

Neuronal Pathway Finding: From Neurons to Initial Neural Networks

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Abstract: Neuronal pathway finding is crucial for structured cellular organization and development of neural circuits within the nervous system. Neuronal pathway finding within the visual system has been extensively studied and therefore is used as a model to review existing knowledge regarding concepts of this developmental process. General principles of neuron pathway finding throughout the nervous system exist. Comprehension of these concepts guides neuroscience nurses in gaining an understanding of the developmental course of action, the implications of different anomalies, as well as the theoretical basis and nursing implications of some provocative new therapies being proposed to treat neurodegenerative diseases and neurologic injuries. These therapies have limitations in light of current ethical, developmental, and delivery modes and what is known about the development of neuronal pathways.

The brain produces approximately one hundred billion cells, including neurons, in the ventricular and subventricular zones during initial brain development (Marshall, Suzuki, & Goldman, 2003). Following neuron migration, neurons send out an axon and are ultimately able to create the rudimentary networks necessary to facilitate information processing and behavioral patterns. The pathway of the visual system has been the most deliberately studied nervous subsystem. Therefore, it presents a working model to gain knowledge of how axons migrate through intermediate regions to find their final targets and set up connections. The aim of this article is to highlight a portion of the research regarding neuronal pathway finding in the visual system and relate it to what is known about neuronal pathway finding throughout the nervous system. Comprehension of this process guides neuroscience nurses in gaining an understanding of normal development of the brain's initial neural networks. In recent years, there has been a thrust of neuroscience research aimed at implanting stem cells and nerve grafts or stimulating axonal regeneration for treatment of nervous system diseases or injuries. Since

these proposed treatments are based, in part, on knowledge about neuronal pathway finding, nurses should understand its theoretical basis.

Axon Formation

From the cell body (soma), an axon (neurite) must develop, and this process is known as polarization. Often several neurites will develop from one cell, but only one will survive and go on to become its axon. Ruthel and Hollenbeck (2000) studied this process to discriminate the preferential growth of one neurite or branch over the others. They considered sibling bias, in which neurites from the same axon take turns to grow, and established that the neurite itself was responsible for regulating the preferential growth of one particular branch. It was conjectured that the modification of transported materials within a particular neurite, affected the microtubules and in due course favored a particular neurite over others.

Growth Cone

From the tip of the neurite develops the growth cone, a filopodial structure. The growth cone is able to move through the environment, sensing structural and chemical cues and laying down the new axon (Fig 1). The tip of the growth cone has several finger-like projections known as filopodium, which are able to advance the axon through the immediate environment and in the appropriate direction. This occurs through the three processes of protrusion, engorgement, and consolidation. In protrusion, the filopodia form finger-like projections in response to environmental cues, and the plasma membrane advances forward. In engorgement, the organelles and cytoplasm move forward, and finally, in consolidation, a new axon section is left behind (Bamburg, 2003; Goldberg & Burmeister, 1989). Within the cytoplasm are neurofilaments, which provide structure, and microtubules, which supply the mechanism for rapid vesicle transport (Schnapp, Vale, Sheetz, & Reese, 1987). Transport to the growth cone is one of the necessary components to maintain the membrane as it changes.

The protein, actin, found in the growth cone membrane and within the cytoplasm, together with tubulin, the structural component of microtubules, provides the means to change the shape of the growth cone structure to move towards, or away from, the environmental cues. Together, actin and the microtubules create focal adhesion (Schaefer, Kabir, & Forscher, 2002). This adhesion, along with the actin polymerization, allows the

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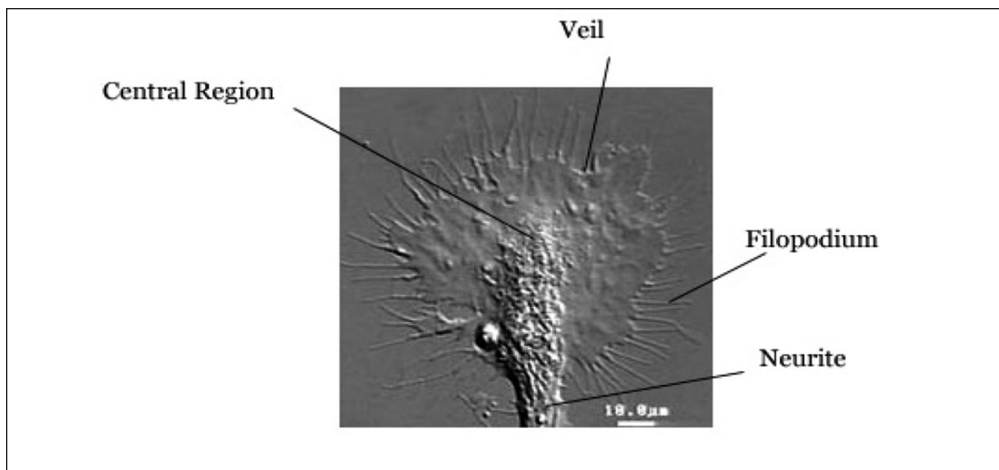


