Refining Synapses

Chapter Nine
Afferent Projection Error during Development

During development there is a constant rearrangement of synaptic connections, new synapses are formed and old synapses may be eliminated.
Afferent Projection Error during Development

- New synapses are formed and old synapses may be eliminated at the same time.
- These changes allow rearrangement of receptive fields and higher accuracy of sensory perception.
- Tightening of receptive fields.

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![Diagram showing receptive field changes over time.](image)
Developmental Plasticity

• Nervous system adapts even while it is developing
  – Partly through activity/stimulation

• Developmental plasticity is very similar to adult learning

Main difference:

• Developmental nervous system is much more plastic and changes are more permanent once they occur
Afferent Projection Error during Development

Two types of projections error occur during development:

1) Axons may project to the correct target but they may spread out too far (lack precision). For example, temporal retinal axons projecting to the anterior tectum may send projections outside their main area of innervations (overshoot the target).

2) Postsynaptic neuron or target receive inputs from the wrong number of afferents. For example at the NMJ.
Synaptic refinement

A) One afferents projects to three neurons but only the correct one remains according to topographic map

B) Neuron receives input from three afferents but only the correct one remains

C) Afferent projects too far and is trimmed back
Afferent Projection Error during Development

Polyneuronal innervations during early embryonic development

- At the NMJ, each muscle fiber receives an input from only one motoneuron but during early development each fiber is innervated by multiple motoneurons

Mature muscle
As the result of projection errors, synapse elimination takes place in many parts of the developing nervous system.

How do we detect loss of synapses occur during development?
Afferent Projection Error during Development

Three main techniques can be used to detect synapse elimination:
1) Intracellular recordings
2) Anatomical studies
3) Study the response of single neurons to afferent stimulation
Intracellular recordings can be used to detect the number of synaptic connections based on the assumption that each axon will evoke one PSP upon stimulation.
Study of Synapse Elimination by Intracellular Recordings

Intracellular recordings can be used to detect the number of synaptic connections based on the assumption that each axon will evoke one postsynaptic potential (PSP) upon stimulation.

At the NMJ stimulation of afferent projections induce a grade response in immature animals that is lost in mature subjects.
Does Synaptic Elimination Occur in Central Synapses?

- In the CNS, neurons can make hundreds of connections. How can we study synapse elimination under these conditions? Since the amplitude of the PSP is too small, intracellular recording cannot provide a clear answer.

- One approach is to count the number of synapses using immunohistochemistry.
Does Synaptic Elimination Occur in Central Synapses?

The process of synapse elimination can be demonstrated by double labeling of commissural neurons in the brain.

If a dye is injected in a particular area of the brain at postnatal day 2 (P2) and then a different dye is injected (at the same site) at P21, we can determine whether synaptic connections were lost by looking for cells labeled at P2 but not at P22.
Multiply Innervated NMJ

- Two neurons (green and purple) are innervating the same NMJ
- See the green neuron expanding
- The purple neuron retracting

[From Walsh and Lichtman, 2003]
Synaptic Elimination in the Developing Visual System

- Retinal ganglion cells send axons to the LGN. Neurons in the LGN then project to layer IV of the visual cortex forming segregated eye-specific termination zones called ocular dominance columns or stripes.

- Injection of radiolabeled proline in the eye indicates that during early development projections from eyes are very widespread.
Synaptic Elimination in the Developing Visual System

- Over several weeks of development the initial widespread innervations breaks into discreet patches that represent eye-specific termination zones.

- Functionally this can also be confirmed by electrophysiological recordings in the visual cortex following light stimulation of the retina.
RGC terminal refinement

A = early in development

A to B – branching is removed from inappropriate layers within LGN

B to C – branching is retracted even within the correct layer

(Adapted from Bur et al., 1994; Braitman and Shatz, 1988)
What factors regulate synapse elimination in the visual system?

- Electrical activity regulates the elimination of inappropriate synapse and the formation of **ocular dominance columns**

- In the retina, spontaneous electrical activity appears before synaptic connections are established in the visual cortex. **Does inhibition of spontaneous electrical activity prevent formation of ocular dominance columns?**
What factors regulate synapse elimination in the visual system?

