MODULE A

ELECTRICAL SYNAPSE

- Current (ions) flows via GAP junctions
- Very fast; almost instantaneous.

CHEMICAL SYNAPSE

- Greater gap b/w pre and post syn mem: *synaptic* cleft
- Neurotransmitter secretion:
    - ➔ Caused due to Ca2+ influx via voltage-gated channels.
    - ➔ Increase in Ca2+= fusion of pre syn and plasma membrane
    - ➔ Releases content.

1. Transmitters synthesised and stored in vesicles.
2. Action potential.
3. Depolarization of pre-syn = Ca2+ voltage gated channels open.
4. Influx of ca2+
5. Ca2+ causes vesicles to fuse w/ pre-syn.
6. Neurotransmitter is exocytosed into the pre-syn cleft.
7. Trans binds to receptor on post syn mem.
8. postsynaptic mem opens/ close -> inhibitory or excitatory response.

WHAT DEFINES A NEUROTRANSMITTER

1. MUST be clearly present w/in pre-syn neuron
    - Can't sec w/o it being there
2. MUST be released bc of depolarization + Ca2+ dependant.
    - Needs ca2+ influx to be released.
3. MUST have spec receptors on the post syn membrane.

Lecture: 1 TOPIC 1

Axons:

- connect to other neurons
- Polarised cells
- Outgoing info
- Presynaptic
- Most common neurotransmitter: GLUTAMATE.
- Active zone
- Synthesise vesicles
- Release neurotransmitters (E info → chemical)

Dendrites:

- Site of synapse
- Incoming info
- Postsynaptic density: increased conc of proteins. High in plasticity.
- Receptors for neurotransmitters (chem info → Electrical info).

Synapse:

- Highly specialized sites of contact.
- Rapid communication of info (millisec)
- Transform electrical info to chemical and back to electrical. (E⟶ C ⟶ E)

SNAREs Protein

- Some on vesicles and post syn mem
- Twirl/**intertwine** and ensure fusion of both occurs.
- Ca2+ enters + induces *Synaptotagmin + synaptobrevin (**on vesicles)*** to intertwine with *syntaxin + SNAP-25 (**on presyn)***
- Important in regulating neurotransmitter transmission
- Does not work properly in Autism

POST-SYN RECEPTORS

- ligand-gated ion channels = rapid
- G-protein coupled receptors = modulatory // regulate things on a longer timeframe.

INHIBITORY

- Medicated by *interneurons**
- ** always inhibitory.*
- Don't release glutamate: -releases GABA* and Glycine
- Don't release glutamate
  - ➔ releases GABA* and glycine
  - ➔ Permeable to Cl-
- Causes hyperpolarization of resting membrane potential.

EXCITATORY

- Mainly glutamate (90%)
- 3 receptors:
  - ➔ AMPA*** ion channels, different channel properties.
  - ➔ NMDA**
  - ➔ mGluR* activates 2nd messengers.

AMPA: only perm to Na+ and K+
NMDA: Mainly perm to Ca2+

- Voltage-gated.
- At rest: Blocked by Mg2+
- Depolarised: Mg2+ unbound and Ca2+ can enter.
- Needs pre & post syn membrane to *DEPOLARISE* to *open*: "coincidence detector"
- Increased level of activation of pre & post; increase in the magnitude of Ca2+ influx.

Lecture: 2

MEASURING SYNAPSE FUNCTION:

- Electrical stimulation of presyn axon = action potential + glutamate release.
- Current flows measured electro physically.

- Gives a measure of synaptic function.
    - ➔ Transmitter release (axons)
    - ➔ Postsynaptic current flow (how much).

FIELD RECORDING/ EXTRACELLULAR RECORDINGS:

- Recording electrode **amongst** synapses formed b/w incoming *axons* + *postsyn neurons.*
- Measures loss of positive ions (mainly Na+) as they move from extra synaptic space to postsynaptic.
- Measures the **strength** of syn from a **POPULATION** of neurons.
- Feedback from large # of cells.
- Loss of positive ion = downwards deflection
- Shows level of synaptic activity/strength

WHOLE CELL (patch clamping) RECORDINGS:

- Electrode attached to **specific** neuron
- Becomes 'part' of neuron
- Measures current flow **into** neurons.
- Gives a measure of the strength of syn from **individual** neurons.
- Anything a neuron does; can be seen electrically.
- Specific info:
    - ➔ AMPA/NMDA receptor activated (what ion channels involved)
    - ➔ excitatory/inhibitory transmission