Fig 1. Growth cone (Image by James Zheng, UMDNJ-Robert Wood Johnson Medical School, NJ; used with permission.)

dispersion and advancement of the growth cone membrane at the leading edge (Mikule, Gatlin, de la Housaye, & Pfenninger, 2002; Steketee & Tosney, 2002). The Rho family of small GTP-binding proteins also aids in controlling the actin molecules when they polymerize or depolymerize. This manipulation of actin molecules allows the axon to advance, retract, turn, or branch.

In addition, the Rho family of proteins controls the retrograde flow of the specific actin protein, F-actin. It is this retrograde flow of F-actin, within the growth cone, that appears to supply the traction needed for the advancement of the growth cone body. Also important are neurotrophic factors, often provided by glial cells, other axons, and glycoproteins located throughout the brain. Neurotrophic factors provide necessary nutrients to sustain the axon (Huber, Kolodkin, Ginty, & Cloutier, 2003; Koleske, 2003; Song et al., 1998; Song & Poo, 1999; Suter & Forscher, 1998).

As the growth cone moves through the environment, receptors on its filopodial structure allow maximum contact with the specific guidance signals, located within the extracellular matrix or the surface of nearby axons (Goldberg & Burmeister, 1989). These guidance signals can be directional, positional, or permissive/modulatory. Directional signals influence the pathway navigated, while positional signals cause the axon to grow, retract, or stop. Permissive or modulatory signals provide neither direction nor position, but instead allow for processes such as axon fasciculation or adhesion to the substrate, which can indirectly aid in the path-finding process. Regardless of the type of guidance signal, the guidance signals must be available at the appropriate time and in the appropriate location to prevent navigational errors (Song & Poo, 2001). Fortunately, many studies have shown that there are often back-up mechanisms for isolated signals that will result in the proper development, despite a single error.

The differential expression of receptors or receptor complexes on the growth cone and their ligand-dependent interactions, along with some intracellular adaptors and mediators, link the external guidance cues with the internal environment of the axon and result in the specificity and polarity of particular axons. Since receptors for axon guidance on the cell surface have at least one transmembrane domain, chemicals and molecules that bind with these receptors can potentially trigger both

cytoskeletal and intracellular signaling cascades within the growth cone filopodia or to the cell body (Dickson, 2002); see Table 1. The intracellular signaling cascades can have a trophic action or modulatory action, each of which results in regulating the axon motility by maintaining stable adhesion (modulatory), causing attraction or repulsion (directional), or giving a stopping or branching cue (positional; Goldberg & Burmeister, 1989; Isbister & O'Connor, 1999; Polinsky, Balzovich, & Tosney, 2000; Song & Poo, 2001).

Retinal Ganglion Cells

Retinal ganglion cells (RGC) are born in a central to peripheral gradient. Newer RGC axons have contact with the axons of older RGC and use these older RGC axons to move along, or possibly fasciculate, with their adjacent axons. This highlights the different neuronal pathway finding mechanisms observed between pioneering and subsequent axons, which are also seen throughout the nervous system. Typically, pioneering axons have larger growth cones to guide themselves to their targets. Subsequent axons have smaller growth cones. These axons often use cell surface adhesion molecules (CAMS) on the pioneering axons, which guide them to their destination. For instance, the RGCs can use laminin-1, which is expressed on the pioneering axons, and these CAMS result in homophilic binding, which occurs when a molecule on one axon binds to a molecule on an adjacent axon. Homophilic binding in retinal axons results in axons that grow in slender bundles (fascicle), from the retina to the optic disc (Brittis, Lemmon, Rutishauser, & Silver, 1995; Burden-Gulley, Pendergast & Lemmon, 1997). Other diffusible factors also influence this process. Chondroitin sulphate, found in the extracellular matrix of the retina, offers a directional clue by inhibiting the axons, directing them away from the peripheral retina and towards the optic disc (Brittis, Canning, & Silver, 1992).

