11 and 12

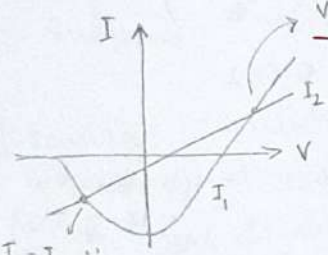
Refer to drg diagrams in Pg. 64

Measuring instantaneous current at V_1 & suddenly V_2

Early current (1.5 ms) is primarily Na (here at $V_1 = -29$ mV) and then suddenly to a range of V_2 while measuring I_1 and I_2. One can estimate IV relationship of Na^+ channels without time dependence

Pg 64 : $I_2 - I_1 = g_{Na}(V_1, t_1) \cdot (V_2 - V_1)$

g_{Na} is const for given V_1 $\Rightarrow$ IV curve is linear: Ohm's law

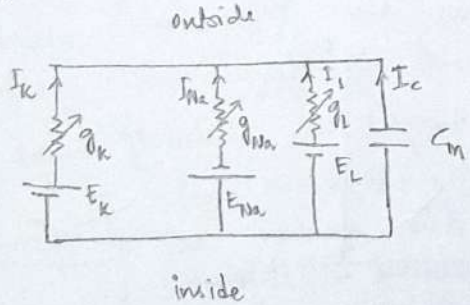


$I_1 = I_2$ ∵ $V_1 = V_2$

* Although current is not 0 — because of other ions, possibly k^+

g_{Na} can be calculated by measuring I_{Na} for some V.

H and H model



Here,

$$\frac{di}{dt} = \alpha_i(1-i) - \beta i \quad \text{where} \quad i_\infty = \frac{\alpha_i}{\alpha_i + \beta_i} \qquad \tau_i = \frac{1}{\alpha_i + \beta_i}$$

First order kinetics - n, m, h

Kirchoff's laws

$$I_m = C_m \frac{dV}{dt} + g_K(V,t)(V - E_K) + g_L(V - E_L) + g_{Na}(V,t)(V - E_{Na})$$

Gate model

$$g_K = \bar{g}_K n^4$$

$$g_{Na} = \bar{g}_{Na} m^3 h$$

Solving the differential eqns —

$$n(t) = n_0 - (n_0 - n_\infty)\left(1 - e^{-t/\tau_n}\right)$$
$$m(t) = m_0 - (m_0 - m_\infty)\left(1 - e^{-t/\tau_m}\right)$$
} Open at depolarisation

$$h(t) = h_\infty + (h_0 - h_\infty)\, e^{-t/\tau_h}$$
} Closes with depolarisation

For this, the steady state values would be —

$$h_\infty \cong 0 \qquad n_0 = m_0 \simeq 0$$

So, substituting, we get —

$$g_K(t) = \bar{g}_K \left[n_\infty^4 \left(1 - e^{-t/\tau_n}\right)^4 \right]$$

$$g_{Na}(t) = \bar{g}_{Na} \left[m_\infty^3 \, h_0 \left(1 - e^{-t/\tau_m}\right)^3 e^{-t/\tau_h} \right]$$

Based on experimental data, we find n_∞ & τ_n and from that, calculate —

$$\alpha_n = \frac{n_\infty}{\tau_n} \qquad \beta_n = \frac{1 - n_\infty}{\tau_n}$$

* When $t \to \infty$

$$g_K(t_\infty) = \bar{g}_K \, n_\infty^4 \quad \Rightarrow \quad n_\infty = \left(\frac{g_K(t_\infty)}{\bar{g}_K} \right)^{1/4}$$

* When $t = \tau_n(V_i)$ # $1 - e^{-1} = 0.632$

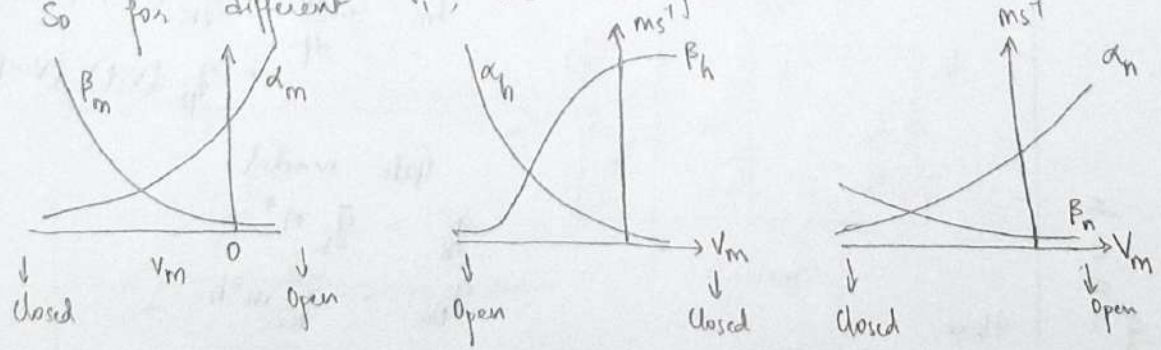
$$g_K(\tau_n) = \bar{g}_K \, n_\infty^4 \, (0.632)^4$$

$$g_K(\tau_n) = g_K(t_\infty)\,(0.632)^4$$

→ From graph, measure t at which $g_K = 0.16 \times g_K(t_\infty)$ — that is your τ_n

If we know $\tau_n(V_i)$ and n_∞, we can find α & β associated with it

So for different V_i, we can find α & β



h and n are ~ 10 times slower than m

Lecture 14 - 20/10

Synaptic transmission and Synaptic plasticity
↳ Molecular basis of neuronal communication, & learning

There's a rich dynamical repertoire of firing by neurons
- Sustained firing by stellate cells
- rapid firing, then slowing down by pyramidal cells
- burst by pyramidal neurons - very imp & useful

This repertoire is governed by the biophysical properties of ion channels and their numbers

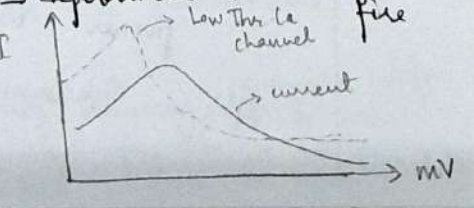
The opening of Na channels & refractory period is because of K^+ channel

There are a huge variety of channels which show a range of voltage & time dependence

→ Sodium channel (slow)
Opens at smaller depolarisation & hence amplifies them
Sustains repetitive firing & bursting

→ Potassium currents
- Greatest diversity of ion channels - huge repertoire
- It has a hyperpolarizing effect ⇒ slows down firing
- These K channels decide the refractory period & act opposite to Na channels

→ Calcium channels ∵ very few Ca channels
Hard to characterize them, but there are mainly 2 types -
- Low threshold Ca current - post-inhibitory ~~rebound~~ rebound
If the membrane goes to v. low / negative potentials, the the neuron will fire a few times & go back to normal - because there Ca channels open slow closing time ⇒ depolarises & makes neuron fire



- High threshold Ca channel

Hyperpolarising current suddenly switched on/off leads to spiking : triggered by inhibitory input
LT Ca channel opens. Ca comes in, this little depolarisation opens some Na channels ⇒ AP.
Response occurs after delay

→ High Thr. Ca current
Very useful for secondary messaging & all kinds of plasti-city
If 'non inactivated' and long-lasting but activated at high depolarisation during AP

→ Ca-activated K channel & adaptation I_{AHP}
Because of this, neurons can have modulatory firing rate
After-hyperpolarisation slow current - calcium dependent
Ca channels open. Ca influx, some K channels open, so cell starts becoming hyperpolarised, so the refractory period increases even as if gets multiple depolarizing currents
The firing freq. decreases - called firing freq. adaptation

Inhibitory connections - allows for robust, synchronous activity of neurons in the brain.
The adaptation (firing freq.) is important in generating rhythms - central pattern generator - through the inhibitory connection.

Propagation of Action potential
- Absolute refractory period of AP makes sure that its unidirectional (orthodromic)
- AP conduction velocity increases with axon diameter
∴ lesser resistance. But large axons would take up a lot of space. So we have myelination = insulation.
Schwann cells - Nodes of Ranvier - saltatory conduction of AP - ↑ conc of Na channels
Distance is 0.2 - 2 mm. Multiple sclerosis is marked by poorer conduction velocities ∵ of damaged myelin

Glia - Schwann cells, oligodendrocytes, astrocytes

Axon

- specialised for transfer of info over long distances
- Axon begins with axon hillock. No ER in axon, proteins come from soma, & its quite different
- Axon branches : collaterals ; length : 1 mm - 1m , Diameter - 1/μ to 1 mm

Axon terminal

- ↳ Bouton : where axon comes in contact with another neuron
- Axon can have many branches : terminal arbor
- Sometimes, axons form synapses at swollen regions along their length & continue elsewhere
- It contains synaptic vesicles. The surface of membrane facing synapse has dense covering of proteins : Active Zone
- Terminal has lot of mitochondria - energy demands

Dendrites ("tree")

They have outcrops called spines — vary based on the strength of synaptic connection ie show plasticity

Size of spines is how long-term information is stored

Dendrites of a single neuron - called dendritic tree

There's a wide variety of shape & size : structure - fn relationship

Its membrane has receptors that detect neurotransmitters

Spine structure is sensitive to type & amt of synaptic activity.

} Last 5 mins of Lec 14

Synaptic transmission (Sherrington)

Process of info transfer b/w 2 neurons

Electric ST - transfer ionic current via gap junction

Membranes are separated by proteins called Connexins.

2 connexins combine to form a gap junction channel

Large bidirectional pore

Electrical synapses are very common in every part of brain.

Advantages : Energy efficient Bidirectional
Fast Sensitive to sub-threshold signals

Found where neighbouring neurons are highly synchronised

Helps neurons to coordinate growth & maturation during development

Chemical ST - more amenable to plasticity / learning
robust to noise

Y.V. important for NT reabsorbed

Synaptic cleft : 20-50 nm to be from the synaptic cleft

REFER SLIDES : history of chemical ST
→ Unreliable - low fidelity
Activity dependent changes in release rate

Lecture 15

Crucial requirements of chemical synaptic transmission

1. Neurotransmitters
2. Release of chemicals through vesicles
3. Small physical distance
4. Receptors on post-synaptic neuron
5. Production of electrical or biochemical response
6. Retrieving the membrane after fusion, removing NT from synaptic cleft
7. Speed : to be useful for sensation & control of movement.

Vesicle packaging and fusion is very important.
Clathryn is crucial for this.
Fusion can be - complete
kiss & run (when rapid firing)

SNAREs - membrane trafficking
↳ proteins allow for one membrane to snare another
Vesicles - v-SNARE, outer membrane - t-SNARE
This helps in membrane fusion - v. crucial so its highly conserved across organisms.

Lec 2 - Aug 18th class 26/10/24

Lecture 2 - Nixon

Introduction to learning and memory

Each neuron can have ~10k - 15k synapses

Neurogenesis - keeps happening throughout life in hippocampus

What is plasticity?

* Plasticity is change in neuronal connections and change in strength of synaptic transmission (synaptic weight)

Human brain is very plastic - it allows us to adapt to changing environment.

* Plasticity can occur due to -
  - change in the environment
  - lesions
* Plasticity is the basis of memory and learning. Learning is the modulation of behaviour based on past experience - its the process by which experiences can change the brain.
* Memory - process involving storage of these changes. An internal representation of past-experiences that is reflected in thought & behaviour.

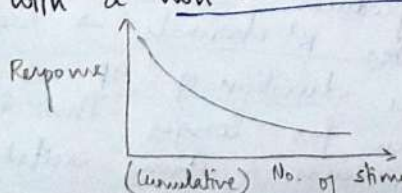
Activity dependent synaptic plasticity - change in synaptic weight based on neuronal activity

* Forms of learning (→ an organism's behavior towards a specific stimuli changes over time)
  - → Associative : when learning is associated with a particular environmental stimulus. Eg. conditioning
  - → Non-associative : learning that is not linked to any particular stimuli

Examples —

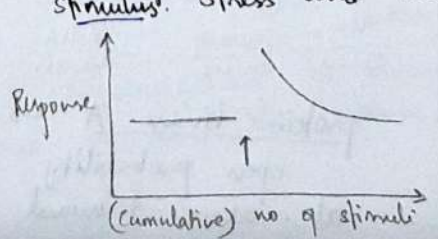
Habituation

Measured response to stimulus decreases after repeated stimulatn with a non-aversive stimulus



Sensitization

The response increases after exposure to an aversive stimulus. Stress condn basically.

