Axon guidance lecture (Dr M Fruttiger)
Last update 02/03/08

The problem
During development newly generated neurons differentiate into different kinds of neurons and take on certain identities before they establish any connections. For example sensory neurons "know" that they are sensory neurons and that they have to target the skin and not a muscle. Thus, based on their identity different neurons will send out processes to their appropriate targets as the embryo develops. How do these outgrowing processes navigate their way through the embryonic body? Does each neuron need to "know" the entire path to the target and how could such an enormous amount of information be encoded in the genome?

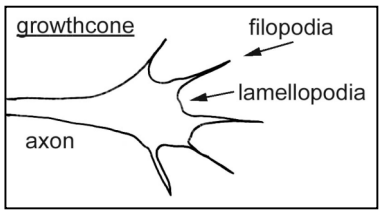
General Principles
There are two basic principles, which simplify the above problem:

1) Intermediate targets:
   Growing axons can use intermediate targets (stepping-stones), which they approach by relatively simple linear growth. Once they have reached the intermediate target they have to "make a choice" before aiming for the next intermediate target. This breaks the entire path into shorter more manageable segments.

2) Selective fasciculation:
   Most growing axons face an environment already full of other neuronal processes which they can simply follow thereby forming axon bundles (fascicles). A developing axon can also switch from one fascicle to another at a given choice points. This selective fasciculation simplifies the navigational problems a growing axon faces.

However, the basic problem remains: how do the first axons (pioneer axons) find their target, and how do axons find their way between intermediate targets?

The answer is, that growing axons respond to guidance signals present in the embryo. Current research is identifying more and more of these signals. They fall into two categories: repulsive and attractive signals. Based on how easily they can diffuse through tissue a further distinction is made: long range and short range signals. Long range signals tend to be diffusible molecules secreted by cells whereas short range signals are non-diffusible and bound to cell surfaces or the extracellular matrix (ECM). The growing tip of the axon is the key structure necessary in interpreting these different signals and is called "growth cone". It consists of a central area (c-region), filopodia and lamellopodia and is highly motile. Shaped like a hand it "feels" its way trough the embryo laying down the axon in its wake. Faced with different signals the growth cone will adapt its growth direction (away from or towards a signal source) or it may simply collapse upon contact with a repulsive signal. This behaviour is based on receptor molecules on the surface of the growth cone transmitting signals to the "motor machinery" inside the growth cone. Filopodia move like fingers "exploring" their environment. These movements are based on actin polymerization and depolymerization. In filopodia actin filaments are organized in bundles whereas in the c-region and in lamellopodia they form an intricate network. The main cytoskeletal protein in axons is tubulin polymerized into microtubules. In the axon shaft these microtubules form a stable cross-linked bundle whereas in the growth cone they splice out and are instable, extending and retracting along actin bundles. Through differential stabilization of actin filaments and microtubules growth cones car



advance, stop, retract or turn. However, the exact molecular mechanisms which control the growth cone cytoskeleton are not well understood yet.

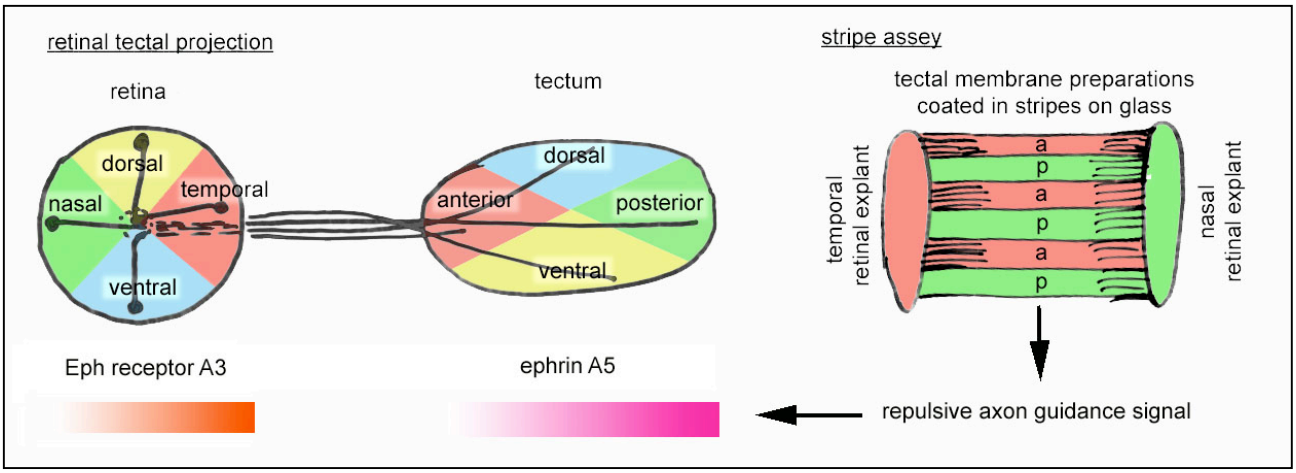
How can axon guidance molecules be studied?

