Synapse Formation

Chapter Eight
Synapse

- Synapse = the connection between neuron and target or two neurons
- Axon grows to target – differentiates into the presynaptic terminal
- Target cell also changes – into postsynaptic terminal
- Both already carrying the components to form the synapse → contact is trigger
Synapses

- Synapses allow neurons to communicate with each other via the release of a neurotransmitter that open ion channels or activate second messenger systems.

- This allows changes in membrane potential and generation of an action potential.
Reticular vs. Neuron Theory

Original theory:
- Nervous cells were all connected physically by their processes

Neuron theory:
- Neurons are now known to be separate cells which meet at synapses to transmit messages
The study of neuronal communication and synapse formation was greatly improved after the introduction of two techniques:

1) electron microscopy that allowed the visualization of synapses at the cellular level, and

2) Intracellular recordings that allowed the study of synaptic function
Synapses

Synapse formation is not a random process, glutamatergic synapses are formed in dendritic spines, whereas GABAergic synapses occur at the cell body and proximal dendrites.

Synapse formation is a complex process that requires appropriate projection of the axon to a specific target followed by the synthesis and targeting of multiple ion channels proteins to the synaptic site. The ability of the growth cone to navigate complex environments and recognize the target is critical for synapse formation.
Synaptic Organization Requires

1. Correct receptors being expressed
2. Synapse at correct locations
3. Which part of membrane should differentiate into the synapse
4. Receptors are matched to appropriate terminal types on presynaptic neurons
5. Control of number of synapses
   • From 1 to 10,000 per neuron
Development of Synaptic Morphology

Addition of synapses in cat visual cortex

- Synapse formation occurs during a critical period of development and occurs in burst.
- During synaptogenesis in the cat visual system there is a significant increase in the number of synapses and the volume of synapses present in each neuron.
Synapse Generalizations

- Neurons and target cells are carrying the building blocks for synapses before contact is made.
- Intracellular signaling induces differentiation upon contact.
- Synapses do not function at "mature" level for quite a while after formation.
  - Change during development.
  - Mature over time.
Development of Synaptic Morphology

Newborn

Adult

Graphical representation of the synapse between the auditory nerve endbulb (EB) and its postsynaptic target (the spherical bushy cell - SBC)

Ultrastructural differentiation of this synapse involves appearance of synaptic vesicles and a postsynaptic membrane density
The Growth Cone And Synapse Formation

When a growth cone comes into contact with the postsynaptic membrane the following changes are observed:

1) Its filopodia retract
2) Membranes on both sides of the synapse become tightly opposed
3) Vesicles are found on both sides of the membranes that will eventually lead to the appearance of postsynaptic densities and synaptic vesicles
Identifying a Synapse

Synapses always have:

1. Small vesicles accumulate in the pre-synaptic cell
2. Narrow cleft of extracellular matrix between the two cells
3. Thickening of the post-synaptic cell membrane
   1. Accumulating proteins
Maturing Synapse

Pre-synaptic

Post-synaptic

(Adapted from Rees, 1978)
Synapse Begins Functioning

- Moment growth cone comes into contact with post-synaptic target → changes begin
  1. Growth cone itself can release neurotransmitter
    1. Released upon contact
  2. Transmission increases over time
  3. Contact increases free calcium within growth cone
Spontaneous Release of Neurotransmitter by Growth Cones

- The growth cone is capable of neurotransmitter release even before synapse formation.

- Early release of NT by the growth cone can be demonstrated using a biological sensor created by excising a membrane patch from a muscle cell with a recording pipette.
Spontaneous Release of Neurotransmitter by Growth Cones

- No ACh-evoked currents is detected when the sniffer pipette is distant from the growth cone.

- An inward current is generated by positioning the sniffer pipette in close proximity to the growth cone.
Transmission increases over time

(A) Diagram showing the recording of a muscle growth cone with a pipet and stimulation with an electrode.

(B) Graph showing spontaneous PSCs over time with a contact marker.

(Adapted from Evers et al., 1989; Xie and Poo, 1988)
Free Calcium

See a calcium increase within growth cone only when *correct target cell* is making contact.
Forming the synapse?

• Remember that when the growth cone reaches the target cell the growth cone slows down
• What is the stop signal?
• Turns out to be Calcium/cAMP
  – cAMP regulates level of Ca++
• Also, cholesterol – delivered from glial cells to thicken synaptic membranes
Calcium Mediates Synaptic Differentiation

- Intracellular calcium concentration can be manipulated in the growth cone with the calcium ionophore A23187.

- Increased intracellular calcium concentration with A23187 slows down the spreading of the growth cone and results in the retraction of lamellipodia and filopodia.
Calcium Mediates Synaptic Differentiation

- Mobility of the growth cone can also be altered by increasing the concentration of cAMP
- Or when exposing neurons to the adenylate cyclase activator forskolin (FSK) and the phosphodiesterase inhibitor theophylline (TH)
- Both of which affect cAMP levels
Adhesion

- Adhesion between growth cone and target cells also change upon contact
- Longer contact = more adhesion

(A) Neuron
Myoball
Growth cone
Contact

(Grade of adhesion)

0
1
2
3

(Adapted from Evans et al., 1983)

(B) Number of cell pairs

Grade of Adhesion

1.5 min contact
15 min contact

(Adapted from Evans et al., 1983)
Adhesion Molecules

• Same adhesion molecules we have already seen that hold cells together:
  – NCAM
  – Laminin
  – Cadherins
(Which are Ca++ dependent)
Cell-adhesion molecules mediate early adhesive interactions

- Synapsin II = adhesive

In dissociated hippocampal neurons, blocking synapsin II will break apart existing synapses and prevent new synapse formation.