Table 1. Overview of Known Chemical and Molecular Factors Involved in Neuronal Pathway Finding in the Visual System

Molecular and Chemical Cues	Effect	Mode of Action	Target	Reference
Cell adhesion molecules (CAMs), i.e., laminin-1 or N-cadherin	Adhesion and attractant	Allow cells to grow along the surfaces of other cells (such as using pioneering axons for subsequent axons)	Growth cone of subsequent axon	Brittis, Lemmon, Ruitishauser, & Silver, 1995; Burden-Gulley, Pendergast, & Lemmon, 1997
Chondroitin sulphate	Inhibitory	Soluble proteoglycan in extracellular matrix	Axons	Brittis, Canning, & Silver, 1992
Ephrins, i.e., Ephrin A5 and A2; mammals have 8 ephrins and 13 Eph receptors	Inhibitory and attractant diffusible proteins; ephrin A5: branching of axons	Diffusible protein: secreted to work locally, as a gradient to work long distance, or membrane bound	Ephrin A B receptors; ephrin receptors	Dickson, 2002
GAP-43	Assist in critical decisions	Amplify environmental signals	Growth cone	Strittmatter, Fankhauser, Huang, Mashimo, & Fishman, 1995
Glial cells or glial wedge	Scaffold trophic support	Secretion of midline attractants and CAMS	Growth cone	Niclou, Jia, & Raper, 2000; Plump et al., 2002
Laminin	Trophic alone and inhibitory when combined with Netrin-1	Diffusible protein	Integrin receptor	Song & Poo, 2001
Netrins	Midline attractant alone and inhibitory when combined with laminin	Diffusible protein; secreted	DCC receptor; Robo receptor	Dickson, 2002
Neurotrophin family, i.e., nerve growth factor, brain-derived growth factor, neurotrophin-3, neurotrophin 4/5	Neuronal survival, proliferation of oligodendrocyte progenitors, down-regulate macrophage's proinflammatory activities, tissue repair after CNS damage	Released from target tissues and neurons	Specific tyrosine kinases receptors	Aloisi, 2003
Semaphorin family (Classes 3 to 7 in vertebrates)	Diffusible proteins: mostly inhibitory, but possibly attractant for some neurons, also a nerve growth factor	Secreted and cell surface molecules	Multimeric receptor complexes on axons or dendrites and can regulate gene expression	Dickson, 2002; Schwamborn et al., 2004
Slit	Large secreted protein	Midline repellent and sensory axon branching or elongation	Robo receptor	Dickson, 2002
Transforming growth factor family protein-7 and growth (TGF), i.e., bone morphogenetic protein-7 and growth protein-7 and growth	BMP-7 and GDF-7 together result in repulsion of axons from midline dorsal spine	Secreted from roof plate of dorsal spine	Two different subunits are bonded together	Yoshikawa & Thomas, 2004
Vicia villosa agglutinin (VVA)	Arborization into specific lamina	Inhibition of axons in deep lamina	Axons	Inoue & Sanes, 1997

One confounding phenomenon is the instance in which two chemicals in the same region can sometimes have a different effect on an axon's response than they would alone. For instance, laminin alone is trophic and netrin-1 alone is an attractant, but together they can modulate an inhibitory response of the axon. This is an example of the bifunctionality of guidance cues, which can be either trophic or modulatory (attractants or repellents) for different types of neurons, at different developmental states, or for different parts of the same neuron. This ability to be bifunctional is determined by the availability of various receptors on the particular axon or growth cone, which trigger different cellular cascades in the intracellular environment (Hopker, Shewan, Tessier-Lavigne, Poo, & Holt, 1999; Song & Poo, 2001). The inhibitory response, from the combination of laminin and netrin-1 in the retina, directs the retinal ganglion cells on into the optic nerve (Fig 2). Here, netrin-1 is expressed alone from neuroepithelial cells at the developing optic nerve head and acts to attract the entering RGC axons (Serafini et al., 1996; Serafini et al., 1994; Shirasaki, Katsumata, & Murakami, 1998).

Optic Chiasm

Another paramount process some axons must perform is the crossing to contralateral hemispheres in the brain. For instance, retinal ganglion cells from the nasal retina must cross the midline of the cortex, through the optic chiasm (Fig 2), to the contralateral hemisphere. Studies in mice have shown that slit proteins offer inhibitory cues to guide their direction. As one study concluded, expression of slit proteins in "wedges" in the anterior and posterior regions of the developing optic chiasm may create guardrails, which act to guide the axons between the wedges, to the

contralateral side (Niclou, Jia, & Raper, 2000; Plump et al., 2002). Another study suggests that at critical decision locations, such as the optic chiasm, chemical signals may need to be amplified to aid in the axon deciphering the appropriate "decision." Strittmatter, Fankhauser, Huang, Mashimo, and Fishman (1995) proposed that growth associated protein-43 (GAP-43) was necessary to immediately amplify environmental signals at the optic chiasm. They found that in mice with a functional GAP-43 mutation, axons remained trapped in the optic chiasm for 6 days. Although they eventually did proceed and develop normally, the authors suggested it was due to other compensatory signals present in the area. They proposed that in the absence of the preferred mechanism, in this case GAP-43, another mechanism could often serve as a backup.

