REVIEW SUMMARY

NEURODEVELOPMENT

Cell migration and axon guidance at the border between central and peripheral nervous system

Tracey A. C. S. Suter and Alexander Jaworski*

BACKGROUND: In vertebrates, the central and peripheral nervous system (CNS and PNS, respectively) are segregated at the cellular level. The CNS encompasses the brain and spinal cord, and the PNS is composed of numerous ganglia and nerves in the body periphery. Each subsystem is characterized by specialized neurons and unique glial cell types critical for neural circuit function. During development, virtually all CNS neurons and glia arise from progenitors located within this subdivision of the nervous system, and the vast majority of PNS-resident cells originate from the neural crest and ectodermal placodes in the periphery. However, it has become evident that at least a subset of peripheral glia is generated in the CNS and migrates into the PNS. Further, whereas most CNS and PNS neurons project axons exclusively within the same subdivision that houses their cell body, hindbrain and spinal cord motor neurons innervate various peripheral targets, and peripheral sensory neurons send axons into the CNS. Therefore, during development, when neurons and glia migrate to their destinations and axons navigate to their targets, the CNS-PNS interface must be permeable to select cells and axons at specific locations but prevent intermixing of most other CNS and PNS components. The cellular and molecular mechanisms that establish this pattern of segregation and selective connectivity are now beginning to be understood.

ADVANCES: Multiple cell types and signaling pathways exert tight control over the movement of cells and axons between the developing vertebrate CNS and PNS. A multilayered barrier surrounds most of the brain and spinal cord to prevent aberrant spillover of CNS and PNS components, but specialized access points called transition zones allow regulated cell migration and axon growth across the CNS-PNS boundary. Studies in various vertebrate species have begun to unravel some of the rules that govern cellular traffic at the CNS-PNS interface. It has become apparent that inhibitory signals from specialized cells located at the CNS-PNS border help to confine migrating cells and nascent axons to one nervous system subdivision; the cues that attract cells and axons to their correct targets are also important for preventing aberrant crossing of the CNS-PNS border. When these signaling pathways are disrupted, transition zones are particularly vulnerable to transgressions by cells and axons that would normally remain within their nervous system compartment. The permissive nature of transition zones is further underscored by the fact that cells in the mature nervous system can occasionally traverse these windows in response to injury. The developmental mechanisms that direct the correct cells and axons toward and across transition zones are still poorly understood, but attractive signals from cells at or beyond the CNS-PNS interface appear to play important roles. Furthermore, axons that need to cross the border are guided there by cues that repel them from inappropriate targets within their nervous system subdivision of origin, and they actively filter out guidance information that would otherwise steer them away from transition zones. Beyond this selective responsiveness to directional signals, these axons also use specialized subcellular structures to penetrate CNS-PNS barrier constituents.

OUTLOOK: The tight regulation of cell migration and axon navigation at the developing CNS-PNS interface is critical for establishing proper neuronal connectivity and allocating functionally specialized cells to the two major nervous system subdivisions. Further investigation of the relevant mechanisms holds the promise to elucidate the full repertoire of cellular interactions, guidance molecules, and signal transduction pathways that control this key dividing line in the nervous system. Because the fundamental division of the nervous system into central and peripheral compartments appears conserved across species, including some invertebrates, continuing to study the CNS-PNS boundary in multiple model organisms will contribute to understanding the evolution of nervous system organizing principles. Moreover, insights into signaling mechanisms at the CNS-PNS interface could aid in the development of therapeutic approaches that rekindle developmental plasticity at transition zones in the mature nervous system and promote regeneration after injury or onset of neurodegenerative disease. ■

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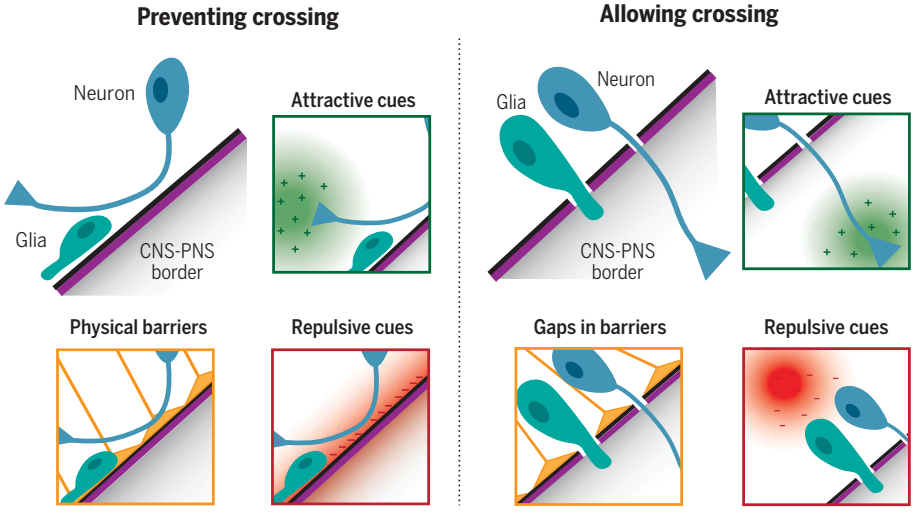

Control of the CNS-PNS boundary. During nervous system development, most glia, neurons, and axons are prevented from crossing the CNS-PNS border, whereas select subsets are allowed to move between the two compartments. Physical barriers and combinations of attractive and repulsive cues control cell behaviors at the CNS-PNS dividing line.

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REVIEW

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Cell migration and axon guidance at the border between central and peripheral nervous system

Tracey A. C. S. Suter[1,2] and Alexander Jaworski[1,2]*

The central and peripheral nervous system (CNS and PNS, respectively) are composed of distinct neuronal and glial cell types with specialized functional properties. However, a small number of select cells traverse the CNS-PNS boundary and connect these two major subdivisions of the nervous system. This pattern of segregation and selective connectivity is established during embryonic development, when neurons and glia migrate to their destinations and axons project to their targets. Here, we provide an overview of the cellular and molecular mechanisms that control cell migration and axon guidance at the vertebrate CNS-PNS border. We highlight recent advances on how cell bodies and axons are instructed to either cross or respect this boundary, and present open questions concerning the development and plasticity of the CNS-PNS interface.