Cell culture and genetic studies have so far have been very successful in exploring the molecules involved in axon guidance. In culture the reaction of live growth cones can be studied when they are confronted with a particular molecule in a defined although somewhat artificial environment. Genetic experiments (e.g. knock out mice) study how a particular gene affects axonal pathway finding in a "real environment" but the interpretation of a particular phenotype is complicated by many unknown, uncontrollable variables. In combination these two approaches have revealed the identity of myriads of axon guidance signalling molecules. They can be grouped into different categories:

Cell Adhesion Molecules (e.g. N-CAM, L1 or Fasciclins)
ECM molecules and their receptors (e.g. collagen, laminin or integrins)
Receptor Tyrosine Kinases and their ligands (trk receptors, neurotrophins, Eph-receptors and ephrins)
Netrins and their receptors
Semaphorins and their receptors

The retinal tectal projection

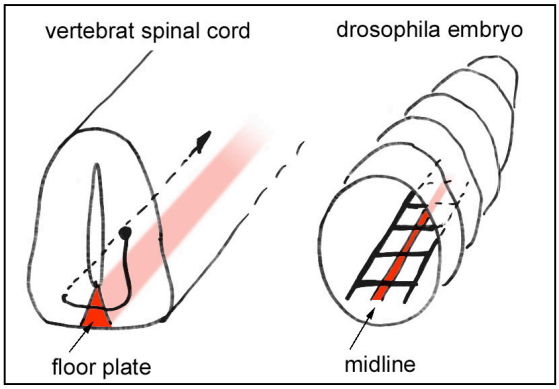
Axons projecting from retinal neurons to the brain represent their retinal topography on the tectum, (their target area of retinal axons in fish, frog and chick) leading to a spatial (inverse) representation



of the retina on the tectum. Neurons located in the temporal area of the retina stop their growth in the anterior part of the tectum whereas neurons from the nasal part of the retina extend through the anterior part to the posterior part of the tectum. This behaviour can be recreated in cell culture by isolating membranes form the anterior and the posterior part of the tectum and coating them as alternating stripes onto a glass slide. Neurons from the temporal retina only grow on anterior membranes and are repulsed by posterior membranes whereas neurons from the nasal retina grow on either membrane preparation. The factor for this repulsive action of the anterior tectum has been identified as RAGS (repulsive axon guidance signal, known today as ephrin A5) and is expressed in an increasing gradient (anterior to posterior) in the tectum (ephrins are the ligands for Eph receptors and are unusual in that they are membrane bound). Retinal neurons express Eph A3 (a receptor for ephrin A5) also as a gradient (nasal to temporal). This means that nasal retinal neurons expressing little Eph receptor A3 are insensitive to ephrin A5 and grow into areas with high concentrations of ephrin A5. Neurons from the temporal retina however, expressing high levels of the Eph receptor A3 are sensitive to relatively low concentrations of ephrin A5 and stop growing in the anterior part of the tectum.

Commissural axons

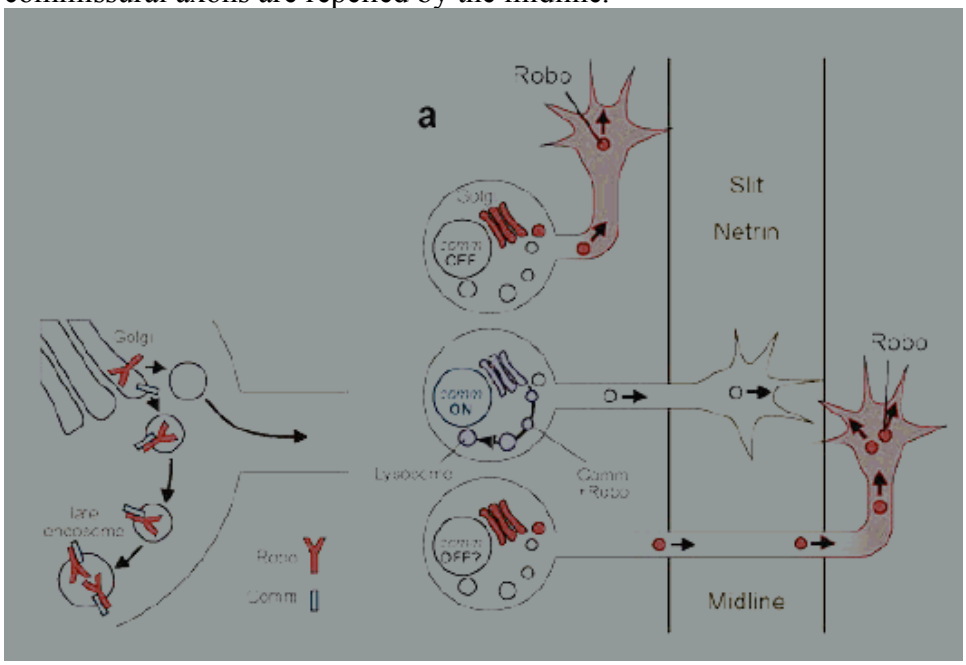
Neurons connecting the two sides of bilateral animals are in evolutionary terms an ancient feature. Axons of commissural neurons cross through the floorplate (in vertebrates) or the midline (in drosophila embryos). On the opposite (contralateral) side they change direction and grow longitudinally (e.g. towards the brain). There are also neurons that do not cross the midline, so called ipsilateral neurons. Thus, axons from commissural and ipsilateral neurons form tracts that run longitudinally on both sides of the midline and that are connected by commissural axons.