MEASURING AMPA RECEPTORS:

- Always opens upon glutamate binding
- Postsynaptic mem is close to *~65mV*
- Current flow can be measured through AMPA receptors
- Downwards deflection (diagram) means a current is coming *INTO* cell.
    - ➔ Regardless of whether it's excitatory or inhibitory.
- EPSC

PLASTICITY:

- Ability of synapse to change their strength in response to specific neuronal info.
- Can be increased or decreased.
- Affects the **size** and **strength** of **current**.
- Studied via hippocampal slice prep

<u>Stimulation that leads to:</u>

↑**in strength = LTP** ⟶ current goes from weaker to stronger
↓**in strength = LTD** ⟶ current goes from stronger to weaker
**both underlie memory formation @ subcellular level.*

HIPPOCAMPUS STUDY:

- Dissect -> keep in oxygenated, artificial cerebrospinal fluid.
- Slices stay viable for hours

- Stimulation of specific axonal pathways + recordings from specific postsyn pyramidal cells in certain area.

Trisynaptic loop: measure synaptic transmission in the hippocampus

1. Perforant path: entorhinal cortex to dentate gyrus* *first site of LTP discovery*.
2. Dentate gyrus neurons (granule cells project *mossy fibres)* to CA3 pyramidal neurons
3. CA3 pyramidal neurons (project *Schaffer collaterals)* to CA1 pyramidal neurons

Lecture 3:

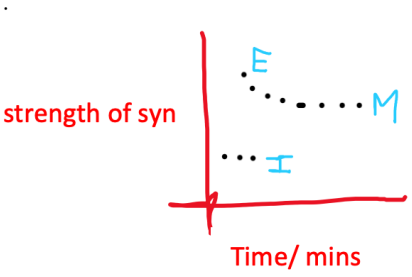
LONG TERM POTENTIAL (LTP):

- Increase in strength
- Only occurs if paired activities of pre-syn & post-syn cells are tightly linked in time.
- Strong post-syn depolarisation occurs w/in 100 m/s of pre-syn release.
- Shows ***input specificity****:*
  - ➔ If one neuron is induced by the stim, the other inactive + uncontacted synapses are unaffected.
  - ➔ Only stimulated synapse are potentiated
  - ➔ Restricted to ACTIVATED cells only.
- ***Associativity****:*
  - ➔ Weak stim doesn't itself cause LTP
  - ➔ One weakly activated pathway + strongly activated the neighbouring pathway = both undergo LTP.
  - ➔ LTP req both depolarisation of post syn cell + pre syn to release glutamate.
  - ➔ ^ like NMDA receptors

❑ LTP= ↑ in syn strength
❑ Measured = amplitude of post syn AMPA receptor current.

3 PHASES:

1. **Induction:** stim that causes ↑ in syn strength. *
2. **Expression:** how synapse strength is ↑
3. **Maintenance:** how the syn strength is maintained.



LTP ***NEEDS* NMDA for induction phase.*

- LTP induction is Ca2+ influx via NMDA receptor dependent.
- ∴ both need to occur simultaneously.

PROPERTIES OF LTP:

- Input specificity.
- Associativity.
- **Saturable:** stimulated neurons can only be stimulated up to a *MAXIMUM* level.

HOW IS LTP INDUCED:

- **TETANIC STIM:** brief burst of ↑ freq stim (100 Hz) for 1 min to presyn axon.
    - ➔ ↑ # of glutamate receptors.
    - ➔ ↑ AMPA receptors.
    - ➔ ↑ in Na+
    - ➔ more/faster depolaristion of post syn neuron.
- **PAIRING:** stim 1 Hz/ sec + postsynaptic depolarization via current injection for 1 min.
- **THETA BURST STIM:** Theta rhythm (6-10 Hz oscillations).
    - ➔ Mimics the freq in the hippocampus.
- *ALL HAVE THE SAME PURPOSE: induce LTP by activating presyn (via glutamate release) and depolarise postman @ SAME TIME.*

