Proceedings of ICBioMed 2025 Symposium: Computational Modelling and Simulation for Biology and Medicine
DOI: 10.54254/2753-8818/2025.LD25940

Molecular Navigation: How Axon Guidance Cues Shape Neural Circuits

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Abstract. Precise long-range axon guidance is essential for neural-circuit assembly, and its failure underlies disorders ranging from corpus-callosum agenesis to epilepsy and autism. Four ligand families, including netrins, slits, semaphorins and ephrins, provide combinatorial attract-repel signals that growth cones decode via receptor repertoires and cytoskeletal dynamics. Recent studies show how co-receptors, stoichiometry and second messengers pivot single cues between attraction and repulsion, yielding context-specific trajectories. Guidance programmes re-emerge in the adult brain during learning and after injury, supplying intrinsic blueprints for regeneration, yet their aberrant reactivation can drive maladaptive sprouting and network hyperexcitability. Therapeutic concepts now in pre-clinical testing include Eph inhibitors, exosome-delivered netrin-1 or Sonic Hedgehog, gene editing of guidance receptors and AI-assisted multi-cue scaffolds. This review integrates molecular and translational advances, linking defined wiring errors to clinical phenotypes and proposing how programmable guidance signals could achieve targeted and patient specific repair of damaged neural circuits, thereby laying a conceptual foundation for next generation regenerative neurology that unites developmental biology, biomaterials engineering, and data driven modelling.

Keywords: axon guidance, neural circuit development, growth cone signaling, guidance cues and receptors, neuro regeneration

1. Introduction

The human brain is one of the greatest wonders of biology, being composed of tens of billions of neurons. And these neurons don’t simply exist together in the brain; they form highly organized synaptic networks that allow us to think, move, feel, and remember. This complexity needs to be based on accurate connections between neurons. In other words, the formation of precise neural circuits depends on the ability of neurons to send out axons that extend over long distances to reach specific targets. This process requires extreme precision in both space and time. Even the slightest deviations in the path of a growing axon can result in misrouted circuits, which, in worst case scenario, may lead to impairments in cognition, behavior, motor function, or perception [1]. The process that makes this possible is called axon guidance. It refers to how projections from neurons, axons, navigate through a complex environment to reach their designated targets. During this process, axons are guided by molecular signals in their surrounding environment that either attract

DOI: 10.54254/2753-8818/2025.LD25940

them, drawing them toward a target, or repel them, steering them away. These signals, termed guidance cues, are a variety of proteins and ligands that bind to receptors on the axon's growth cone, which is the motile tip of the axon [2].

Axon guidance is more than just forming local connections between neighboring neurons. The cues that are involved in this process ultimately help construct large-scale brain structures, such as the corpus callosum that connects the two hemispheres, or the retinotectal projection, which carries visual input from the retina to the brain. During development, commissural axons must cross the brain's midline to reach their targets on the opposite side. Axon guidance cues ensure that these axons cross only once and do not cross back again. These molecular cues also prevent axons from growing into unwanted regions and help organize neural connections into patterns that would have specific sensory or motor functions. Moreover, the influence of axon guidance doesn't end after birth. Although the most crucial wiring events occur during embryonic development, the adult brain still retains a significant amount of plasticity. Axon guidance molecules continue to exist later in life, especially during periods of neural remodeling, learning, or after injury. In fact, when the brain is damaged, neurons may attempt to reconnect using the same molecular cues that guided them during early brain development [3]. For this reason, understanding axon guidance is also key to understanding neural repair and regeneration.