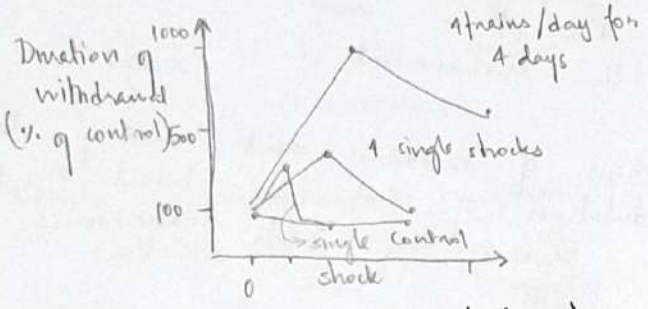


Model organism - Aplysia californica

* About 20,000 neurons - 2,000 in 10 ganglia
Different neurons can be identified based on size, pigmentation & position in NS

* 2 important behavior - Siphon withdrawal reflex
Gill withdrawal reflex

* A light touch to the siphon makes the gill withdraw.
But this reflex decreases after habituation.
If the tail of the animal is shocked (aversive stimulus), a touch to the siphon produces a longer & larger withdrawal reflex. (lasts 1 hour)

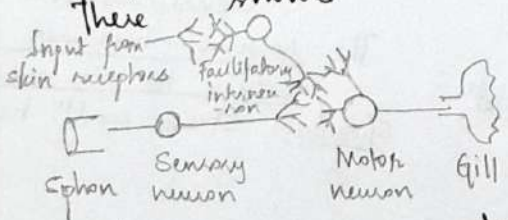


* Physiologically, this is what happens during habituation:
repeated stimulation of a neuron decreases the neurotransmitters released by pre-synaptic compartment.
This is because voltage-gated Ca^{2+} channels at the axon terminal become less sensitive to the incoming AP, which decreases the release of NT.

31/10

Nixon lec 3 - 27/10

Physiological basis of sensitization.
The aversive stimulus occurs at the tail, but the response is heightened in gill withdrawal reflex.
There should be a circuit involved in this



Facilitatory neuron releases serotonin which activates the production of adenylyl cyclase (AC) in sensory neuron.
AC produces cAMP, which activates protein kinase A → phosphorylates K^+ channels → reduces open probability → increases duration of AP → Ca^{2+} channel at axon terminal remain open for longer. Thus motor neuron receives a strong excitatory signal

Classical conditioning - Pavlov (1904)

Conditioning stimulus - ringing of a bell while giving food

Conditioned response - salivating
↳ after learning to associate the stimulus with food.

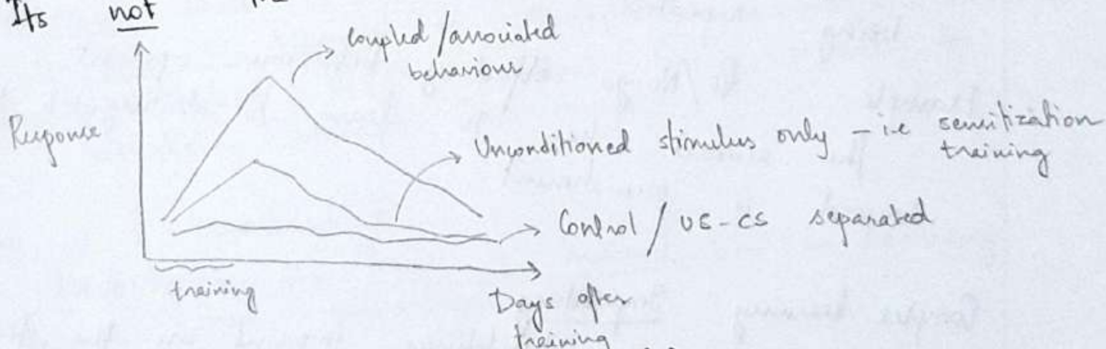
Unconditioned stimulus - elicits a response without training

The CS can be appetitive or aversive

→ Excitatory conditioning : first event (blue stick) predicts the occurrence of 2nd event (cat odor, in this case)

→ 2nd order Pavlovian conditiong - if we introduce another stimulate (red light) before the conditioned stimulus, then this is also learned by association

→ Classical conditioning in Aplysia ??
Associating tactile stimulus with electric shock at the tail
Once the organism has learned this, only tactile stimulus will produce a large response.
Its not the same as sensitization.



→ Molecular basis of conditioning in Aplysia

- CS opens Ca^{2+} channel & Ca^{2+} rushes in. (due to sensory neuron
- US releases serotonin → AC. Presence of Ca^{2+} increases the affinity of AC ⇒ more cAMP production. → phosphorylates K^+ channels.
(Ca-Calmodulin complex)
- Organisms learns the coincidence of Ca^{2+} ions & cAMP production.
- With phosphorylated K^+ channels, repolarization is delayed, leading to more Ca^{2+} influx → more NT release
∴ of higher cAMP during training?

(Left margin:) ↳ AC detects the coincidence of ↑ serotonin release & serotonin levels
level → varies b/w US and US + CS

Operant conditioning - BF Skinner (1938)

Based on Thorndike's (1905) Law of Effect -
responses that produce a satisfying effect in a particular situation become more likely to occur again in that situation, and responses that produce a discomforting effect become less likely to occur again in that situation.

Skinner box - changing behaviour by the use of reinforcement, given after desired response.

Lecture 4 - 28/10 31/10

Skinner box - the rat has to press a lever to get food or stop electric shock

- An association is made between a behaviour and a consequence (positive or negative) for that behaviour
- 'Operant' because - active behaviour that operates upon the environment to generate consequences
- If the behavior is followed by positive reinforcement, then that behaviour is strengthened
- Neutral operants: responses from the environment that neither increase or decrease the prob. of a behaviour being repeated.
- Example: Go/No-go olfactory behaviour operant
  The animal has to learn to distinguish b/w reward & punishment.

Complex learning: Imprinting

Konrad Lorenz: new hatchlings imprint on the first (moving?) object that they encounter.
This imprinting happens only during the critical period.
With goslings, he showed that imprinting didn't occur after 1 day.

Imprinting - complex learning in which the development and maturation of the brain is modified by sensory experience in a critical period

long term potentiation & Long term depression — measure of synaptic strength

Brain plasticity

Types of synaptic plasticity —

1. Neuroplasticity — changes in neural pathways & synapses due to environment, behaviour, injury etc.

2. Developmental plasticity
   Changes that occur as a part of growth & development, especially when the young brain is being bombarded with sensory information.
   Important, tied to our understanding of how CNS encodes sensory information & controls behaviour

3. Physiological level
   Change in efficacy of synaptic connectivity
   Best studied in vitro brain slices using electrophysiological methods & stimulation of identified circuits
   for these studies, we need to have methods to quantify the change or plasticity. (LTP and LTD)
   Brain slices can be stored in CSF at low T for 4-5 hrs

4. Anatomical level
   Addition & removal of synaptic connections b/w neurons
   Ideally studied in vivo, intact brain circuits by visualising synaptic connections.

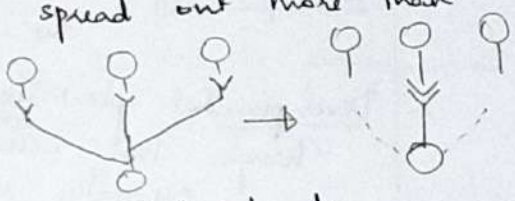
Why is it important?
Plasticity is the basis of individuality — learning, memory and neurodegeneration.

Synaptic refinement
There is a great turnover rate of synapses. Why?
Strength of synaptic transmission also changes dramatically.
This helps in making new connections and retaining & strengthening relevant connections / circuits.
We can detect / measure plasticity by measuring a neuron's computational abilities. The change in pattern of APs are a sensitive assay of ~~plasti~~ refinement

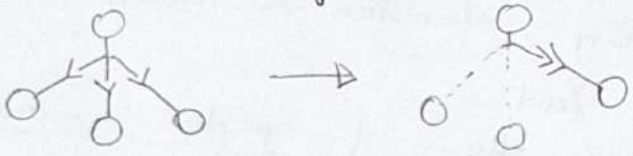
"Neurons that fire together wire together" - Hebb's rule by K Schatz
Central dogma : correlated activity across synapses or lack thereof shapes the connectivity across neurons.

Neural refinement during development

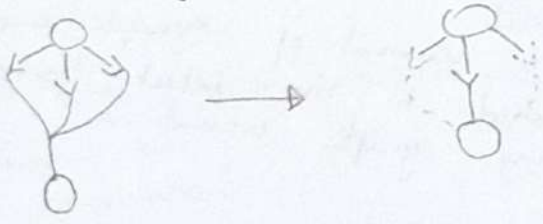
* Refinement of topography
  Individual axons in early life spread out more than they do in adult.
  Eg: Olfactory system



* Refinement of convergence
  A developing neuron has greater no. of inputs than matured neuron. Eg. mammalian NMJ



* Refinement of postsynaptic compartment
  Elimination of terminals from one region of the postsynaptic neuron to another
  Eg: Medial Superior olive in auditory brain stem - inhibitory terminals are eliminated from the dendrite, gradually restricted to cell body.



2/4/24

Lec 5 - Nikon
Are functional synapses getting eliminated during development?
If can be done through electrophysiology & optogenetics

Basics of electrophysiology
Voltages are recorded using glass capillaries with a sharpened metal electrode.
A small glass pipette is used to create suction on the membrane & record in different ways.

PTO

★ Whole cell recording – cytoplasm is continuous with pipette interior

Inside out recording – exposed to air, cytoplasmic domain accessible

Outside-out recording – extracellular domain accessible

★ The voltage can be recorded while keeping either voltage or current constant – patch clamp recording

★ Also, depending on the membrane, you get to measure different channels. Single channels can also be measured

→ Single neuron gets inputs from several pre-synaptic neurons. Membrane potential is a summation of all input signals – and this can be recorded.

→ Assumption : each axon evokes post synaptic potential (PSP) when stimulated and PSPs summate linearly.

→ We can record pre & post synaptic potentials in immature and mature neurons. Stimulation of pre-synaptic neurons leads to different PSPs be in early & late stages of development.

→ Convergence (ratio of afferent axons per post synaptic neuron) varies greatly in the neurons. There's no general rule for % lost or time taken.

→ Conclusion : functional synapses are eliminated during development.

Synaptic refinement during development

To what extent is it influenced by sensory inputs?

Wiesel and Hubel – visual system in cats.

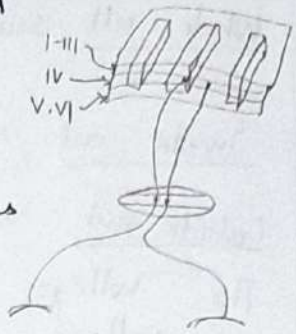
In mammals, retina maps onto the lateral geniculate nucleus (equivalent to tectum) and then onto visual cortex

In frog, connections are entirely contralateral most of the time

In mammals, inputs from each eye goes ipsilaterally & contralaterally at same time

Synaptic refinement in visual system of cat

Ocular dominance columns



The 2nd order neurons from LGN project to Layer IV where they form segregated, eye-specific termination zones called ODC or "stripes."

Layer 4 neurons receive input from one eye or the other - monocular.

Most of the cortical neurons (except Layer IV) are activated by both eyes - binocular

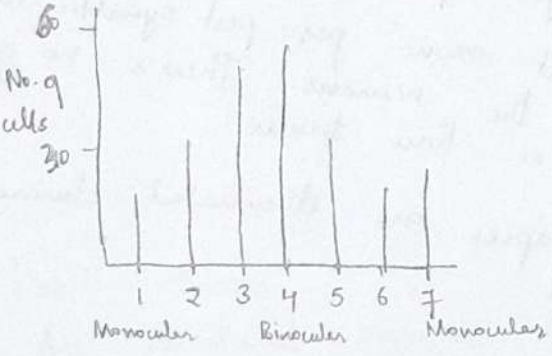
Trans-neuronal labelling

They mapped this by injecting H^3-proline (radioactive) in the retina. It was picked up by sensory neurons then, through anterograde axonal transport, all the downstream neurons were labelled with radioactive proline. It was detected using silver emulsion (probe)

Experiments

→ Both eyes open

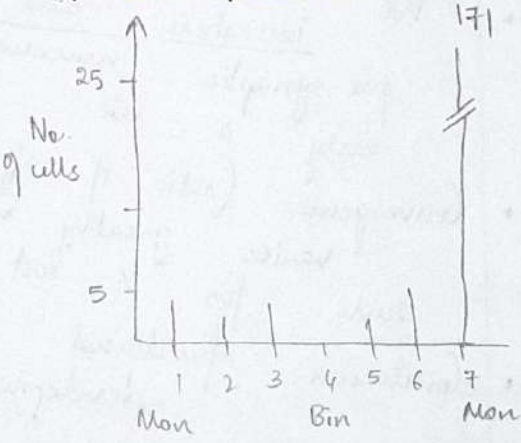
Ocular dominance histogram



Ocular dominance

1 - exclusively contralateral 4 - no observable difference 5 - exclusively ipsilateral

→ One eye closed

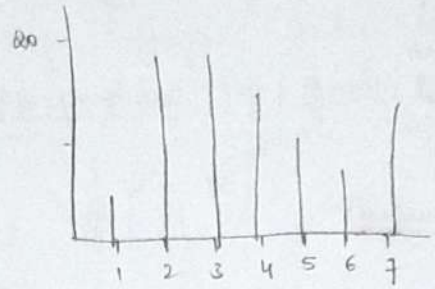


Ocular dom. histogram

The size of columns in layer 4 also changes based on deprivation.

Lecture 6 - Nixon

→ What would happen if both eyes were closed



The no. of responding cells are much much less than other cases.

