Synapse Formation

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Course News

Coffee Hour

Friday (Nov 16) 8:30- 9:30am

Surdyk’s Café in Northrop Auditorium

Stop by for a minute or an hour!

Course News

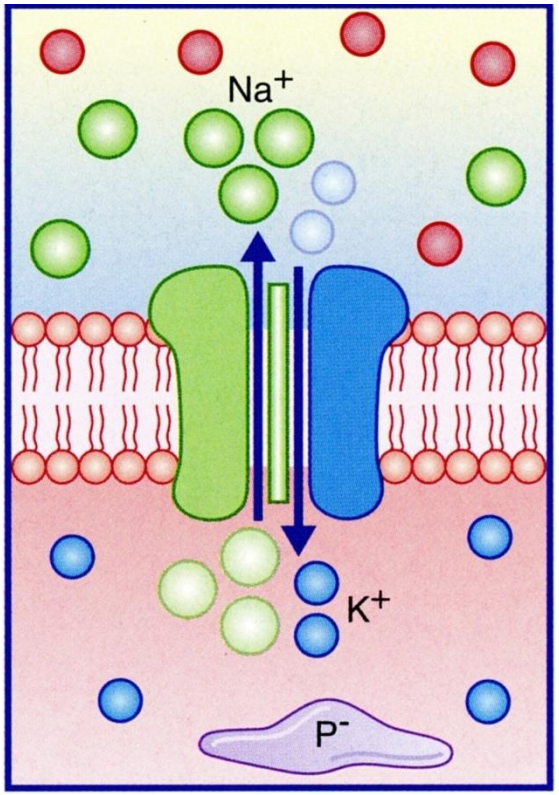
Exams will be returned in class on Friday!

Electrical Properties of Neurons

- A neuron at rest, that is a neuron receiving no synaptic input, maintains a higher concentration of K^+ and a lower concentration of Na^+ and Cl^- in its cytoplasm than outside the cell.

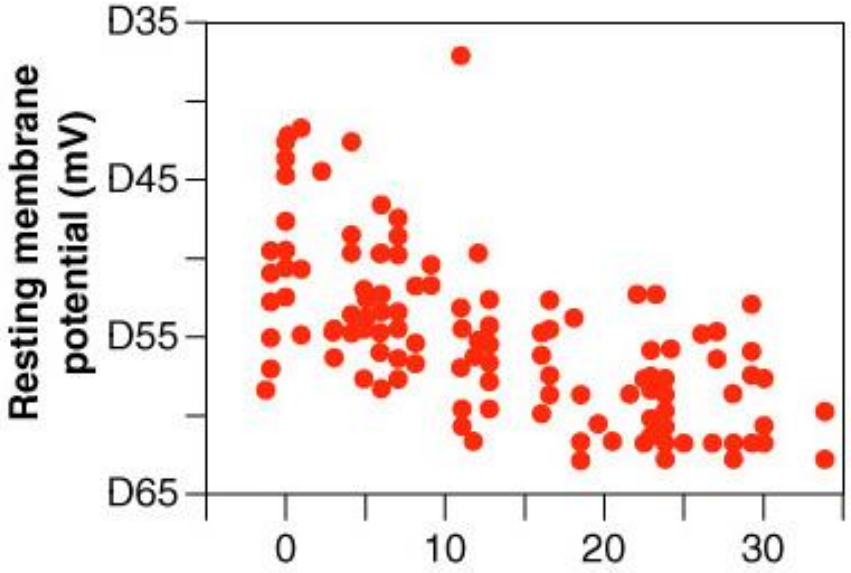
- A sodium-potassium pump and other ion channels maintain this ion differential.

- The ‘resting membrane potential’ can be measured with electrodes on the inside and outside of the cell; this is typically -65mV.



Developmental of the Electrical Properties of Neurons

- The resting membrane potential of neurons becomes more negative as development progresses.
- Glia are partly responsible for the change in the resting potential of local neurons. Glia regulate extracellular potassium in the CNS. (Extracellular K^+ goes from 35mM in the early developing brain to 3mM in the adult brain.)

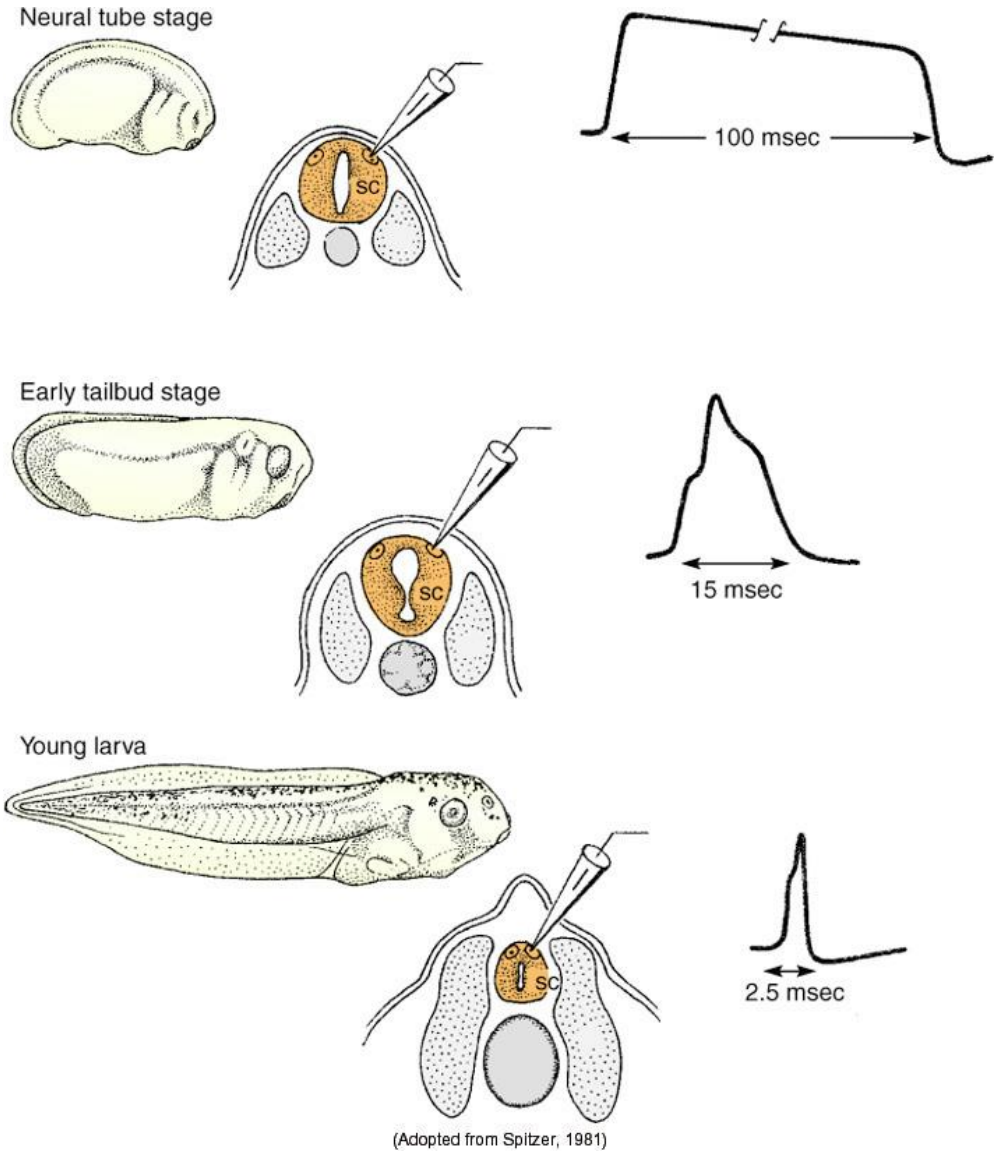


Developmental of the Electrical Properties of Neurons

- Ion channels of neurons change with maturation. Voltage-gated calcium channels mediate the first action potentials. Later, voltage-gated sodium channels predominate. Finally, voltage-gated potassium channels add a delayed rectifier function to the action potential.

  (Action potential of frog Rohon-Beard cells at different ages of development)

- Axons can have action potentials before they form synapses.



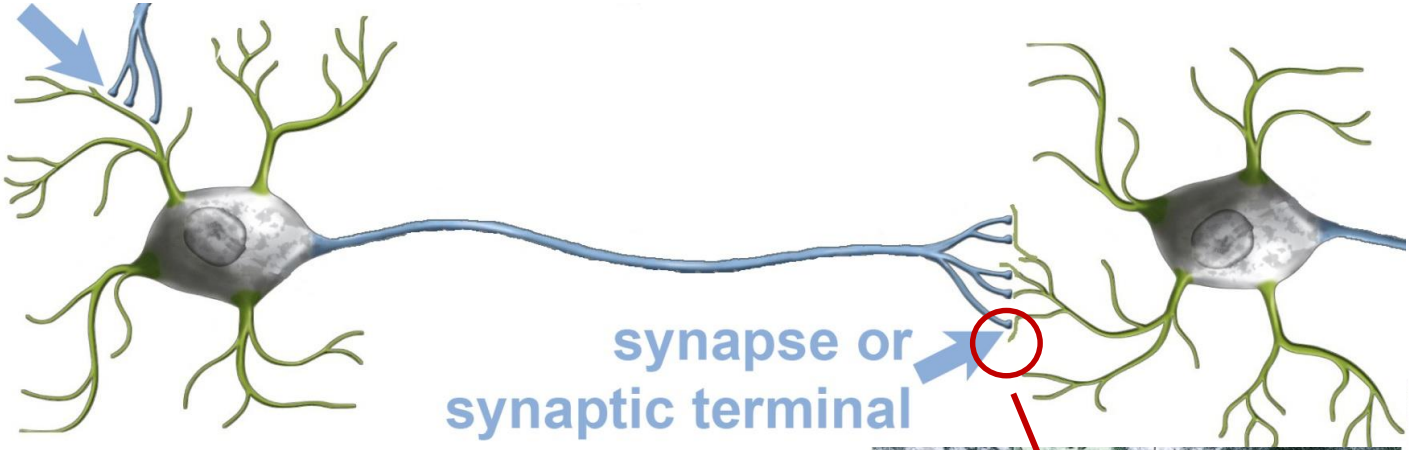
Developmental of the Electrical Properties of Neurons

- The density of ion channels in the membrane of neurons increases as a cell matures.
- Synapse formation and activity (action potentials) are required for full electrical maturation of neurons.