Inhibition of spontaneous electrical activity in the retina with the sodium channel blocker TTX prevent formation of ocular dominance columns - innervation is spread out throughout cortex.
What factors regulate synapse elimination in the visual system?

- Spontaneous electrical activity in the retina of ferrets can be recorded in isolated preparations using a microarray of electrodes or intracellular recordings.

- Retinal explants or the entire retinogeniculate pathway can generate spontaneous electrical activity in vitro.
What factors regulate synapse elimination in the visual system?

- How does spontaneous electrical activity result in the formation of ocular dominance columns?

- It appears that the ability of action potentials to arrive at the cortex with certain delay may trigger separation of axonal projections in layer IV.

- Therefore dependent on time of innervation.
Activity regulates connections

- Visual manipulation experiments
- Test what affects activity, lack of activity or changing the activity will have on synaptic connections
- Normally each neuron in Layer 4 of the visual cortex will receive projections from one eye or the other – **monocular**
- Neurons outside of layer 4 will receive projections from both eyes – **binocular**
Regulation of Synaptic Connections by Electrical Activity

What factors regulate synaptic connections? Use-dependent changes appear to play an important role in remodeling of the nervous system.

This can be demonstrated by recording from monocular and binocular neurons in the cat visual system.
Extracellular recordings with an electrode that passed tangentially through the cortex indicated that the majority of neurons are binocular in a normal cat.
Regulation of Synaptic Connections by Electrical Activity

However, following closure of one eye (also known as monocular deprivation) can result in a significant change in the ratio of binocular/monocular neurons in the visual cortex. Under monocular deprivation, the majority of neurons become monocular.
Regulation of Synaptic Connections by Electrical Activity

Yes, does disuse weaken the synapses from the deprived eye? Closure of both eyes (binocular deprivation) does not result in any changes in the ration of binocular vs. monocular neurons.

In this case the majority of the neurons are binocular.

This suggests that the total amount of evoked activity does not predict the strength of a synapse.
Regulation of Synaptic Connections by Electrical Activity

- The strength of a synapse is determined by the differences in the amount of synaptic activity. This is also known as the **competition hypothesis**.
Regulation of Synaptic Connections by Electrical Activity

The strength of a synapse is determined by the differences in the amount of synaptic activity. This is also known as the competition hypothesis.

In monocular deprive animals cortical neurons receive two different amounts of stimulation. In control or binocular deprived animals there is no change in the synaptic activity received from each eye.
Regulation of Synaptic Connections by Electrical Activity

To confirm the competition hypothesis, kittens were raised with artificial strabismus (misalignment of the eyes caused by manipulating one of the ocular muscles).

Under this condition visual stimulation activates different positions in the retina so cortical neurons are not activated at the same time. This procedure creates a phase shift in cortical activation coming from different eyes.
Regulation of Synaptic Connections by Electrical Activity

- Shift activation of cortical neurons results in the majority of neurons to respond to activation of one eye or the other - monocular activation
- This suggest that synapses from each eye must be activated at the same time in order to maintain a strong functional contact with the postsynaptic neuron
Competition Hypothesis

• Competition hypothesis states that synaptic activity alone is not what determines synaptic connection

• More important is the difference between amount of activity in each competing neuron
  – Activity levels are similar = binocular

• Timing also matters – synapses need to firing at same time to keep strong connection with both neurons (binocular)
Misaligning Synapses

A = normal cat
• Visual cortex is normally innervated by neurons of all columns

B = cat with misaligned eyes (strabismus)
• Visual cortex is only innervated by neurons within same column
  – Monocular
Regulation of Synaptic Connections by Electrical Activity

Electrical activity (use) also regulates the elimination of polyneuronal innervations at the NMJ. In the rat, polyneuronal innervations is eliminated between postnatal dates 10-15.

