Neural development its all connected
Developmental biology is understanding how organisms develop (form)

From: Kimmel et al. Stages of embryonic development of the zebrafish
Dev. Dyn. 203:253-310, 1995
Why should we care about development biology?

1. We can understand the cause of many birth defects.

2. Developmental biology combines cell biology, genetics, biochemistry, evolution, and molecular biology.

3. Stem cells are important and are going to be more important. Developmental biology can teach us how to use them.
How do you build a nervous system in an animal?

1. Learn how tissue is instructed to become nervous system.
   Neural induction

2. Learn how the nervous system is patterned to generate distinct neuronal cell types.
   Neural patterning

3. Learn how neurons send axons and dendrites to proper locations to form synapses with correct neurons.
   Neural circuits
How do you build a complex nervous system?

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   **Neural circuits**
Developmental stages of *Xenopus laevis*
Zygote → Cleavage → Eight-cell stage → Cleavage → Blastula → Cross section of blastula

- Blastocoel
- Endoderm
- Ectoderm
- Gastrula
- Blastopore

Gastrulation
Fate map of *Xenopus* suggests neural “induced” early

How could you determine if the “purple” cells inherently know that they should be neural or if they are instructed to be neural?
Spemann-Mangold organizer and neural induction
Signals released from Spemann-Mangold organizer

Morphogens diffusible signals
Neural induction: telling cells to become neurons

http://www.mun.ca/biology/desmid/brian/BIOL3530/DEVO_05/ch05f04.jpg
sox2 expression is a molecular marker of the neural plate
Xenopus (frog)  Drosophila (fruit fly)
Nerve cord forms on the ventral side of *Drosophila* embryo

But similar molecular program is regulating where the nervous system will form!
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   **Neural circuits**
Basic anatomy and regionalization of nervous system underlies distinct functions

- Breathing - Head and neck
- Heart rate - Shoulder
- Wrist and elbow
- Hand and Finger
- Sympathetic tone (temperature regulation), Trunk muscles
- Hips/Pelvic region
- Knees
- Knees and Foot
- Bowl / Bladder

Wnt8 expression suggests it may regulate A-P patterning.

Kelly et al., (1995) Development
Over-activating Wnt8 posteriorizes *Xenopus* embryos

Kiecker and Niehrs (2001) *Development*
Wnt Antagonists anteriorize the neural plate

Kiecker and Niehrs (2001) Development
Summary of anterior-posterior neural patterning

Low Wnt

Telencephalon
Diencephalon
Metencephalon
Myelencephalon
Spinal cord

High Wnt

Prosencephalon (forebrain)
Mesencephalon (midbrain)
Rhombencephalon (hindbrain)

DKK

http://www.uni-heidelberg.de/md/izn/researchgroups/niehrs/niehrs_fig2.jpg
Distinct domains with sharp borders form by local refinement of gradient information.
At this point we have

1. induced a plate of cells to become neuronal

2. told all the cells within that plate their relative position along the anterior-posterior axis
The neural plate folds and closes to form the neural tube.

Dorsal-Ventral patterning in the neural tube generates distinct domains that give rise to specific neuronal types.

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UNC5 (Hedgecock et al., 1990; Serafini et al., 1994; Keino-Masu et al., 1996; reviewed by Barallobre et al., 2005), Slits/Robos (Seeger et al., 1993; Kidd et al., 1998, 1999; Brose et al., 1999; Li et al., 1999; reviewed by Brose and Tessier-Lavigne, 2000) and Semaphorins/Neuropilins/Plexins (Luo et al., 1993; Chen et al., 1997; He and Tessier-Lavigne, 1997; Kolodkin et al., 1997; Winberg et al., 1998; Takahashi et al., 1999; Tamagnone et al., 1999; reviewed by Fujisawa, 2004). It also has become clear that secreted factors best known for their role as morphogens, such as Sonic hedgehog (Shh; Trousse et al., 2001; Charron et al., 2003; Bourikas et al., 2005), FGFs (McFarlane et al., 1995, 1996), BMPs (Augsberger et al., 1999) and Wnts (Lyuksyutova et al., 2003; Yoshikawa et al., 2003), are key regulators of axon guidance decisions (reviewed by Bovolenta, 2005; Charron and Tessier-Lavigne, 2005).