Structural Characteristics of Mature Synapses

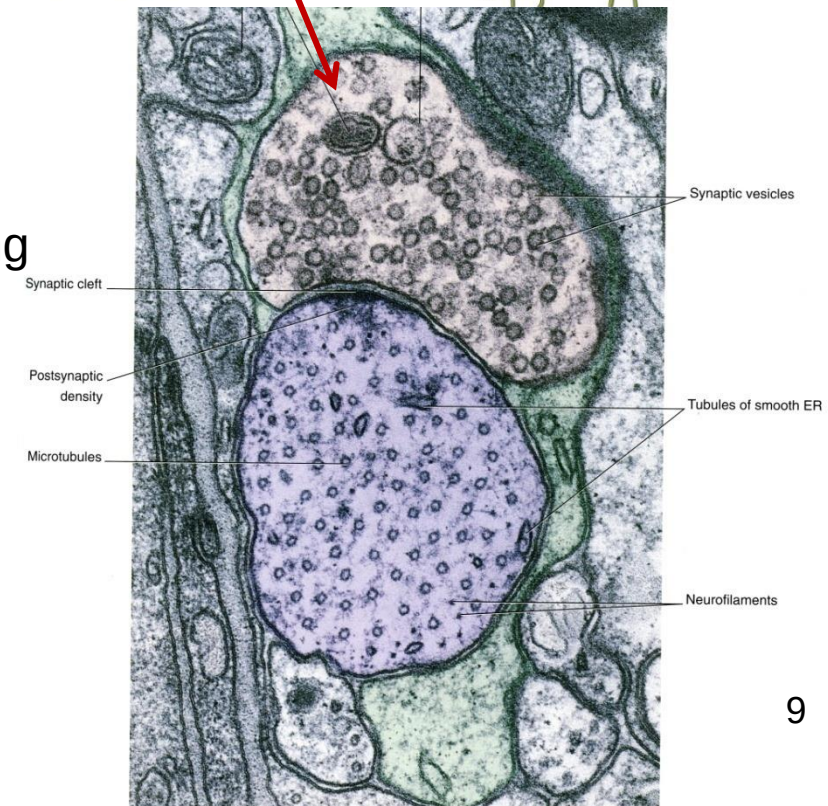
- Most communication between neurons and their target cells is via chemical synapses.
- The number of synapses on a single neuron varies from one to more than 10,000.

Structural Characteristics of Mature Synapses



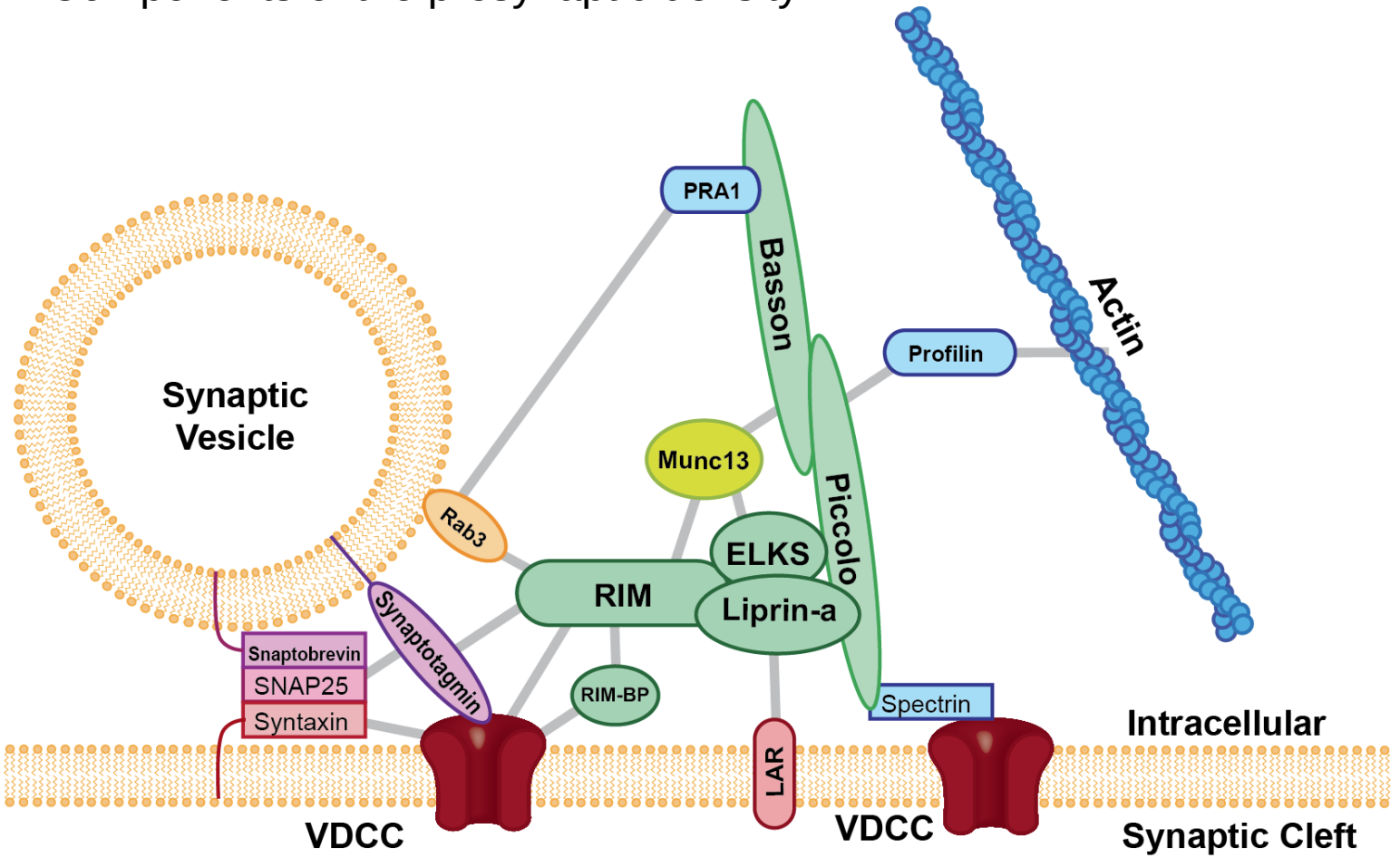
Structure of a typical synapse:

- Presynaptic terminal
    - Synaptic vesicles containing neurotransmitter
    - Presynaptic density
- Synaptic cleft
- Postsynaptic element
    - Neurotransmitter receptors
    - Postsynaptic density



Structural Characteristics of Mature Synapses

- Components of the presynaptic density:

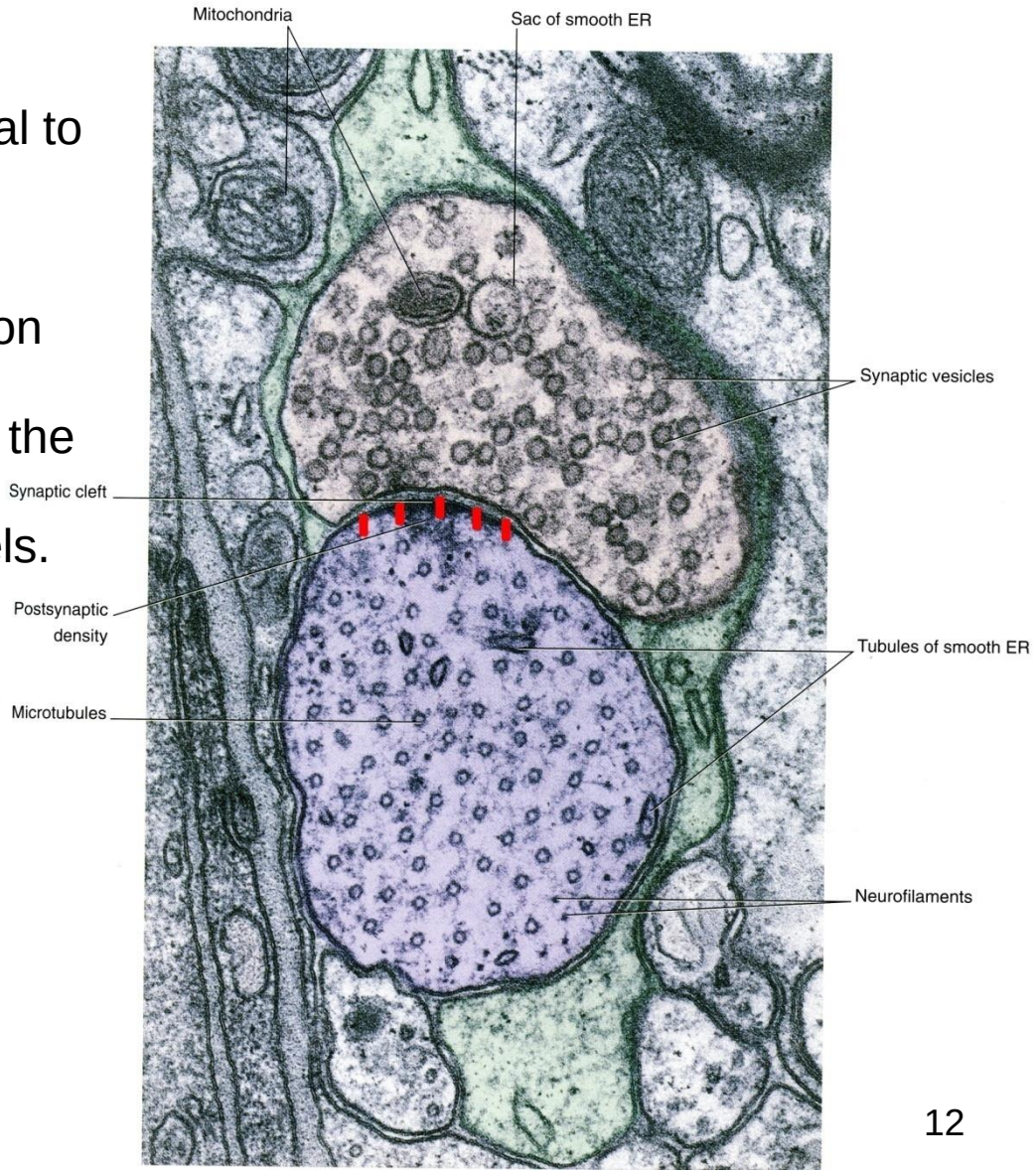


Structural Characteristics of Mature Synapses

- Synaptic communication requires release of a neurotransmitter from the presynaptic cell that activates receptors on the postsynaptic cell.
- Some common neurotransmitters include:
  - Acetylcholine (excitatory at neuromuscular and many parasympathetic synapses)
  - Noradrenalin (excitatory at many sympathetic synapses)
  - Glutamate (main excitatory transmitter in the CNS)
  - GABA (main inhibitory transmitter in the CNS)