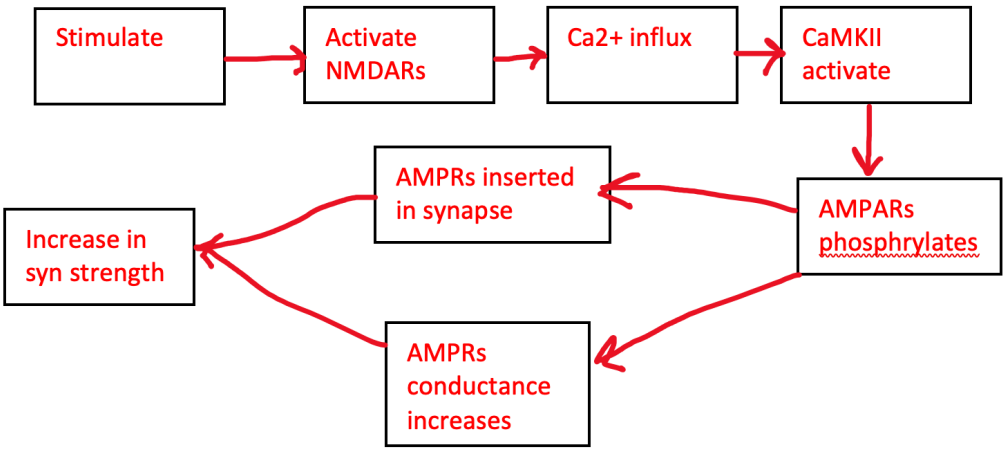
AMPA RECEPTORS + CaMKII:

- CaMKII = calcium calmodulin dependant protein kinase II.
    - ➔ Phosphorylates. (↓ after Ca2+ influx)
    - ➔ CaMKII phosphorylates AMPA receptors.
    - ➔ CaMKII injection in post syn is adequate to induce LTP
    - ➔ Makes up 2% of total proteins in neurons.
    - ➔ S831p is a major site of CaMKII phosphorylation.

❑ Makes conduction of existing AMPA receptors.
- Phosphorylation ↑ AMPA receptor conductance.

❑ ↑ in # of AMPA receptors @ post syn neuron.
- ↑ synapse strength ∴ ↑ in LTP.

LTP SUMMARY:



Lecture 4:

LONG TERM DEPRESSION (LTD):

- Induces ↓ in syn strength.
- ↓ in size of amplitude of AMPAR current.
- Prevents saturation
- ***LFS:*** low freq stimulation

HOW IS LTD INDUCED:

- Extended period of ↓ freq (1 Hz) over a long period of time @ presyn axon.
  - causes depression in the population of post syn neurons.
- LFS NOT accompanied by large depolarisation.

PROPERTIES OF LTD:

- Input specific: only neurons stim w/ 1Hz are depressed.
- NMDAr induced Ca2+ influx dependent.
  - application of NMDA blockers (eg. APV) during LTD induction blocks LTD.

Ca2+ INFLUX TRIGGERS LTP & LTD:

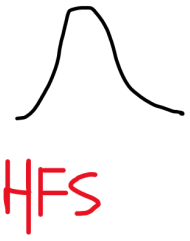
- **When LTD induced:**
  - ➔ Post syn is WEAKLY depolarised; ↓ Ca2+ influx.
  - ➔ LTP = more depolarised ∴ more Ca2+ influx.
- LTP or LTD = depends on LEVEL of Ca2+ influx vida NMDAR.

PHOSPHATASES (LTD):

- Prolonged levels of ↓ Ca2+ levels activates protein PHOSPHATASES.
- Opposite action of protein kinases.
  - ➔ Dephosphorylation of AMPAR.
  - ➔ Leads to removal of AMPAR @ post syn. **unanchored, endocytosed back into cell via clathrin-dependent endocytosis.*
  - ➔ ↓ # of AMPAR
  - ➔ ↓ current.

LTP: = ↑ depolarisation
= ∴ ↑ Ca2+ influx **(HFS)**

LTD: = ↓ depolarisation
= ∴ ↓ Ca2+ influx **(LFS)**.
= long, slow stimulation.
= phosphatase activation → dephosphorylation of AMPAR → loss of high conductance AMPAR.



PLASTICITY & MEMORY:

- LTP & LTD can be induced in awake, behaving animals.
- Contralateral hippocampus provided excellent control.
- LTP/LTD can be induced w/ brief trains of stimulation.
  **mimic real life currents.*
- Robustly expressed in all major pathways.
- Agents that block LTP & LTD also prevent/ block spatial learning. (*presence of NMDA blockers)*
- Genetic removal of CaMKII gene prevents LTP + induces learning deficits in rodents.
  Smart mouse = ↑# CaMKII & NMDAR.
- LTP & LTD lasts for long periods of time (months).

Lecture 5: TOPIC 2

BRAIN CELLS & RECEPTORS:

GLIA, aka neuroglia: create the myelin sheath.

