Lecture 20 – Patterning Neuronal Connections.

1. How “billions and billions” of synaptic connections in an adult nervous system come to be correctly wired is an immensely complex problem at first thought. Conceptually the problem can be reduced to several general developmental principles:

   a) **Development proceeds by progressive developmental restrictions.** An initial *pluripotent* cell makes a progressive series of developmental choices, each choice progressively restricting the range of possible final phenotypes for the fully *differentiated* cell. Thus, the pattern of connections (and shape) exhibited by an adult neuron reflects its history of developmental choices made as it grew and differentiated.

   b) **Selective adhesion determines the specificity of tissue and cellular associations.** Cell- and tissue-specific chemical cues found in the plasma membrane, mediate selective adhesion.

   c) The basic **scaffold of the future adult nervous system** is created at the **earliest stages** of embryonic development, and serves as the structural template for the future nervous system. While the embryo is still small, single **pioneer neurons** grow axonal projections, following chemical cues provided by **guidepost cells** to establish the initial scaffold. Diffusional cues are easily established across broad regions of the embryo while it is small, which can profoundly pattern global features of the later (larger) embryo. **Growth cones** at the tips of growing axons integrate chemical cues and direct the growth of axonal projections. The adult nervous system results from new neurons adding to the initial scaffold. New axonal projections navigate across the embryonic scaffold through the process of **selective fasciculation.**

2. One useful vertebrate model for the study of axonal guidance is the **retinotectal map** of axonal projections made by retinal ganglion cells to the optic tectum. The adult retinotectal map follows an orderly spatial pattern of projections that creates a **retinotopic map** of the visual world. Ganglion cells along the nasal/temporal axis of the retina project along the posterior/anterior axis of the optic tectum. Similarly, ganglion cells along the ventral/dorsal axis of the retina project along the medial/lateral axis of the tectum. Is this orderly map of retinotopic projections mediated by gradients of chemical cues along the tectum, which are “read” by projecting axons? Yes, almost certainly.

3. **Chemoaffinity hypothesis** proposed by Roger Sperry, based on classic experiments performed with frogs (1956). This hypothesis states that axons differentially recognize chemical cues displayed by target cells, and this selective recognition is the basis for guiding proper neuronal connections. In his experiment, the eye of a frog is isolated (severing the original connections to the tectum), then rotated 180° and reimplanted. In the frog, the retinal
ganglion cells are able to regenerate axons that project back to the tectum, and re-establish functional synapses. Result…the visual world for these frogs is subjectively inverted! Behavioral evidence: when tempted by a tasty fly in its upper visual field, the hapless experimental frog lunges downwards. This wholly inappropriate behavior can be explained if regenerating axons of the retina grow back to their original location in the tectum. Recall that the eye has been inverted 180°. Visual stimulation of the upper visual field now stimulates photoreceptors at an inappropriate location in the inverted retina (dorsal vs ventral), and thus in the analogous inappropriate location in the tectum (lateral vs medial), leading to an inappropriate perception (down vs up)! This interpretation can only work if the regenerated connections of retinal ganglion cells in the inverted eye are reestablished at their originally specified locations in the tectum. Restated differently, since the physical orientation of the experimental eye is inverted relative to the visual world, the retinotopic projection from the eye to the tectum must be unaltered, if the frog is to perceive the world as upside-down. Because the frog behaves as if its entire visual world is inverted, this suggests a very precise, global mechanism directing the innervation of the retinotectal map. Sperry proposed that spatial gradients of chemical cues expressed by tectal cells likely mediate this process during development.

4. Experimental support for the chemoaffinity hypothesis came from classic cellular association assays performed with dissociated cells from vertebrate and invertebrate tissues. When dissociated cells of one tissue-type are mixed with dissociated cells of a different tissue-type in cultures, cells of the same tissue-type tend to preferentially reaggregate. For example, dissociated epidermal cells tend to reaggregate with other epidermal cells, and mesodermal cells with other mesodermal cells. This is even observed with dissociated cells from two species of sponges (traced by different pigmentation). Thus, differential adhesion appears to be an ancient mechanism used by cells to generate ordered multicellular tissue assemblies. Neurons adapted this ancient mechanism to guide axonal outgrowth. Interestingly, immune cells may also have adapted the same molecular mechanism for recognizing and binding antigens.