It has been shown that the floor plate (or midline in drosophila) secretes attractive signals that draw axons towards it. In vertebrates **netrin** and **sonic hedgehog** have such a function. In mice that lack the netrin gene (so called netrin knockout mice) commissural axons do not cross the floorplate and many commissural structures (e.g. corpus callosum) are missing. In flies sonic hedgehog is not involved in midline crossing but netrin is. Further, so far unknown, factors must exist because commissure formation is only partially disrupted in flies that lack netrin. However, the presence of an attractive signal in the midline/floor plate raises some fundamental questions:

1) Why are commissural axons attracted towards the midline but ipsilateral neurons (non-crossing neurons) ignore the signal?
2) If the midline is so attractive, how can commissural axons move away from it once they have crossed?

This puzzle is solved by a repulsive factor, **Slit**, that is also secreted from the midline. Its receptor, **Robo** (short for roundabout), is present on growth cones from ipsilateral neurons and guides the axon away from the Slit source. In commissural neurons Robo is produced but prevented from reaching the cell surface by **Comm** (short for commissureless). So the growth cone can't "see" slit and is attracted towards the midline by netrin. In flies that lack Comm no commissures are formed because commissural axons are repelled by the midline.



(Figure from Dickson B.J and Gilestro G.F. (2006) Annu Rev Cell Dev. Biol 22:651-675)

After crossing, commissural neurons downregulate Comm (and upregulate Robo), which repels the growth cone from the midline to prevent back crossing. Consequently, in flies missing Robo axons cross backwards and forwards across the midline going "round and round" (hence the name roundabout). In flies that lack Slit ipsilateral and contralateral axon tracts collapse into a single structure along the midline. Vertebrates also have Slits and Robos which seem to perform very similar functions in floorplate crossing. However, Comm was not found in vertebrates (where its function of antagonizing Robo seems to be carried out by Robo3). Nevertheless it is remarkable that at least some of the signalling molecules (Netrin, Slit and Robo) involved in guiding commissural axons are highly conserved throughout the animal kingdom.

After successfully crossing commissural axons join one out of three (fasciclin II positive) fascicles. Neurons expressing just robo1 join the fascicle closest (medial) to the midline, neurons with robo1 and robo3 join the next (intermediate) fascicle and neurons with robo1, 2 and 3 will join the third (lateral) fascicle. Thus, the more robo receptors a neuron expresses the more sensitive it will be to slit and the further away it will grow away from the slit secreting midline (this is also the case for ipsilateral neurons). Note that similar to the retinal tectal projections, the amount of receptor on a growing axon will determine how far it will grow in a gradient of a repellent signalling molecule. So, growth cones respond not only to different guidance molecules but can also use gradients of individual guidance molecules for navigation.

An additional level of control lies within the growth cone itself. It has recently emerged that attractive guidance molecules such as netrin can become repulsive under certain conditions, an effect termed "response conversion". There is evidence that the level of cAMP (an intracellular second messenger molecule) inside the growth cone is responsible whether a particular guidance cue is repulsive or attractive.

Further reading

Text books:

Gilbert, chapter 13, pp 425-439 (sixth edition).
Kandel, Schwartz and Jessel, chapter 54, pp1063-1086 (fourth edition).
Walpert pp 352-360 .
Alberts et al., Molecular Biology of the Cell (fourth edition), pp1228-38

Review articles:

Dickson B.J and Gilestro G.F. (2006) Regulation of Commissural Axon Pathfinding by Slit and its Robo Receptors. Annu Rev Cell Dev. Biol 22:651-675.
Dickson B.J. (2002) Molecular Mechanisms of Axon Guidance. Science 298: 1959-1964
Yu T.W. and Bargmann C.I. (2001) Dynamic regulation of axon guidance. Nature Neuroscience 4: Suppl:1169-1176.
Tessier-Lavigne M. and Goodman C.S. (1996) The molecular biology of axon guidance. Science 274: 1123-1133.