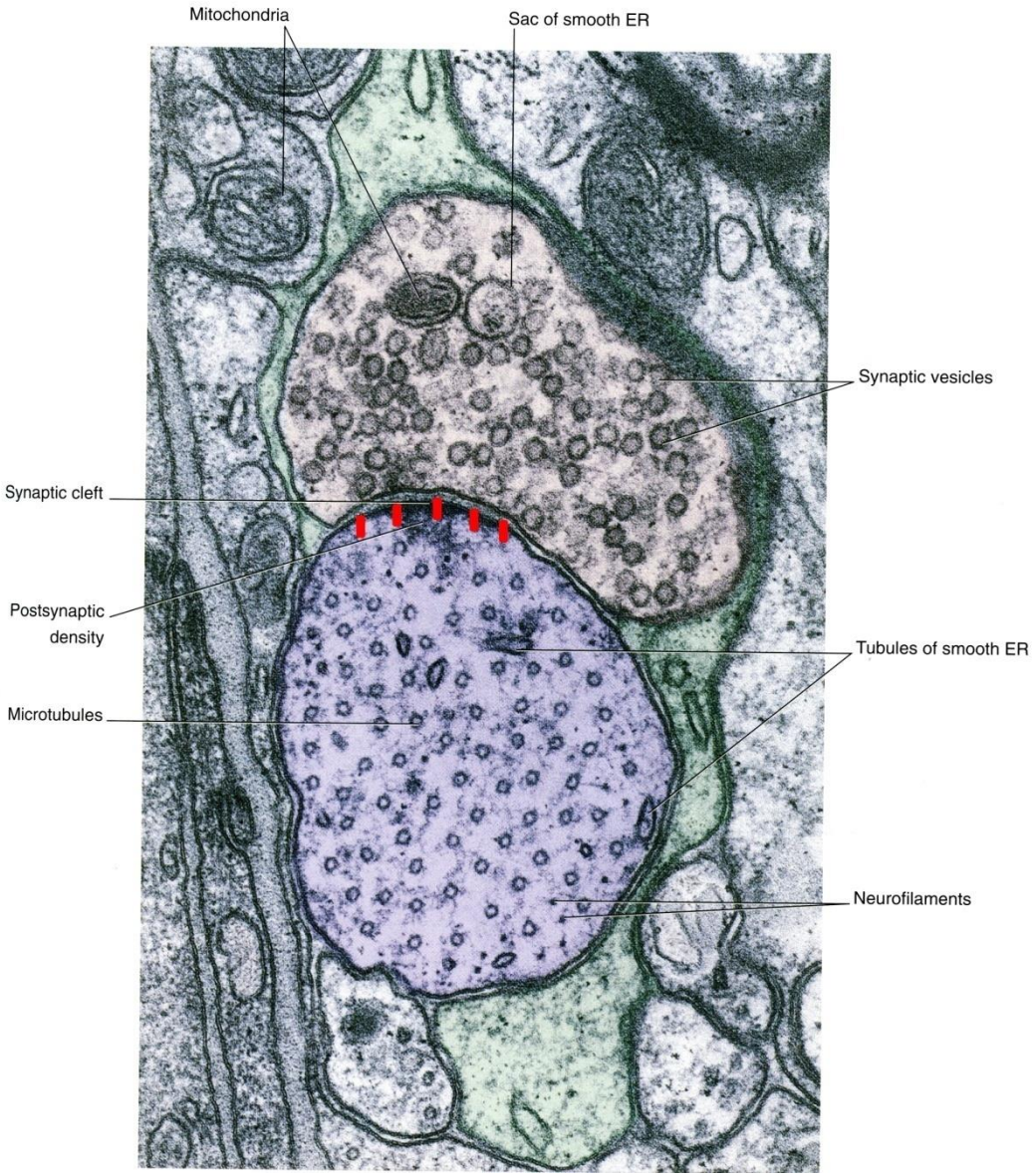
Structural Characteristics of Mature Synapses

- Neurochemical communication requires the postsynaptic terminal to have the proper receptor for the neurotransmitter.
- Neurotransmitter receptors are ion channels or initiate a second messenger signaling cascade in the postsynaptic cell that results in opening or closing of ion channels.
- The transmitter-receptor pair determines whether the active synapse will excite or inhibit the postsynaptic cell.



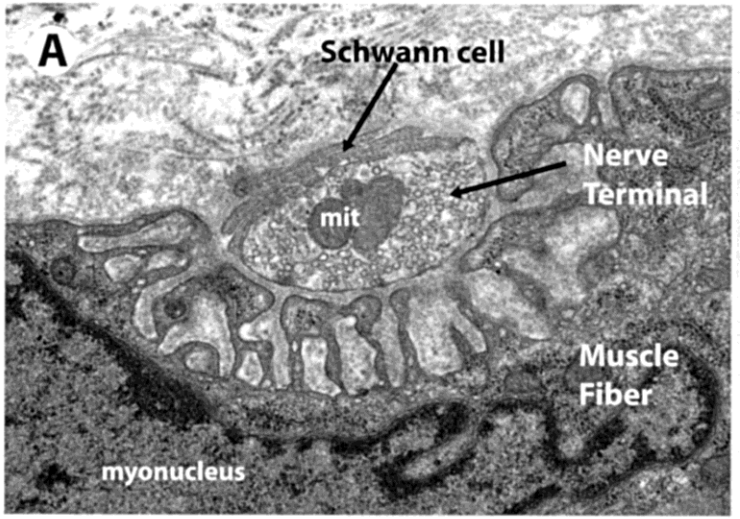
Structural Characteristics of Mature Synapses

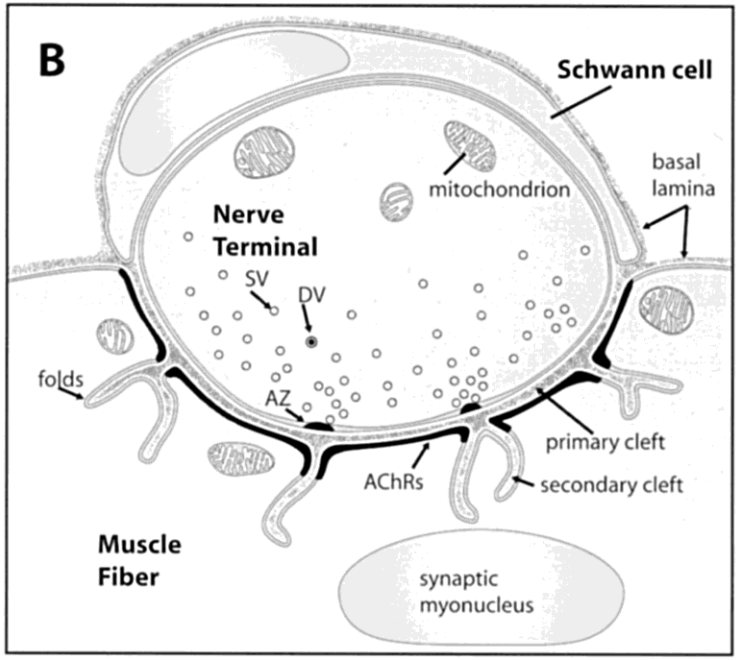
- Synapses are often surrounded by astrocytes.
- Astrocytes remove and recycle neurotransmitter.
- They ‘insulate’ synapses from one another.
- They regulate the extracellular ionic environment of the neurons.



Structural Characteristics of Mature Synapses

- Neuromuscular junction (NMJ):
  - terminal of a motor neuron axon
  - non-myelinating Schwann cell
  - folded membrane of a myofiber
  - Ach receptors in the myofiber membrane
  - synaptic cleft containing acetylcholinesterase & numerous ECM molecules
  - myofiber nucleus



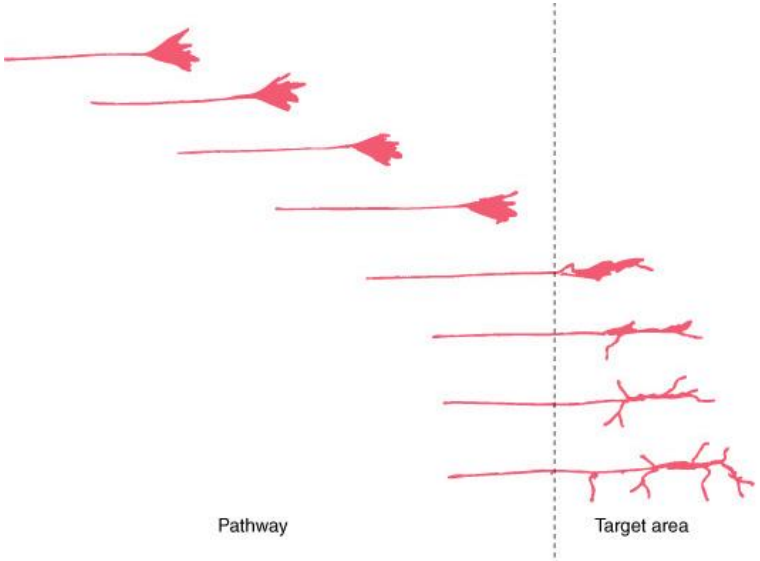


Generalizations Regarding Synaptogenesis

- Formation of synapses is a process called synaptogenesis.
- Neurons prepare for synaptogenesis well before contact is made between cells.
- Signaling between the pre- and postsynaptic cell induces synapse maturation.
- Synapses mature structurally and functionally over time.

Structural Changes during Synaptogenesis

- Growth cones slow & change morphology upon entering their target cell population.



- Contact with neurons in the target initiate the slowing.

  Axons of pontine neurons in culture grow rapidly on a substrate of cerebellar glial cells, and grow slowly when cerebellar granule cells are included in the substrate. Contact between the growth cone and granule cell is required for the slowing.