Superior Colliculus

The major midbrain structure that is targeted by ipsilateral temporal RGC axons and contralateral nasal RGC axons is the superior colliculus (Fig 2). A retinal topographic map is crucial within the superior colliculus because when the retina sends an afferent signal of a visual target of interest or the auditory system processes a sound of interest, the eye must be guided to that target, accurately and in microseconds. This is accomplished through circuitry that is able to calculate the precise location. Vicia villosa agglutinin (VVA) has been shown to initially direct axons entering the optic tectum (superior and inferior colliculus in humans) by acting as guideposts. After this initial entry, ephrin A5 plays a role in further guidance. In wild type mice (no mutation), ephrin A5 has a graded expression from rostral to caudal. Another ligand, ephrin A2, is expressed in high levels in the superior

colliculus with low or non-detectable levels in the most rostral and caudal regions. Together, these two ligands provide both directional and positional cues through receptor recognition, which aid in maintaining the topographic regions of the superior colliculus. Once the axons are situated in their appropriate region of the superior colliculus, VVA then guides the arborization of the axons into the specific lamina within the superior colliculus (Inoue & Sanes, 1997). However, there may be other cues, which are not yet known and are also responsible for the ordered map in the

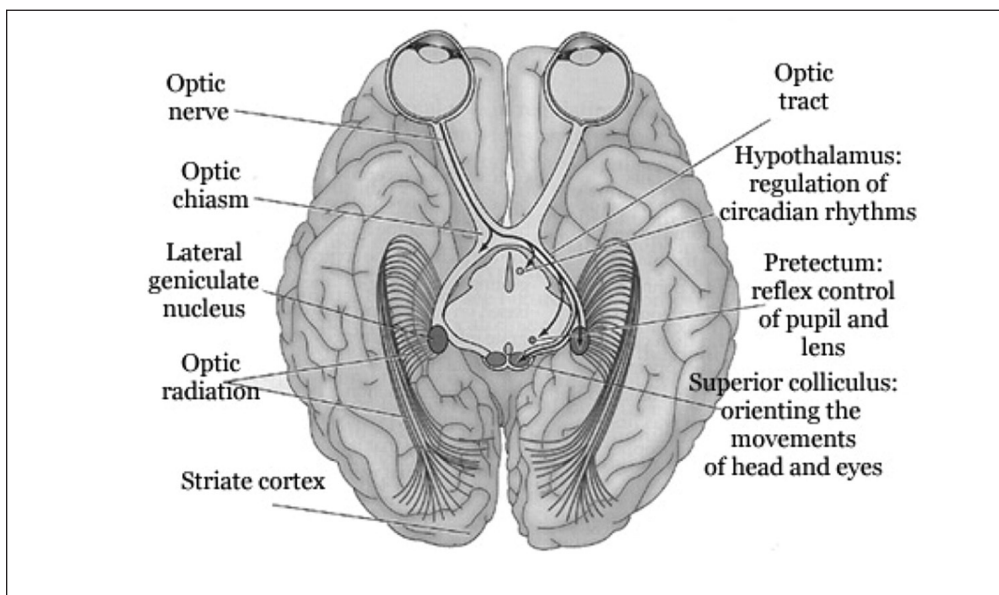


Fig 2. Central projections of retinal ganglion cells (Note: From Neuroscience, Fig 12.2, p. 253, by Purves et al. Sunderland, MA: Sinaner. Reprinted with permission.)

absence of one factor. For example, in the absence of ephrin A5 there seems to be some topographic order to the retinal ganglion cells. This highlights the redundancy within the developing nervous system to maintain important circuitry (Flanagan & Vanderhaeghen, 1998).