5. How does one identify molecules mediating axonal guidance? Two basic approaches:

a) Biochemical fractionation and isolation of “axonal guidance factors”, employing a functional assay. This approach best exemplified by Friedrich Bonhoeffer’s lab (Max Planck Institute for Developmental Biology, Tuebingen, Germany). Membrane preparations from different regions of the tectum applied to a dish were found to mediate differential adhesion by growing axons from retinal ganglion cells, in culture. Using this functional
assay, tectal membrane preparations were fractioned and purified for an “axonal guidance factor”.

b) **Molecular genetic** approach, using a genetically tractable organism and a histological screen for mutants affecting axonal guidance. This approach exemplified by Corey Goodman’s lab (UC-Berkeley) with *Drosophila*. Embryonic lethal screen (similar to screens for segmentation genes) were conducted to identify mutants affecting the morphology of *commissures and longitudinal fascicles* in the early embryonic nervous system; many of these genetic mutants affected genes mediating normal axonal guidance. Mutant genes could then be identified, by molecular genetic cloning techniques.

6. Both approaches reveal a large collection (likely incomplete) of secreted and membrane-associated molecules with common structural features. These molecules are constructed in modular fashion, and can be grouped into 4 basic classes:

a) **Laminin, fibronectin** and other secreted glycoproteins (proteins with covalently attached sugar groups) associated with **extracellular matrix**.

b) **Cadherins** and **catenins**, associated with Ca^{2+}-dependent homophilic interactions and tight junctions. Cadherins are membrane proteins with an “adhesive” extracellular domain and an intercellular domain that binds catenins. Catenins bind to actin and thus anchor cadherins to cytoskeletal elements. Catenins may also serve a dual role as **intracellular signaling** proteins (in the Wnt signaling pathway).

c) **Cell adhesion molecules (CAM)**, containing IgG-like domains, similar to those found in immunoglobulins expressed by immune cells. IgG domains mediate cellular recognition and binding in both cases. This is the largest class of guidance molecules, containing both membrane-associated and secreted members. May mediate **attractive or repulsive** cues.

d) **Receptor tyrosine kinases (RTK)** and **receptor phosphatases (RP)**. These molecules contain an “adhesive” extracellular domain, a single membrane-spanning domain, and a catalytic intracellular domain that may either attach phosphates (kinases) or remove phosphates (phosphatases) from other proteins. These molecules thus may serve a **dual** function for selective adhesion, and for transmitting contact-dependent instructive signals via **intracellular signaling pathways**.

7. Functionally four classes of guidance signals can be distinguished:

a) **Diffusible chemoattraction**. (secreted, the CAM netrin for example)

b) **Diffusible chemorepulsion**. (secreted, the CAM semaphorin II for example)

[**Diffusible cues mediate long-range signals.**]
c) **Contact-dependent attraction.** (membrane-associated, the CAM fasciculin II for example)

d) **Contact-dependent repulsion.** (membrane-associated, the RTK Eph for instance)

[Contact-dependent cues mediate short-distance signals.]

8. Correct developmental specification for wiring “billions and billions” of neurons may rely upon innumerable individually tailored developmental programs for each neuron. Each developmental program may be mediated by an unique combination of chemical/adhesive cues, dynamically provided by large families of related guidance molecules, modularly constructed, specifying first global, then progressively restricted local cues. These genetic cues interact with selective elimination of “inappropriate” connections, resulting from experience or environmental factors, to sculpt the ultimate complexity and specificity of the adult nervous system.

References:


Axonal Guidance

1. **Pioneer neurons** construct the earliest scaffold of the nervous system, following extracellular chemical cues.

2. Multiple chemical cues are *integrated* by **growth cones**, including **long-range diffusible cues** (secreted molecules) and **short-range contact mediated cues** (membrane-associated).

3. Chemical cues may be **attractive** or **repulsive**.

4. Chemical cues may mediate *both selective adhesion* and **intercellular signaling pathways**.

5. Axonal guidance molecules are encoded by multiple ancient conserved gene families, including large classes with structural similarity to **immunoglobulins**.

6. Final axonal pathways are likely specified by **unique combinations** of molecular cues expressed by growing neurons, glia and target cells.

7. Human mutations of axonal guidance genes may underlie many hereditary neurological conditions affecting complex cognitive functions.