In the vertebrate nervous system, the central nervous system (CNS) and peripheral nervous system (PNS) are characterized by functionally specialized neurons and unique glial cell types. The CNS is composed of the brain and spinal cord, and the PNS encompasses the sympathetic, parasympathetic, and enteric subdivisions and ganglia containing various sensory neurons with their associated nerves and glia. Virtually all CNS-resident neurons and glia arise locally, and the vast majority of cells in the PNS originate from the neural crest and ectodermal placodes in the periphery. However, at least a subset of glia is generated in the CNS and migrates into the PNS. Further, most CNS and PNS neurons send axons to targets within the same subdivision that houses their cell body, but motor and sensory axons project out of and into the CNS, respectively, to allow CNS-PNS communication. Therefore, although intermixing of most CNS and PNS components is prevented by cellular interactions at the boundary between the two nervous system compartments, this barrier must be permeable to select cells and axons at specific locations during development. The mechanisms that restrict and facilitate cell migration and axon growth across the CNS-PNS interface have long remained elusive, but recent research has begun to uncover some of the underlying developmental principles. In this review, we first describe the anatomy of the CNS-PNS border before summarizing our current understanding of how the behavior of neurons and glia at this dividing line is controlled. We also highlight promising research directions regarding the formation and function of this boundary.

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Cellular constituents of the CNS-PNS boundary

The surface of the CNS is a mostly uniform, impenetrable barrier preventing the movement of neurons, glia, and axons between the CNS and surrounding tissues, including the PNS. This boundary is formed by various cellular and extracellular components, including radial glia and astrocytic endfeet (forming a structure called the glia limitans), the meninges, and a specialized extracellular matrix (ECM) (Fig. 1). However, during development, specific access points called transition zones are selectively permeable for subsets of cells and axons, allowing for connectivity between the CNS and PNS. Transition zones are regions of the nerve rootlets protruding from the neural tube surface where CNS and PNS tissues meet and partially interdigitate (Fig. 1, B to D). They are characterized by local disruption of some of the CNS-PNS barrier components and the presence of dedicated cell types—boundary cap (BC) cells in mice (Fig. 1, A to C) and motor exit point (MEP) glia in fish (Fig. 2A)—which help to regulate CNS-PNS access.

Radial glia and astrocytes

During development, neuroectodermally derived neural progenitors, termed radial glia, form elongated processes that extend from the ventricular to the pial surface of the brain and spinal cord (Fig. 1, A to C). Radial glia are not only the essential, primary contributors to CNS neurogenesis and gliogenesis, but also serve as a scaffold for migration of their cellular offspring away from the ventricular zone (*1*). Additionally, radial glia aid both in the architectural organization of the CNS and in establishing the CNS-PNS boundary. At the pial surface, radial glia processes contact the basement membrane, which surrounds the CNS (see below), and their endfeet form a tight physical barrier essential for preserving the separation of the CNS and PNS compartments (*1*). As proliferative activity ceases, radial glia gradually disappear or, in rare instances, directly transform into astrocytes with a similar morphology. Concurrently, astrocyte precursors, which arise from radial glia in the ventricular zone, begin to migrate from their birthplace, and astrocytic processes fill the gaps vacated by radial glia endfeet at the pial surface (*2*, *3*). Ultimately, astrocytes completely replace radial glia to form the glia limitans at the CNS-PNS border (Fig. 1, C and D), but the timing of this substitution remains poorly understood. At mature transition zones, where axons have crossed the CNS-PNS boundary, the peripheral portion of the transition zone apparatus contains a funnel-shaped sleeve of glial processes, which surround and separate each axon (*4*–*6*).

Meninges and basement membrane

The basement membrane is a thin, protective layer of ECM molecules that surrounds most tissues in the body, including the neural tube (Fig. 1). It is largely composed of laminins, collagens, nidogens, and proteoglycans, and it further contains a variety of signaling molecules that interact with the core ECM constituents and instruct numerous cellular behaviors (*7*). The CNS basement membrane is produced predominantly by radial glia and the mesenchyme surrounding the neural tube, and it plays an integral role in neural development. It provides an adhesive surface for radial glia endfeet and helps to control neural crest cell migration and axon growth at the CNS-PNS boundary. After neural crest delamination, the basement membrane forms a complete seal around the neural tube surface (*8*, *9*) (Fig. 1).

The meninges are membranes of connective tissue enveloping and protecting the entire CNS (Fig. 1). Three layers compose the mature meninges: the pia, arachnoid, and dura mater (*10*). During development, the meninges surrounding the forebrain arise from cranial neural crest cells, but the origin of the meninges surrounding the caudal CNS had long been controversial. Over time, consensus emerged that spinal cord meninges are not neural crest derived and instead form by condensation of mesodermal mesenchyme around the neural tube (*11*–*13*). The initial, primitive meningeal layer, termed leptomeninges, is located in direct apposition to the outer basement membrane of the neural tube (*8*, *10*). During development, the meninges fulfill multiple functions: they control the migration and positioning of various neuronal populations, regulate radial glia proliferation and survival, and organize and maintain the basement membrane (*10*, *14*, *15*). They also produce signaling molecules that appear to control axon behavior at the CNS-PNS interface (*16*).

Boundary cap cells and motor exit point glia

Transition zones allow movement of select cells and axons between the CNS and PNS (*6*, *17*). They