- Oligodendrocytes
- Astrocytes
- Microglia: immune sys cells.

MICROGLIA CELLS:

- Immune cells (innate immune system)
  - ➔ monocytic/myeloid origin.
- Express diff immunological markers.
- Brain's "professional phagocytes"
  - ➔ Extremely good/ experts.
  - ➔ Designed to recognise debris
  - ➔ ↑ # of receptors.
- Mobile.
- Secrete proinflammatory cytokines; may present antigens (APC).
- Types:
  1. Perivascular
  2. Foamy
  3. APC via MHC
  4. Activated (inflam)
  5. phagocytic/ non-phagocytic.
  6. Ramified.
- CD163- scavenger receptor.
- Elude to cellular function; but highly context dependent.
  - ➔ Some microglial cells have a subset

POTENTIAL THERAPEUTIC TARGET:

1.
   a) Phagocytosis
   b) inflammatory response
   c) Suppression of APC activity
2.
   a) Microglia = functionally heterogeneous
      - Target specific,
      - APC vs phagocytic functioning.
   b) Drugs targeting the brain ⇉ get across BBB.

→NO CURRENT DRUGS

ASTROCYTES:

- Support for neurons: **always @ synapses**.
  - ➔ Neurotransmitter: clearance/levels/production.
  - ➔ Nutrient supply, H2O removal.

➔ O2 provider.

- Maintenance/ support of the neurovascular unit.
  - ➔ Important source of neurotrophic factors.
  - ➔ Progenitor qualities/ capabilities.
- Have immunological functions too (innate immunity).
  - ➔ During neuroinflammation.
- Actively migrate towards injury.
- Produce cyto/chemokine*.
  - ➔ MCP-1, IR10, IL-8, MIP1

    **chemokines = attract other immune cells.*
- Produce neurotrophic factors → help in protecting neurons from cell death
- Express **GFAP** - glial fibrillary acid protein.
  - ➔ Used to stain for astrocytes.
- Astrocytes wrap around neurons = forming complex network.
- Loss of neuronal support cells = :(
- Loss of vascular integrity = :(
- Produce neurotrophic factors
  - ➔ CDNF *important growth factors for neurons
  - ➔ NGF
  - ➔ FGF
  - ➔ VEGF
- Important for neuroinflammation
  - ➔ Very plastic/dynamic cells

Lecture 6:

DISEASES:

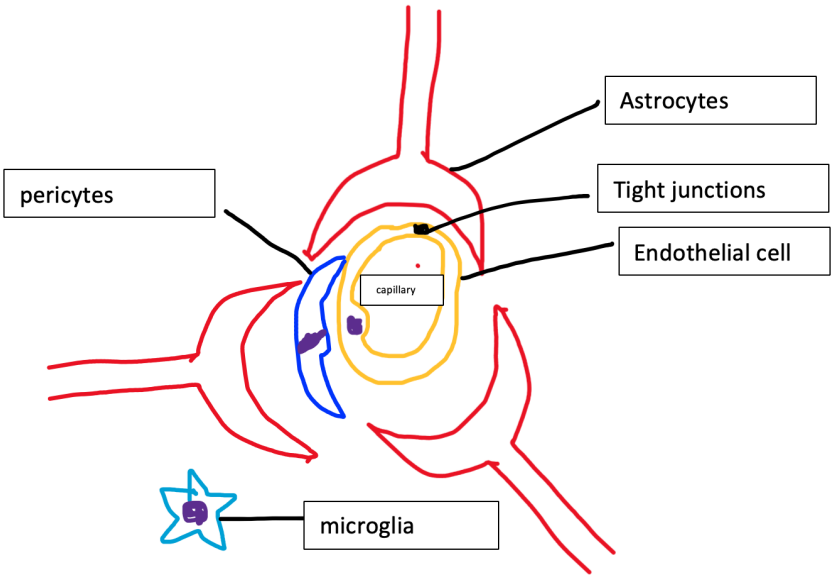
- Neurons = alzheimers
- Astrocytes = astrocytoma
- Microglia = anti MG antibiotics in AD patients.
- Endothelial cells = stroke, vascular disease
- Oligodendrocytes = infections, multiple sclerosis

NEUROVASCULAR UNIT + B.B.B:

- Brain's highly vasculated
- Small capillary: 20µm
- Large vessels: 100-150µm
- Every neuronal cell is w/in 200µm of vessels