Thalamic Connections

The lateral geniculate nucleus (LGN), of the thalamus, receives input from both eyes. However, the axons from each eye terminate in distinct layers. Thus, each layer is controlled by one of the eyes (Hubel & Wiesel, 1968). Retinal axons are also organized in a topographic fashion as they project to the dorsal lateral geniculate nucleus (dLGN). Braisted et al. (2000) showed that dorsal thalamic axons favor the chemoattractant netrin-1 by demonstrating the disorganized and abnormal pathways that develop without it. Other ligands, ephrin A2 and ephrin A5, are expressed in dLGN and seem to match the gradient of ephrin receptors in the retinal axons, based on their nasal or temporal origin. For instance, the nasal retinal axons do not have receptors for ephrin, a repellent, and the temporal retinal axons do. When both axons reach the dLGN, the nasal axons are able to enter the anterior portion, where ephrins are high, because without the receptor, they do not respond to the repellent. The temporal axons end up entering the posterior portion or the dLGN, where ephrins are low, because with the receptor, they do respond to the repellent. Then as the topographic map is refined, ephrins are down-regulated (Feldheim et al., 1998).

Afferent Cortex Projections

The thalamus sends afferent axons to the cortex where these axons terminate in one of the six lamina of the cortex. Most typically afferent axons terminate in layer IV (Lopez-Bendito & Molnar, 2003). In the visual system, higher level processing occurs in the striate cortex, also known as the primary visual cortex (Fig 2). Visual system axons projecting from the thalamus may use glial cells as initial guidance as they enter the cortex and travel to the striate cortex. However, further guidance comes from within some of the specific layers of the brain, most specifically the subplate of the cortex (Kanold, Kara, Reid, & Shatz, 2003). In rats, Molnar, Adams, and Blakemore (1998) stated that axons remain in the intermediate and subplate zones for several days, at which point they make a sharp 90-degree turn and invade the cortical plate. They also concluded that while pausing in the subplate, axons organize themselves before they proceed to layer IV of the cortex. In addition, from their observations they concluded that the cells of the preplate layer formed a scaffold, which the thalamic axons climbed to their final target.

Krug, Smith, and Thompson (1998) determined how the topographic map is maintained by the geniculocortical projections. They used retrograde labeling to test for nearest neighbor analysis, which is the possibility that

neighboring cells are alike, and therefore, a topographic map is maintained. At postnatal day 2, they found no topographic map; in fact, axons arrived in disorder. However, by postnatal day 6 the nearest neighbor analysis showed a topographical map formed in the adult state. They proposed that through active terminal rearrangement small numbers of arbors projected into the wrong area, withdrew, and then projected to their proper target. The researchers also stated that axons probably sort themselves out in the subcortical plate, before they reach layer IV.

Castellani, Yue, Gao, Zhou, and Bolz (1998) studied the selective effects of ephrin A5 on cortex assembly within the layers. The ephrin A5 receptor is located in cortical axons that terminate in layers 2, 3, and 5 of the cortex and the ligand ephrin A5 is expressed in layer 4. They used cortical explants that were cultured with stripes of a control and also an ephrin A5 membrane. What they found was that axons, which contained the receptor for ephrin A5 (layers 2, 3, and 5 axons), bundled tightly when crossing the ephrin A5 membrane and unbundled when crossing the control stripe. These same axons also reduced their growth rate in response to the ligand ephrin A5. When the layer 6 axons, which do not contain the receptor, crossed the control stripe and membrane, they showed no preference. However, the layer 6 axons did show a promotion of branching and the layer 4 axons branched more widely in response to ephrin A5. Therefore, these findings suggested that ephrin A5 controlled axonal guidance through receptor recognition in layers 2, 3 and 5, but did not control axonal guidance in layer 6 axons. However, the ephrin A5 did promote branching behaviors of axons in layers 4 and 6. This highlights the bifunctional effects, directional versus positional, that the same ligand can have on axons due to receptor recognition.

What positional signals are present to stop the migration of axons and then cause branching that results in synaptic connections? Yamamoto, Higashi, and Toyama (1997) used cortical explants of striate cortex to observe the stop and branch behaviors. They reported that the axons stopped in layer IV regardless of which direction the investigators manipulated them to enter, which suggested that a molecular signal between layer IV and adjacent layers may give the cue to stop, while a neurotrophic factor in layer IV may be the reason for the branching observed. Szebenyi, Calaway, Dent, and Kalil (1998) proposed that axonal branching is cued in regions where pausing behaviors of axons were observed. They suggested that it was the deposit of filopodial or lamellar protrusions that left cues, which later prompted the axon to branch from these new active sites. They stated that as the growth cone passed near the target, its receptors recognized the target, paused to deposit the cueing mechanism, and then branched to subsequently reach the target.

Cell surface molecules and diffusible proteins have been proposed to explain this phenomenon of synapse development. Diffusible factors from the pre- and post-synaptic cells can allow these two cells to communicate prior to synapse development, while cell surface molecules and/or diffusible proteins can allow communication between the pre- and post-synaptic cells during synaptic development. This two-way communication between pre- and post-synaptic cells allows for specificity of synaptic development (Goda & Davis, 2003).