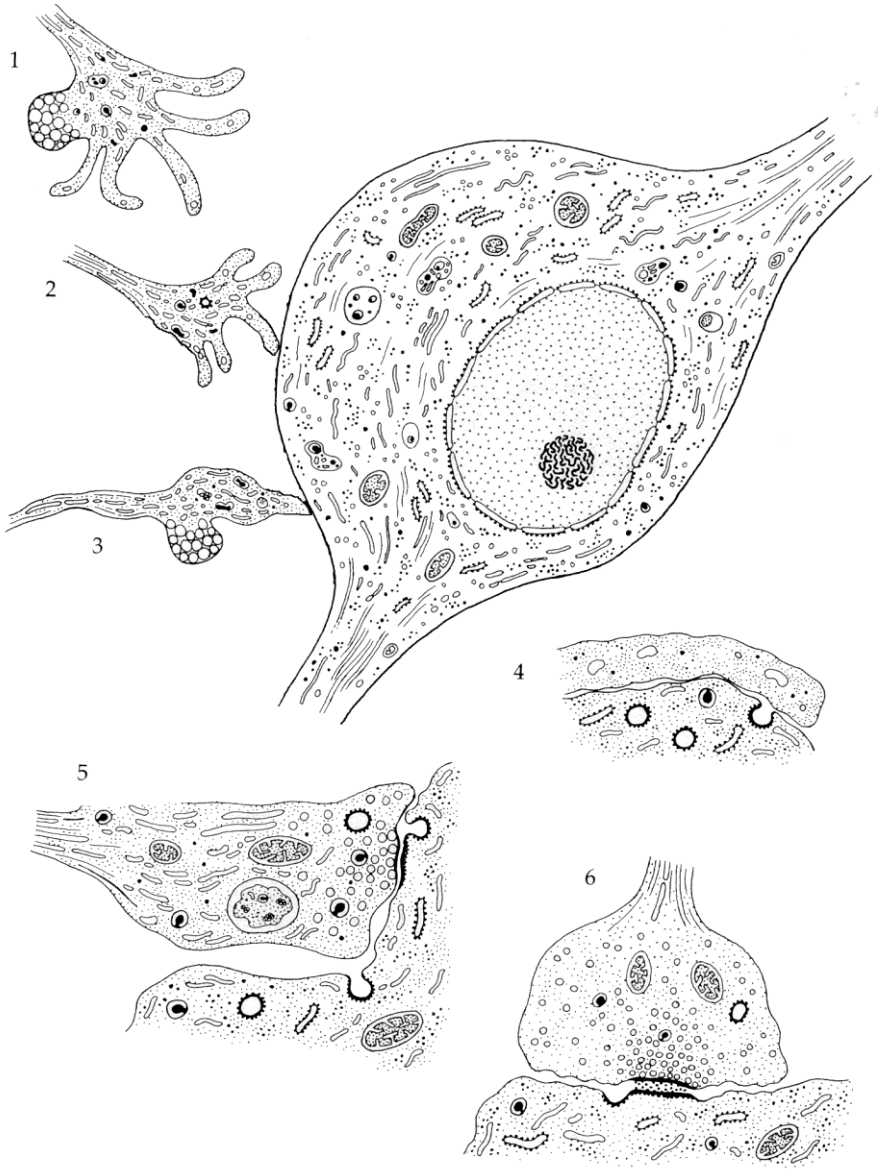
Structural Changes during Synaptogenesis

- The postsynaptic cell often extends a small process to receive or possibly find a growth cone of an axon.

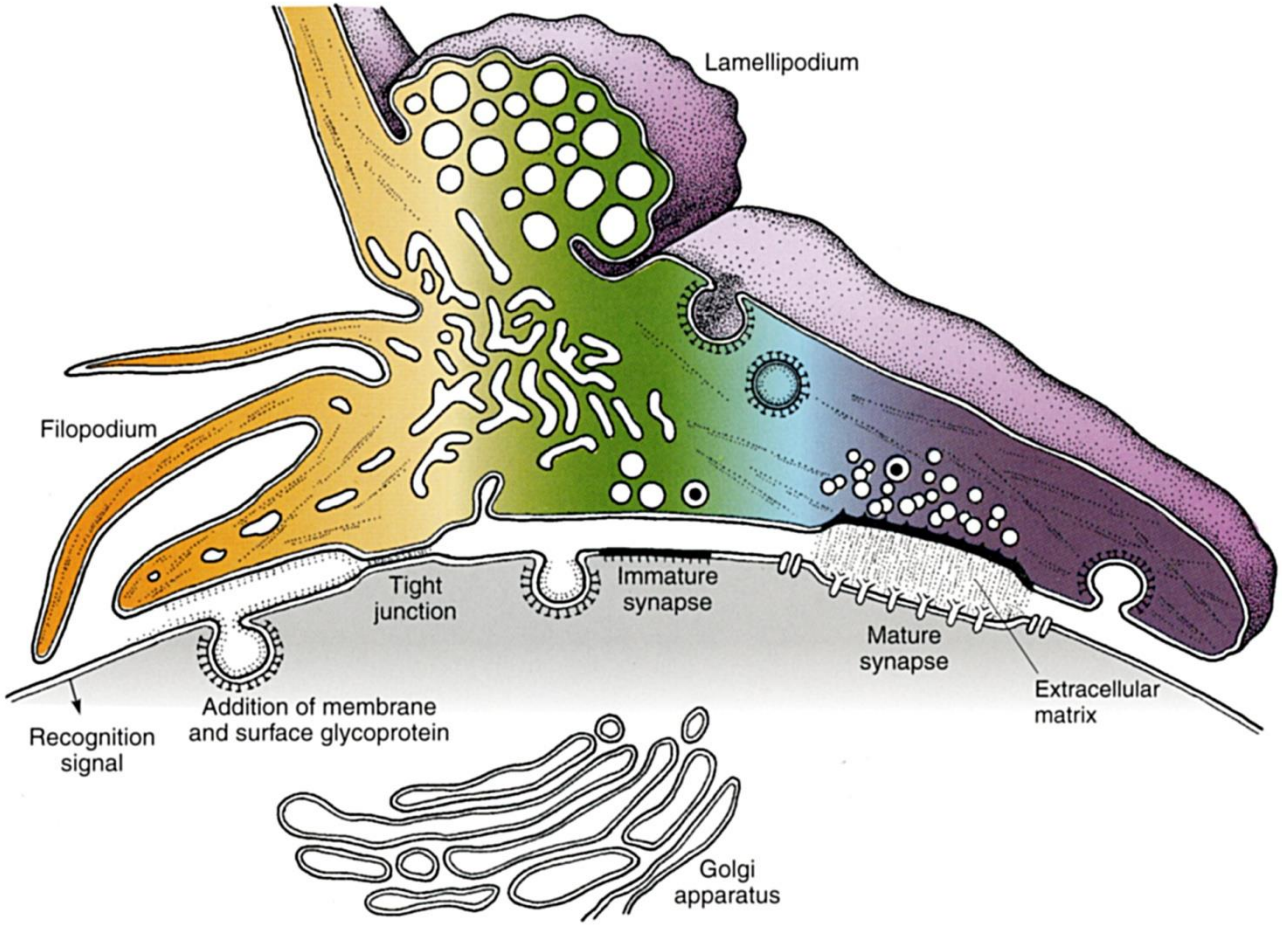
  (e.g. In spinal cord, ~75% of the synapses form on ‘dendritic growth cones’.)

Structural Changes during Synaptogenesis

- Growth cones that come in contact with an appropriate postsynaptic cell lose their filopodia.
- Small adherens junctions form between the pre- and postsynaptic cell.
- Coated vesicles appear on both sides.
- Coated vesicles fuse with the membrane at the site of adhesion.
- ECM appears in the cleft.
- Synaptic vesicles accumulate on the presynaptic side of the junction.



Structural Changes during Synaptogenesis



Non-Target Synaptogenesis

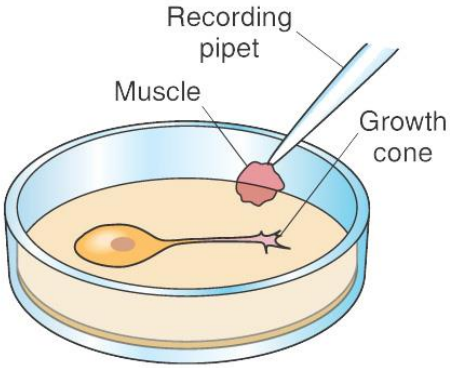
- Primitive synapses have been found transiently between axons and glial cells suggesting that axons sample their environment.
- Mature synaptic morphology only develops between appropriate pre- and postsynaptic elements indicating that bidirectional communication is required for synapse maturation.

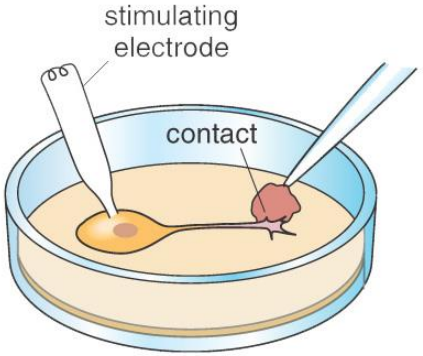
Development of Synaptic Function

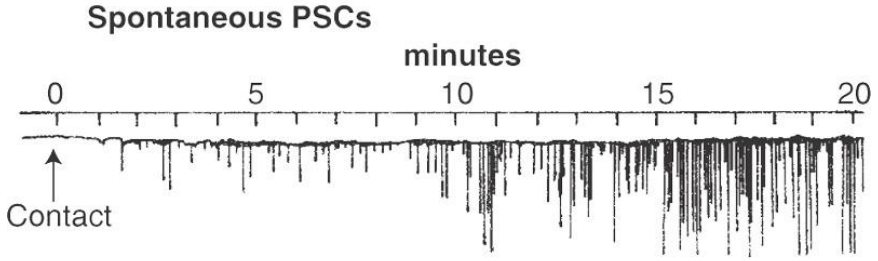
- Synapses develop physiologically before they develop anatomically.
    - Growth cones can release neurotransmitter.
    - Growing axons can have action potentials.
    - Postsynaptic cells have a low density of transmitter receptors diffusely spaced across the cell prior to receiving synapses.