Much work is being done currently to relate the function of these molecules to the formation of specific axonal pathways.

This review will focus on our current understanding of the mechanisms directing axon guidance in the developing visual system. Due to its relatively simple anatomy, ease of analysis and stereotypical projection pattern the developing optic pathway has proven to be one of the most useful models for studying axon guidance decisions. Much of our understanding of the actions of specific guidance cues has come from studies of this system. Here we will highlight recent progress in unravelling the precise repertoire of guidance signals required for optic pathway development. The transcriptional regulation of these guidance signals and factors that modulate the response of growth cones to specific cues also will be discussed.

The developing optic pathway

Following their differentiation RGCs extend their axons into the optic fibre layer (OFL) at the inner surface of the retina where they grow in a highly direct, radial fashion towards their exit point from the eye, the optic nerve head/disc. From here, they enter the optic nerves and extend towards the ventral midline of the diencephalon (developing hypothalamus) where the two nerves meet at an invariant position along the anterior–posterior axis of the brain to form the optic chiasm, a major brain commissure. In species with eyes located laterally all axons cross the midline at the chiasm whereas in animals with binocular vision a proportion of axons originating in the temporal region of the retina do not cross but instead project ipsilaterally. Irrespective of their behaviour at the chiasm, the RGC axons then project dorsally within the optic tracts towards their targets in the midbrain and thalamus (Fig. 2). At the optic nerve head, chiasm and on reaching their target, RGC growth cones increase in size and adopt highly complex morphologies tipped with multiple filopodia and lamellipodia (Bovolenta and Mason, 1987; Holt, 1989; Godement et al., 1994; Hutson and Chien, 2002). This behaviour is indicative of growth cones encountering novel environments or faced with a choice of pathway selection (Mason and Wang, 1997). Thus, the developing optic pathway can be considered as a series of discrete segments intersected by these intermediate targets/decision regions. The mechanisms directing RGC axon pathfinding in each of these regions has been studied in a wide range of vertebrates ranging from fish through to mammals (Table 1). Despite differences in the overall organisation of the RGC axons as they navigate through the optic pathway in different organisms, particularly at the optic chiasm, these studies have revealed a high degree of conservation in the underlying guidance mechanisms (e.g. Nakagawa et al., 2000; Herrera et al., 2003; reviewed by Jeffery and Erskine, 2005). Information gathered from different species will therefore be considered as a whole in this review.

Fig. 1. Schematic diagram of growth cones growing in the absence (A) or presence (B, C) of guidance cues. A: Growth cones extend dynamic filopodia and lamellipodia. These structures are formed from F-actin, which is organised as bundles in filopodia and a meshwork in lamellipodia. This actin-rich region forms the thin peripheral (P) domain of the growth cone with the thicker central (C) domain being composed of microtubules and organelles. Microtubules are tightly bundled together in the neurite shaft but splay out in the growth cone. In a gradient of a repellent (B) filopodia and microtubules are lost selectively on the side of the growth cone facing the gradient resulting in repulsive turning. In a gradient of an attractant (C) filopodia and microtubules are stabilised selectively on the side facing the gradient resulting in turning towards the signal.
The basics of axon guidance

- The growing region also needs to adhere to a substrate to stabilize the outgrowth and allow progression in one direction.
Common axon guidance cues

- Netrin-1 with DCC: Attraction
- Slit and DCC: Repulsion
- Netrin-1 with Robo: Attraction silenced
- Metalloprotease and γ-secretase: DCC silencing
- Netrin-1 with DCC: Attraction

(c) Sema3A and Sema3F: Repulsion
- Npn1 and Npn2: Repulsion
- Plexin: Repulsion
- Sema3C: Repulsion

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the responsivity of the axons to netrin remains to be determined. Several other factors, including reverse-signalling by EphBs acting as guidance cues, BMP receptor 1B and NrCAM also contribute to the targeting of RGC axons to the optic disc and subsequent exit from the eye (Birgbauer et al., 2000, 2001; Liu et al., 2003; Zelina et al., 2005).