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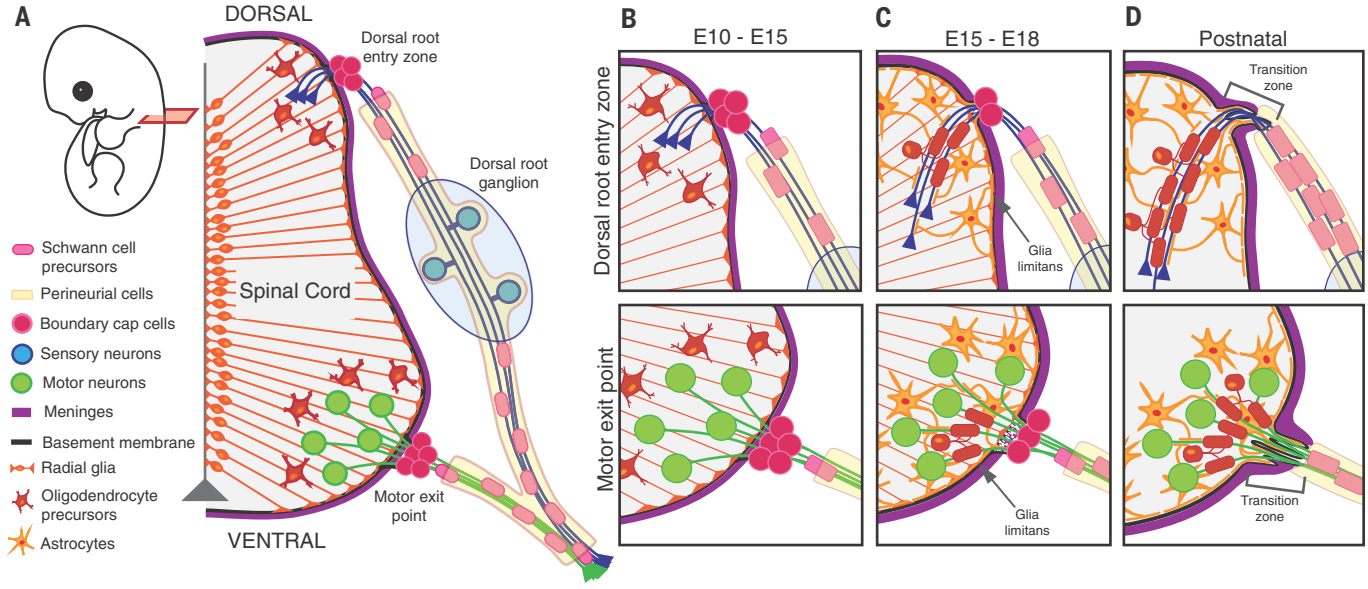

Fig. 1. Anatomy of the CNS-PNS border during development. (**A**) Schematic of mouse embryo indicating the plane of the spinal cord sections shown on the right. Depicted are the various constituents of the CNS-PNS border. (**B** to **D**) Developmental changes in cellular composition and arrangement of spinal cord transition zones.

are best characterized in the spinal cord, where most motor axons leave the CNS through MEPs and dorsal root ganglion sensory axons enter the CNS at the dorsal root entry zone (DREZ) (Fig. 1). The importance of these transition zones is highlighted in human patients with Friedreich ataxia, in which DREZs are disorganized and exhibit intrusions of CNS and PNS components into the inappropriate compartment (*18*). In rodents and chicks, BC cells, a transient population of multipotent neural crest cells, play an integral role in the establishment and maintenance of transition zones during development (*19*). BC cells migrate in close association with the neural tube surface before arresting at axon entry and exit points (*20*, *21*) (Fig. 1, A to C). BC cells at DREZs and MEPs have distinct gene expression profiles, and ventral, but not dorsal, BC cells appear to extend protrusions into the neural tube, suggesting that they each fulfill specialized functions (*19*, *22*). Moreover, the timing of BC cell arrival relative to axon exit and/or entry differs between MEPs and DREZs. BC cells arrive at the presumptive DREZ before sensory axons, consistent with the idea that they could guide sensory axons into the spinal cord; in contrast, motor axons emerge from the spinal cord before BC cell clustering at MEPs, suggesting that nascent motor axons might recruit BC cells to MEPs (*8*, *21*). After axon growth through MEPs and DREZs has concluded, BC cells differentiate into multiple peripheral cell types (*19*). Although BC cells associate with all exit and entry points of hindbrain and spinal nerves (*19*, *20*), it remains elusive whether an analogous cell population is present at the transition zone for olfactory sensory axons.

Zebrafish do not possess a neural crest–derived equivalent of BC cells. Instead, MEP glia, a specialized subset of myelinating glia whose gene expression signature combines CNS and PNS characteristics, originates in the CNS and localizes to the proximal portion of motor axon tracts just outside of the neural tube (*23*). To date, no similar cell type has been identified at sensory axon entry points. MEP glia in fish localize to the same region that ventral BC cells do in birds and mammals and similarly function to maintain the integrity of the CNS-PNS boundary, but they appear to do so through distinct mechanisms (*23*, *24*). This suggests that these cell types represent convergent evolutionary solutions to a common problem in CNS-PNS border development across fish and amniotes.

Positioning CNS and PNS glia

With the exception of microglia, all glia of the CNS and PNS arise from neural ectoderm during development. We focus here on the behavior of these neuroectoderm-derived glia at the CNS-PNS interface and refer the interested reader to other excellent reviews on the development of microglia, which originate in the embryonic yolk sac and, shortly after onset of neuronal differentiation, migrate into the developing CNS by breaking through the neural tube basal lamina using matrix metalloproteases (*25*, *26*). CNS-resident astrocytes and oligodendrocytes are born in the neural tube, whereas PNS Schwann cells, satellite glia, BC cells, olfactory ensheathing glia, and most perineurial glia arise from neural crest progenitors in the periphery. By contrast, MEP glia and a small subset of perineurial glia are CNS derived and enter the PNS through transition zones. However, in the intact, mature nervous system, astrocytes and oligodendrocytes are not observed in the PNS and peripheral glia do not enter the CNS. This segregation is likely of functional importance, e.g., peripheral Schwann cells produce basement membrane, which would interfere with cellular interactions in the CNS (*27*, *28*). Nevertheless, transition zones remain plastic enough to adapt to environmental perturbations or developmental defects that deplete the glial pool in one compartment, allowing Schwann cells, oligodendrocytes, and astrocytes to cross the CNS-PNS boundary and partially compensate for the absence of their missing counterpart. Thus, the CNS and PNS environments are seemingly able to support the survival and differentiation of glial cells originating in the other compartment (*24*, *29*, *30*). The mechanisms that permit and restrict movement of glia between the CNS and PNS are now beginning to be understood.

