L11: NEUROTRANSMITTER RELEASE

Lecture Outcomes:
- *Explain key aspects of neurotransmitter release*
- *Explain how to measure neurotransmitter release*
- *Explain different steps of the vesicle cycle*

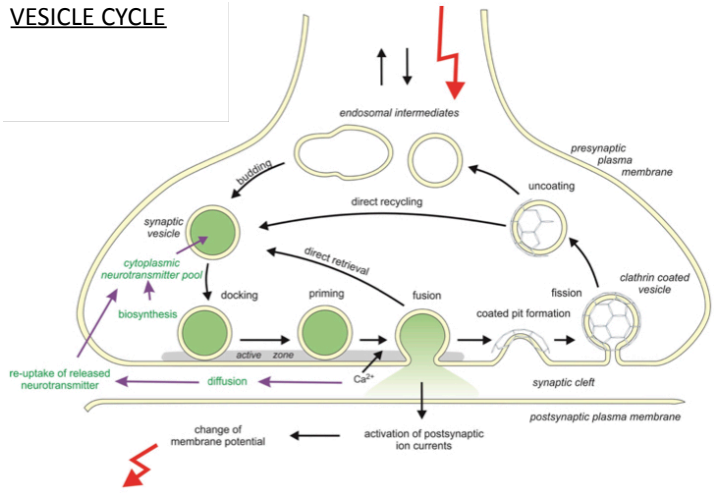
STORAGE AND RELEASE OF SYNAPTIC VESICLES

- The **active zone** (synaptic cleft) is the release site of vesicles – has a a greater concentration of vesicles and Ca^{2+} channels.

DETECTION OF TRANSMITTER RELEASE

- Measure **membrane capacitance** which determines how quickly the membrane potential can respond to a change in current – the greater the size of the membrane, the greater its ability to hold charge (↑ capacitance).
- Pre-load vesicles with a fluorescent dye and image vesicular release (exocytosis).
- Use electrochemical probes to detect breakdown products in the synaptic cleft – amount of NT release.

VESICLE CYCLE



- Proteins necessary for the vesicle cycle are produced in the soma ⟶ transported down the axon, **targeted** towards the axon terminal ⟶ **tethered** to the cytoskeleton ⟶ "filled" with NT ⟶ **docked** very close to the cell membrane (near synapse) ready for release ⟶ depolarisation ⟶ Ca^{2+} influx ⟶ triggers vesicle to merge with cell membrane ⟶ **release** contents into synaptic cleft.
- Membrane and transmitter recovery/breakdown.
- Vesicle replenishing and recycling.

TWO POPULATIONS OF VESICLES

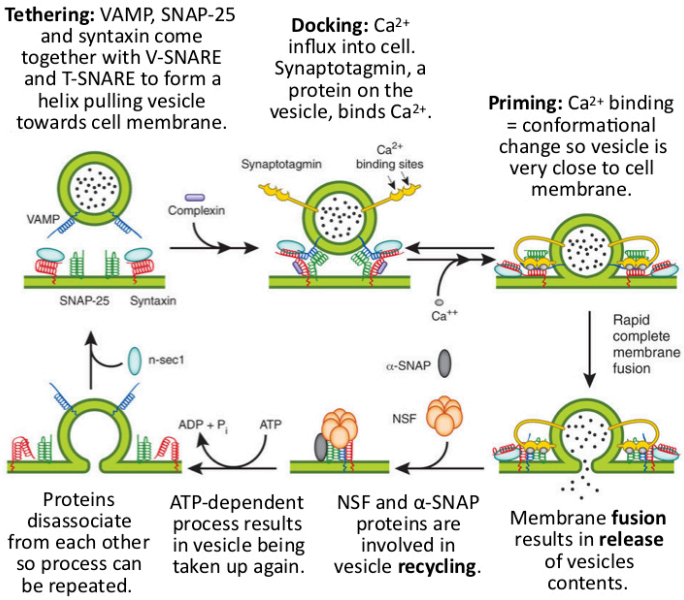
- **Storage pool or reserve pool (RP)**:
  - Only recruited at higher frequencies of nerve stimulation
  - Located further away from the active zone
  - Bound to microtubules (actin) by synapsin I
- **Readily releasable pool (RRP)**:
  - Docked vesicles ready for immediate release
  - Located near active zone (defines release probability = p)

Reserve Pool to Readily Releasable Pool

- High frequency stimulation ⟶ large Ca^{2+} influx in presynaptic membrane ⟶ Ca^{2+} activates protein CaM kinase II ⟶ phosphorylates synapsin (unbinds vesicles from cytoskeleton) ⟶ vesicles migrate (along the cytoskeleton?) to active zone ready for release.
  - Process requires large amounts of Ca^{2+} in a large area.
- However, the amount of Ca^{2+} influx sufficient to induce only release is very small.
  - Ca^{2+} influx can be immediately effective because only the local concentration has to rise (nanodomain/ microdomain).
  - Concentration is ~100µm and dissipates/disappears fast.