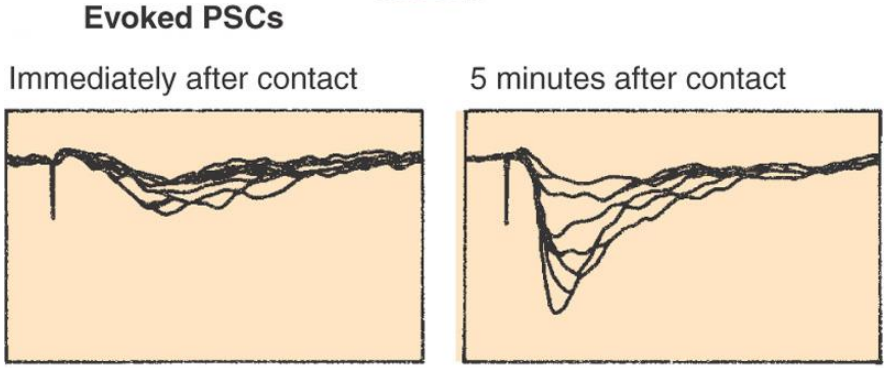
Development of Synaptic Function

- In tissue culture studies where contact between a growth cone and target cell was monitored with a microscope while recording activity in the postsynaptic cell, activity was observed within seconds after contact. Significant evoked activity could be elicited within minutes.



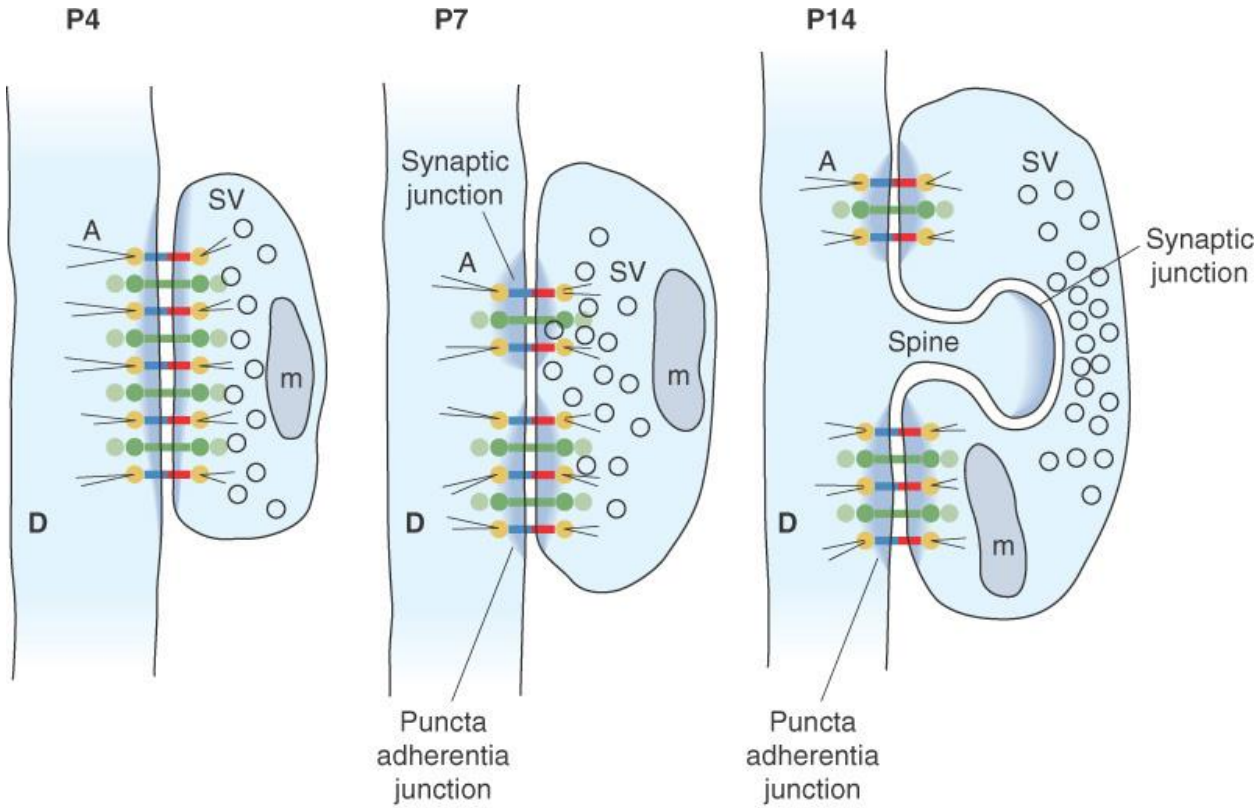






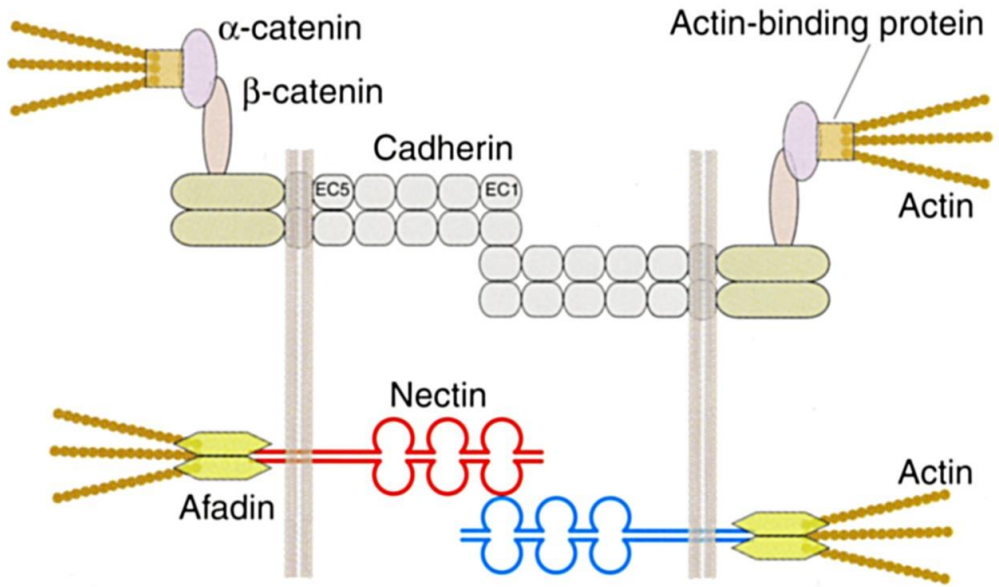
Synapse Assembly

- Multiple cell adhesion molecules mediate the formation of adherens junctions between growth cones and target cells.
- The area of tightest adhesion moves to the sides of the synapse as it matures.



Synapse Assembly

- Cell adhesion molecules involved in synapse formation:
  - cadherins – 20, calcium dependent, homophilic
  - protocadherins – 80, mostly homophilic
  - synCAMS (Ig-like) – 4, heterophilic binding
  - Ig-like CAMS – mostly homophilic
    - nectins, sidekicks, dscams, etc.
- Many CAMs interact with the actin cytoskeleton.

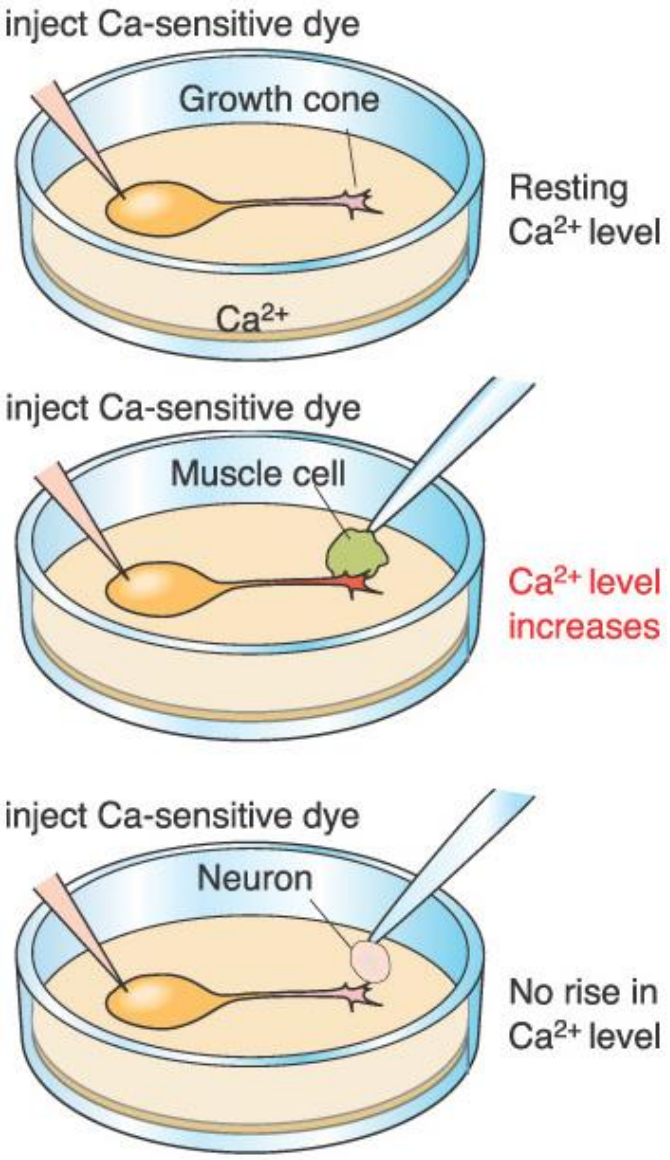


Synapse Assembly

- The pairing of pre- and postsynaptic cell adhesion molecules contributes to the specificity of connections.

Calcium and Synaptogenesis

- Calcium levels increase in growth cones immediately after contact is made with an appropriate postsynaptic cell.
- Calcium does not increase when a growth cone contacts a non-target cell (i.e. glial cell).
- If calcium is increased in a growth cone experimentally (e.g. inserting a calcium ionophore in the membrane), then the growth cone will exhibit early presynaptic characteristics.