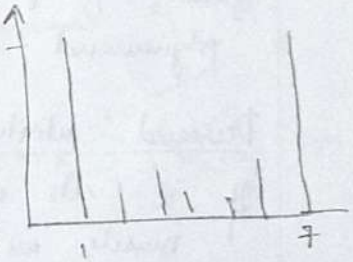
This led to competition hypothesis - the total amount of activity doesn't matter, but the differences in activity determines synaptic strength.

Compt. hyp: total amt of evoked activity doesn't predic whether a synapse will be strong or weak Difference in amt. of activity in pathway from one eye relative to the other seems to determine the strength of projection

→ Kittens with artificial strabismus (misalignment of eyes)

The surgery misaligned the eye, so visual stimuli activates different parts of retina & cortical neurons are rarely activated by both eyes at the same time.

It seems that timing of synaptic activity must be involved in allowing inputs to remain connected functionally to cortical neurons



Not just amt. of activity

→ Synaptic refinement during development

To know what happens to intracortical circuits, dye was injected in visual cortex to carry out retrograde labelling.

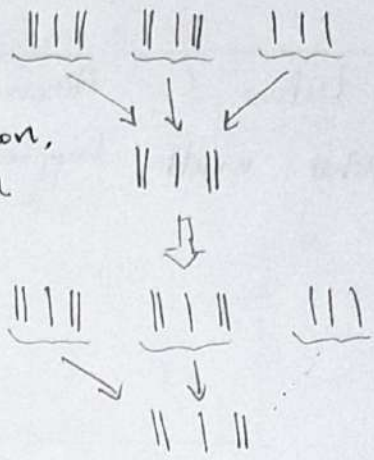
Normal: dye was retrogradely transported by neurons in both ocular dominance columns

Strabismus: (??) dye was transported only by neurons that shared the same ocular dominance

So visual experience influences intrinsic cortical projections *

(56) → Synaptic strengthening as per Hebb's rule

Neurons whose spiking activity is highly temporally correlated with firing pattern of post-synaptic neuron, those synapses are strengthened



Un-correlated neuronal connections are weakened overtime and lost.

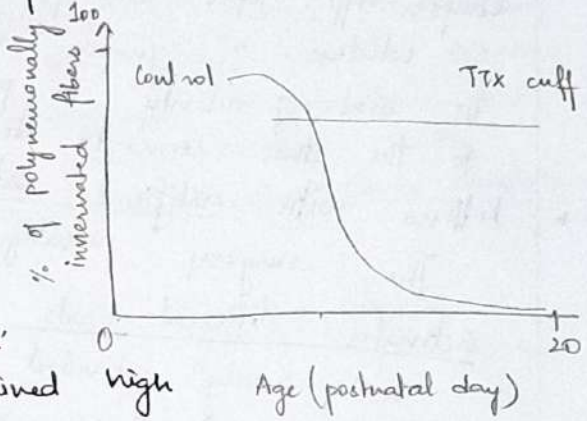
This is called: Spike time dependent plasticity

activity

→ How does lack of, affect refinement

At rat nerve-muscle jxn, polyneuronal innervation declines b/w 10-15 days.

When polyneuronal innervated fibers were prevented from firing by a TTx cuff, polyneuronal innervation remained high



Decreased activity prevents the elimination of synapses at NMJ

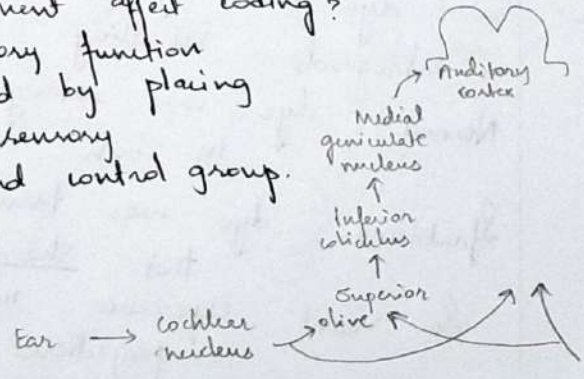
If 2 sets of motor axons innervating the same muscle were stimulated in synchrony, then after the sensitive period, the muscle would be innervated with 2 neurons.

10/11

Lecture 7 - Nixon

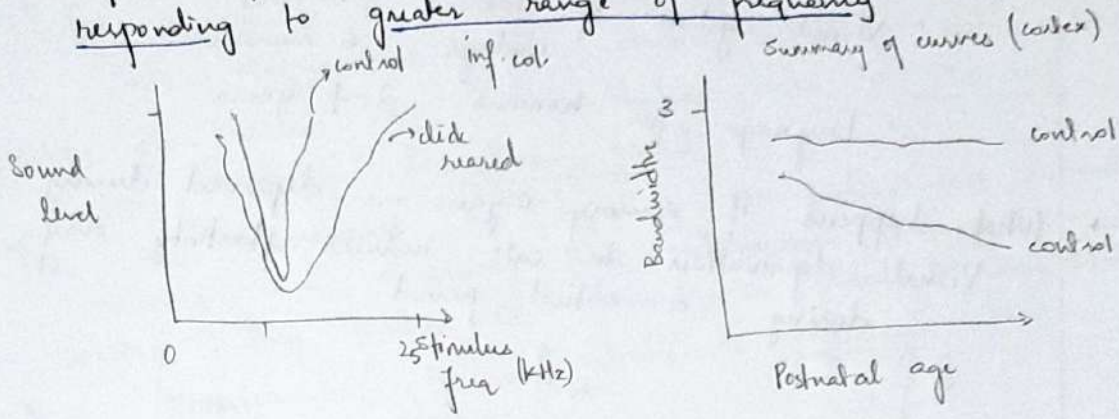
→ How does sensory environment affect coding?

It was studied in auditory function of rats. This was studied by placing animals in an enriched sensory environment, dampened and control group.



Rearing environment : clicks or noise pulses

Because of this, more neurons are firing synchronously for long time. So the freq. tuning curve of of higher order neurons is broader – i.e they're responding to greater range of frequency



→ Effects of environmental enrichment

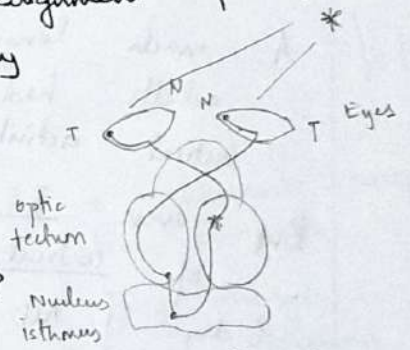
If the animals have an enriched environment, then early-life stressed animals recovered from their learning-deficit caused by stress.

→ Activity contributes to topography and alignment of maps

Remapping in frog visual pathway

Frogs reared in dark, then single point activation in dark

Direct retinal projections from contralateral eye continue to form a precise map but indirect projection via nucleus isthmus is poorly organized



When one eye (contralateral) was shifted by 180°, the contralateral projection was the same (∵ it's based on molecular cues), whereas the indirect projection twists and convolutes to join the contralateral projection – because it depends on activity

Normal binocular neuron

→ Critical period

Its a defined period in development during which some forms of neuronal plasticity are observed

The critical period can vary based on the organism and the sensory inform system

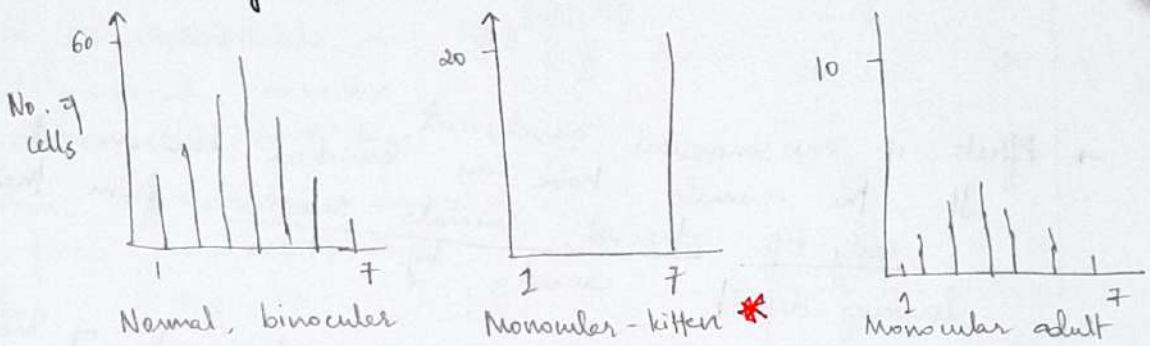
Eg: • If rodent whiskers are destroyed before postnatal day 5, then associated barrels dont form

• Visual system of cat - 3 months
monkeys - 6 months

• Language for humans : 2-4 years

→ What happens if sensory organs are deprived during CP?

Visual deprivation in cats induces plasticity only during a critical period



A much longer period of monocular deprivation in adults had little effect on ocular dominance (although cortical activity had diminished).

But even 3 days of MD produced significant shift of cortical innervation in favor of non-deprived eye

6 days of MD showed starker results than 3 days.

→ Synaptic inhibition & critical period OD plasticity

When juvenile mice are monocularly deprived, there's a shift towards non-deprived eye

But there's virtually no change in adult MD.

Interestingly GAD65 -/- mutant (GABA synthesizing enzyme is absent)

Adults show similar shifts as that of juvenile MD — as if this critical period has been extended.

⇒ Inhibitory synaptic connections are important for critical period plasticity. Effect of MD can be initiated much earlier in development by increasing inhibitory transmission

NMDAR – glutamate ionotropic (Ca^{2+}) receptor – it maybe blocked by Mg^{2+} ion, which only goes away when neuron is sufficiently depolarised

→ Spontaneous activity & refinement
↳ electrical activity is present in the nervous system even in the absence of visual or auditory simulation

- Spontaneous activity regulates formation of stripes
  Cat reared in the dark had LGN afferents segregating into stripes ⇒ visually driven activity is not required
- When all retinal APs were blocked with a TTX cuff, then stripe formation failed
- This was discovered by injecting 3H-proline into retinal neurons and observing their anterograde transport – whether they reached OD columns, or failed to show stripes
- Thus, spontaneous retinal activity is sufficient to form stripes

There are temporal & spatial patterns to spontaneous activity
In the cortex, large-scale waves of activity have been found to move slowly from caudal to rostral during first postnatal week

11/11/2024

Lecture 8 – Nixon

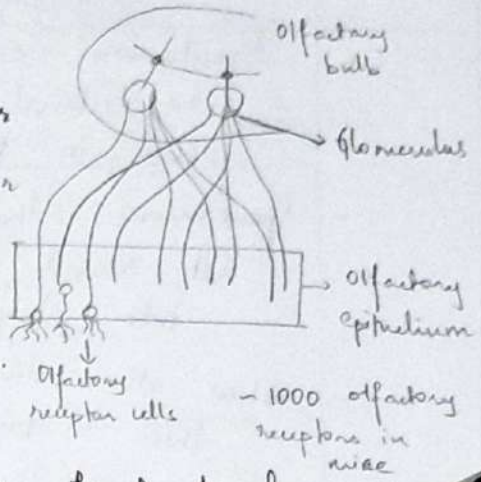
Involvement of intracellular calcium
There are voltage gated Ca channels & glutamate-receptor channel – NMDA receptor channel (cation channels).

- When a third eye is implanted in Frog embryo, tectum becomes co-innervated by 2 eyes, forming stripes. similar to primary visual cortex. (ocular dom. col)
- When NMDA receptors were blocked, stripes were not formed & when NMDA agonist was used, the stripe formation was enhanced (NMDA blocked ⇒ refinement blocked)
- hypothesis: depolarising synaptic potentials open voltage gated Ca channels & Ca^{2+} dependent proteolytic enzymes are recruited to demolish non-active terminals, leading to refinement.

Does plasticity occur in sensory periphery?
What is the impact of associative learning?

Organisation of rodent olfactory system

Olfactory coding is a combinatory recording ie single molecule can trigger several receptors; different molecules can trigger the same receptor



★ Spatiotemporal representation of odorants#
Using different imaging techniques, we can visualise olfactory bulb activity.

★ Imaging technique : Synaptophluorin
Its a pH dependent GFP variant fused to luminal side of vesicle-associated membrane protein (VAMP) whose fluorescence increases at the synapse upon vesicle release. When inside the vesicle, fluorosence is quenched

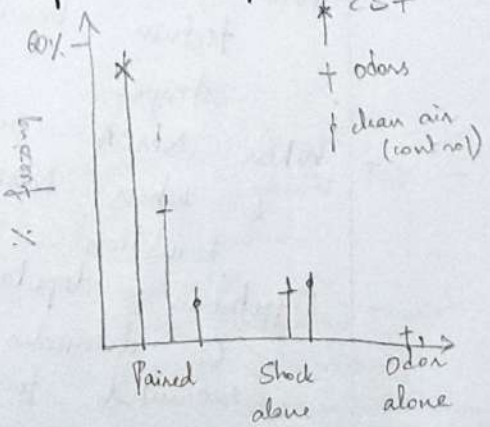
When vesicle fuses, synapto-pHluorin is exposed to neutral environment and the pre-synaptic terminal becomes brightly fluorescent

Olfactory fear conditioning
- Synaptophluorin was expressed in olfactory neurons to image them simultaneously while training the animals
- Trial based, discriminative olfactory fear conditioning paradigm where 3 conditions were — odor alone
shock alone
paired (cs+)

There animals were imaged or behaviourally tested after learning

1 2 3 4 5 6 7 8 9
Imaging/ behavioural | Trng 2/ Rest | Training | Test

Test is done in a different environment but similar to the one in which they were trained



Green light - blood vessel # Intrinsic optical signalling imaging from the olfactory epithelium, neurons project to the glomerulus in a specific mirrored pattern
Red light - activated glomeruli
Using different wavelength lights, activity of neurons can be visualised

Fear learning induced plasticity in odorant-evoked nerve output

Individuals were imaged before & after training.