VESICLE TETHERING, DOCKING, AND PRIMING (SNARE)

- **SNARE proteins,** connected to the vesicle and the cell membrane, have the ability to bind to one another and form a helix. Conformational changes in the helix can pull the vesicle and membrane together.
  - V-SNARE (synaptobrevin/VAMP) = bound to vesicle.
  - T-SNARE (syntaxin) = bound to target (cell membrane).
- However, many other proteins are also involved:

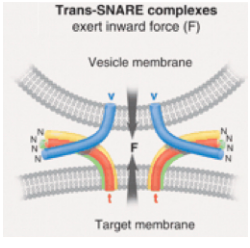


Calcium Triggered Release via Synaptotagmin

- Ca^{2+} binds to C2A domain, whereas C2B domain is required for maximal association with syntaxin.
- It has been shown that NT release is reduced by mutations that prevent binding of synaptotagmin (and several other proteins) to syntaxin.

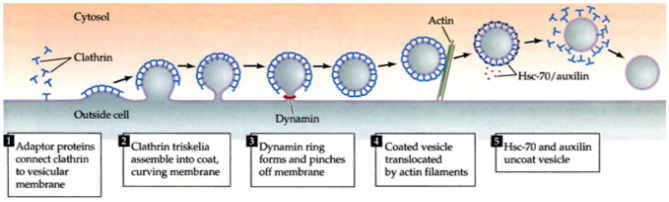
Membrane Fusion

- SNARE complex (including synaptotagmin after Ca^{2+} binding) "pulls" membranes together, forcing them to *fuse*, creating a fusion pore for transmitter release. They are *NOT* thought to be a pore.

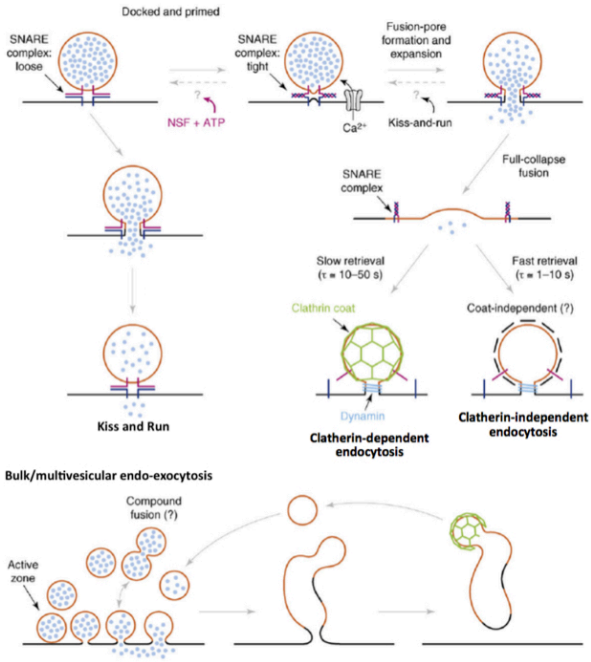


VESICLE RECYCLING

- Fusion increases surface area of plasma membrane nerve terminal, needs to be recycled.
  - ↑ surface area = ↑ capacitance.
  - Vesicle recycling can be monitored by a decrease in capacitance.
- **Clathrin proteins** are involved in vesicle recycling.
  - Adaptor proteins connect clathrin to membrane ⟶ **clathrin triskelia** assemble into a coat ⟶ **dynamin** pinches off the vesicle from the membrane ⟶ coated vesicle is translocated to the cytoskeleton by actin filaments ⟶ **Hsc-70** and **auxilin** uncoat the vesicle.



Other Ways to Recycle Vesicles



SYNAPTIC SPECIALISATION (MULTIQUANTAL RELEASE)

- **Ribbon synapse (cochlear hair cells):** ribbon tethers multiple vesicles ready for release at once – always have a response in post-synaptic membrane = high speed synapses.