- Addition of synapsin II allows synapse formation
Semaphorin II expression block synapse formation

- In *Drosophila*, different motor axons innervate sema II-expressing muscle cells (33) and sema II-negative muscle cells (6,7).

- Misexpression of sema II in muscles 6 and 7 prevent RP3 motor axon from making contact with target. Notice that as sema II expression decreases, axons are able again to make contact with muscle fibers 6 & 7.
Who comes first?

- Presynaptic sites display rapid molecular development from a growth cone into a full presynaptic site.
- Within 45 minutes of contact 90% of growth cones are expressing pre-synaptic proteins.
- In same time period only 30% of target cells are expressing post-synaptic proteins.
- Therefore seems that pre-synapse is first.
Pre-synaptic Receptors

- Pre-synaptic cells express receptors for neurotrophins
- Post-synaptic cells express ligands
Pre-synaptic Receptors

- Receptors: Frizzled and beta-Neurexin-1
- Ligands: Wnt and Neuroligin-1
- When receptor binds ligand \( \rightarrow \) growth cone changes into pre-synaptic site
Who comes first?

• If pre-synaptic sites require ligand from post-synaptic sites then isn’t this a case of the chicken coming before the egg?

• Why not?

• Because entire target cells are releasing these ligands

• Not just post-synaptic sites

• Then once pre-synaptic scaffold is formed this induces changes in post-synaptic site
Neurotransmitter receptor clustering

- Most obvious hallmark of synaptogenesis is the clustering of neurotransmitter receptors in **post-synaptic** site
- Pre-synaptic site releases neurotransmitters
- Post-synaptic site has receptors that pick up neurotransmitters
- Pre-synaptic sites induce receptor clustering
Clustering of ACh receptors during development

- Many neurotransmitter receptors are expressed before synapse formation. However, receptor aggregation is the most important hallmark of synapse formation.

- Receptor aggregation following synapse formation have been extensively studied using the neuromuscular junction (NMJ) as a model.
Clustering of ACh receptors during development

- Labeling of nicotinic ACh receptors in the muscle fiber indicates that E17 nACh receptors are spread out over the muscle surface.
- In the adult muscle fiber, nAChRs are concentrated (cluster) over the synaptic area.
- Muscle deinnervation results in spreading of nAChR back over the entire muscle fiber.

(Adapted from Anderson and Cohen, 1977; Cohen and Weidner, 1980)
Clustering of ACh receptors during development

- nAChR clustering involves lateral migration of receptors already present in the membrane.
- As well as specific targeting of newly synthesized receptors to the contact site.
Clustering of ACh receptors during development

- Clustering of nAChR is cell specific
- Clustering of nAChR can be studied in vitro by co-culturing isolated motoneurons and myotubes. If myotubes are co-cultured with DRG or sympathetic neurons, clustering fail to occur
- Is clustering dependent on cholinergic transmission or the presence of a specific clustering complex at the NMJ?
Clustering of ACh receptors during development

- Is cholinergic transmission (synaptic activation of nAChR) required for receptor clustering?
- Clustering of nAChR is not inhibited by $\alpha$–bungarotoxin.
- Blocks transmission
- This suggests that cholinergic transmission is not required for receptor clustering
What is the nature of the clustering signal? The extracellular matrix contains a factor that induces nAChR clustering.

Cutting the motor neurons and the removing the muscle cells will destroy the synapses as well as any AChR clustering.

However as long as the basal lamina remains then AChR clustering will reform when muscle cells regenerate.
Clustering of ACh Receptors Requires Agrin

- At the neuromuscular junction the clustering signal is generated by Agrin.
- Agrin alone can induce clustering
- Antibodies against this protein indicate that it is present in the nerve terminal, muscle and basal lamina.
- Is Agrin induced receptor clustering derived from the neuron or the muscle cell?

From Kroger & Schroder, 2002
Clustering of ACh Receptors Requires Agrin From Pre-synaptic Cell

- Block Agrin coming from chick only
  - Specific antibody
- When Agrin comes from chick neurons normally get AChR clustering
- When Agrin coming from chick neurons is blocked get no clustering
- When Agrin coming from chick muscle is blocked have normal clustering
- Therefore Agrin must come from neuron
Agrin in nAChR Clustering

- Agrin induces AChR phosphorylation prior to clustering
- Agrin-induced AChR phosphorylation precede aggregation of nAChRs
Clustering of ACh receptors

- Role of agrin and the muscle-specific kinase (MuSK) in clustering of nicotinic Ach receptors
- Agrin is released by the presynaptic terminal and activates a receptor complex that includes MuSK
- At the intracellular side of the postsynaptic membrane, rapsyn is required for agrin-mediated clustering
AChR cluster in wildtype cells along the nerve In MuSK -/- AChR does not cluster Therefore MuSK is needed
Clustering of ACh receptors

Agrin phosphorylation of MuSK leads to clustering of nAChR, rapsyn and other proteins at the NMJ

From Burden, 1988
Clustering of ACh receptors

Beside agrin, MuSK and rapsin other molecules including dystrophin-associated α–dystroglycan, acetylcholinesterase and s-laminin also seem to concentrate at the site of contact. Their role in receptor clustering is still under investigation.
Any Questions?

Read Chapter Eight