Regulated glial crossing between the CNS and PNS

The long-standing idea that some peripheral glia might originate in the neural tube has been confirmed by recent work in zebrafish and mice, raising the question of how these cells penetrate the CNS-PNS boundary. In zebrafish, MEP glia leave the neural tube through MEPs and myelinate motor axons in spinal cord–proximal regions of ventral roots (*23*). Although MEP glia have not been observed in birds or mammals, at least one glial cell type appears to emigrate from the CNS into the PNS as part of normal development across vertebrates: a subset of perineurial cells (Fig. 1) (*31*, *32*). Perineurial glia envelop peripheral nerves to protect them from toxins and infection, regulate extracellular ion concentrations, and provide metabolic support (*33*). Although most perineurial cells originate from the neural crest, the CNS-proximal aspect of the ventral root perineurium in both zebrafish and mice is derived from Nkx2.2^{+}

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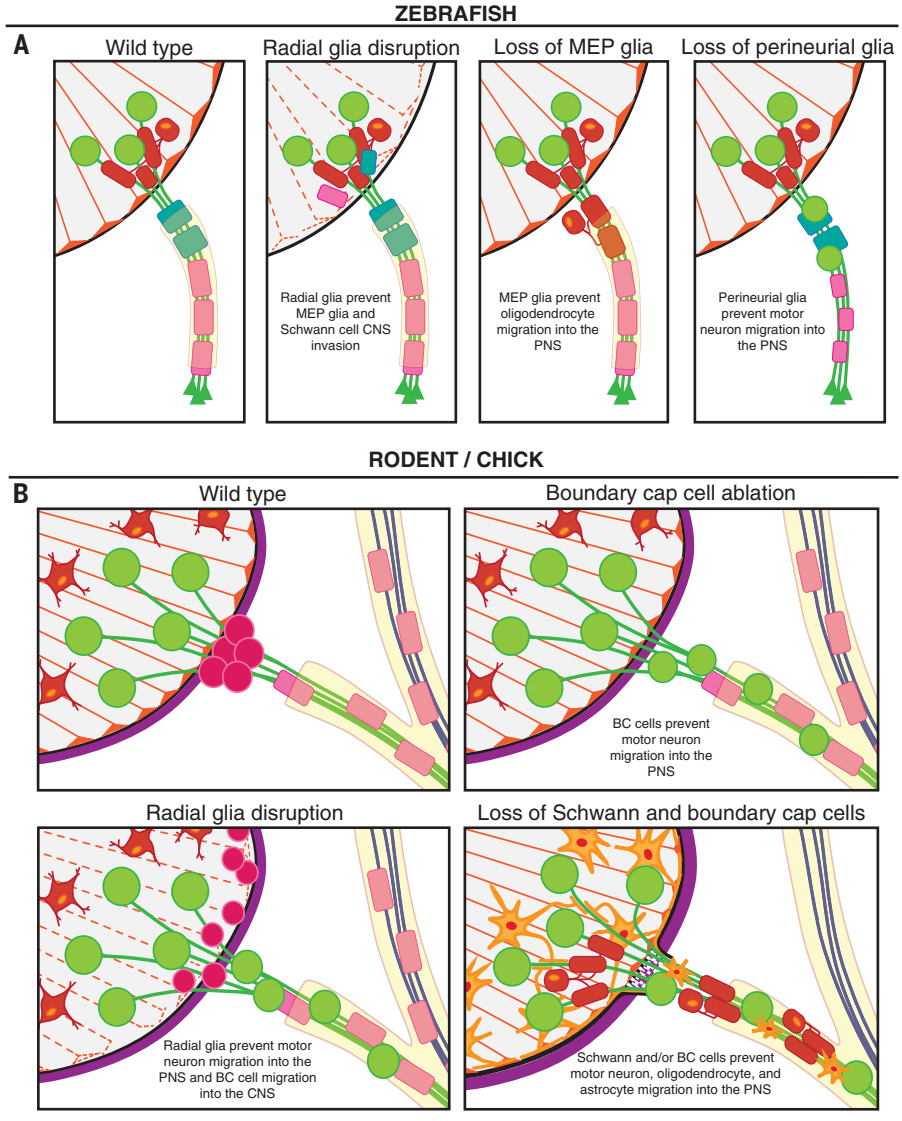

Fig. 2. Migration of neurons and glia across the CNS-PNS border. (**A**) Schematic of wild-type zebrafish motor exit point, followed by depictions of the effects resulting from disruption or loss of various cell types. (**B**) Schematic of wild-type rodent/chick motor exit point, followed by three examples of events occurring after the disruption and/or loss of a particular cell type. Text within each panel summarizes the effect on CNS-PNS segregation.

progenitors in the ventral spinal cord. These CNS-derived perineurial glia leave the spinal cord through MEPs and join their peripherally generated counterparts to form a continuous sleeve around motor nerves (*31*, *32*).

Transition zones also allow peripheral glia to transiently enter the CNS during development. Live-imaging studies in zebrafish have demonstrated that a small number of $Sox10^+$ neural crest–derived peripheral glia enter the spinal cord through MEPs after motor axon exit but before MEP glia emigration. After a few hours, all of these PNS-derived cells relocate back into the periphery and remain restricted to the PNS afterward (*34*). This transient migration of peripheral glia into the CNS has not been observed in higher vertebrates, raising questions about its evolutionary conservation and functional significance.

Together, these recent studies have uncovered a selective permeability of transition zones for glial migration during development, but the specific mechanisms that permit and instruct the movement of MEP glia, $Nkx2.2^+$ perineurial cells, and $Sox10^+$ peripheral glia across the CNS-PNS border have not yet been elucidated. However, these migrations appear to be restricted to the time before radial glia endfeet form a continuous seal at MEPs, suggesting that gaps between these endfeet are a prerequisite for glial crossing between the CNS and PNS.

Preventing aberrant intermixing of CNS and PNS glia

The vast majority of CNS and PNS glia remain confined to their respective compartment of origin. This raises the question of how aberrant intermixing of CNS and PNS glia is prevented, and experimental manipulations that erode this segregation have begun to provide insights into some of the relevant mechanisms. In zebrafish, ablation of MEP glia causes oligodendrocytes to emigrate from the spinal cord through MEPs and myelinate motor axons (Fig. 2A). Even during normal development, oligodendrocyte processes constantly probe the peripheral space outside of the spinal cord, but these cellular extensions retract upon contacting MEP glia (*23*). Contact-mediated inhibition from MEP glia therefore appears essential for preventing oligodendrocyte exit from the fish spinal cord, even though the precise molecular mechanism remains elusive. In mice, BC cells appear to fulfill a similar role, as oligodendrocytes exit through MEPs and DREZs after genetic ablation of the BC (*29*), but it is unknown whether this function involves direct physical contact between oligodendrocytes and BC cells (Fig. 2B). Interfering with Schwann cell differentiation or survival in fish and mice by genetic deletion of *Sox10* or *ErbB3* also leads to oligodendrocyte (and in mice astrocyte) emigration from the CNS through transition zones (*23*, *35*, *36*) (Fig. 2B). However, because these manipulations also affect MEP glia and BC cells, it is not entirely clear whether Schwann cells help to confine glia to the CNS, but the presence of CNS glia in peripheral nerves from a human patient lacking Schwann cell myelin is consistent with this idea (*29*). Lastly, pharmacological inhibition of A2a adenosine receptors or blockade of neurotransmitter release in zebrafish results in ectopic oligodendrocyte migration through MEPs without affecting MEP glia development (*37*). Although the precise mechanism underlying this effect has not been elucidated, this finding suggests that, in addition to interactions with peripherally located glia, neuronal activity helps to prevent oligodendrocyte migration across the CNS-PNS border.