Ocular Dominance Columns: Highly Specific Organization Within the Cortex

In the visual system, afferent axons in layer IV of the primary visual cortex terminate in an alternating series of eye-specific zones (left or right eye) known as ocular dominance columns. Several studies have proposed that molecular cues and receptor recognition on the thalamic axons, cortical cells, or both were responsible for the directional and positional cues that caused the separation of axons into columns within layer IV of the cortex (Crowley & Katz, 2000). One study explained that contralateral connections (nasal) are dominant during the early developmental period and these connections influence initial separation into columns, but during postnatal week 4, visual experience is important for strengthening and establishing selective connections (Crair, Gillespie, & Stryker, 1998). This view is consistent with that of Crair, Horton, Antonini, and Stryker (2001), who argued that ocular dominance columns in cats are established long before activity-dependent competitive processes are effective. They proposed that the formation of ocular dominance columns and the selective processes of strengthening connections are two distinct and time-separated processes. This highlights the fact that many initial connections are not sufficient for behavioral processes that will come later. In this case, postnatal visual experience refines and strengthens the cortical connections that will result in the highly specific final network necessary for vision.

Callosal Connections

The corpus callosum connects the right and left hemispheres of the brain and is an excellent model for studying the development of cortical connections (Olavarria & Hiroi, 2003). In the visual system, the cells of the primary visual cortex of each hemisphere communicate with each other by sending their axons medially, through the corpus callosum, to the opposite hemisphere (Olavarria, 2001). How these projections cross the midline has been explained by Shu and Richards (2001), who proposed that midline glial cells influenced directional cortical axon growth by expressing slit-2, which repelled the ipsilateral axons away from the midline because they are not meant to cross. Another study showed the involvement

of semaphoring proteins (Zou, Stoeckli, Chen, & Tessier-Lavigne, 2000). Shu and Richards stated that the glial wedge, in its correct orientation, might provide attractive guidance cues through a contact-mediated mechanism and the chemoattractant netrin, which is recognized by the family of receptors known as deleted in colorectal cancer (DCC) receptors. The DCC receptors are also known to upregulate the Robo receptor on the axon surface. The Robo receptor is responsive to the repellent protein, slit, also expressed by the midline, but not netrin (Kidd, Bland, & Goodman, 1999; Stein & Tessier-Lavigne, 2001). Thus, the axons cross the midline after being attracted by netrin, and then the upregulation of the new receptor causes the axon to project alongside the midline, but never recross or linger there, as now the axon is responsive to slit and not netrin. This illustrates how a guidance cue can modulate the expression of receptors, which can adapt the response of the axon to local guidance cues (Song & Poo, 2001; Stein & Tessier-Lavigne).

General Concepts of Pathway Finding

Although specific genes, chemicals, molecules, environmental influences, or critical periods may differ between species, studies across the genomes of species indicate that the genetic mechanisms, which direct embryonic brain development, are amazingly conserved (Santini, Boore, & Meyer, 2003). Neuronal pathway finding is highly specific because the organizer and transcriptional genes, which regulate development within the specific regions of the brain, in turn, induce several local genes, which influence ligand and receptor expression, or development of local structures, and these ultimately guide the neurons within that area (Pasqualetti & Rijli, 2001).

Several kinds of local cues influence the pathway of the neuron: CAMS, diffusible proteins (attractants, repellants, neurotrophins), and glial wedges. Diffusible proteins can create long-range cues that direct axons from distances by creating gradients. Also, many of the diffusible proteins have bifunctional roles to change the axon direction, influence position, or execute functional needs.

The growth cone provides the gateway from the extracellular matrix to the intracellular environment of the axon. However, it is the axon's receptor expression and specificity, as well as its intracellular protein synthesis, that allows for modification of the axon behavior (Steward, 2002). Environmental cues can also change the receptor expression on the axon and thus change the axon's response to the environmental cues available in that local region. The growth cones of the pioneering axons are larger and more complex than the subsequent growth cones, signifying the complex environmental cues they must sort through (Bak & Fraser, 2003).

Cortical connections are remarkably specific. These connections and the neuronal response properties determine the specificity. Interneuron connections, on the other

hand, connect distant cells and neighboring cells, which are not aligned by their network. These differences in connectivity, between cortical and interneuron connections, may provide clues to how various stimuli are processed (Alonso, 2002).