Synapse Assembly

- Coated vesicles deliver the preassembled synaptic apparatus into the membrane at the site of adherens junctions.
- Ca^{++} initiates vesicle fusion.
- Presynaptic coated vesicles contain:

  synaptobrevin and other proteins for vesicle fusion

  neurexins

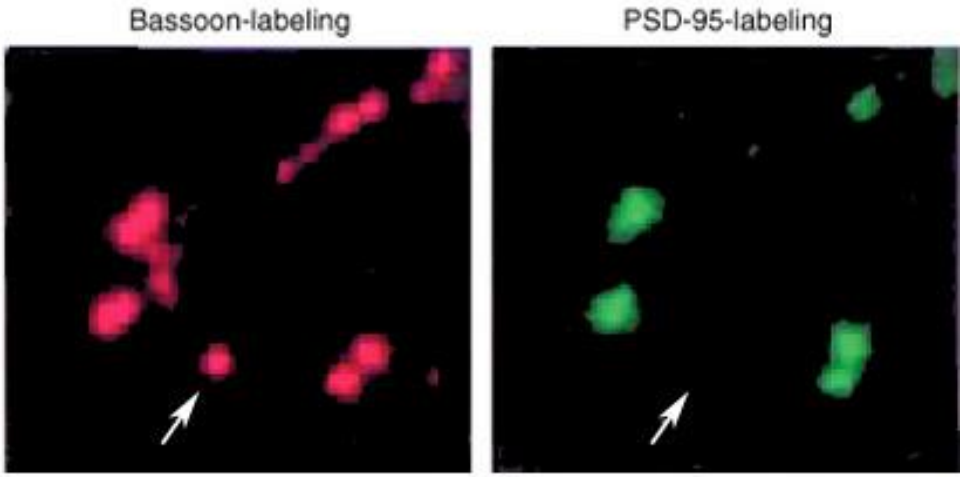
  cytoskeleton-associated proteins

  calcium channels

- As few as 2-3 vesicles are sufficient to establish a functional presynaptic terminal.

Synapse Assembly

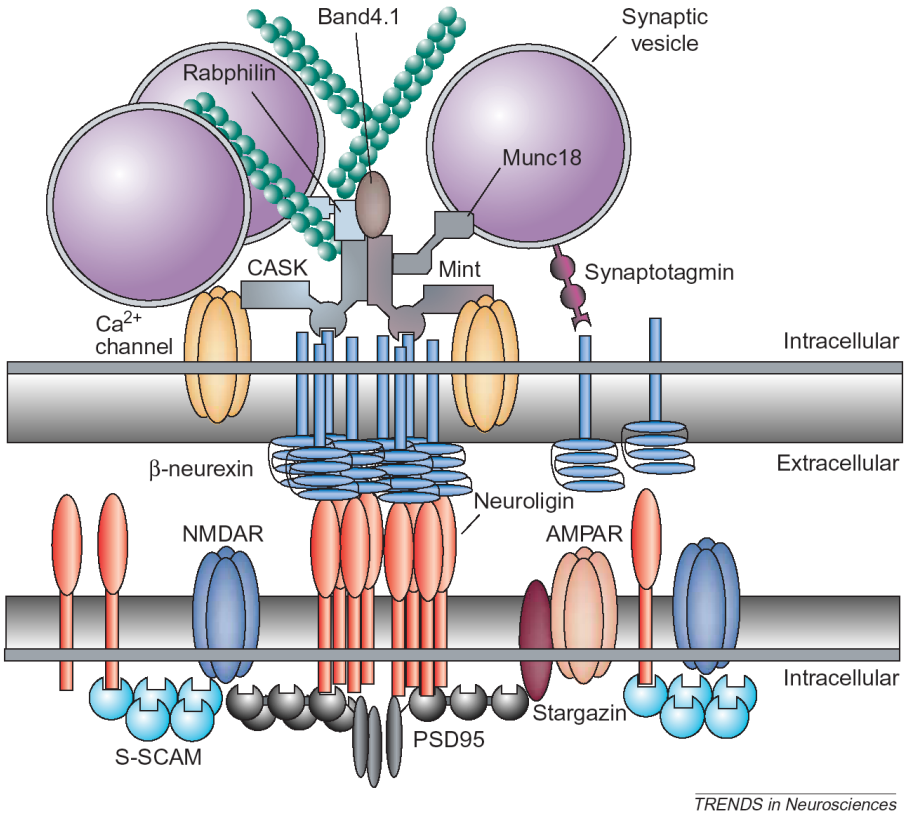
- Presynaptic proteins appear at the site of contact soon after contact is made. Postsynaptic proteins appear slightly later.



Synapse Assembly

- As the synapse matures, adhesion in the active zone is mediated by neurexin (presynaptic) and neuroligin (postsynaptic). These integral membrane proteins anchor a number of synaptic scaffolding proteins inside the cell (via PDZ domains).

  e.g. Neuroligins bind PSD95 (and other related scaffolding proteins); PSD95 binds neurotransmitter receptor proteins.



Synapse Assembly

- There are 3 mammalian neurexin genes. Each codes for an α- and a β-neurexin from different promoters (plus alternative spliced varients).
- There are 5 mammalian neuroligin genes. (The 5th is on the Y chromosome.)
- Both neurexin and neuroligin genes have alternative splicing, raising the possibility of 1000's of protein variants.
- Neuroligin-1 is in excitatory synapses, neuroligin-2 and -4 are in inhibitory synapses and -3 is in both.
- Neurexins can have ligands other than neuroligins (e.g. dystroglycan).

Synapse Assembly

- Misexpression of neuroligin-1 (postsynaptic) in a non-neuronal cell can initiate presynaptic differentiation in an axonal growth cone that makes contact with the cell. Neuroligin-coated beads in contact with neurons in culture result in clustering of presynaptic proteins.
- Expression of neurexin (presynaptic) in a non-neuronal cell can initiate postsynaptic differentiation.
- Overexpression of neuroligin-1 in neurons resulted in a significant increase in the number of synapses.
- Knockouts of neuroligins or neurexins in various combinations results in fewer synapses or lethality.

Synapse Assembly

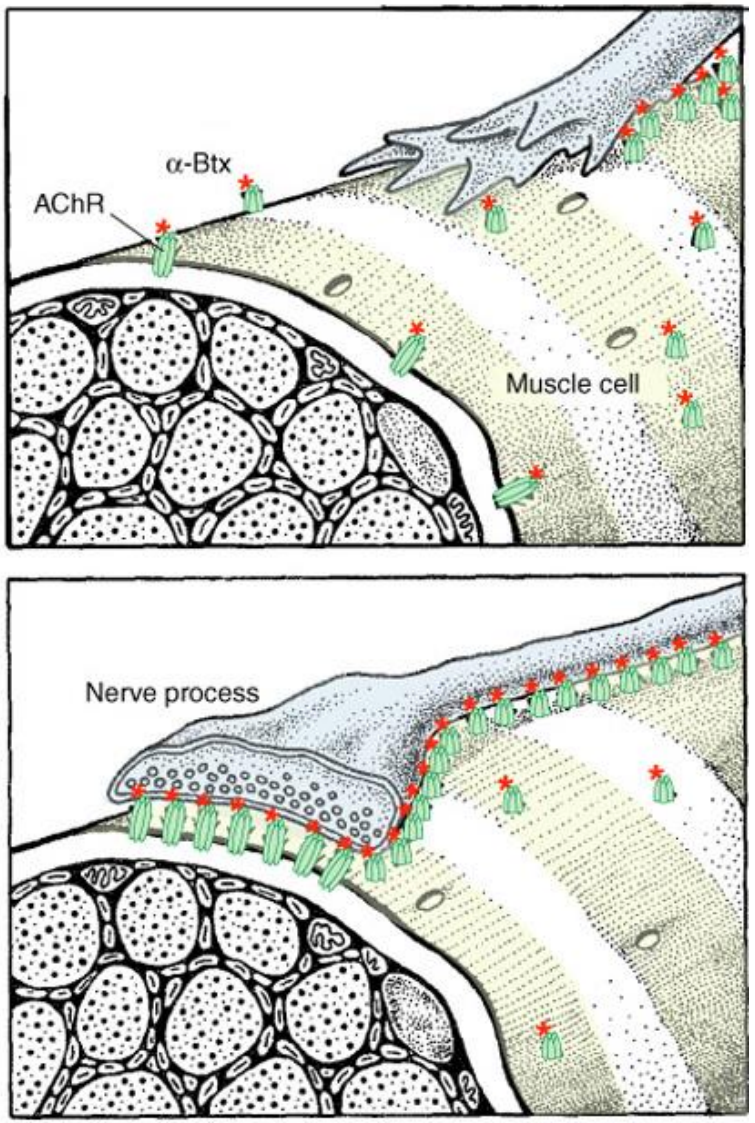
- Several neuroligin mutations have been identified in some patients with autism, mental retardation, or schizophrenia, which suggests that synaptic structure/function may underlie these conditions.

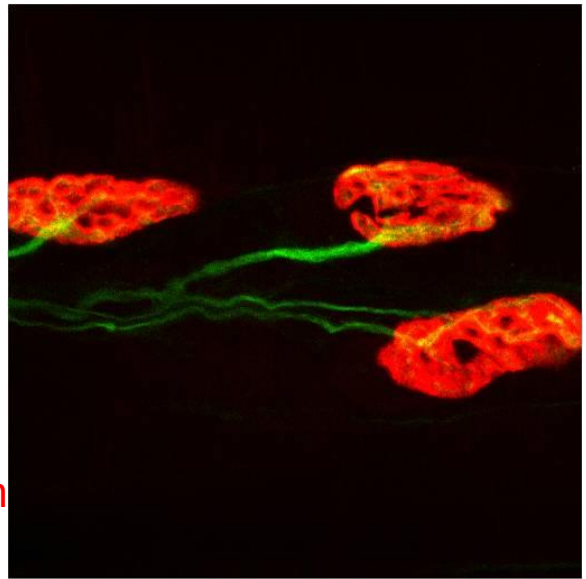
Review of Some Synaptic Associated Proteins

presynaptic	neurotransmitter	inside vesicles
	piccolo bassoon rim SNAP25	for vesicle fusion
	synaptophysin synaptotagmin synaptobrevin (VAMP)	vesicle protein
	dynamin	for vesicle recycling
cleft	neurexin neuroligin cadherins protocadherins	anchor/adhesion
postsynaptic	PSD93/95 S-SCAM	anchor
	neurotransmitter receptors	

Receptor Clustering at the Neuromuscular Junction

- Prior to arrival of an axon, acetylcholine receptors are distributed diffusely across the muscle fiber membrane (visualized with α-bungarotoxin).
- After a motor neuron axon makes contact with the muscle fiber, receptors become concentrated at the point of contact (i.e. the developing synapse).
- Receptor clustering is evident about three hours after contact is made.