PNS-resident glia also need to be prevented from entering the CNS. Radial glia appear to fulfill an essential function in this process. As mentioned, in zebrafish, $Sox10^+$ peripheral glia freely cross between the CNS and PNS at the MEP until radial glia endfeet form a continuous barrier (*34*). When radial glia are selectively ablated, peripherally located glia, including MEP glia, continue to migrate into the spinal cord throughout later stages of development (*34*) (Fig. 2A). Similarly, in mice, genetic inactivation of the chemokine CXCL12 or its receptor, CXCR4, leads to the formation of gaps between radial glia endfeet in the developing spinal cord, resulting in immigration of BC cells into the neural tube through DREZs and MEPs (*38*) (Fig. 2B). Together, these studies support the idea that radial glia endfeet prevent peripheral glia from invading the CNS. Lesion studies in the adult rodent spinal cord suggest that astrocytic endfeet fulfill the same function after the disappearance of radial glia. After spinal cord injury, Schwann cells often invade the CNS and remyelinate axons at the lesion site while retaining their original compartment identity

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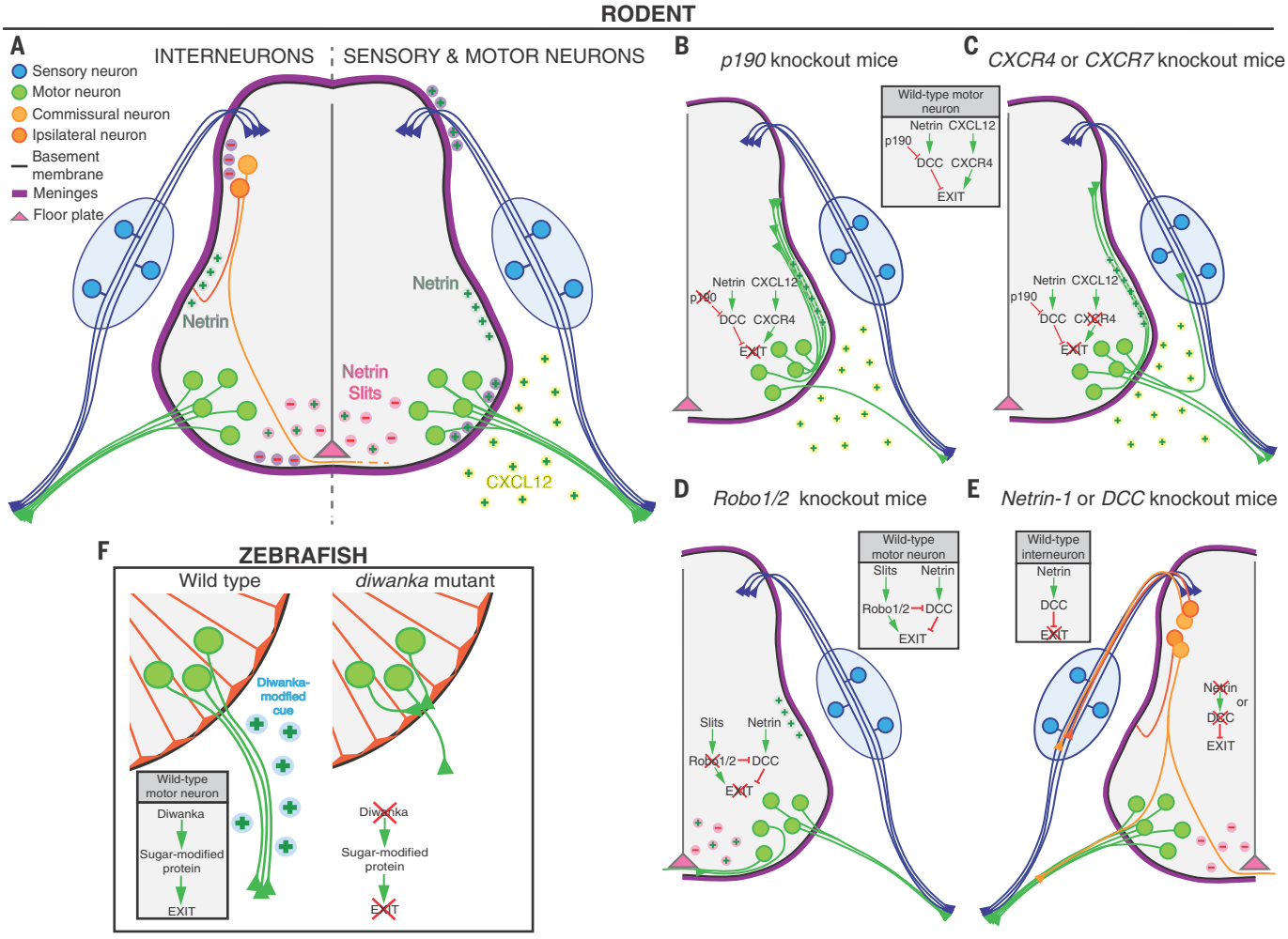

Fig. 3. Aberrant axonal crossing of the CNS-PNS border. (**A**) Schematic of rodent spinal cord and relevant axonal populations. Colored circles signify attractive and repulsive guidance cues, with colors indicating tissue of origin, e.g., purple circles represent meninges-derived cues. (**B**) Failure of motor axon exit due to gain of attraction to basement membrane–associated Netrin-1. (**C**) Failure of motor axon exit due to loss of CXCL12 attraction toward the periphery. (**D**) Failure of motor axon exit due to loss of responsiveness to spinal cord–intrinsic repulsive guidance cues. (**E**) Misprojection into the PNS by axons that are normally confined to the CNS caused by loss of attraction to their appropriate targets. (**F**) Peripheral attraction is necessary for motor axon exit in zebrafish. Gray boxes depict the wild-type signaling pathway, and notes within panels show how these pathways are perturbed.

and myelination characteristics (*39*–*41*). Early work attributed this migration of peripheral glia into the injured spinal cord to the disruption of the astrocytic endfeet barrier (*41*–*44*). However, Schwann cells also enter the spinal cord and remyelinate CNS axons in multiple rat lines in which CNS myelin is eliminated but the glia limitans appears intact (*30*). This indicates that CNS myelin provides inhibitory signals that prevent Schwann cell invasion of the CNS. Myelin-associated glycoprotein is one such potential signal, as it inhibits the migration of Schwann cells and induces their death through the p75 neurotrophin receptor (*45*). Overall, the precise interplay of mechanisms that restrict and, in the case of injury, trigger Schwann cell crossing of the CNS-PNS border remain poorly understood. However, these spinal cord injury studies unmask a notable plasticity in the allocation of glia to the CNS and PNS compartments, endowing the nervous system with the capacity to repair itself.