When a TTX cuff is placed around the nerve, removal of polyneuronal innervations is delayed.
Removal of polyneuronal innervations at the NMJ can be accelerated by increased electrical activity. Electrical stimulation of the sciatic nerve accelerated the loss of polyneuronal innervations. Therefore activity speeds up normal synaptic elimination.
Sensory Stimulation Regulate Synaptic Rearrangements

- If electrical activity regulates synaptic strength and elimination, then sensory coding is regulated by experience.

- Experiments in the central auditory system using sound and electrical stimulation have demonstrated changes in sensory coding due to electrical activity.
Sensory Stimulation Regulate Synaptic Rearrangements

In the central auditory system, neurons respond to a limited number of frequencies because the auditory nerve fibers from the cochlea project topographically to the CNS.
Sensory Stimulation Regulate Synaptic Rearrangements

- Afferent innervations can be characterized by plotting the range of sound frequencies against the sound intensities that evoked a threshold response. This is called a frequency tuning curve.

- If a narrow area of the cochlea project to a central neurons, the frequency tuning curve recorded from that neurons will be very narrow.
Sensory Stimulation Regulate Synaptic Rearrangements

- If a central neuron receives projections from a wider area of the cochlea its frequency tuning curve will be wider.

- Does the temporal pattern of neural activity influences the development of frequency tuning?
Sensory Stimulation Regulate Synaptic Rearrangements

- When mice were reared in a sound environment consisting of repetitive clicks (monotonous stimulation) the frequency tuning curve becomes broader.

- This suggests that when afferents are exposed to identical patterns of stimulation they are unable to segregate along the frequency axis.
Sensory Stimulation Regulate Synaptic Rearrangements

- Similar rearrangement of afferent innervations can be obtained in cats that have their cochlea removed and where hair cells are artificially stimulated by electrical pulses

- Again broad frequency tuning curve (in pink)
Synaptic Rearrangement

• Co-activated synapses establish strong connections during development
  – Broader curves than normal

• Hypothesis = when all afferents have identical activity they do not segregate normally – instead they innervate the same region

• After a certain point in development these experiments will no longer have any affect
  – Therefore only plastic during development
Environmental Stimulation Regulate Synaptic Connections

- In many parts of the brain, neurons respond to stimuli of a specific orientation or to a moving stimulus in a particular direction.

- When kittens are reared in specific visual environments, recordings from brain neurons indicate that the majority of neurons respond to the stimuli present during rearing.
Environmental Stimulation Regulate Synaptic Connections

Strobe lights:
- Cats cannot respond to movement, only stationary objects

Horizontal or vertical stripes:
- Selectively respond to the stimuli they were reared with – either horizontal or vertical stimuli accordingly
- Therefore rearing environment will influence neuronal connections
Often axons project to a particular area forming topographic maps. Electrical activity can regulate the alignment of topographic maps generated on the same target by two different sources of axonal projections.
Electrical Activity and Alignment of Sensory Maps

In the frog, the tectum receive two retinotopic projections: one from the contralateral eye and one from the ipsilateral eye via the nucleus isthmus.

These two projections are aligned so the same area of the tectum receive information from both sides of the head.

Rotation of one eye disrupt this connection between two sides but it can be restored over time.
Topographic Map:

- Map can only align if frog is still developing.
- If misalignment of frog’s eye happens too late in development the two different sites in the tectum will not align.
  - Double vision.
- Also – frogs have two types of axons:
  - Direct axons will be correct even if frog is raised in complete darkness/misalignment.
  - Indirect axons will NOT be organized.
  - Therefore some axons are activity dependent and other axons are NOT activity dependent.
Owls

- Alignment of timing of sound and visual space
  - To more accurately locate squeaking prey

(Adapted from DeBello et al., 2001)
Prism reared

- Owls are raised wearing prism glasses so that everything will be 23 degrees off.
- Their auditory map will readjust to match the apparent visual map.

(Adapted from DeBello et al., 2001)
Adjusting Topographic Map

B shows normal map of sound within optic tectum

C shows how auditory map adjusts within tectum by rearranging neurons
Any Questions?

Read Chapter Nine