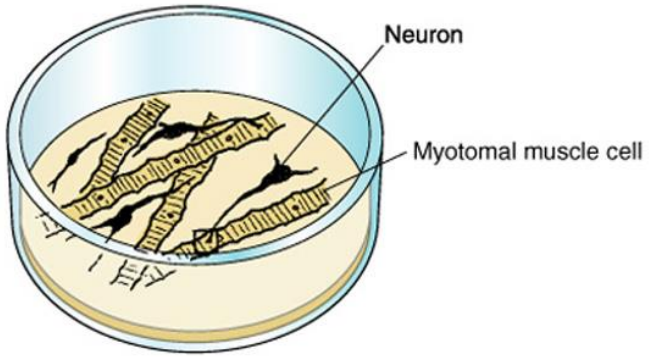


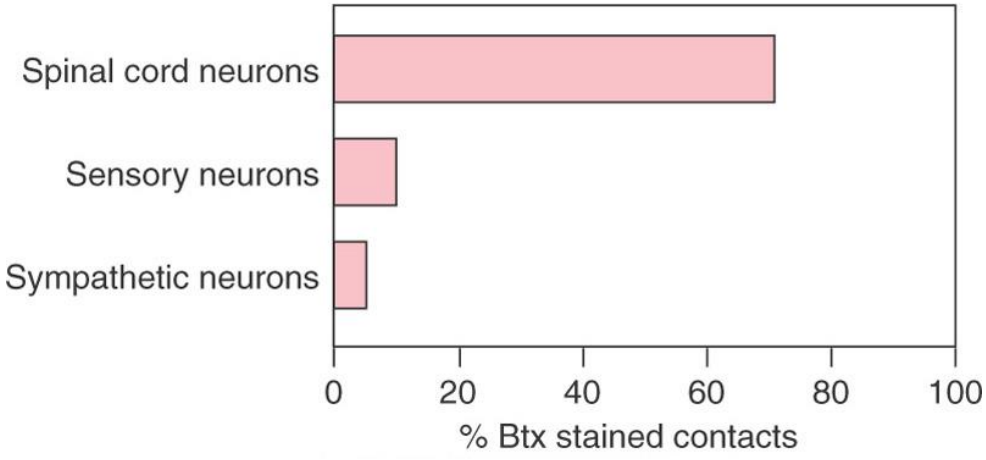


red = fluorescently tagged α-bungarotoxin
green = motor neuron

Receptor Clustering at the Neuromuscular Junction

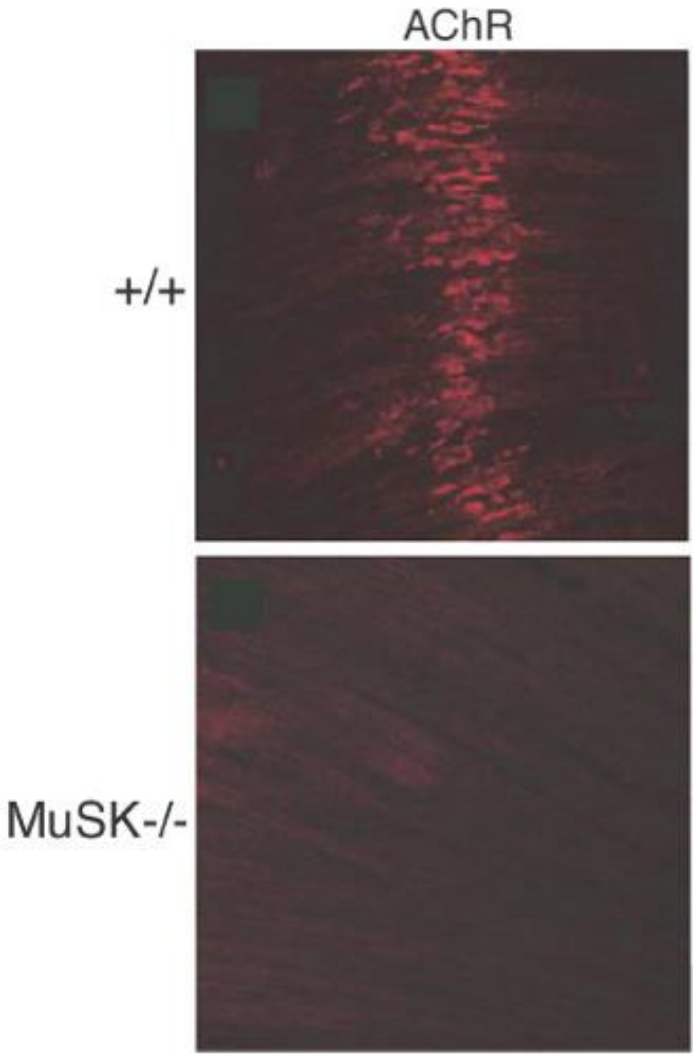
- Only motor neurons can initiate receptor clustering in muscle fibers. (i.e. There is specificity in synaptogenesis.)
- Blocking neurotransmission (e.g. with curare) does not block receptor clustering. (i.e. The transmitter does not initiate clustering.)
- If the target cell is denervated, receptors disperse. (i.e. The axon is essential for maintaining receptor clustering.)





Receptor Clustering at the Neuromuscular Junction

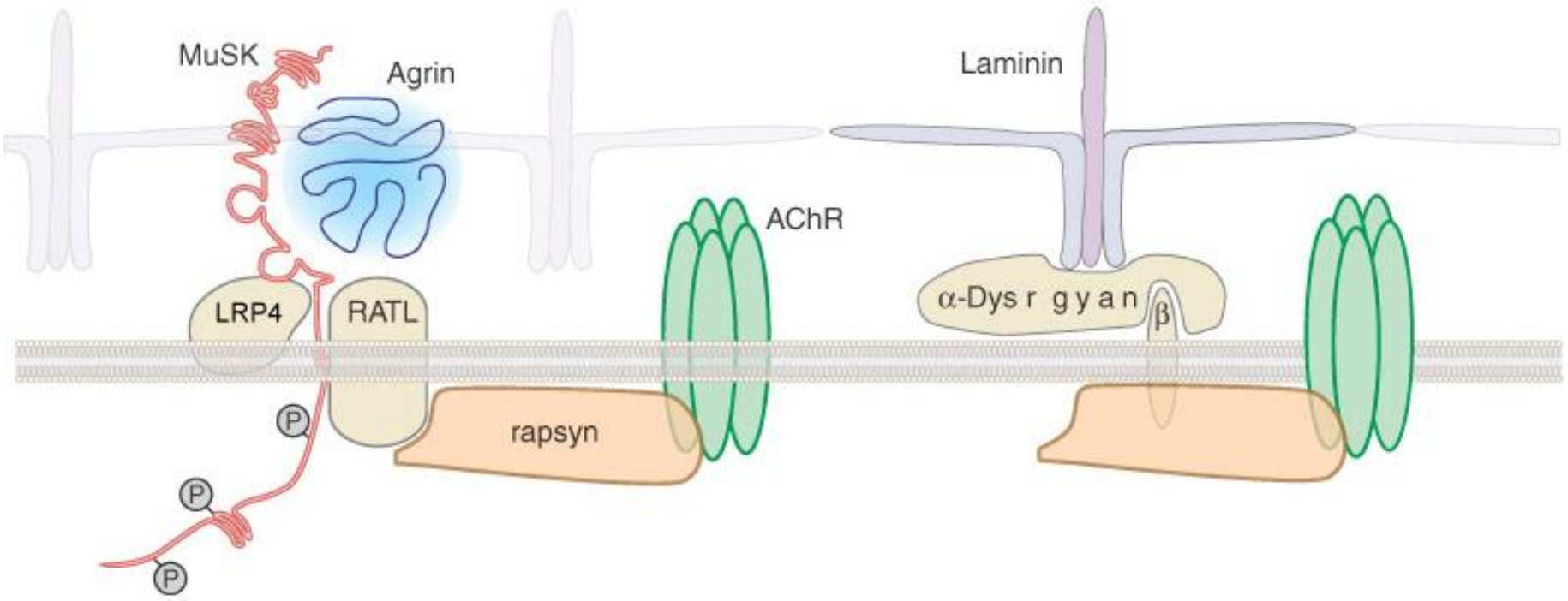
- Agrin, an ECM molecule, released by the motor neuron axon at the neuromuscular junction initiates receptor clustering. Motor endplates do not form normally in agrin knockout mice.
- Agrin is a large heparan sulfate proteoglycan expressed in many tissues. Only motor neurons (and adult Schwann cells) express agrin with the critical 19 amino acid sequence that induces AChR clustering. This form is called z-agrin.
- Muscle Specific Kinase (MuSK) is expressed by the muscle at the neuromuscular junction. No receptor clustering in Musk knockout mice.



(Adapted from Lin et al., 2001, Nature 410: 1057–1064)

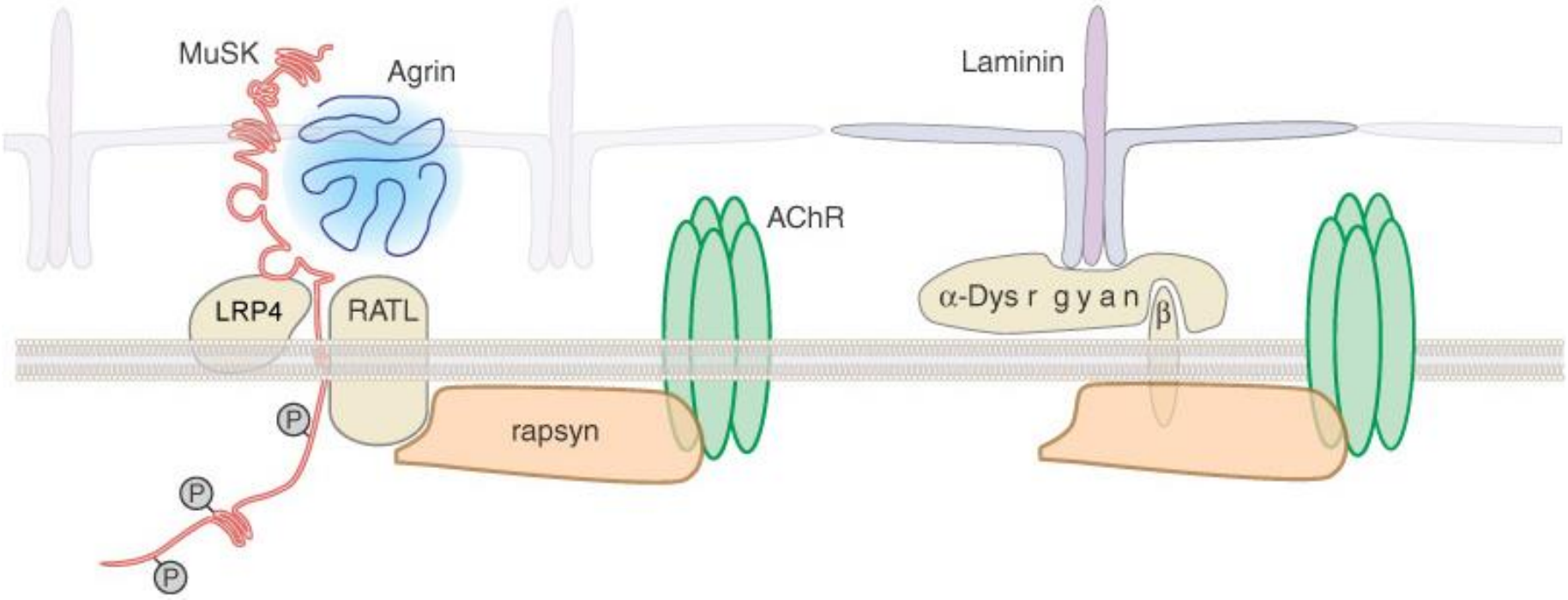
Receptor Clustering at the Neuromuscular Junction