Due to the importance of axon guidance system, failures in this system can have serious consequences. Errors in axon guidance are known to cause a lot of neurological disorders. Structurally, mutations in genes that encode guidance molecules or their corresponding receptors can lead to major brain malformations, such as failure to form the connections between the brain's hemispheres. Functionally, misrouted neuron circuits may cause conditions such as autism spectrum disorder, schizophrenia, and epilepsy [4]. In those cases, neurons can form connections, but they connect to the wrong targets, leading to distorted neural communication. This makes axon guidance an important area for therapeutic research. If researchers can understand how to manipulate these guidance signals, they may be able to guide axons to regrow correctly after neuron circuits get damaged or misrouted. Further research in this area can give us ways to moderate axon regeneration and restore lost functions. This paper explores the molecular foundations of axon guidance, examines how these systems shape neural development, and evaluates their relevance to human neurological disorders. By understanding how axons are guided to find their targets, we may also discover ways to repair and rebuild the brain when it gets damaged.

2. The four major families of molecular guidance systems

Guiding an axon from its point of origin to the target can be an arduous task, during which the axons must traverse a busy and dynamic environment using molecular cues and signaling pathways. The molecular cues can be grouped into 4 main families of molecular signals: netrins, slits, semaphorins, and ephrins. Each of these families play a certain role in determining how an axon navigates, including when it will grow, when it will turn, and when it will stop.

Netrins were one of the first axon guidance cues discovered. They were identified for their ability to attract commissural axons in the spinal cord. For many years since it was first discovered, scientists believed that netrin-1 was secreted by the floor plate of the spinal cord to draw axons across the midline. More recently, however, researchers found that netrin-1 is produced by the neural progenitor cells that migrate along the path of the axons and not just cells of the floor plate [5] [6]. This discovery creates a surprising twist for the traditional netrin model. Netrins, through their sole receptors DCC and Unc5, have been shown to demonstrate a measure of switching behavior. When DCC is only expressed on an axon, netrin-1 is attractive. However, with the addition of Unc5 on the

DOI: 10.54254/2753-8818/2025.LD25940

axon, netrin-1 now becomes repulsive. The switching behavior allows one molecule to have more than one role dependent on the context of the tissue. Tissues are noted for expressing their unique set of distinct netrins that can also function in processes beyond axon guidance, such as synapse formation, angiogenesis, and regulating dendritic growth [7].

While netrins allow axons to reach the midline of the nervous system, slit proteins prevent axons from crossing the midline more than once. Slits are secreted repulsive cues that bind to Robo receptors on the axon. After crossing the midline, Robo1 and Robo2 are activated to help push the axon away from the midline and avoid looping back [8]. Interestingly, axons express a unique receptor called Robo3 prior to crossing the midline. Robo3 will block the repulsive effect of slits to allow the axons to cross. After crossing, Robo3 expression is reduced and Robo1/2 takeover to complete the 'do not return' message. Disruption to this system has detrimental effects. Mutations in ROBO3 can lead to the rare disorder Horizontal Gaze Palsy with Progressive Scoliosis (HGPPS). The issue is that the axons did not cross appropriately in the brainstem [9].

Another major group of guidance cues is the semaphorins, which tend to act as repellent cues. One of the better characterized members is Semaphorin 3A (Sema3A) which gives information to axons on where not to go, by making them turn or stop growing altogether. Semaphorins bind to plexin receptors and in most cases with the help of neuropilins to induce the internal changes the axons cytoskeleton [10]. Semaphorins are of great importance to fine-tuning connections during the late developmental period. They are known to be involved in pruning for example, removing extra branches of axons; as well as regulating how many dendritic spines grow [11]. This is not the end of the semaphorin roles, many of their influences go as far as their initially proposed 'repellent' actions postnatally, predominantly in regions of the brain associated with learning and memory such as the hippocampus and cortex. Semaphorin misregulation has been implicated in certain disorders, such as epilepsy. Sema3A was many times found to be upregulated in seizure-prone regions, indicating it may induce aberrant rewiring and increased brain excitability [12].