SOME THINGS TO THINK ABOUT

- Neurotransmission at synapses of the CNS shares the fundamental features of neuromuscular signalling, however, there are several important differences:
  1) Synaptic boutons are much smaller and contain only one or a few active zones.
  2) The mechanism of transmitter release may vary between synapses or at a particular synapse, with both complete exocytosis and kiss-and-run release of transmitter through a transitory fusion pore. This can cause variation in quantal size and vesicle diameters.
  3) Multiquantal release (constant activation).

L12: SYNAPTIC DEVELOPMENT AND PRUNING

Lecture Outcomes:

- *Explain steps in synapse formation*
- *Explain the role of different pre-synaptic and post-synaptic factors in synapse formation/maturation*
- *Explain process of receptor clustering and factors involved*
- *Peripheral vs central synapse formation*
- *Explain steps and factors involved in synapse elimination*
- *Explain the role of activity in synapse elimination*
- *Explain Hebbian modification of synapse strength*

BASIC STEPS OF SYNAPSE FORMATION

1) Formation of a selective connection between axon and target – directed innervation.
2) Differentiation of growth cone into nerve terminal.
3) Elaboration of post-synaptic apparatus.

SYNAPSE FORMATION

- **Synapse:** specialisation for cell-to-cell communication.
- Once an axon finds its target, changes occur in both pre- and post synaptic cells.

Pre-synaptic cell	Post-synaptic cell
– Vesicle clustering – NT synthesis and release – Cytoskeleton changes – Formation of active zones – Concentration of mitochondria to synapse	– Clustering of NT receptors (scaffolding proteins) – Morphological changes: • Postsynaptic density – CNS • Membrane involutions – NMJ

NEUROMUSCULAR JUNCTION (NMJ)

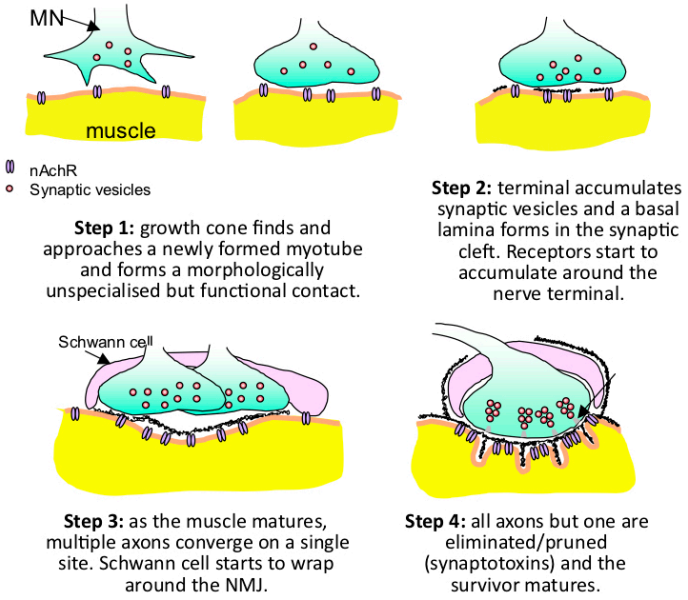
- NMJ is the best studied system for synapse formation.
- Experiment in frogs (Glicksman and Sanes, 1983):
  - Frogs easily regenerate motor neurons. Muscle damage and denervation – myofibres decompose but the basal lamina survives (staining acetylcholinesterase produced shows regrowth occurs at the same location).
  - Nonetheless, axons regenerate, contact basal lamina and acquire clusters of synaptic vesicles and membrane-associated dense patches that resemble active zones.
  - Suggests involvement of basal lamina.

BASAL LAMINA

- Sheet-like **basal lamina** is part of the extracellular matrix surrounding muscle fibres – consist of collagen and proteins.
- One such protein is **Laminin,** produced by the postsynaptic membrane, involved in **synapse maturation**.
- Synaptic basal lamina is rich in **acetylcholinesterase**.
- **Synaptic laminin** (s-laminin) may play a signalling role.
- There are multiple forms of laminin. Knockout of laminin results in abnormal synapse formation.
  - α2-deficient = junctional folds are smaller.
  - β2-knockout = no junctional folds/no pruning (multiple motor neurons end on muscle fibre = paralysis).

SYNAPSE FORMATION AT NMJ

- Synapse formation proceeds in discrete steps:



Step 1: growth cone finds and approaches a newly formed myotube and forms a morphologically unspecialised but functional contact.

Step 2: terminal accumulates synaptic vesicles and a basal lamina forms in the synaptic cleft. Receptors start to accumulate around the nerve terminal.

Step 3: as the muscle matures, multiple axons converge on a single site. Schwann cell starts to wrap around the NMJ.