Compared to all other paradigms, tested in different conditions, Paired/CS+ had an increase in amplitude of signals

After associative learning, the activity of glomeruli in olfactory bulb is modulated

→ Learning mediates in long term functional plasticity of olfactory sensory outputs.

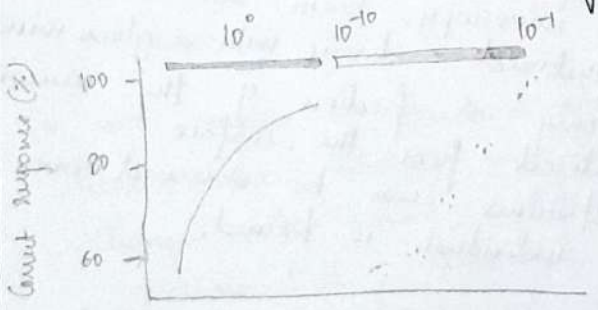
* Intrinsic optical signal imaging in awake mice

Experiments conducted in 3 groups: Trained, Exposed, Naive

* Training paradigm: Go/No-go olfactory conditioning where one odour is associated with reward (water) while the other is nothing

* Initially, mice were trained on 1% solution. Then they were exposed to v. dilute → dilute conc. of odors to see if learning had occured

Correct response: licking for odour 1
no licking for odour 2



Then they were exposed to another pair of odours where learning improved with ↑ in conc

Also, reaction time decreases at higher conc (10^{-3}) when they have learned

* Olfactory learning enhances sensory neuron input strength.

This was determined by imaging the brains of trained, exposed & naive mice after the training

The (increased) activity of glomeruli (?) can only be seen when conc is more than the threshold conc required for learning to occur.

can't be quantified

* Also, the activity of neurons in trained animals were more at all times as compared to untrained ones

These studies clearly show that plasticity occurs at sensory periphery.

The trained individual learns the un-rewarded odor as well as the rewarded odor.
To make sure that there's no internal bias, half of individuals are rewarded for odor 1 & other half for odor 2

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Lecture 9. Nixon

Structural plasticity

Synapses can occur b/w axon-dendrite, axon-axon & dendrite-dendrite
Structural plasticity is an important part of memory formation

Monitoring structural changes

1. Ex vivo / in vitro imaging - looking a brain slices
Changes in structure recorded in an interneuron over 2 hours — very dynamic even though brain is kept artificially alive
External stimuli are mimicked to make this happen
~~Here,~~ In the video - neurogenic olfactory neuron is undergoing structural changes without stimuli

2. In vivo imaging : looking at brain through a window
Using advanced microscopy, brain can be visualised in an anaesthetized brain with a glass window
But non-invasively, only a fraction of the neurons can be visualised from the surface
The change in structure can be observed over weeks as the individual is trained.

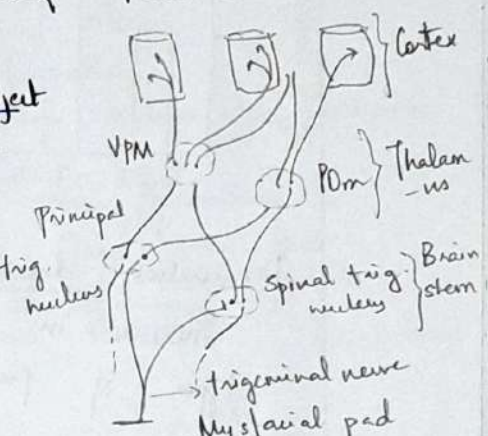
3. In vivo imaging : miniaturised microscope with tubular lens to study neural basis of behaviours
The lens records calcium activity which means activated cell bodies fluoresce.
But finer axon / dendritic recording is not possible
Eg: recording the activity of place cells in rats

Whiskers in rodents

They are a great system to study structural changes in touch perception.

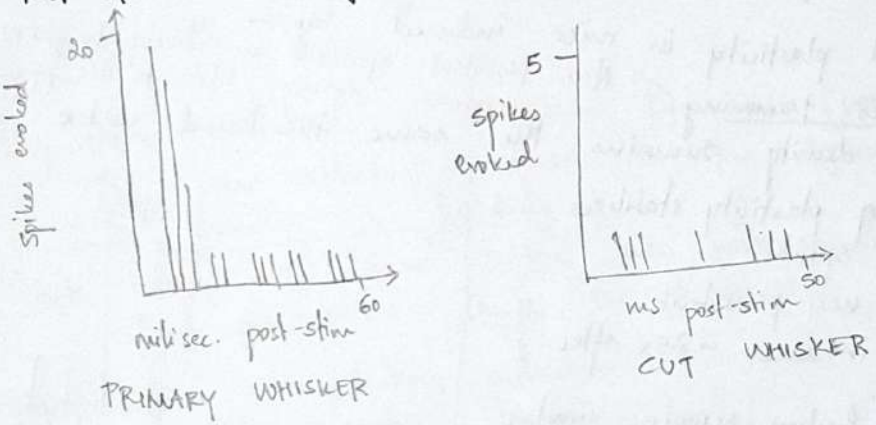
The neurons from each whisker project to individual barrels in the cortex

This allows us to study changes/ different form of stimuli in the same brain.



Sherrington ← Receptive feild : region in sensory periphery within which stimuli can influence the electric activity of sensory cells

→ Post-stimulus histogram



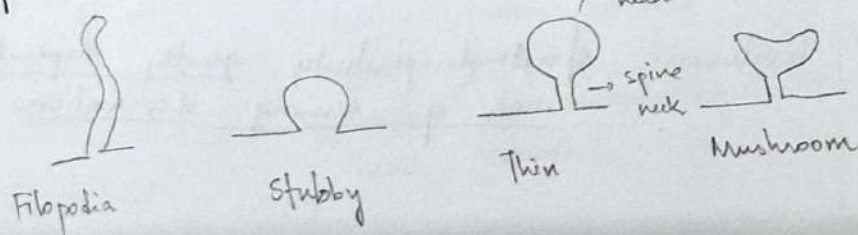
→ In vivo imaging of structural plasticity

Neurons were labelled with GFP in Thy1 transgenic mice

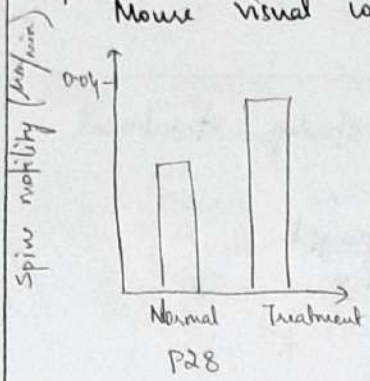
Changes at axonal & dendritic level in thalamocortical, intracortical & layer 5,6 pyramidal cells can be visualised (but the no. of neurons that can be visualised precisely in a brain are less/limited)

→ Sensory deprivation affects structural plasticity

Structures



Expt: Binocular at different time periods
Mouse visual cortex, layer 5 neurons expressing GFP
Dendritic spines show motility (extension - retraction)



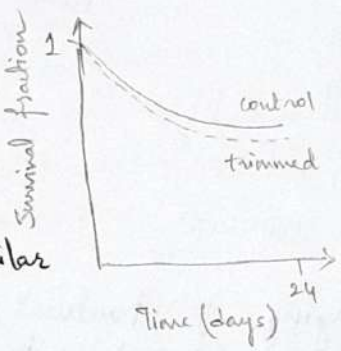
P14 P21 P28 P42 – developmental stages
Binocular deprivation increases spine motility by 60% at p28 (critical period)
from P14 (?)

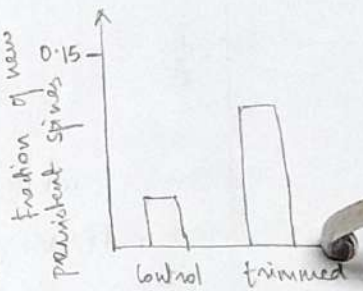
Deprivation doesn't alter the structure of protrusions at any age
Increase in motility at P28 is evident in all types of protrusions – f, s, t, n

Spine turnover rate during the critical period
Turnover rates were highest in young mice and not visibly affected by deprivation

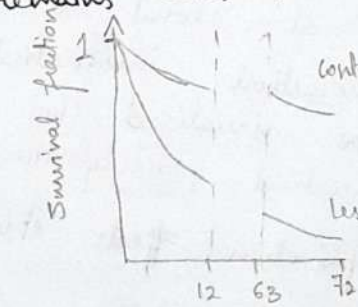
Structural plasticity in mice induced by –
* Whisker trimming → New persistent spines are likely to grow & previous spines likely to disappear
Spine density remains the same in barrel cortex

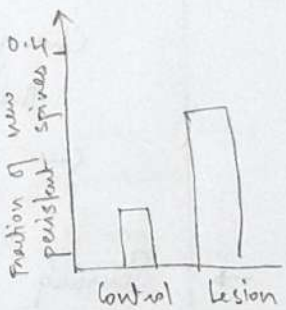
Induction of plasticity stabilizes new spines
Fraction of new persistent spines increases ~2.5x after trimming
Survival fraction remains similar





* Lesions in the retina
Again, spine density remains constant
Most spines are lost & replaced by new persistent ones.





Conclusion: Structural plasticity greatly depends on the mode of sensory deprivation.

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Lecture 10 - Nixon

→ Learning dependent spine formation during sleep

Sleep is important for memory formation

Yang et al. - how sleep modulates spines after learning

Rotarod

After training mice on ~~rotaboard~~ (?) spine formation & elimination in prim. motor cortex. They saw that spine formation was greater in trained mice while there was no difference in spine elimination → for 2 days

They found that sleep depivation significant reduced rate of spine formation in both low forming branches (LFB) and high FB.

Stages of sleep can be tracked by EEG

Long term Potentiation & Long term Depression (LTD)

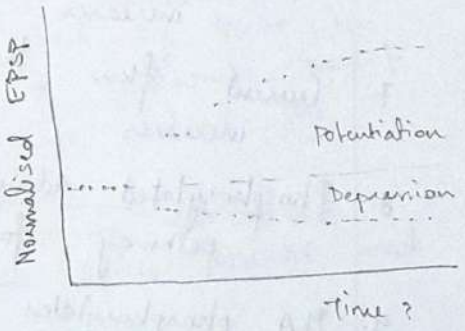
They are cellular processes in the synapse involved in memory & learning.

Both require calcium influx into post synaptic neuron for expression. They're the physiological substrate for memory

They produce opposite effects on synaptic excitability

Potentiation : Long lasting activation stimulated by high frequency signals. (~ 50 Hz)

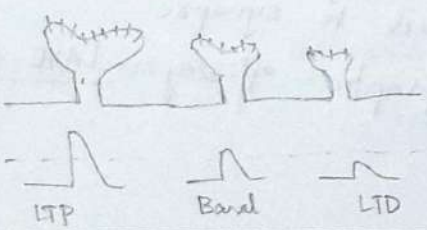
Depression : Long-lasting decrease in activity caused by low freq stimuli (1 Hz for 15 min)



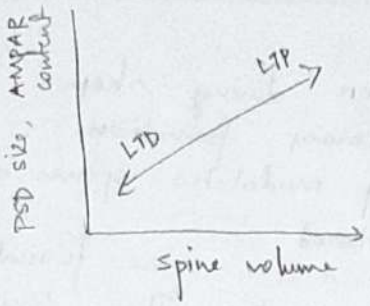
LTD/LTP shape structural organisation of synapse

The spine grows/shrinks and the no. of receptors are also modulated

Cofilin (protein involved in actin dynamics) plays an important role in modulating spine head volume



Post-synaptic Density (PSD) size, spine volume & receptor AMPAR content correlate positively with each other



Post-synaptic mechanisms of LTP induction

1. There's an increase in release of NT. Glutamate binds to ionotropic glutamate receptors in ps neuron, so there's correlated activity b/w pre & post synaptic neuron
3. There's calcium influx, strong EPSP is generated, which results in backpropogating AP, further increasing Ca conc in cell.
4. Calcium is released from ER (intracellular store) through a series of receptors - signalling molecules.
5. Elevated calcium contributes to kinase active states & AC
6. Active kinase (CaCaMK) acts of AMPAR receptors, and increases their activity by phosphorylating them
7. Current flow through phosphorylated AMPA receptors increases : Early Phase LTP
8. Phosphorylated adenyl cyclase (AC) activates cAMP dependent pathway for protein-kinase A (PKA) activation
9. PKA phosphorylates CREB to trigger gene transcription
   CREB : cAMP response element-binding protein
10. Gene transcription & translation triggers LATE LTP
11. New AMPA receptors are inserted into the synapse
    # Not all receptors are newly translated, some are translocated from spine neck to synapse
12. Increased AMPA-R increases synaptic efficacy : LATE LTP

Some expts in mice hippocampus

Spatiotemporal specific knockouts : NR1 subunit of NMDAR receptor

Regional restriction of gene expression using promoter/suppressor region.