The ephrin–Eph system is distinct from netrins or slits because it operates through contact between cells rather than through long-distance gradients. When a neuron with an Eph receptor makes contact with a neighboring cell that expresses an ephrin ligand, both cells will be signaled simultaneously. This bidirectional signaling creates sharp boundaries between brain regions and determines axon targeting. One classic example of ephrins is in the context of the retinotectal map where retinal neurons project their axons to specific locations in the brain. Neurons that express high levels of Eph receptors avoid areas with high expression of Ephrins leading to correct topographic mapping [13]. Eph-ephrin interactions, as previously mentioned, are not just limited to development, but they continue to shape the adult brain. They can impact synaptic plasticity, which demonstrates the strength in connections made by neurons with one another, and are also related to memory. Misregulation of ephrin signaling has also been associated with epilepsy and neurodegenerative diseases [12].

3. Developmental dynamics and signal integration

Axon guidance is not static. As the brain grows and changes, the cues and receptors are also subject to change and along with them, also change their corresponding influence. Furthermore, the outcome of guidance is determined not just by the identity of the molecules but also by when, where and how they are expressed. Essentially, one axon can express different combinations of receptors over the course of its growth. For example, it may express DCC early on to follow a netrin gradient but later it may express Robo too. The timing of this receptor "switch" is critical because if it switches too early or late the axon could go down the wrong path, or not reach the final destination.

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This brings to light why it is so important to control the expression of an individual gene during development, because we want it to happen at the right time without disturbing other programs of development.

Receptors do not act in isolation either; many receptors require co-receptors or binding partners to enable them to do their job. For example, while the functions of DCC, are modulated by APP (Amyloid Precursor Protein) via intracellular signaling [3]. APP also works in the realm of Alzheimer's Disease, so maybe the interactions between individual signals could connect the dots between developmental guidance and brain health later in life. Another example of co-receptors are neuropilins, which work as co-receptors in semaphorin signaling that switches the behavior of their primary receptors. These complexes demonstrate a way that neurons can fine-tune their behaviors based on the same guidance cues.

Additionally, cells can even internally regulate the action of their receptors by either internalizing the receptor or cleaving the receptor into non-functional fragments. This possibility offers a way in which neurons can "turn off" cues at critical decision-making steps. In this role, internalization and/or cleavage also extends the number of options available to regulate behavior by allowing the cell to filter and refine their behavior at times other than just feeling their environment. One of the more exciting discoveries in axon guidance has been those regarding the potential roles of exosomes. Exosomes are secretory vesicles produced by neurons, that contain proteins, RNA, and possibly other signaling molecules to be used at a later time. Axon guidance cues, including netrin and semaphorin can be delivered through exosomes which could offer a targeted source of signals that are also stable [14]. This kind of communication may be especially helpful during regeneration, where accurate connection is required to reconnect damaged circuits. Unlike classical signaling, which calls to all the cells simultaneously, exosomes can be directed to certain places, and even deliver combinations of molecules in one package.

4. Axon guidance and human diseases

4.1. Congenital malformations

The axon guidance system has central importance during development, but is also a major player in disease. When the guidance systems fail, the consequences can change the structure and function of the brain. One example is Horizontal Gaze Palsy with Progressive Scoliosis (HGPPS); a genetic syndrome due to mutations in the ROBO3 gene. These patients have developmental impairment of the brainstem connections which leads to compromised eye movements and spinal deformity [9]. There is another example of how one common gene related to a guidance receptor can impact function of the entire body; mutations in DCC or Unc5 can block axons from forming the corpus callosum, the structure connecting the left and right hemisphere of the brain, and lead to mirror movement disorders where one side of the body is doing the same unintended movement as the other side [4]. These disorders convey the strong link between axon guidance and gross anatomy of the brain.

4.2. Neurodevelopmental and psychiatric phenotypes

Axon miswiring is also implicated in autism, schizophrenia, and intellectual disability. These disorders are often associated with altered connectivity of regions in the cortex, hippocampus, or thalamus. There is a report that many of the genes contributing to these disorders extend from the axon guidance system [1]. While these disorders are multi-factorial, the importance of the misrouted

DOI: 10.54254/2753-8818/2025.LD25940

axon as a consistent theme remains. Our work also illustrates how seemingly minor developmental mistakes can lead to wide-reaching long-term functional consequences.