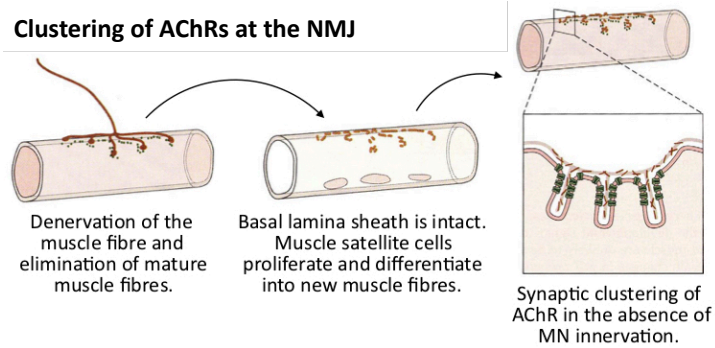
Step 4: all axons but one are eliminated/pruned (synaptotoxins) and the survivor matures.

- Not all processes that occur during synapse formation are dependent on other factors, some factors are already present independent of other driving factors – nerve and muscle cells can assemble synaptic components individually.
  - Example: a myotube produces ACh receptors independently of post-synaptic elements being present.

SYNAPSE MATURATION AT NMJ

- In an adult, the distribution of receptors is greater at the synapse compared to the rest of the fibre.
- However, during development, AChRs are distributed diffusely on the muscle surface. Nerve innervation triggers redistribution of AChRs.
  1) Translocation of surface AChRs.
  2) Transcriptional activation of AChRs in the nuclei at the synaptic region.
  3) ↓ AChR expression at non-synaptic sites = ↓ density of AChRs outside of synapse; ↑ gene expression at synapse = ↑ AChRs at synapse.

Clustering of AChRs at the NMJ



Denervation of the muscle fibre and elimination of mature muscle fibres.

Basal lamina sheath is intact. Muscle satellite cells proliferate and differentiate into new muscle fibres.

Synaptic clustering of AChR in the absence of MN innervation.

Agrin

- Agrin is a pre-synaptic modulator that plays a role in the clustering of AChRs.
- Agrin is released by nerve terminal and acts through MuSK and rapsyn (both post-synaptic) to aggregate AChRs at NMJ.
- In the absence of agrin, few AChR clusters are formed.

Neuregulin

- Neuregulin is expressed and secreted by nerve cells (pre-synaptic). It stimulates synthesis of AChRs at synaptic sites via erbB kinases (post-synaptic).
  - When neuregulin binds, downstream effects on the nuclei change transcription/translation of AChR RNA.

Neural Activity

- Nerve activity and denervation suppresses AChR expression (transcription) at non-synaptic sites.
- No activity results in upregulation of mRNA outside the synapse and down-regulation at the synapse.

PRUNING

- Initially, all motor neurons begin to grow towards muscle fibres – create more than needed (polyneural innervation).
- During development, some of neurons/synapses get **pruned** so each muscle fibre only receives input from one particular motor neuron.
- Reason for over-production is still unknown – possibilities:
  - Not enough genes to hardwire information.
  - Ensure all muscle fibres are innervated.
  - Epiphenomenon of over-eager motor neurons.

SYNAPTIC ELIMINATION

- Synapse elimination is a step of NMJ maturation.
- Segregation ⟶ invasion/retraction ⟶ receptor loss ⟶ ↓ synaptic strength/quantal content ⟶ final retraction bulb.
- Most AChR loss occurs before axon retraction.

Role for activity competition

- Remove some motor neurons, all muscle fibres are still innervated; remove original innervation, other motor neurons take over.
  - Example: monocular depreciation (Hubel and Wiesel).
  - Synapse elimination during crucial period = large effect, elimination during adult period = minor effect.
- Active synapses can destabilise inactive ones – maintenance vs. punishment signal.

FIRING PATTERNS (SYNCHRONY VS. ASYNCHRONY)

- *Early* in development, gap junctions are *present*, so the maintenance and punishment signals have no effect.
- *Later* in development, when the gap junctions *disappear*, motor neurons do not communicate so some neurons retract and eventually there is only a single neuron left.

HEBBIAN MODIFICATION

- Describes a basic mechanism for synaptic plasticity, where an ↑ in synaptic efficacy arises from the presynaptic cell's repeated and persistent stimulation of the postsynaptic cell **– "neurons that fire together wire together"**
- NMDA receptors: competition, synchronicity, feedback mechanisms of neurotrophic factors from post- synaptic element support the survival of neurons that fire together.