Knocking out NR1 subunit blocks the action of NMDAR

Recombination of 2 lines causes deletion of gene encoding NR1 in cells of CA1 region (in hippocampus)

They are called conditional knockout animals

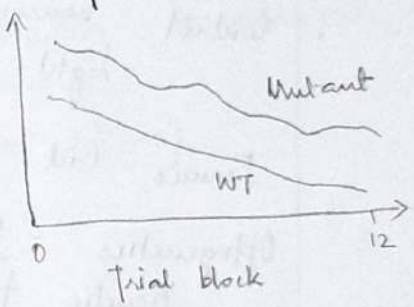
In NR1 knockouts, LTP and spatial memory is impaired

Spatial learning paradigm : mice are released from one point in a water trough, and they have to return to that platform using reference objects.

WT animals performed better in all respects than the knockout mutants.

Even their learning was impaired

No LTP was induced and this reflects in their learning



Why does NMDAR matter?

Hypothesis : NMDARs act as coincidence receptors

At resting state NMDARs are blocked by Mg^{2+} ion.

Weak activation i.e low conc of glutamate won't generate high enough EPSP to relieve the Mg^{2+} block.

High conc of glutamate i.e strong activation which leads to high EPSP opens NMDAR and allows influx of cations.

∴ NMDAR acts as a detector of coincidence of pre & post-synaptic activity.

→ Multi-sensory decisions — CNS encodes

Lecture 11 - Nixon 18/4

Neural basis of behaviours : Optogenetics and more

Causality is elusive in neuroscience because brains are phenomenally complex

It's nearly impossible to figure out how many and which neurons are involved in a particular behaviour, say twitching of a whiskes

How do neurons talk to each other? - Action Potential and Synaptic Transmission.

How do we study causality?

- Monitor neuronal activity while behaving
- Using genetically encoded fluorescent reporters
- Control neuronal activity using genetically addressable light activated tools (actuators)

Francis Crick had pre-empted the possibility of optogenetics.

Optogenetics - Diesseroth 2006

Genetic targeting of specific neurons or proteins optical technology for imaging or control of targets within intact, living neural circuits

Light responsive molecules

ChR2 - Na channel that opens at 470 nm

VChR1 - Na channel that opens at 535 nm

NpHR - Cl^- channel that opens at 589 nm

These channels have been isolated from algae & microbes

They can be used to depolarise or hyperpolarise a cell

Gain or Loss-of-function phenotypes of specific neuronal cells can be created

Electrical stimulation can't distinguish b/w different types of neurons. Optogenetics allows targeted and precise stimulation of neurons

* Why Optogenetics? * Advantages

- In vivo readouts, non-invasive / less invasive compared to electrode
- Fast depolarization / hyperpolarization of neurons in specific circuits
- Precise control over cell populations
- Spatial and temporal specificity
- Bidirectional modifications: causal positive and negative proof with cell specificity
- Can study the morphology of targeted neurons using fluorescent labelling

24/4

Lecture 12 - 23/11

If is important to deduce the circuitry to establish the causality of some behaviour

- Channelrhodopsin - excitatory (Na^+, K^+, Ca^{2+} in)
- Halorhodopsin - inhibitory (Cl^- in)
- Archaerhodopsin - inhibitory (H^+ out)

Using these ion channels, bidirectional control of neurons is possible

Specificity in optogenetic experiments

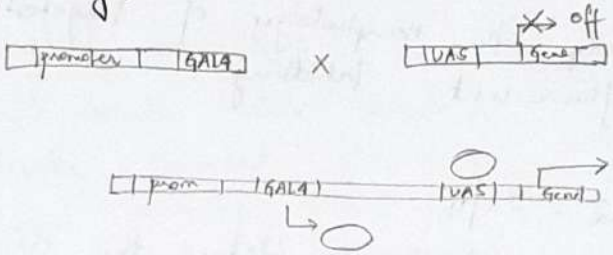
Virally encoded opsin is injected to a specific site, then there's viral expression, and finally light delivery of light through laser excites/inhibits the neuron.

6 modes - local somata
recombinase / promoter-dependent
projection targeting
projection termination - synaptically connected cells can be targeted
Combinatorial local somata
Combinatorial projection

First discovery : Rhodopsin - regulated Ca current in chlamydomonas

Chlamydomonas responds to flashes of light by changing its swimming direction.
They express rhodopsin as functional photoreceptors — currents are recorded in response to light flashes on the eyespot
Photocurrents are Ca-dependent and suppressed by Ca^{2+} channel inhibitors.

This was first used to control drosophila behaviour
UAS-Gal4 system was used



- They targeted dopaminergic neurons (TH GAL4 : UAS P2X2)
  P2X2 — ligand gated ion channels : ionotropic purinoreceptor
- The ligand (ATP) was made inactive by adding photo removable blocking groups
- Photostimulation of dopaminergic neurons caused changes in locomotor activity & locomotor patterns

Something about caspain — ?? (~26 mins)

Locomotor activity (% of time walking) and locomotor pattern both increased post stimulation using phototrigger. Locomotor speed was about the same

Non-functional phototrigger showed no changes in any activity. & we can conclude that activity of these neurons is essential for certain behaviours

In mammalian neurons
Channelrhodopsins were inserted in mammalian hippocampal cell cultures first and showed these cells fire in response pulses of light (intensity, frequency, duration etc had to be optimised).

Repeatability

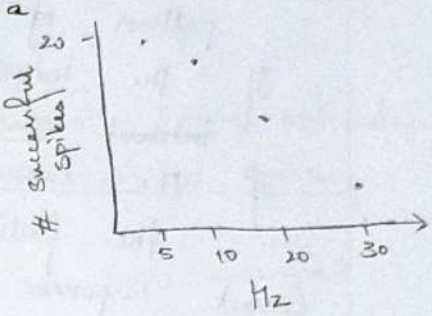
One neuron, repeated pulse series 10 ms duration, 100 ms interval
repeatability was close to 100%.

Three neurons, same light pulse — very high (~100%) repeatability

So, light pulses can reliably generate AP in neurons

ChR2 can mediate spiking across a physiologically relevant range of firing frequencies

But across all frequencies, there were ~0 excess spikes.



Controlling behaviour using ChR2

Hypocretin (orexin) — a neuropeptide synthesized in lateral hypothalamus that plays a key role in arousal state

Loss of function — leads to narcolepsy

So they wanted to check if hypocretin is required for waking from sleep.

ChR2 expressed in hypocretin neurons of LHT in mice

Stimulation of ChR2 reliably generated APs

After stimulation, the sleep-to-wake tendency/ratio decreased at different frequencies of light (5-30 Hz)

24/11

Lecture 13 - Nixon

cont...

Effect of Hcrt receptor antagonist on latencies of light induced wake events

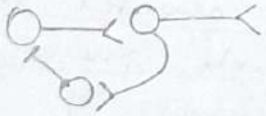
Essentially, sleep-to-wake ratio increased with increase in concentration of the antagonist.

This shows that Hcrt is required for wake induction

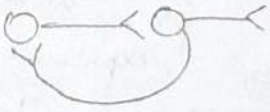
Optogenetics as a tool to study timing of neural responses
Signals from olfactory glomerulus can transmit information using identity, intensity and temporal cues
Precise control of simulation intensity.
The reaction to stimulation by optogenetics of glomerulus was (ultimately) the same as the reaction to the actual odor, after some trials.
The stimulation is synced with the breathing pattern of the mice
If the intensity of stimulation was increased, or the ~~intensit~~ contrast b/w Go-Nogo stimulation or if the latency in stimulation increased, then the fraction of correct response increases.
Correct response — licking water in response to odor.

→ Using halorhodopsin channel — loss of function
There are microcircuit motifs — they occur very frequently in neural circuits.
Nucleus accumbens (NAc) — involved in reward-related behavior
Cholinergic neurons — enhancing inhibition of inhibitory striatal medium spiny neurons?

Feedback inhibition



Feedback excitation



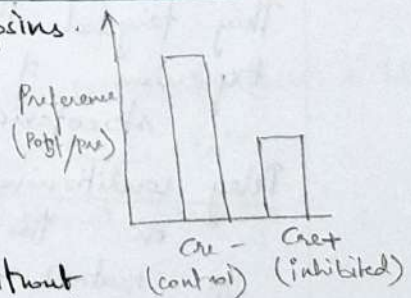
Striatal disorders — Parkinson's
Cholinergic neurons in NAc make up < 1% of local neurons
Medium Spiny neurons (MSN) — 95% of population & constitute the output of NAc.
They tagged choline acetyl transferase (ChAT) and the halorhodopsin expressed in these cholinergic neurons
Stimulating HR should result in hyperpolarisation, and stimulating ChR should depolarise the cell.
These responses were confirmed in brain slices.

→ How are cholinergic neurons modulating activity of MSN?
First, record the output of MSN by itself.
Then record its activity after stimulating ChAT
and they observed that inhibitory spikes had increased.
When GABA receptor antagonist was used, the inhibitory spikes decreased (⇒ Cholinergic neurons used GABA for inhibition). This was done in brain slices.

Bidirectional changes — in vivo observation
Recording was at population level, using optrodes.
★ Photostimulation of cholinergic neurons inhibited spiking of 81% of MSNs
Here ChR of cholinergic cells were activated.
★ Halorhodopsin stimulation
optogenetic photoinhibition of ChAT interneurons enhances spiking in 76% of MSNs in vivo.

→ We know that this is an important circuit for reward related behaviour. To study this,
Conditioned Place Preference (CPP) paradigm was used —
there's an association of a certain environment with drug treatment, and a neutral place with no treatment.

→ Along with giving cocaine, the ChAT neurons were inhibited by stimulating halorhodopsins.
They saw that these animals had lesser preference to go to the place associated with cocaine.



→ Acute silencing of ChAT interneurons disrupts drug related learning without affecting conditioning in the absence of drug.
So control over this microcircuit could be used to disrupt effects of drug abuse without affecting appetitive or aversive learning/responses in general

Lecture 14 - Nixon - 25/11 5/12/21

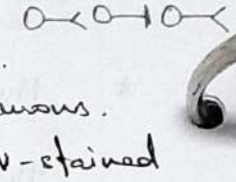
Obsesive Compulsive Disorder

Can we use optogenetics to control mental disorder?
First we need to dissect the neural circuitry & then employ optogenetics.

Paper: Optogenetic stimulation of lateral Orbitofronto-striatal pathway suppresses compulsive behaviours.

Sapap3 - mutation that causes excessive grooming; a repetetive behaviour.

Here too, a thalamo-cortical circuit was studied. They focused on Fast-spiking striatal interneurons (FSIs) which mediate fast feed-forward inhibition of MSNs in response to cortical activation.



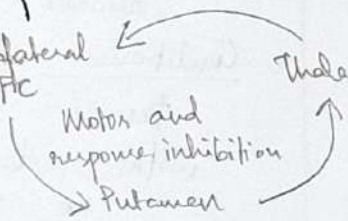
Most FSIs are Parvalbumin-containing interneurons.

In sapap3 — there were significantly lesser PV-stained cells.

These Fast-spiking GABAergic interneurons make up a v. small % of striatal neurons. but they play crucial role modulating cortical input and inhibiting MSNs

Different striatal loops have different functions

Repetetive action occurs bcuz of malfunction in circuit involved in motor & response inhibition.



They targeted the orbito-fronto-striatal system Ventral cognitive circuit.

Expression of ChR in lateral OFC neurons, using stereotaxic injection.

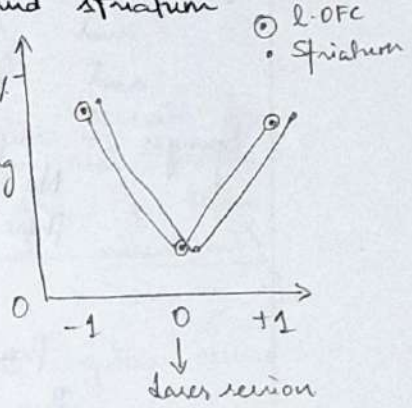
Delay conditioning paradigm: a tone paired with water drop on the snout after 1.5s, which triggers grooming.

In mutant, it causes repetetive grooming.

As training progresses, in WT mouse, grooming in response to the tone ~~in~~ to decreases.

But in sapap3 mutants, grooming occurs ~~even~~ in response to the tone, even in absence of water

To identify the circuit, they recorded from various neurons in between the tone & water drop.
They found that striatum neurons have increased activity in mutant in the late training phase.
So we modulate these striatal neurons.
Optogenetic stimulation of FSI decreases the spiking activity of MSNs.
Optogenetic stimulation of lateral OFC and striatum alleviates compulsive grooming.
% of grooming and grooming duration both decrease when l-OFC & striatum were photostimulated.