Controlling neuronal cell body and axon behavior at the CNS-PNS interface

Neurons that arise in the CNS do not enter the PNS, even though their cell bodies often migrate over long distances within the brain and spinal cord. Similarly, peripherally born neurons remain confined to the PNS, with one notable exception: Gonadotropin-releasing hormone (GnRH) neurons migrate from the olfactory placode into the brain (*46*). Although the vast majority of axonal projections are confined to either the CNS or PNS, a substantial number of axons grow across the CNS-PNS interface to connect the two subdivisions. Motor neurons are located in the hindbrain and spinal cord, and their axons leave the CNS through MEPs to innervate peripheral muscles and ganglia. Neural crest–derived somatosensory neurons localize to dorsal root ganglia and multiple cranial ganglia and project axons into the CNS through DREZs (Figs. 1 and 3) or various cranial nerves. Gustatory and audiovestibular information is similarly carried into the CNS by sensory neurons, which arise from the cranial neural crest and otic placode, reside in specialized ganglia, and project through cranial nerves. Lastly, olfactory sensory neurons are born in the nasal placode, reside in the olfactory epithelium, and send axons through the cribriform plate into the olfactory bulb. The mechanisms that selectively allow some neurons and axons to cross the CNS-PNS border while preventing most others from traversing this boundary are only now beginning to be understood.

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Migration of select neurons across the CNS-PNS border

GnRH neurons are currently the only known neuronal population that migrates across the CNS-PNS boundary, raising the question of what allows them to accomplish this unique feat. However, they reach the CNS as part of a larger "migratory mass," which contains additional, as yet unidentified cells that express neuronal markers (*46*, *47*). Failure of GnRH neurons to populate the hypothalamus causes hypogonadotropic hypogonadism, underscoring the functional importance of their journey into the CNS (*47*). GnRH neurons originate peripherally in the nasal placode and translocate along bundles of olfactory, vomeronasal, and terminal nerve axons to leave the olfactory pit. These neurons then use this axonal scaffold to cross the developing cribriform plate and enter the brain, either through or just ventral to the olfactory bulb, at which point they continue migrating toward their final destination in the hypothalamus (*46*, *47*). Association with axons that project from the nose into the CNS is instructive for GnRH neuron migration, as genetic manipulations that cause misrouting of these axons before penetrating into the brain noncell autonomously prevent GnRH neurons from entering the CNS (*46*–*49*). The comorbidity of anosmia and hypogonadotropic hypogonadism in patients with Kallmann syndrome has been commonly interpreted as a link between olfactory sensory axon guidance and GnRH neuron migration (*46*, *47*). However, recent evidence strongly suggests that most GnRH neurons follow terminal nerve fibers, whereas only a small subpopulation migrates along olfactory and/or vomeronasal axons (*50*). In addition to following these axons, which are steered toward the brain by various guidance molecules, GnRH neurons appear to also require their own chemoattractive cues to migrate into the CNS. Hepatocyte growth factor and CXCL12, which are expressed by mesenchymal cells along the GnRH neuron migratory route and increase in concentration toward the olfactory bulb, have been implicated as two such cues; however, their precise mechanism of action remains elusive, and it is not entirely clear whether these factors do indeed control GnRH neuron migration directly without affecting axon guidance (*51*–*53*). Therefore, GnRH neurons appear to require attractive guidance cues and a correctly targeted axonal substrate for their migration across the CNS-PNS boundary. The complete repertoire of guidance cues, receptors, and cellular mechanisms that regulate GnRH neuron entry into the CNS awaits identification.

Prohibiting CNS exit of neuronal cell bodies

How are the vast majority of neuronal cell bodies contained within either the CNS or PNS? Motor neurons are the only pan-vertebrate, CNS-resident neurons with axons that project into the PNS. This renders their cell bodies particularly vulnerable to accidental CNS exit, and multiple mechanisms prevent motor neurons from leaving the neural tube by helping to uncouple cell body translocation from axon extension. Both fish and mammals depend on CNS-derived perineurial glia to contain motor neurons within the spinal cord, and the radial glia endfeet barrier and additional signaling from BC cells help to solidify this confinement in mammals (Fig. 2B). In *Reelin* knockout mice, radial glia endfeet in the spinal cord fail to form a continuous barrier along the basement membrane, and motor neurons emigrate from the spinal cord through MEPs (*54*). Thus, radial glia endfeet appear to prevent motor neuron exit from the CNS, but it is unclear whether this is mediated by inhibitory signals or the formation of a physical seal at MEPs. Ablation of BC cells also causes motor neuron CNS exit through MEPs in both chicks and mice (*55*) (Fig. 2B). At least two BC-derived signals appear to mediate this function of confining motor neurons to the CNS: the transmembrane Semaphorin Sema6A (*56*, *57*) and the Netrin family member Netrin-5 (*58*). Knockdown of *Sema6A* in chick BC cells or genetic deletion in mice causes motor neuron emigration from the spinal cord; however, the identity of the receptor(s) mediating the effect of Sema6A on motor neurons remains controversial, as conflicting RNA interference evidence in chick embryos implicates either PlexinA1 or PlexinA2, whereas knockout studies in mice implicate the class III Semaphorin receptor Neuropilin-2 (*56*, *57*). Mice lacking Netrin-5, which is expressed by BC cells, or the Netrin receptor DCC, expressed in motor neurons, also exhibit motor neuron emigration through MEPs. This supports the idea that BC-derived Netrin-5 restricts motor neuron exit from the CNS through DCC (*58*). Sema6A and Netrin-5 might well function as BC-derived repellants that prevent the migration of motor neurons into the PNS, but this mechanism of action has yet to be confirmed. CNS-derived perineurial glia in the proximal ventral roots further help to confine motor neurons to the CNS. When the development of these cells is perturbed by inactivation of *Nkx2.2*, motor neuron somata leave the CNS through MEPs in both fish and mice (Fig. 2A) (*31*, *32*), but the mechanisms through which perineurial glia control motor neuron positioning are still unclear. Multiple studies in mice have shown that inactivating motor neuron–specific transcription factors, including HB9, Islet1, and Islet2, causes motor neuron cell body migration into peripheral nerves without affecting BC cell clustering at MEPs or radial glia endfeet morphology (*59*–*62*). These findings suggest that these transcriptional regulators control the expression of genes required for motor neurons to sense guidance cues that keep their cell bodies within the CNS. The identity of the relevant misregulated effector molecules remains elusive, but components of the repulsive Semaphorin-Neuropilin and Slit-Robo signaling pathways have been implicated as downstream mediators of Islet1/2 in preventing motor neuron emigration into the PNS (*62*). Together, these studies show that motor neurons rely on multiple, nonredundant signaling mechanisms to remain within the CNS.