NVU

- endothelial cells + pericytes + astrocytes + perivascular macrophages
- Tight junctions between endothelial cells

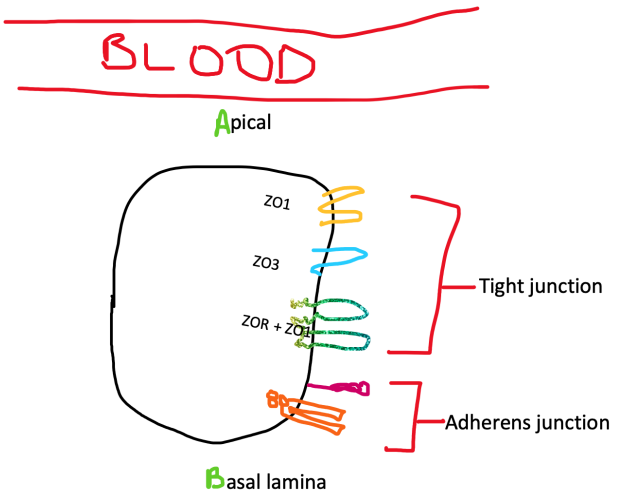


- Side facing blood = apical

TIGHT JUNCTIONS: selective barrier

- Occludin
- Claudin
- ZO-1 (zonula occludens)
- JAMS (junctional adhesion molecules)

*form paracellular space



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B.B.B & NEUROINFLAMMATION:

- Neurons don't survive well during inflammation
- T-cells survey (@ low frequency) for inflammation, infection, cancer
- Cytokines, IL-1/ TNF-α (by microglia)
  - ➔ Upregulate cell adhesion (apical side) molecules
  - ➔ Immune cells have to go through vasculature L.T.S.D
  - ➔ **L**eukocyte-endothelial interaction
  - ➔ **T**ethering/rolling
  - ➔ **S**trong adhesion
  - ➔ **D**iapedesis/extravasation
- VCAM-1 & ICAM-1 important endothelial cell adhesion molecules
  - ➔ Promotes strong interaction w/ blood leukocytes
- Important for brain immune cells

Lecture 7

ENDO Cell Adhesion Molecules interacting w/ LEUKOCYTE CAM:

ENDO CAM

- ICAM-1
- VCAM-1
- E-Selectin
- P-Selectin
- CD99
- CD31

LEUKOCYTE CAM

- CD11a, CD11b, CD11c (LFA)
- CD49D/ CD29 (VLA4)
- CD15 & CD162
- CD24 & CD162
- CD99
- CD31

MULTIPLE SCLEROSIS:

- Autoreactive disease ⟶ T-cells destroy *oligodendrocytes (produce myelin sheath for neuronal axons)*
- Immune cells almost “absent” in normal CNS but in large numbers in M.S
- M.S = chronic inflammation

- Vessels enlarged + inflamed
    - ➔ Express ↑ amounts of adhesion/cytokines
- T-cells ⇒ antigen specific to *myelin.*

DRUGS & B.B.B:

- Difficult to get through (lipophilic molecules can easily get through)
    - ➔ a) passive diffusion
    - ➔ b) ABC transporter EFFLUX
    - ➔ c) solute carriers SLC
- Smaller drugs eg. *paracetamol/NSAIDs* can readily get through BBB
    - ➔ Caffeine: adenosine R-antagonist
    - ➔ Alcohol: NMDA/GABA receptor
    - ➔ Cocaine: Blocks dopamine transporter
    - ➔ Cannabis: CB1/CB2 receptors + others
- Larger drugs = excluded
- Most drugs excluded by endothelial export transporters & BBB tightness.

Lecture 8: TOPIC 3

REVISION FROM MEDSCI142:

- **Left Hemisphere:** dominant for language
- **Right Hemisphere:** spatial awareness, face recognition etc
- **Precentral gyrus:** 1° motor cortex
- **Postcentral gyrus:** 1° sensory cortex ⟶ peripheral sensations
- **1° visual cortex:** take sight in + organise the visual field
    - ➔ Rest of occipital lobe: understand visual field
- **1° auditory cortex:** periphery sound ⟶ pick up sound + orders it according to tone
    - ➔ Tonotopic rep of sound
- **Wernicke's area:** figures/understands WORDS
    - ➔ Interprets sound // tonopic rep
- **Temporal association cortex:** Memory + anger
- **Supramarginal Gyrus:** reading
    - ➔ *Eye movements* + understanding
- **Angular Gyrus:** Writing