

How are interactions between neural cells established and maintained?

The human embryo is a collection of clusters of non-specific cells, which then develop into the tissues of the adult human. In particular immature, non-specific neuroblasts must be differentiated into the highly specialised cells of the nervous system, each with a unique structure, function and synaptic interactions (Watson 2004).

The whole function of the nervous system relies on the synaptic and other interactions between neural cells being developed appropriately and maintained throughout life. This account covers the initial development of specific neural cells from immature precursor stem cells and also the methods by which the growing specialised neurons grow along the correct routes to form their interactions with other cells, both neuronal and non neuronal.

Establishing neural cells from stem cells

Progenitor or stem cells are non-specific immature cells that are able to differentiate and form specialised cells in the adult organism. There are relatively few types of neural stem cells, with the potential to differentiate into many different types of specialised neural cells, depending on specific, localised requirements. Multipotent (able to form many types of daughter cells) neural stem cells were first identified in the early 1990s (Imitola et al. 2004) and subsequent research has focussed on specific examples of these immature progenitor cells.

Neural stem cells can give rise both to different types of mature cell, as a result of asymmetric cell division, as well as identical daughter cells via simple symmetric cell replication (Gage 2000). The exact form of the adult cell is controlled by the location of progenitors during differentiation, as well as by diffusible factors acting from a more distant site, usually the final location to which the differentiated cells will migrate.

Signalling molecules influence the differentiation of neural stem cells. For instance valproic acid (VPA) actively suppresses glial differentiation, instead encouraging the development of adult neurons, via upregulation of the neurogenic basic helix-loop-helix transcription factor (Hsieh, Gage 2004). The mesenchymal stem cell (MSC) is derived from bone marrow and able to self regenerate and differentiate into several different types of cells in vivo (Kondo et al. 2005). MSCs are able to form both neuronal and glial cells; the difference relating to the presence or absence of voltage gated ion channels; indicative of the potential to develop into a full neuronal cell. Furthermore the presence of specific signalling molecules in the cell milieu can influence the development of specific types of neuronal cells. One such example involves the sonic hedgehog and retinoic acid signalling molecules, which have been shown to cause MSCs to define neurons adapted for the peripheral nervous system (Kondo et al. 2005). It is believed that this guidance of development occurs via the promotion of specific transcription factors appropriate to the adult neuronal cells. The diffusible signalling molecules would switch on the transcription factors.

Axon guidance

A growing axon has an area of active tissue on the tip, known as the growth cone (Watson 2004). It is the interactions between the growth cone and the surrounding structures and chemicals that govern the route along which the axon grows. Chemotactic guidance is the name given to guidance derived from close physical contact between the growth cone and structures it comes into contact with. For instance, physical obstacles such as bones or cartilage may prevent the axon from continuing along a specific route. Instead the filopodia (thin membrane extensions) would literally feel around for a route that the axonal growth could take. In the case of growth of the axons of the optic nerve, the growth of retinal neurites is influenced by the presence of laminin and fibronectin. Axons interact with these extracellular matrix components differently according to their origin; with some adhering more strongly to laminin, with others showing greater allegiance to fibronectin (Watson 2004). Hence some retinal neurites cross the optic chiasm whilst others do not.

Chemotactic guidance does not, however, operate in isolation, instead being linked to the presence of chemical signalling molecules, many of which are similar to those affecting the initial stem cell differentiation.

The idea that the gradient of chemical substances secreted by a target cell might be responsible for the ability of growth cones to find their way to a target cell was initially proposed by Ramon y Cajal more than a century ago (Ming et al. 2002). These chemotropic (literally movement in response to a chemical factor) factors are present in the extracellular matrix and act to either attract or repel the growth cone, along a chemical gradient. One example is netrin-1, which causes isolated xenopus spinal neurones to turn towards the greater concentration. However too high a concentration of netrin-1 (eg 5ng/ml-1 compared to 5 (g/ml-1) appears to desensitise the neurones, which no longer exhibit turning on a gradient (Ming et al. 2002). Netrin-1 (also known as unc-6) is also required by the worm C-elegans in order for differentiating neuroblasts to migrate along the dorsoventral axis; the final direction determined by interactions between the neuroblasts and specific receptors (Hatten 2002).

Crossing the midline a detailed example

In the fruit fly drosophila the genes roundabout (deficient mutant - robo), commissureless (deficient mutant - comm) and slit (deficient mutant - sli) work together to influence axonal growth across the CNS midline (Kidd, Bland & Goodman 1999). Axons may cross the midline and become commissural or remain ipsilateral as longitudinal axons. Figure 1 below illustrates the effect that removal of each of the 3 chemotropic factors has on the appearance of longitudinal and commissural axons within the CNS axon scaffold, described as follows:

Wild type (all genes thus chemotropic factors intact) shows neat crossing of the midline, resulting in 2 commissures per segment and balanced commissural and longitudinal axons.

comm - no axons cross the midline, all remain longitudinal.

sli - axons enter the midline but then continue as longitudinal axons, within the midline.

robo - axons cross and recross the midline resulting in excessive commissural axons and very few longitudinal axons.

The authors concluded that slit acted as a short range repellent of axons with respect to the midline, thus was a chemotropic factor (Kidd, Bland & Goodman 1999). Subsequent research has shown that robo is actually repelled from the midline in response to slit, which has been found to be a large protein expressed by midline glia (Kraut, Zinn 2004).

Neuronal and Glial cell interactions

Much recent research into the methods by which developing neurons form interactions with other neural cells as well as the glial support cells, has come from study of insects such as drosophila. Glia are crucial in the correct development of the developing eye disc in insects. The diffusible factors decapentaplegic and hedgehog both strongly influence glial proliferation, with decapentaplegic also having a role in glial motility. Glia motility is particularly crucial as axons will extend and grow in the correct direction in response to diffusible factors but will not enter the optic stalk without the chemotactic guidance of glia (Oland, Tolbert 2003).

Maintaining the connections in the adult nervous system

It has recently become apparent that, as a neuron matures, its responsiveness to signalling molecules such as netrin-1 changes (Salinas 2003). There is logic to this, as a maturing axon would expect to receive specific guidance cues to establish connections with other cells; whereas a mature axon would not seek to continue to migrate towards the cells, rather wishing to maintain accurate connections. As there is a need to replace dead cells throughout life it would not be appropriate to completely switch off signalling molecules such as netrin-1, as every new axon would need to migrate towards the correct connections, but would then benefit from becoming effectively immune to the signalling capabilities of netrin-1 as there would be no need for further migration.

Conclusion

The importance of accurate migration from the stem cell germinal site to the area of axon-target interaction relates to the need for accurate synaptic connections in the adult nervous system. Organisms with a more simplistic nervous system have a low extent of migration, whilst the vertebrate nervous system involves a high degree of migration (Hatten 2002). Thus the eventual nervous system depends heavily on the accuracy of the guidance provided by both the signalling molecules and the chemotactic scaffold.

Whilst there is immense potential in understanding how stem cells develop, in terms of replicating this in order to replace damaged cells in the human adult; thus far the research underlying the regulation of endogenous stem cells is in its infancy and mechanisms are poorly understood. Further, the method by which differentiated cells are able to accurately locate their target connections is also an area of current mystery.