- Low density lipoprotein receptor-related protein 4 (LRP4) in the muscle binds Agrin and recruits it to MuSK.



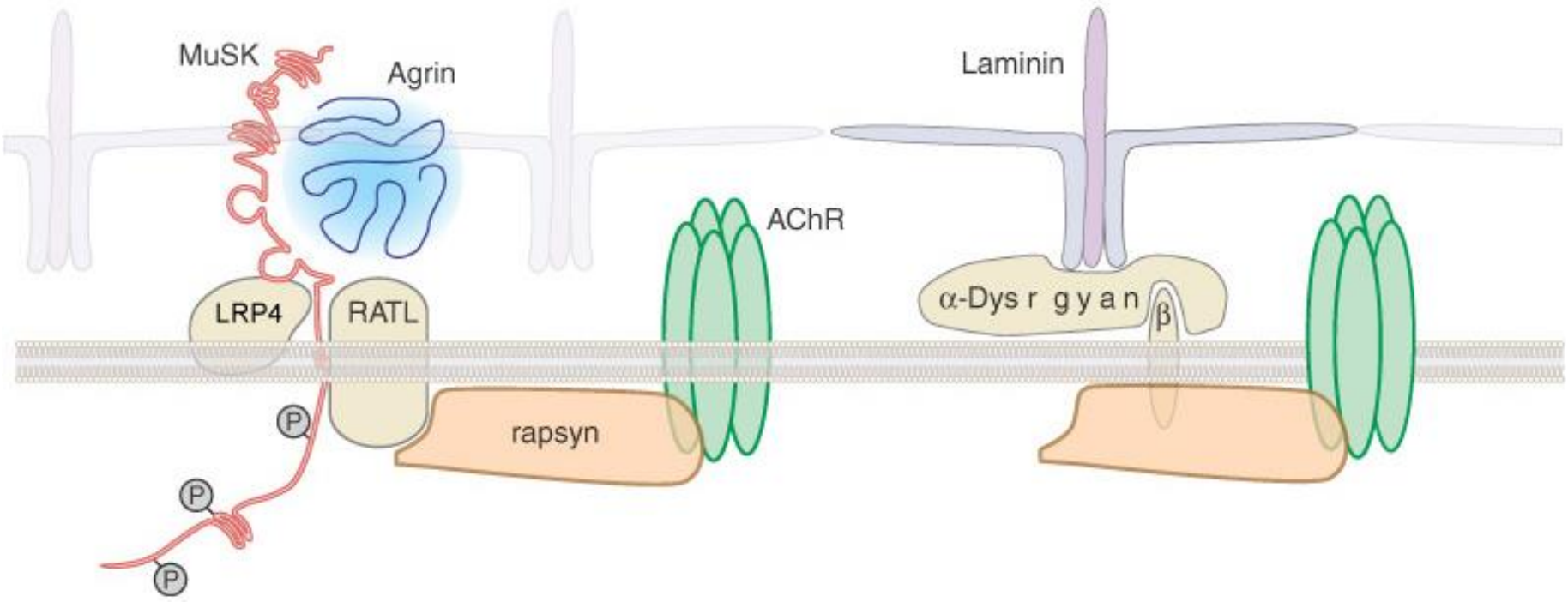
Receptor Clustering at the Neuromuscular Junction

- Agrin activates MuSK
- MuSK phosphorylates AChR subunits.
- Inhibition of the kinase activity prevents receptor clustering.
- Phosphorylated AChR binds rapsyn.
- Rapsyn clusters AChRs.



Receptor Clustering at the Neuromuscular Junction

- Rapsyn anchors the AChR and dystroglycan. Rapsyn is required for AChR clustering.
- S-laminin (merosin) is an ECM glycoprotein concentrated in the basal lamina of the neuromuscular junction. S-laminin anchors the motor neuron terminal to dystroglycan.



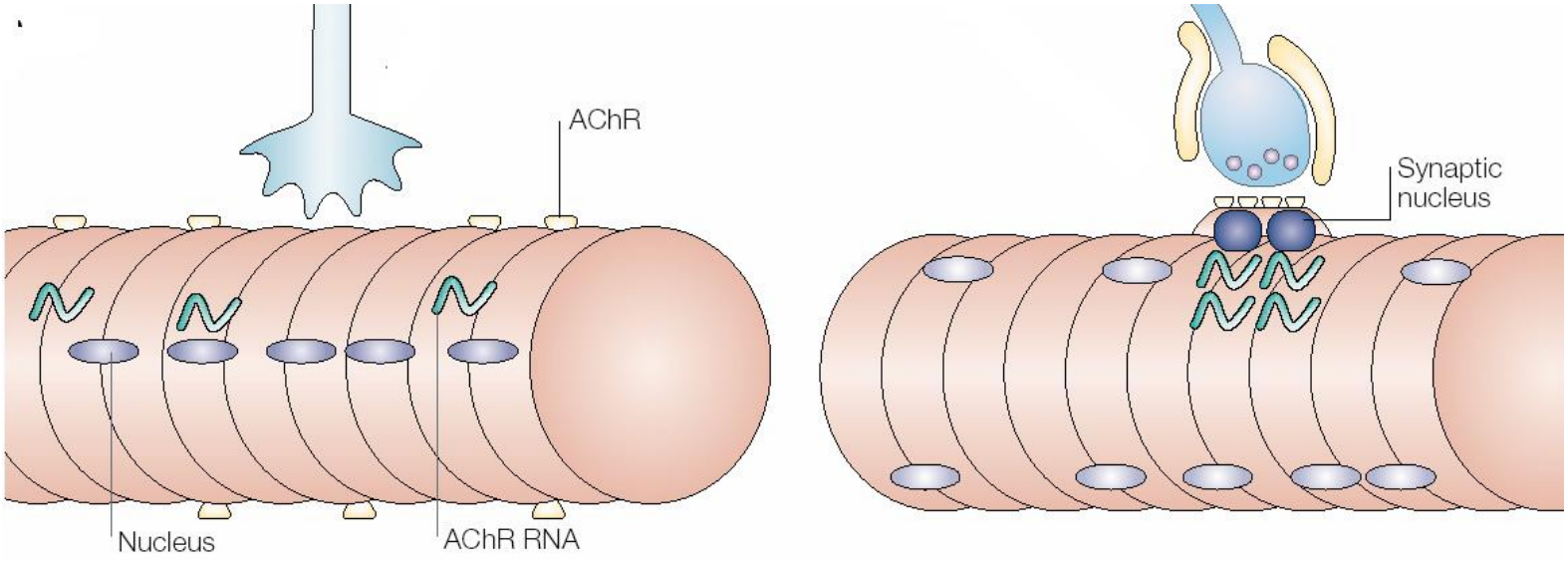
Receptor Clustering at the Neuromuscular Junction

- Myasthenia gravis is an autoimmune disease characterized by sever muscle weakness due to poor neuromuscular communication. Patients have antibodies to AChR or MuSK.

- Muscular dystrophy is another family of autoimmune diseases that affects muscle. The most common forms, Duchennes muscular dystrophy, is due to antibodies to dystrophin, which is anchored to dystroglycan.

Receptor Clustering at the Neuromuscular Junction

- In addition to clustering of AchR, nuclei accumulate at the NMJ, and transcription for AChR and other NMJ proteins is elevated in these nuclei.



Receptor Clustering in the CNS

- In the CNS, glutamate and GABA initiate clustering of each of their receptors specifically.
- Four agrin splice variants are expressed in brain. There is some evidence that they are important for receptor clustering in CNS neurons.
- MuSK isoforms also are expressed in the CNS.
- Narp is an ECM protein present in the synaptic cleft of glutamatergic synapses. Narp appears to be required for AMPAr clustering in the postsynaptic cell.
- Neurexin (presynaptic) and neuroligin (postsynaptic) are required for receptor clustering.
- Scaffolding proteins like PSD95 are essential for receptor clustering in the CNS.

Neuregulins and Transmitter Receptor Expression

- Expression of neurotransmitter receptor increases as the synapse matures.
  - Neuregulins are expressed by some neurons and released at synapses.
  - erbBs are neuregulin receptors. erbBs are receptor tyrosine kinases that activate the transcription factor CREB via the MAPK pathway.
  - CREB activation increases expression of neurotransmitter receptor.

Neurotrophins and Synaptogenesis

- Neurotrophin, particularly BDNF in the CNS, increases synapse maturation and synapse number.

Glia Contribute to Synapse Development

- Astrocytes are required for synapse maturation in the CNS:
  - Cholesterol from local astrocytes is essential for synapse maturation.
  - Thrombospondin from local astrocytes activates α-2/δ-1 on neurons and is required for excitatory synapse formation in the brain. α-2/δ-1 is part of the calcium channel complex.

    [A commonly used analgesic and anti-epileptic drug, gabapentin, blocks the α-2/δ-1 receptor.]