The initial rudimentary connections formed during pathway finding tell only part of the story, as "experience" or plasticity is known to later refine the number and the strength of connections. Many more connections may be initially formed than are necessary, but are later pruned back and enhanced. Plasticity is a continuous process that refines synapses and strengthens the overall networks, but is too extensive of a topic to be covered in this article.

Nursing Implications of Newly Proposed Treatments

Although technology has offered huge strides in understanding this process, there continue to be huge gaps. For instance, cells with stem cell-like qualities have been found in the adult brain. However, the differentiation of these cells for treatment purposes can be restricted by temporal and spatial cues. It is still not clear whether these restrictions are inherent, due to their cell specific origin, or influenced solely by environmental cues. Research directed at answering these questions may lead to treatments in which stem cells are differentiated outside the body, and then grafted into their respective brain region,

This finding may hold more promise for injuries or early demyelination, in which axon degeneration has yet occurred.

where this particular cell type is found. *Pluripotent* (undifferentiated) cells can be placed in the brain in their undifferentiated state, where environmental cues can influence their differentiation (Tai & Svendsen, 2004; Temple, 2001). Preliminary biologic studies using pluripotent stem cells recovered from human embryos and fetal tissue implanted for restorative treatments have had preliminary small-scale clinical success. However, the ethical debate over the use of these sources has led to the search for other sources from both adult neural origins and adult non-neural origins, such as stem cells from bone marrow. Preliminary studies using cells from adult non-neural origins showed some sources produce populations of cells with a capacity for plasticity and neuronal differentiation, but further studies are producing conflicting and sometimes irreproducible findings (Tai & Svendsen).

Studies using mouse embryonic stem cells transplanted into rat Parkinson disease models at low concentrations had successful differentiation into dopaminergic neurons, which improved motor function of the animals. However, formation of teratomas in 20% of the transplanted animals resulted in death and the additional lack

of graft survival in 24% of those who received grafts means that the full mechanisms involved are not understood. In human trials, the transplanted dopamine cells fail to establish the appropriate axonal circuitry, which seems to some extent to result in dyskinesias (Freed et al., 2001; Hagell et al., 2002; Piccini, 2002). The growth limitations, which seem to occur in the adult brain, do not also occur when differentiated neurons are grown in culture (Lie et al., 2002). This finding seems to emphasize the fact that endogenous signals produced in the adult brain currently limit transplant therapies.

Cell transplants aimed at targeting multiple sclerosis, which results in multiple demyelinating sites, have had difficulty reaching the multiple sites of injury. However, when mouse models have been injected with adult-mouse donor cells intravenously or the donor cells have been injected directly into the brain ventricles, the injected cells have shown the ability to migrate intraparenchymally to the injured sites and cause host stem cell differentiation into oligodendrocytes (80%) and a limited amount of donor cell (20%) differentiation into the myelinating cells. Despite these single-study promising results, this study was not able to show any functional recovery that resulted from both the demyelination and axon degeneration. The newly formed oligodendrocytes may remyelinate the axons, but do not have the ability to repair the axon degeneration. This finding may hold more promise for injuries or early demyelination, in which axon degeneration has yet occurred. This finding also means that, currently, grafted neural stem cells are responsible for only a limited amount of direct neuronal replacement. However, they seem to have a promising role in regulating host endogenous repair, which is potentially due to an associated release of growth factors and other trophic substances (Tai & Svendsen, 2004).

This finding has led to excitement in the possible treatment of ischemic tissue, which can initiate endogenous *progenitors* (precursor cells) from the periventricular region and parenchyma in select tissues. Migration and differentiation of endogenous progenitors was further enhanced with the addition of select growth factors. However, clinical application of growth factors is not currently safe, as this could also lead to overgrowth of new cells and potentially tumor formation (Tai & Svendsen, 2004).

Provocative new research is also aimed at learning how to overcome inhibitory features so that axon grafts or axon regeneration might someday be successful. Some studies are using olfactory ensheathing cells on transplanted nerves, because olfactory tissue does not respond to inhibitory factors that other tissues respond to, illuminating the environmental influences that stimulate or inhibit adult neurogenesis of distinct brain and spinal cord cell populations. However, only partial functional recovery occurs in transplanted olfactory ensheathing cells, if at all, and depends on location and

the nature of the lesion (Hallbergson, Gnatenco, & Peterson, 2003; Jones, Sajed, & Tuszynski, 2003; Nieto-Sampedro, 2003).

Bench-science studies also continue to enlighten current clinical treatment options, offering novel approaches in the early period of disease or after injury, to prevent some of the inflammatory, excitotoxicity, and inhibitory cascades that cause glial scars and secondary injury, prevent axon survival, or prevent regeneration of transplanted tissue (Nieto-Sampedro, 2003).