Guidance at the optic chiasm

After leaving the eye, RGC axons run into the developing optic stalk, where they are curtailed to the optic pathway by inhibitory Sema5A signalling (Oster et al., 2003; Fig. 3B), and grow toward the brain where they enter at the ventral-most aspect of the diencephalon. There, axons from the two eyes cross over each other to form a characteristic X-shape known as the optic chiasm (Fig. 2). The factors determining the invariant place at which the chiasm forms are beginning to be elucidated (Table 1).

Shh, in addition to be expressed at the retina, is present along the entire axial midline of the chick embryo prior to the arrival of the RGC axons. As the first RGC axons approach this area, Shh is downregulated specifically at the optic recess level, a spatiotemporal change that, by alleviating a block on RGC axon extension, is critical for chiasm formation (Trousse et al., 2001). In Pax2 null mice and the equivalent noizebra fish mutant, the chiasm fails to develop and this is associated with persistent expression of Shh in the optic recess (Torres et al., 1996; Macdonald et al., 1997). This suggests that, in contrast to the retina (see above) Shh acts in the chiasmatic region as an inhibitor of RGC axon extension. In vitro, Shh has a dual effect on RGC axons depending on its concentration (Kolpak et al., 2005). Thus, one possibility for this differential responsivity of RGC axons in the retina and chiasm is different levels of Shh expression. Alternatively, intrinsic changes in the RGC axons as they navigate through the optic pathway, for example in cyclic nucleotide levels (see below) or receptor expression (Bourikas et al., 2005) could modulate the response. Further studies will be required to elucidate the basis for this change in Shh function in the retina and chiasm. Slit molecules also are expressed in the diencephalic area and, through inhibitory signalling via Robo2, outline the precise position along the midline neuroaxis at which the optic chiasm develops (Erskine et al., 2000; Fricke et al., 2001; Hutson and Chien, 2002; Plump et al., 2002)( Fig. 3B).

In binocular species, once RGC axons are in the right position and approaching the midline, they have to decide whether to cross or remain uncrossed. Work in Xenopus indicated that ephrin-Bs play an important role in inducing divergence at the midline (Nakagawa et al., 2000). Further studies in mice expanded upon these findings and demonstrated that ephrinB2/EphB1 signalling is crucial for the formation of the ipsilateral projection (Table 1). EphrinB2 is expressed by chiasmatic radial glia at the time ipsilateral axons are turning at the midline and is not only sufficient but also necessary for the formation of the ipsilateral projection (Williams et al., 2003; Fig. 3B). A receptor for ephrinB2, EphB1, is expressed highly in the same region. Slit molecules also are expressed in the diencephalic area and, through inhibitory signalling via Robo2, outline the precise position along the midline neuroaxis at which the optic chiasm develops (Erskine et al., 2000; Fricke et al., 2001; Hutson and Chien, 2002; Plump et al., 2002)( Fig. 3B).

Axon pathfinding in the Superior Colliculus

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Summary:

1. Neural induction is caused by inhibition of BMP by CHD and NOG

2. Anterior neural fates arise during induction and the action of a WNT8 gradient posteriorizes the neural tissue.

3. Opposing gradients of SHH and BMP pattern the dorsal-ventral axis of the spinal cord.

4. Regulating attraction and repulsion guides axons to their target regions.

5. Local interactions allow for synapse formation and plasticity over time.

A common theme is that gradients pattern neural tissue.

Any one cell has 3 dimensions of patterning: A-P, D-V, and Time.