In fish and amphibians, but not in amniotes, an additional population of CNS neurons projects axons across the CNS-PNS border: Rohon–Beard sensory neurons. These neurons reside in the dorsal spinal cord and send axons into the periphery, but the mechanisms that contain their cell bodies within the CNS are unknown.

Behaving as a mirror image of motor and Rohon–Beard neurons, peripheral sensory neurons project axons into the CNS. The only documented instance of neurons aberrantly entering the CNS occurs in mice lacking Six1 and Six4—loss of these transcription factors impairs differentiation of dorsal root ganglion neurons and causes their migration into the spinal cord through the DREZ (*63*). It is not known whether this reflects impaired responsiveness of sensory neurons to inhibitory signals or pleiotropic effects on the structure of the CNS-PNS border.

When motor or sensory neuron somata aberrantly cross between the CNS and PNS, they always do so through transition zones, suggesting that these exit and entry points are more permissive for neuronal migration than the rest of the CNS-PNS boundary. Consistent with this idea, transition zones in the hindbrain are also vulnerable to ectopic migration of nonmotor neurons out of the CNS. Rhombic lip–derived pontine neurons migrate long distances underneath the hindbrain pial surface, where they must maneuver around multiple cranial nerve exit points before reaching their final location (*64*). Netrin-1 is present in the basement membrane at the pial surface and functions as a guidance substrate for pontine neurons, which express DCC. When *Netrin-1* or *DCC* are deleted, pontine neurons aberrantly leave the hindbrain through cranial nerves and enter the PNS (*65*, *66*). Because these defects arise despite normal BC cell localization and radial glia morphology, this suggests that Netrin-1 chemotactic or haptotactic activity keeps pontine neurons on the correct path to promote their retention in the CNS (*65*, *66*) and supports the idea that CNS neurons are vulnerable to exiting into the PNS when their normal migratory trajectories are disrupted.

Transgressions of the CNS-PNS boundary by neurons at sites other than transition zones are rare and appear to require a more dramatic breakdown of the barrier. Defects in radial glia or basement membrane formation in the cerebral cortex result in overmigration of neurons into the marginal zone and subarachnoid space of the meninges (*67*–*73*), indicating that these components of the CNS-PNS border keep neuronal cell bodies confined to the cortex. Disruptions of the radial glia scaffold in the spinal cord have not been reported to cause similar nonspecific breaching of the CNS-PNS border outside of transition zones, but the reasons underlying this difference between cortex and spinal cord are unclear.

Allowing the right axons to connect the CNS and PNS

To project across the CNS-PNS interface, axons must penetrate between radial glia endfeet and

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through the basement membrane and meninges (Figs. 1 and 3A). At the time when motor and sensory axons navigate through MEPs and DREZs, respectively, radial glia endfeet form an incomplete barrier with numerous gaps (*8*), and olfactory sensory axons enter the olfactory bulb through small fenestrations in the basement membrane (*74*). This suggests that gaps in the CNS-PNS barrier might be required to permit axon growth through transition zones. However, recent work has also highlighted the importance of transient morphological and functional changes in sensory and motor axon growth cones for crossing the CNS-PNS border. When pioneer axons reach prospective CNS exit and entry points, they pause, reorganize their growth cones into a structure called an invadopodium, and secrete matrix metalloproteases to digest the ECM and puncture through the basement membrane (*75*, *76*). Therefore, axons can themselves create gaps necessary for crossing the CNS-PNS barrier. When the radial glia endfeet barrier at the DREZ is removed, sensory axon growth cones enter the CNS without transforming into invadopodia (*75*), suggesting that invadopodium formation is triggered by contact with radial glia or radial glia–derived ECM and is only required for neural tube entry when the CNS-PNS border is intact.

Local disruption of the CNS-PNS boundary is likely a prerequisite for axon crossing at transition zones, but instructive cues are needed to guide axons toward and across the CNS-PNS interface. Consistent with this notion, multiple studies indicate that motor axons exit the CNS in response to signals provided by peripheral tissues. In zebrafish, a myotome-expressed glycosyltransferase, LH3, is required for motor axon exit from the spinal cord, embryonic motility, and survival. LH3 (encoded by the *diwanka* gene) likely functions by adding sugar modifications to myotomal type XVIII collagen, which are needed for this ECM molecule to direct pioneer motor axons into the spinal cord periphery (Fig. 3F) (*77*, *78*). In mice, the CXCL12-CXCR4 signaling pathway has been implicated in motor axon exit from the spinal cord. CXCR4 is expressed by motor neurons, whereas CXCL12 is expressed by the meninges and mesenchyme surrounding the spinal cord (Fig. 3A), and many motor axons fail to leave the spinal cord in *Cxcl12* and *Cxcr4* mutant mice, instead projecting either medially to the ventricular zone or dorsally to the DREZ (*79*) (Fig. 3C). This suggests that CXCL12 functions as an attractive peripheral cue that promotes motor axon exit. Guidance molecules that direct sensory axons to DREZs and into the spinal cord remain elusive, but BC cells have been implicated as a likely source of such cues (*21*). Only slightly more is known about signaling mechanisms that promote olfactory sensory axon entry into the brain (*74*, *80*). The only clear-cut example of a molecule that guides these axons across the CNS-PNS border is the secreted Semaphorin Sema3A, which is expressed in the olfactory bulb. In *Sema3A*-knockout mice, most olfactory and vomeronasal axons fail to cross the CNS-PNS boundary and instead accumulate at the cribriform plate or misroute dorsally into meningeal tissue, but how exactly Sema3A steers olfactory axons in this system is unclear (*81*). Additional cues that direct motor and sensory axons to their transition zones, as well as the tissues producing these cues, remain to be identified. Recent in vitro studies provide evidence that the developing mouse spinal cord meninges secrete diffusible, as yet unidentified chemoattractants for somatosensory and motor axons, which could help to guide these axons to the CNS-PNS interface (*16*) (Fig. 3A).