Patients and their families will want the latest information on available treatments. They may not understand that many of these proposed treatments have only been done in animal models and are a long way from the clinical setting or in the early stages of clinical trials and not

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widely available. There needs to be a balance among the reality of current knowledge, the associated risks, and patients' and families' enthusiasm for the latest treatments and cures. Nurses can offer education and build support systems by facilitating awareness of the options available in their community or other communities. Nurses can also offer reliable sources for continued updates. Currently, Web resources offer the most up-to-date information:

1. The Stem Cell Research Foundation, www.stemcell-researchfoundation.org
2. The National Institute of Neurologic Disorders and Stroke, www.ninds.nih.gov (Follow links for neuroscience news or search under disorders.)
3. The Canadian American Spinal Research Organization, www.csro.com/home.html
4. The Spinal Cord Injury Information Network, www.spinalcord.uab.edu

Developmental Implications

Many of the mechanisms during neuron pathway finding are redundant and resilient. Thus, severe aberrations must occur for major developmental abnormalities to be observed. Abberations would be best explained by mutations in organizer or regulatory genes, without a compensatory overlap. Since organizer and regulator genes affect a cascade of developmental processes within regions of the brain, genetic mutations would affect many critical processes (Fransen, Van Camp, Vits, & Willems, 1997; Pasqualetti & Rijli, 2001). However, there is often heterogeneity of expression in these genetic abnormalities and more mild aberrations, which can affect single processes such as the neuron pathway finding process, might explain some of the ranges of social, cognitive, and

developmental abnormalities that are seen clinically and not completely understood (Hirose & Mitsudome, 2003).

Knowledge continues to be gained about critical or susceptible periods during development, which can be enhanced or exacerbated by internal or environmental processes, such as the early embryologic and early neonatal periods. As understanding of these critical periods and any potential environmental teratogens is improved, preventive patient education in the prenatal period will be improved. Studies within molecular biology and genetics are advancing the understanding of how genes influence normal brain development. This in turn is elucidating the molecular basis for congenital malformations and abnormalities in the brain at a much faster pace. This hastened knowledge will surely lead to proposed treatments to predict, prevent, and manipulate the developmental period. Nursing leaders need to sit at the table of ethical discussions now, to educate themselves, and to consider the ethical concerns should developmental manipulation become a reality in the future.

Nursing leaders need to consider potential ethical dilemmas now before technology has advanced to the state at which manipulation of the developmental process is possible. What are the benefits and risks if the developmental process is engineered to prevent all developmental anomalies? Will these treatments be available to all parents all over the world or will only a select few have the economic advantages? Does the current state of world health warrant such high expenditure for this treatment or could the money be spent more wisely and fairly? What will become of those children who did not have the benefit of prenatal diagnosis and manipulation as they become more rare in societies?

Summary

As knowledge of the development of neuronal pathways continues to grow, it also elucidates potential treatment modalities for neuronal degenerative diseases and injuries. Molecular and genetic technological advances have propelled research interest in the areas of stem cell, axon grafts, and axon regeneration, for novel treatments. What has been learned is that where these treatments are proposed in humans, there must be a neurotrophic environment that allows for survival, as well as directional and positional cues to guide the axons through their intermediate environment and establish synaptic connections. However, some neurotrophic, directional, and positional proteins have critical periods. There are also known inhibitory signals in the central nervous system, following myelination, which can prevent an environment conducive to these treatments (McDonald & Sadowsky, 2002; Properzi, Asher, & Fawcett, 2003; Schwab, 1990).

Research is now elucidating the role of stem cells in endogenous repair of damaged tissues in the brain and spinal cord, which may lead to promising therapies.

Neuroscience nurses need to stay abreast of developments as patients and their families want to know the latest treatments available, in some cases even if not locally available. A firm understanding of the normal development of neuronal pathways will aid nurses in discerning the strengths and weaknesses of current research in this area. In addition, it will help nurses educate their patients and families to understand the limitations of current knowledge and to weigh all benefits and risks. It will also elucidate the potential ethical dilemmas that may result if this technology is developed to the point at which the developmental period can be manipulated. Nurses can play a leadership role in the advocacy for social justice and world health.

Acknowledgments

The author acknowledges Jaime Olavarria, MD PhD, Associate Professor of Psychology, at the University of Washington for his review of the initial manuscript.

The author was supported by National Institute of Nursing Research, Predoctoral Biobehavioral Research Fellowship, NIH Grant number 5 T32 NR07160-04.

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