Disadvantages of optogenetic techniques.
- Optic fibers — their use in deeper parts of brain is invasive
- Channelrhodopsins have to be expressed in particular neurons, which can be cumbersome.
- If we're not careful with optimising the energy delivery, the blue light might cause some damage to the neurons.

Other techniques —
* Calcium imaging — place cells
* Imaging using GCaMP — fusion of GFP, Calmodulin (CaM) & M13
CaM - postsynaptic density protein that binds Ca^{2+}.
Ca^{2+} binds to CaM — causes conformational change that causes GFP to fluoresce.

Lecture 15 - Nixon - 30/11 6/11/24

Neurodevelopment

Different stages are involved -
- Neural induction
- Birth & differentiation of neurons
- Polarity, segmentation of nervous system
- Migration of neurons to their destination
- Axonal growth and guidance to their post-synaptic targets
- Changes in synapses

→ In evolutionary tree of life, all animals except flagellates and sponges have recognizable neurons with excitability and long processes

Sponges - simplest metazoans
- No specialised sensory cells or nerve cells
- There are 'independent effectors' or myocytes that have sensori-motor functions, but not electrically excitable.
- They control the size of water pores & hence the water flow
- They have 25 genes similar to humans, involved in functioning of nerve synapses

In Cnidarians - nerve net
In higher orders, which have bilateral body plan, the NS becomes more & more centralized.

→ Early embryology of metazoans

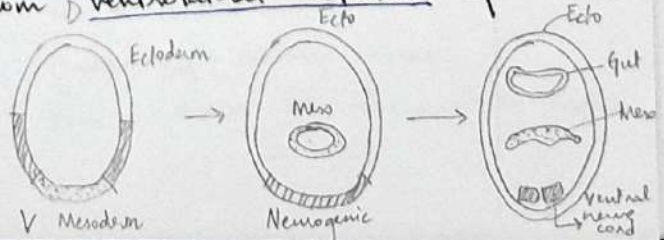
Zygote → Blastula —Gastrulation→ Gastula

When egg is fertilised, they are polarised - animal & vegetal pole
After series of cell division (without cell growth), it becomes blastula where cells rearrange (gastrulation) into outer ectoderm, middle mesoderm and inner endoderm
This is where neuronal development starts.

→ CNS Development in Drosophila

Major part is derived from ventrolateral ectoderm of blastula

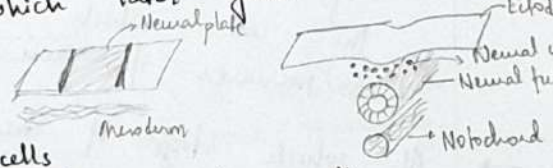
At beginning of gastrulation, ventral furrow begins to form.



So neurogenic region moves from —
Ventrolateral side → Ventral furrow → Ventral midline
In ventral midline, it forms ventral nerve cord (CNS)
The signalling between mesoderm & neurogenic ectoderm influences the fate of these cells.
This leads to neuroblast formation where this neurogenic tissue gives rise to neurons & glia. These cells condense at certain region to form ganglia or brain.

→ Delamination
Its the process through which neuroblasts separate from ectoderm first. These cells enlarge and move out of the layer. There neuroblasts divide to form a Ganglion Mother Cell (GMC) and Nb. (another neuroblast).
Each GMC divides to form a pair of neuron or glia.
In drosophila, further development of nervous system occurs during metamorphosis.

→ CNS development in Xenopus
Polarity : Vegetal - yolk concentrated, Animal - yolk free
In blastula, (~128 cells), a group of cells - involuting marginal zone or IMZ - involute or grow into the embryo and go on to form mesodermal tissue They induce the overlying ectoderm cells to develop into neurogenic tissue called the neural plate, which later gives rise to neurons and glia



Neural plate rolls up to form a neural tube with neural crest cells above it, separate from ectoderm The mesoderm cells underneath condense to form the notochord

→ How does delamination happen?
Some achaete-scute (as-c) genes or proneural genes are involved in this process.
In WT, one of the cells from proneural cluster of ectoderm cells becomes a neuroblast cells.
In proneural mutant, no neuroblasts in CNS and PNS were observed.

In neurogenic mutants (Notch & Delta), many neuroblasts delaminate instead of just one from a patch (like in WT).

→ Lateral inhibition

Just before delamination, a group of cells in ectoderm expres proneural genes (as-c), forming proneural cluster.
One of the cells at the center expresses higher level of these genes and through lateral inhibition begins to block the expression of as-c in cells around it.
So, only one cell is left expressing proneural genes, and it delaminates to form a neuroblast.

Don't confuse this lateral inhibition with that of circuits — where an excitatory neuron activates inhibitory neurons which in turn inhibits lateral excitatory cells.

Notch signaling pathway

- Binding of Delta to Notch (transmembrane protein) causes it to cleave the intracellular part — Notch ICD
- Notch ICD interacts with Suppressor of Hairless (Su(H)) and together they form a Tx factor.
- This Tx factor turns on the expression of downstream target genes, specifically Enhancer of Split (E(spl)).
- E(spl) proteins are suppressors of As-c genes, so they block further neural differentiation and reduce levels of Delta expression.

In proneural cluster, cells express As-c and components of Notch pathway. Lateral inhibition is a positive feedback loop: More As-c expression ⇒ more Delta expression in the cell, which blocks neighbouring cells and in turn, increases its own until it delaminates

→ At which stage of development does neural lineage start?
Induction of neural tissue : Spemann organizer

* When dorsal ectoderm was cultured from pregastrula & gastrula stage, the cells differentiated into epidermis and neural tissue respectively.
Neural lineage arises during gastrulation.

* Does mesoderm induce the ectoderm to become neural tissue?
The dorsal lip of one embryo (pigmented) was dissected and transplanted to the interior of another embryo.

An entire second body axis (brain, spinal cord & eyes) developed (whose tissue mostly came from host ie non-pigmented)

⇒ Grafted blastopore cells have the capacity to induce neural tissues from region of ectoderm that wouldn't give rise to neural tissue

So this dorsal lip acts as a neural inducer, as well as an organizer of body axis. So, this is called the Spemann organizer.

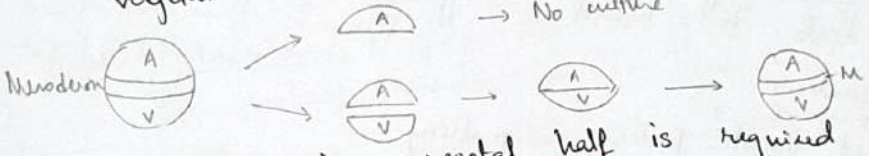
→ Molecular nature of neural inducer.

★ From 1930s to 1950s there were over 100 studies to characterize the mechanism.

- Extract active factor from blastopore cells
- Trying out candidate molecules for inductive activity
- Testing other tissue for inductive activity

There approaches were not very successful.

★ Formation of mesoderm requires some interaction b/w animal & vegetal halves - Nieuwkoop.



Some induction from vegetal half is required for the formation of mesoderm.

★ Noggin as neural inducer. (Harland et al 1993)

When embryo is exposed to -

UV radiation : ventralized embryo - dorsal axis fails to develop

Lithium chloride : hyperdorsalized embryo

When mRNA from hyperdorsalized embryo was injected into UV treated one, that embryo developed normally.

Isolated the cDNA from the organiser which coded for a secreted protein called Noggin.

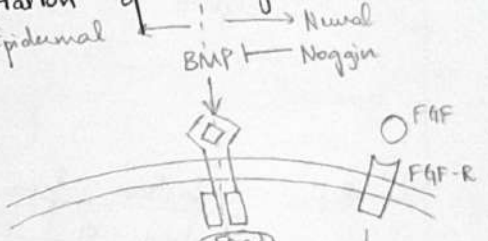
When noggin was supplied to animal cap, it induced neural genes even without induction of mesodermal tissue

Noggin protein +  → 

* Chordin as neural inducer (DeRobertis isolated chordin) - Sasai et al. 1994
  Chordin is expressed when neural induction occurs.
  Overexpression of chordin in ventral part of embryo caused a secondary axis to form.

* Conservation of neural induction
  Functional homolog Ortholog of chordin in drosophila - short gastrulation / sog gene
  Expressed in ventral side of fly embryo
  In null mutants of sog, epidermis expands & neurogenic region is reduced.
  Microinjection of sog reverts the effects of mutation
  In drosophila, sog interacts with a gene called dpp, a TGF-like protein related to vertebrate genes known as Bone morphogenic protein (BMPs). (??) BMP belong to TGF-β family
  TGF-β : transforming growth factor
  dpp = BMP4, Sog = chordin
  In dros. sog inhibits dpp to induce neural tissue in ventral side

* "Default model" of neural induction
  Noggin, chordin, cerberus, follistatin etc are released from IMZ.
  They bind to BMP4 and interfere with activation of BMP-receptor in ectoderm, thereby blocking the anti-neuralizing effects of BMP4.
  This leads to neural differentiation of neurogenic ectoderm



* FGF signaling
  Fibroblast Growth Factors - signalling molecules maybe important
  BMP induces → Smad inhibits Zic1. Zic1 & Zic3 are required for neural plate to form.
  FGF signaling inhibits Smad (through phosphorylation) and activates transcription of Zic3 (through ERK pathway)
  So, BMP inhibition by Noggin etc. and FGF activation are both important components of neural induction.

Lecture 16 - Nixon - 1/12
Neurodevelopment
Vertebrate nervous system
Tripartite division of neural tube — forebrain, midbrain, hindbrain
This is highly conserved. They can be observed as 3 brain swellings in early development.

Molecules involved during segmentation?
→ Hox genes — control patterning along anterior-posterior axis
↳ highly conserved, code for homeodomain class of Tx factors.
Different Hox genes are expressed in different regions along this axis. — same order in dros & mouse.
Hox gene position on the chromosome is correlated with its expression along the axis.

When Hox gene activity is altered —
★ Rhombomere identity is determined by Hox gene
↳ segments in the hindbrain
Each rhombomere gives rise to specific cranial nerves that control muscles in the head
When Hoxa1 gene is knocked out, Rhombomere 4 is lost, reduced 5.
abducens nerve is not present — there's a lot of rearrangement, because that Hox gene decides the boundaries of the segment.
★ Otx2 — important for development of mouse fore brain & head
Mutants lack r3 (anencephaly) & most of head region.

Retinoic acid (RA) signaling controls Hox gene expression
- Complex of RA + RA-receptor moves to nucleus and regulates gene expression by interacting with promoters, called RA Response element (RARE)
- A gradient of RA is maintained along axis ⇒ very high RA levels in posterior portion.
- Excess RA inhibits Hox genes & development fails. So its a powerful teratogen ↓ in anterior section

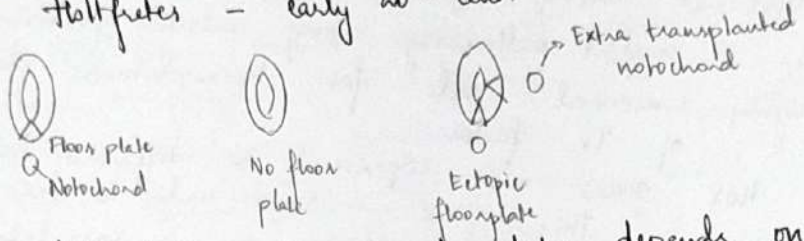
(112) ⇒ Dorso-ventral patterning

In vertebrates, along with neural tube, ventral side forms the floor plate, whereas dorsal side is flattened to form roof plate.

A distinct fissure forms b/w dorsal & ventral parts of neural tube - sulcus limitans (4th ventricle)

Pattering : dorsal - sensory neurons
ventral - motor neurons

→ Notochord is imp. for ventral floorplate development
Hallfuter - early 20th cent.



So differentiation in neural tube depends on factors derived from adjacent tissue.

Notochord signal - Sonic hedgehog (shh) and ventral differentiation
It was discovered in drosophila. Shh from notochord sets up the ventral patterning.

When intermediate tissue from neural tube was cultured along with Shh, then it acquired ventral markers.
- floor plate + motor neurons.

Dorsal patterning signals

→ Liem et al - when intermediate tissue was co-cultured with ectodermal tissue, it acquired neural crest markers.
When BMP 4/7 was added to intermediate tissue, they also induced dorsal markers

Shh and BMP have antagonistic signals & they set up opposing gradients that control differentiation of spinal cord

More organising centers in the brain
⇒ Midbrain-hindbrain border expresses signals important for organization of the brain.
Like Spemann Organizer
Molecules - Wnt1, en1, fgf8

Mesencephalon - metencephalon boundary
↓ midbrain ↓ cerebellum

easy immuno-staining

When mid-fore border tissue was transplanted (from quail) to another forebrain (to chick), ectopic midbrain and cerebellum developed.

⇒ Generation and Migration
Neural tube - CNS Neural crest cells - PNS

* Neural crest cells - multipotent progenitors (neuroblasts)

Signals important for deciding fate of NCC —
Different types of cells - sensory, sympathetic, parasympathetic, schwann, smooth muscle, melanocyte glial cells etc.