Although attractive signals appear instrumental in guiding motor and sensory axons across transition zones, multiple studies have highlighted that these axons also need to avoid navigating to inappropriate targets en route to CNS exit and entry points. In *Robo1/2* double-knockout mice, a subset of motor axons fails to reach the MEP and instead projects across the spinal cord midline (Fig. 3D) (*82*–*84*). Robos can mediate axon repulsion from their Slit ligands while suppressing DCC-mediated attraction to Netrin-1 (*85*, *86*), and the motor axon guidance defect in *Robo1/2* mutant mice is therefore likely a result of reduced repulsion from midline-derived Slits and/or increased Netrin-1 attraction. Similarly, the Rho-GTPase antagonist p190RhoGAP was recently shown to suppress Netrin-DCC attractive signaling in motor axons, thereby allowing motor axons to ignore attraction to basement membrane–associated Netrin-1 and project into the periphery through the MEP (*87*) (Fig. 3B). This pathway seems to function in parallel to the CXCL12-CXCR4 pathway to collaboratively steer motor axons out of the spinal cord (*79*, *87*). In summary, motor axon exit from the CNS requires suppression of attractive signals within the spinal cord in addition to attraction to the MEP and peripheral tissues. Consistent with a direct role for repulsive signaling in directing axons of CNS-resident neurons to their exit points, Rohon–Beard sensory axons in embryonic zebrafish require Sema3D, which is expressed in the spinal cord roof plate, to leave the CNS (*88*). Similar pathways that steer peripheral sensory axons to their CNS entry points have so far remained elusive.

Lastly, the location of spinal cord MEPs appears sensitive to signals that change motor neuron cell body or axon positioning. In both mice and zebrafish, loss of CNS-derived perineurial glia after *Nkx2.2* deletion causes not only motor neuron emigration but also aberrant motor axon exit from the CNS at sites lateral to the MEP (*31*–*33*). Owing to the additional expression of Nkx2.2 in ventral spinal cord neurons, it is unclear whether these changes result exclusively from effects on perineurial glia. Mutations in *Netrin-1* or *DCC* affect motor neuron cell body positioning and cause the MEP to shift dorsolaterally, whereas mutations in *Slit* genes or *Robo1/2* result in a ventral shift. When both signaling pathways are inactivated simultaneously, the MEP remains in its normal position, suggesting that push/pull signals from spinal cord midline–derived Netrin-1 and Slits dictate MEP location (*84*, *89*). Therefore, the positioning of transition zones is, at least partially, shaped by the axons that cross the border.

Preventing the wrong axons from leaving the CNS

How most axons are forced to remain within either the CNS or PNS has not been extensively investigated, but several studies support the existence of mechanisms that actively prevent CNS axons from projecting into the PNS. In vitro experiments demonstrate that the meninges secrete repulsive axon guidance molecules for dorsal spinal cord neurons and suggest that these still unidentified cues could aid in preventing axons from aberrantly exiting the CNS (*16*) (Fig. 3A). Moreover, in *Netrin-1* knockout mice, axons of commissural and ipsilaterally projecting neurons in the spinal cord, as well as pontine neuron axons in the hindbrain, aberrantly exit the CNS through transition zones (*65*, *66*, *90*–*92*) (Fig. 3E). Similar defects are observed in mice lacking the Netrin receptors DCC or Unc5c; however, in *Unc5c* knockout mice, CNS axons invade the DREZ but do not fully exit the CNS (*90*). Analysis of these mutant lines suggests two possible mechanisms through which Netrin-1 could prevent CNS axons from projecting into the PNS: (i) drawing axons away from transition zones by attractive signaling and (ii) creating an inhibitory environment at the DREZ (*90*). The full complement of mechanisms that prevent axons from crossing the CNS-PNS border remains to be uncovered.

Outlook

Multiple cell types and signaling pathways exert tight control over the movement of cells and axons between the developing vertebrate CNS and PNS. A multilayered barrier surrounds the brain and spinal cord to prevent aberrant intermixing of CNS and PNS components, and specialized transition zones allow regulated cell migration and axon growth across the CNS-PNS boundary. Studies in various vertebrate species have begun to unravel some of the rules that govern cellular traffic at the CNS-PNS interface; it appears that these findings frequently arose fortuitously through chance discovery of instances in which cells and axons aberrantly crossed between the two subdivisions of the nervous system or failed to do so in cases when they should have. Tellingly, for the overwhelming majority of aberrant CNS-PNS boundary transgressions, defects are restricted to transition zones. This underscores the permissive nature of these access points, the importance of multiple mechanisms to regulate cell migration and axon growth at these sites, and their plasticity in response to physical injury or developmental defects. It also highlights the fact that, aside from transition zones, the CNS-PNS border appears to be very robust, likely using numerous, at least partially redundant mechanisms to restrict aberrant movement across this boundary. More directed research efforts to understand the CNS-PNS interface promise to elucidate the full

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repertoire of cellular interactions and molecular signaling pathways that control this key dividing line in the nervous system. These future studies will likely also provide additional insights into the roles of transition zones in response to nervous system injury and regeneration. Finally, some of the cell types and molecules controlling axon guidance at the *Drosophila* CNS-PNS boundary have been uncovered (*93*–*97*), and continued study of this interface in multiple model organisms, including invertebrates, will contribute to our understanding of the evolution of CNS-PNS segregation and connectivity.

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Cell migration and axon guidance at the border between central and peripheral nervous system

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Neurons negotiating boundaries

Barriers around the brain and spinal cord separate central from peripheral nervous systems, yet the two systems are interlinked. Suter and Jaworski review what is known about how cells, axons, and signals negotiate the boundary zone. Understanding what goes wrong in boundary transgressions reveals the inner workings of multiple, partially redundant mechanisms built during development that separate the two compartments in adulthood.

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