4.3. Aberrant reactivation of axon guidance cues in epilepsy

In epilepsy, the homeostasis cues may be reactivated in the wrong areas, thus triggering excessive or misdirected axon growth. For example, Semaphorin 3A and ephrin-B3, are frequently found at higher levels near seizure-prone regions in the brains of patients than those who do not display seizure [12]. These molecules can direct sprouting of axons in areas that they do not belong leading to hyperactive looping that is indicative of a seizure. The role of inflammation also appears to be tuned to guidance signals that change the stability of the networks involved, thus further compromising the brain's attempts to stabilize the networks in an epileptic brain. This presents some novel thinking about how we may treat seizures, not just focusing on the targeted neurotransmitters but now also includes addressing structural connectivity or the creation of stability.

4.4. Emerging therapeutic avenues

As researchers learn more about the axon guidance system, new and advanced therapies which flip these cues are in development. One research direction is through either making drug or biologics to block receptors to assist in axon regeneration. For example, it has been determined that blocking Eph receptors had a positive impact in terms of axons growing past the injury in spinal cord injury [13]. Another interesting idea is exosome delivery. Researchers like Yu et al are investigating how to engineer those to carry useful cues such as netrin-1, or sonic hedgehog (SHH) and deliver them onto specific injury in the brain or spinal cord [15]. It is suggested that this may be a more natural way to promote regrowth as they don't trigger an immune response in the brain to fight this change. Though these plans are still in the works, they represent a major shift of in perspective in terms of brain repair. Rather than patch the damage, they would elaborate on rebuilding the circuits naturally with the cues developed to build them in the first place.

5. Conclusion

The genesis of precise neural circuits is contingent upon developing axons successfully reaching the proper targets relying on a tightly controlled process called axon guidance. Axon guidance consists of a vast array of molecular cues, including netrins, slits, semaphorins, and ephrins, that each work through specific receptor and ligand interactions. They will guide growing axons toward or away from each cue by eliciting intracellular signaling pathways that alter the growth cone cytoskeleton. Each family of cues serves a unique role in axon guidance; however, there are often differences among families and there are also overlaps among cues. There is an intricate and robust coordination of cues to create a constellation of guidance signals that neurons conform to as they establish the long-distance connections that allow us to receive sensory stimuli, coordinate movement, and form cognitive functions.

Although most literature focuses on the role of axon guidance molecules during early brain development, many of the same cues are expressed and reused later during periods of synaptic remodeling, learning, and regeneration due to injury. For example, once there is neural damage, and the axons attempt to regrow, they may use the same molecular cues that were originally a part of their initial formation. This functional reuse indicates that axon guidance is a dynamic lifelong process contributing not only to brain plasticity but also to brain repair. If the axon guidance process

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becomes dysregulated, whether from genetic mutation or by environmental factors, it may lead to structural and functional consequences for the organism. Many clinical conditions connected to abnormal axon pathfinding and subsequently abnormal circuit formation such as HGPPS, mirror movement disorder, autism, and epilepsy underscore the clinical significance of learning about these axon guidance processes.

Emerging regenerative-medicine approaches that harness defined axon guidance cues open diverse therapeutic avenues. Physician-engineered exosomes, CRISPR-based gene correction, and ideal biomimetic scaffolds can all deliver axon guidance signals directly to the damaged region. The continuing challenges are spatial, temporal, and combined delivery of the signals. To begin tackling issues associated with optimizing cue delivery, important future-initiated research opportunities will include clinical use of high-resolution live imaging; development of integrated or holistic cue-based models that incorporate multiple cues; and the utilization of computational methods. Integration of AI and single-cell profiling could pave the way for personalized regenerative approaches that will allow patients to be assessed based on their own molecular positioning. Ultimately, if we can deepen our knowledge about the developmental and late-stage axon guidance cues, then the dynamic nature of axon guidance might not only reveal how the brain constructs itself, but may also ultimately reveal how the brain heals.

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