BMP 2/4 - Sympathetic neurons
Glial Growth Factor (GGF) - represses neurogenesis, promotes schwann cell
TGF β - smooth muscle cell

These differentiations were first visualised with radioactive thymidine. Now deoxy bromouridine is used to label neurogenic cell population (BrdU)

* Cerebral cortex neurons
They're generated in an inside-first, outside-last manner
When is the subtype identity of these neurons acquired?
Progenitor cells from ventricular zone of young animals were transplanted to older ones.
Early cell stage - migrate to deep layers (layer 2/3), following host cell fate
Late cell stage - cells maintained their original identity.
⇒ Young cells remain sensitive to time-dependent signals, but older cells are committed to their fate

* What controls the cortical progenitors to differentiate or remain proliferative
Orientation of plane of cleavage - & consequent sym/asymmetric division - correlates with the fate of the cell
Vertical cleavage - symmetric fate - 2 neurons
Horizontal cleavage - asymmetric fate - 1 progenitor, 1 neuron

Lecture 17 - Nixon - 2/12/21

Neurodevelopment 2

How does activity affect survival of neurons?

Changing size/activity of muscle target influences survival

Limb bud amputation - no neurons survive on that size 50% on the normal side

Extra limb transplant - 75% neurons survive on extra-side

Paralysis - neuromuscular transmission blocked by curare 75% survival - this reduces the extent of death

Rita Levi-Montalcini & Stanley Cohen - Nobel for nerve growth factor.

Developing neurons compete for a limited supply of neurotrophic factor (NF) provided by target tissue. Successful neurons survive while others die

Nuerons that don't get NF die by apoptosis.

NF deprivation triggers caspase by this pathway

* NGF → Bcl-2 → Apaf-1 → Caspase → Cell death *

↓ NGF → This ↑ leads to apoptosis

Axon guidance

In giraffe, axons travel meters before terminating in correct area

Different views —

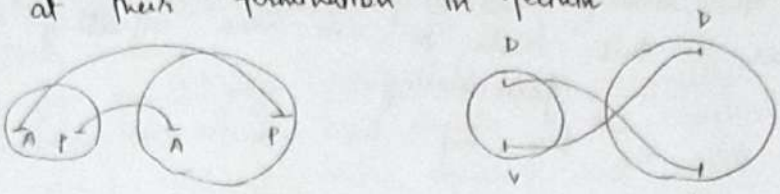
Molecular view - JN Langley

Resonance of electrical activity - Paul Weiss proposed that much of axonal growth is random & apt. connections survive where electrical activity of axon and target tissue matches.

Stereotropism - axons grow along some mechanical support (like blood vessels, cartilage in embryo).

Today, resonance & stereotropism are not considered important Molecular view is based on expts by Roger Sperry

Axons from retina form a topographic representation of retina at their termination in tectum



Because of this, projection is inverted when the eyeball was rotated in its socket, this connection reversed ⇒ projection got wonky

The Growth Cone

The extension / tip of a neurite seeking its synaptic target encounters various types of guidance cues—

1) Growth promoting molecules in ECM
2) Adhesive cell surface molecules on neuroepithelial cells promote growth
3) Fasciculation – when one axon meets another
4) Chemoattractant directs the axon
5) Contact inhibition – intermediate target bears repellant molecules
6) Soluble inhibitory molecule biases axon's trajectory
7) After contact with target, axon growth cone terminates & begins to form a terminal arbor.

→ Cues from neuroepithelium guides formation of optic tract.
Portion of neuroepithelium was grafted from a donor embryo and transplanted in the same place, but in different orientations.
In each case axon grew towards the part of the graft that was originally caudal.

→ ECM molecules promote neurite growth
Laminins: major components of basal laminae and account for much of axon outgrowth influence
In a grid, the axon grew along collagen-corridors.
The interaction of integrins with actin-talin in intracellular domain advance axon growth along membrane-matrix adhesion.

→ Cell adhesion molecules Cadherins & IgG promote neurite outgrowth (CAMs)

They are membrane proteins that bind to actin / kinase activities on intracellular domain & bind to similar proteins on other cells (homophilic) causing aggregation.

Eg — this leads to one axon attracting another and fasciculating.

But how are they imp for neurite outgrowth?

→ Netrins mediate chemotropic responses of neurons to intermediate targets

Floor plate is an intermediate target (with netrins) of spinal commissural axons. Netrins exert a tropic effect through their receptors on these axons

→ Inhibitory signal : binding of ephrins to eph kinases

Membranes were prepared from anterior & posterior tectum

Posterior retinal axons selectively grew on anterior membrane because of chemorepellant on posterior membrane (ephrin).

When Ephrin kinase on growth cones is activated, its an inhibitory signal

→ Chemorepulsive signal from semaphorins

When collapsin / semalophorin was applied, the filopodia collapse & recover after 10 mins. When applied on one side, the growth cone turned, because cytoskeleton on one side collapses.

Synapse formation

↳ its characterized by post-synaptic density — ion channels, receptors, cell adhesion proteins, kinases & phosphatases, signalling molecules etc

Steps in development of a neuromuscular junction

1. Growth cone approaches myotube — unspecialized but functional contact
2. Vesicles accumulate at terminal & basal lamina forms in synaptic cleft
3. Multiple axons converge to a single site
4. All axons but one are eliminated, & survivor matures

Recall basics of electrophysiology

→ Nerve & muscle cells assemble synaptic components on their own

Electrode tip coated with outside-out patch of muscle membrane containing high density of AChR receptor.

Simulate isolated cultured motor neurons and record from electrode—the patch with AChR receptors is activated because motor neurons release ACh even in absence of myotubes.

→ Components of basal lamina at synaptic site helps organize the nerve terminal

Following nerve damage, motor axons regenerate and all the new synapses are formed at original synaptic sites, even after muscle fibers had been removed.

Regenerating axons differentiate into terminals when they come in contact with basal lamina.

Components of synaptic basal lamina — selective reinnervation & differentiation of nerve terminals

→ Neural activity represses AChR receptor synthesis in non-synaptic area

Denervation and paralysis resulted in AChR expression in non-synaptic sites.

→ Cytoplasmic proteins mediate clustering of receptors at central synapses

Gephyn — connects glycine receptors (GEPHYRIN)

PSD-95 — clustering of NMDA glutamate receptor

Gephyn mutant showed scattered glycine receptor

How does axon damage affect neuronal circuits?

- After axotomy, nerve terminal degeneration occurs rapidly
  So does distal stump (Wallerian degn), myelin sheath.
- Phagocytic cells invade
- Chromatolysis of cell body — nucleus moves to an eccentric position
- Presynaptic processes on the neuron withdraw, ensheathed by glia
- Inputs to & targets of injured neuron can atrophy even degenerate

Restoration of function requires synaptic regeneration

Peripheral nerve grafted to the brain provide a permissive environment for central axons to regenerate their target and form synapses.

Rat : optic nerve ↔ sciatic nerve. Some retinal ganglion cells regenerated & recordings were made from superior colliculus.

Adult neurogenesis – hippocampus & olfactory bulb

Rido – question paper, example problems

(A)

B13154 - Neurobiology

Neuroanatomy - list of nuclei

M 1. Nucleus gracilis & nucleus cuneatus - junction box (in brain stem) through which all sensory neurons pass to go to thalamus

M 2. Cardiovascular centres - Medulla

Vagus - Nodose ganglion - Nucleus of ST - Nucleus ambiguus - Vagus

M, P 3. Respiration

Pre-Botzinger complex - central pattern generator

Pneumotaxic & apneustic centre - Pons

Dorsal & Ventral respiratory groups - Medulla

M, P 4. Raphe nuclei - Seratonin - projections into limbic area

P 5. Locus ceruleus - norepinephrine (80% loss in alzheimer's)

Midbrain

6. Oculomotor nucleus - III cranial nerve - Edinger-Westphal nucleus → Optic radiation

7. Substantia nigra - Dopamine (70% loss in Parkinson's)

8. Red nucleus - motor coordination / Medial & lateral geniculate nucleus (94) Thalamus

9. Ventral tegmental Area - also Dopamine - innervates ventral striatum, cerebral cortex and hypothalamus → Auditory pathway

Thalamus

10. Basal ganglia - caudate, putamen, substantia nigra, globus pallidus, nucleus accumbens, subthalamic nucleus

Controls voluntary action, behaviour, emotion, learning etc

11. Hypothalamus - made up of a series of nuclei

12. Suprachiasmatic nucleus - biological clock

Ventrolateral nucleus - PMA (cortex) to cerebellum

More neuroanatomical terms

Cortex - 6 layers

Post central gyrus - primary somatosensory (3b)

Pre-central gyrus - primary motor (Also supplementary & Pre Motor Area)

Broca's area (44, 45) - generating language

Wernicke's area (22 - left temporal) - comprehension

Cortical homunculus (Penfield)

Association region

Hippocampus – Dentate gyrus + Cornu Ammonis – Archicortex (3/4)
Olfactory bulb – Paleocortex (3) – olfactory neurons project into mucus layer

→ Visual circuit

Optic chiasma : Ipsilateral (+,+) – Contralateral (+,–)
(Temporal ↑, Nasal ↑)

4 projections – Suprachiasmatic nucleus – circadian rhythm
(TNTNTN) → Lateral geniculate nucleus – Optic radiation to Occipital lobe
Alternating
Prefectum – reflex control of pupil
Superior colliculus – focusing on a moving object

* Pupillary light Reflex Pathway (ANS)
* Retino-hypothalamic tract – SCN

→ Auditory pathway

Spiral ganglion —(Auditory nerve)→ Dorsal & Ventral Cochlear Nucleus → Superior Olivary nucleus —(Lateral lemniscus)→ Inferior colliculus → Medial Geniculate Nucleus → Auditory Cortex

Dorsal & Ventral Cochlear Nucleus, Superior Olivary nucleus: Medulla
Inferior colliculus: Midbrain
Medial Geniculate Nucleus: Thalamus
Auditory Cortex: Neocortex

→ Dopamine releasing pathways

* Substantia nigra → Dorsal striatum (caudate, putamen)
Basal ganglia motor loop – ✕ for movement
* Ventral tegmental area → Ventral striatum & cortex
Pleasure and learning.

→ Autonomous nervous system (ventral root)

Sympathetic	Parasympathetic
– Thoracolumbar outflow	– Craniosacral outflow
– Chain of sympathetic ganglia st. preganglionic < postganglionic	– Preganglionic > Post ganglionic
– Accelerates heartbeat & other stress responses	– Relaxes heartbeat & acts antagonistically to restore homeostasis
– Postganglionic is adrenergic (except skin)	– Both pre & post ganglionic fibres are cholinergic

* Receptors

Cholinergic – Nicotinic : ANS ganglia, adrenal medulla : ⊗ Tubocurarine
Muscarinic : Effector targets of PNS, sweat glands

Adrenergic – Effector targets of SNS
Alpha – ++ NE, + E ⇒ constricts smooth muscles
Beta – + NE, ++ E ⇒ relaxes smooth muscles

→ Meninges - Piamater, Arachnoid, Duramater
Ventricles - Lateral (2), 3rd & 4th
Lined by Ependyma cells
Choroid plexus - produces 500 ml of CSF each day

Blood brain barrier - gap junction tight
capillaries surrounded by astrocyte foot
mitochondria more numerous

→ Cerebellum
Coordination (timing and force) of movement
Anterior & posterior lobe, divided into folia
3 cerebellar penduncles in the pons - support & relay info
Deep Cerebellar Nuclei - white matter (inside)
Dentate nucleus
Emboliform nucleus
Globose nuclei (2 pairs)
Fastigial nucleus
Cerebral cortex - grey matter - Molecular, Purkinje, Granule cell

* Cellular geometry
Purkinje cells - v. large cell body - its dendritic tree is flat & oriented perpendicularly to several folia
Granule
Molecular cells - T shaped - run along a folia talking to several purkinje cells.

* Neurocircuits
- Climbing fibers : Red nucleus - Inferior olive - Cerebellum
Olivocerebellar fibers that innervate deep nuclei and P. cells
- Mossy fibers : Cerebral cortex - ventrolateral nucleus - Pons - Cerebellum
Pontocerebellar fibres that innervate deep nuclei and molecular cells
granule

Both climbing & mossy fibres are excitatory whereas Purkinje axons are gabaergic ⇒ inhibitory
Deep nuclei integrate & compute all inputs.

Suhitha's part

Paramecium : * Posterior bump – K^+ channels – more -ve – swims rapidly
* Anterior bump – Ca^{2+} channels open – Ca rushes in ⇒ more +ve
– Cilia beat backward rapidly
– If depolarisation is above certain threshold, it triggers a larger, ungraded response
– Recovery : Ca-dependent K^+ channels & active transporters to eliminate Ca^{2+}.

Action potential — • Ungraded ie triggered by anything above threshold (no noise) ↵
• Doesn't degrade (long distance)
• Fixed size & duration

Reverse potential : the electric potential difference that exactly balances the ionic conc. gradient

→ Laws

1. Fick's Law of diffusion

$$J_{diff} = -D \frac{\partial [c]}{\partial x}$$

Diffusion takes place down conc gradient & its directly proportional to magnitude of conc grad

2. Ohm's law of drift

$$J_{drift} = -\mu z [c] \frac{\partial V}{\partial x} \qquad \partial_{el} = -\mu z [c] \text{ : electrical conductivity}$$

3. Einstein's relation – Both drift & diffusion face the same frictional force at thermal equilibrium

$$D = \frac{kT\mu}{q} = \frac{\mu RT}{F}$$

4. Space-charge neutrality – $\sum_i z_i^c e [c_i] = \sum_j z_j^a e [c_j]$

→ Nernst-Planck Equation (NPE)

$$J = -\left[\mu z [c] \frac{\partial V}{\partial x} + \frac{\mu kT}{q} \frac{\partial c}{\partial x}\right] \quad \text{Ionflux form}$$

$$j = -\left[\mu z [c] \frac{\partial V}{\partial x} + \frac{\mu RT}{F} \frac{\partial c}{\partial x}\right] \quad \text{Molar form}$$

$$I = -\left[\mu z^2 F [c] \frac{\partial V}{\partial x} + \gamma z RT \frac{\partial c}{\partial x}\right] \quad \text{Current form}$$

* Nernst equation : $V_{in} = \frac{RT}{zF} \ln\left(\frac{[c]_o}{[c]_i}\right)$

* Pumps & transporters : 1. Na-K pump ($3Na \rightarrow$ $2K \leftarrow$)
  2. Na-Ca exchanger (Na in, Ca out)
  3. PMCA & SERCA (takes Ca^{2+} out of cytoplasm)

* If no active transporters and membrane is permeable, then membrane potential is the Nernst potential of ions.

* Donan equilibrium : for C^{+m} and A^{-n} — $V_{in} = V_C = V_A$

$$\frac{1}{m} \ln\left(\frac{[C]_o}{[C]_i}\right) = \frac{1}{n} \ln\left(\frac{[A]_o}{[A]_i}\right)$$

* Membrane permeability (when conc. decreases linearly i.e $\frac{d[c]}{dx} = \frac{\beta \Delta c}{l}$)

$$P = \frac{D\beta}{l} = \frac{\gamma RT\beta}{Fl}$$

→ GHK (Constant Field Model)

Take equation for I from NPE. Define $y = I - \frac{\gamma z^2 [c] V}{l}$

$\frac{dy}{dx}$ → find the expression for I

Current equation : $I = \left(PFz\Sigma \left(\frac{[c]_i - [c]_o e^{-\Sigma}}{(1 - e^{-\Sigma})}\right)\right)$ $\quad \Sigma = \frac{zFV}{RT}$ $\quad P = \frac{\gamma\beta RT}{lF}$

$$I = I_{out} - I_{in}$$

Voltage equation : $V = \frac{RT}{F} \ln\left(\frac{P_K[K_o] + P_{Na}[Na_o] + P_{Cl}[Cl_i]}{P_K[K_i] + P_{Na}[Na_i] + P_{Cl}[Cl_o]}\right)$

→ Membrane conductances

Chord : $G_m = \frac{I_i}{V_m - E_i}$ $\quad$ Slope : $G_m = \frac{dI}{dV_m}$

Linear membrane : chord = slope

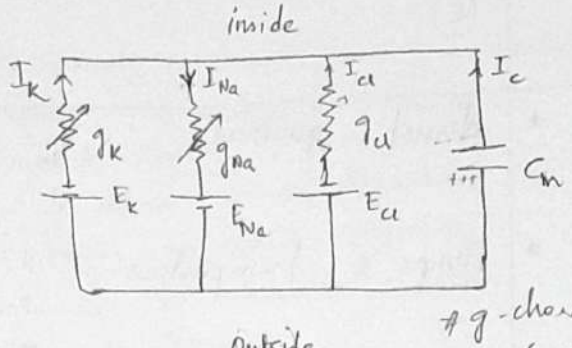
Non-linear membrane : Chord : $G = \frac{F(v)}{V_m - E_i}$ $\quad G = F'(v)$: slope

(I is a fn of voltage [and time]) → Instantaneous & steady state

→ Parallel conductance model

$$V = \frac{g_K E_K + g_{Na} E_{Na} + g_{Cl} E_{Cl}}{g_K + g_{Na} + g_{Cl}}$$

at steady state ($I = 0$)



g-chord conductance

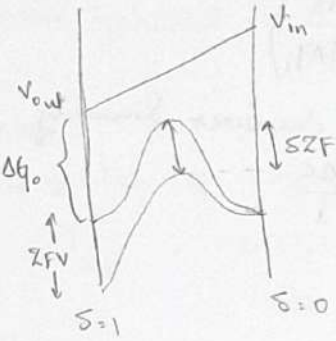
Current equation

$$I = C_m \frac{dV}{dt} + g_{Na}(V - E_{Na}) + g_K(V - E_K) + g_{Cl}(V - E_{Cl})$$

→ Energy barrier model

In general, $\beta[c]_0 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \beta[c]_i$; Arrhenius eqn: $k = A e^{-\frac{E_a}{RT}}$

Mass action law: $J = k\beta[c]$



$$I = zF(J_{out} - J_{in})$$

$$I = zF\beta k_0 \left([c]_i e^{\delta zFV/RT} - [c]_0 e^{-(1-\delta)zFV/RT} \right)$$

When $\delta = 0$ ⇒ Barrier inside ⇒ Inward rectified

$\delta = 1$ ⇒ barrier outside ⇒ Outward rectified

→ Gate model

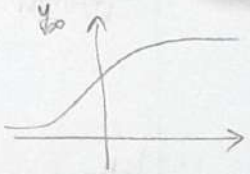
Assumptions: Ions flow down conc gradient

Each channel is voltage dependent – described by single barrier

Rate of opening & closing: Open $\underset{\alpha(V)}{\overset{\beta(V)}{\rightleftharpoons}}$ close : 1st order kinetics

(y) (1-y)

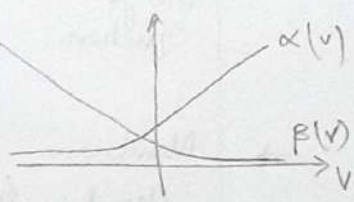
$$y_\infty = \frac{\alpha}{\alpha + \beta} \qquad y(t) = y_\infty - (y_\infty - y_0) e^{-(\alpha+\beta)t}$$



P ind. gates ⇒ $Y(t) = [y(t)]^P$

$\alpha = \alpha_0 e^{\delta zFV/RT}$ $\quad \beta = \beta_0 e^{-(1-\delta)zFV/RT}$

If $\alpha_0 = \beta_0$, $\quad y_\infty = \frac{1}{1 + e^{-zFV/RT}}$



→ Hodgin-Huxley analysis of Giant Squid Axon

Voltage clamp experiments: $\frac{dV}{dt} = 0$

Early Na+ current ; late k+ current.

Instantaneous conductances are same — so IV relation is linear

Based on parallel conductance. I is additive & changing conductances are given by—

(D)

$$g_K(t) = \bar{g}_K n^4 = \bar{g}_K \left(n_\infty \left(1 - e^{-t/\tau_n}\right)\right)^4 \quad \because n_\infty \sim 0$$

$$g_{Na}(t) = \bar{g}_{Na} m_\infty^3 h_0 \left(1 - e^{-t/\tau_m}\right)^3 e^{-t/\tau_h} \quad \because n_0 \sim h_\infty \sim 0$$

	m	h	n
Speed	Fast	slow	slow
Ion	Na	Na	K
Opens at	Depolarisatn	Polaris	Depolarisatn

From data, find n_∞ and $\tau_n \longrightarrow g_K(t_\infty) = \bar{g}_K n_\infty^4$

$$\alpha_n = \frac{n_\infty}{\tau_n} \qquad \beta_n = \frac{1 - n_\infty}{\tau_n}$$

$$g_K(t = \tau_n) = \bar{g}_K n_\infty^4 (0.632)^4$$

$$= g_K(t_\infty) \times 0.16$$

Neurobiology B13154 - Summary Pt. 2

Plasticity - change in neuronal connections & strength of synaptic transmission

① Habituation - response to non-aversive stim. decreases
② Sensitisation - response after aversive stim. increases

① Decrease in release of NT after repeated stim
② Facilitatory neurons release Serotonin → AC → cAMP → prot. kinase A → phosphorylates K^+ channels (reduces open prob.) → increases AP dur → Ca^{2+} ch. at axon terminal remains open

Classical conditioning
CS opens Ca^{2+} channels ⇒ Ca^{2+} rushes in
US releases serotonin → AC, which detects coincidence of serotonin & Ca^{2+}. AC levels vary b/w US & US+CS
AC → cAMP → phosphorylated K^+ ch → more Ca^{2+} influx

Operant condtn : association b/w behaviour & it's consequence (Law of effect)
Imprinting - complex learning.

Types of synaptic plasticity —

Neuroplasticity
- Physiological p
- Anatomical p.

Developmental p.
- Synaptic refinement — Topography, Convergence, Post synaptic compartment

Meaning plasticity — record changes in PSP (which are additive)

Synaptic refinement in ODC of cat
- Both eyes open : bell shaped
- one eye closed :
- both eyes closed :
- artificial strabismus :

Synaptic strengthening : temporally correlated spike time dependant plasticity

(2)

Lack of activity — prevents elimination of synapses

Enriched environment — response to greater range of stimulus
stress recovery

Activity contributes to topography — eye reversal in frog

Critical period — period in development during which some forms of neuronal activity plasticity occurs

- Monocular depriv. in kitten — shift in cort. innervation
- GAD 65 mutant — no GABA — adults show similar shifts
  ⇒ inhibitory connections are v. important

Spontaneous activity — sufficient for stripes

NMDAR — when blocked, no refinement. (frog 3 eyes, tectum stripes)
when they open, Ca^{2+} rushes in, Ca-dependent proteolytic enzymes degrade non-active terminals

Plasticity in periphery — Glomeruli (olfactory)

* Fear conditioning w/ smell — after training, there was an increase in amplitude of signals ⇒ activity modulated

* Olfactory learning enhances sensory neuron input strength
  Go/No-go paradigm — improved w/ ↑ conc, activity increased

Structural plasticity

- Whisker removal in mice — new persistent spines likely to grow, old ones disappear, same spine f.
- Binocular deprivation — increases spine motility, doesn't alter structure or turnover rate
- Spine formation greater in trained mice (Rotarod). Sleep deprivation decreased this rate

LTP/LTD — long lasting activation/decrease in activity
↳ shapes the synapse as well

1. NT release — Glut. receptors open
2-3 Strong EPSP → backpropogating AP ⇒ more Ca^{2+}
4. Intracellular Ca released from ER
5. ↑ Ca contributes to kinase activity
6. Kinase (CaCaMK) phosphorylates AMPAR & AC — ↑activity
7. Current flow through AMPAR ↑ : Early LTP
8. AC → cAMP → PKA
9. PKA → CREB → gene transcription
10. Gene Tx — triggers late LTP
11. New AMPAR inserted in synapse
12. This increases synaptic efficacy i.e. Late LTP.

LTP mutant - NR1 (NAMPAR) knockouts - impairment of spatiotemporal memory.

NMDAR - coincidence detector - need high EPSP to remove Mg block

Optogenetics

ChR2 - 470 nm VChR1 - 535 nm NpHR - 589 nm

Precise control, specificity, fast response, bidir

- Drosophila - P2X2 dopaminergic neuron mutant - light → more locomotor activity
- Mouse - hypocretin - sleep to wake ratio decreased after light
- Timing of neurons - glomeruli
- Halorhodopsin - loss of function
  - ChAT neurons → MSN (inhibit through GABA)
  - Cocain - condtn place preferance decreased when ChAT inhibit
- OCD - sapap3 mutant
  - Fast-spiking striatal interneurons (FSI) → MSN
  - Delay conditioning paradigm: water, tone
  - optogen stim of lateral OFC alleviates compulsive grooming

Disadv: invasive, cumbersome, harmful

Neurodevelopment

Drosophila: ventrolateral → ventral furrow → delamination → ventral midline

as-c gene clusters - v. imp

Lateral inhibition (only one cell) through Notch signaling pathway

Delta + Notch → Notch ICD + SuH → Tx factor → E(spl) → as-c

Xenopus: IMZ cells induce overlying ectoderm - neurogenic tissue - neural plate → neural tube + crest cells

Spemann organizer - dorsal lip - neural inducer + body axis organizer

Neural inducer molecule - Noggin, Chordin

UV - ventralized LiCl - hyperdorsalized

In drosophila: Sog inhibits dpp to induce neural tissue in ventral

Noggin → BMP4 → Smad → Zic1 → neural genes

FGF → ERK → Zic3 → neural genes

Segmentation : Hox genes, rhombomere

Otx 2 - head

Retinoic Acid 

Dorsoventral patterning : Ventral : floor plate - motor,
dorsal : roof plate, sensory

- Notochord imp for ventral - Shh
- Epi Ectodermal tissue - BMP 4/7 - dorsal patterning

Midbrain - hindbrain - important marker

Neuroblasts - generation & migration

BMP 2/4 GGF, TGF β

Cerebral cortex neurons - inside-first, outside last
vertical / horizontal cleavage

Survival of neurons

NGF → Bcl 2 ⊣ Apaf 1 → Caspase → Cell death

Molecular view of axon growth.