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REVIEW

Making sense out of spinal cord somatosensory development

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ABSTRACT

The spinal cord integrates and relays somatosensory input, leading to complex motor responses. Research over the past couple of decades has identified transcription factor networks that function during development to define and instruct the generation of diverse neuronal populations within the spinal cord. A number of studies have now started to connect these developmentally defined populations with their roles in somatosensory circuits. Here, we review our current understanding of how neuronal diversity in the dorsal spinal cord is generated and we discuss the logic underlying how these neurons form the basis of somatosensory circuits.

KEY WORDS: Dorsal spinal cord development, Neuroepithelium, Transcription factor networks, Vertebrate neural tube, Nociception, Pain, Thermosensation, Pruriception, Itch, Mechanosensation, Cutaneous, Touch, Proprioception

Introduction

Somatosensation collectively refers to the bodily senses of nociception (pain), thermosensation (temperature), pruriception (itch), mechanosensation (cutaneous/touch) and proprioception (limb and body position). These senses are largely relayed and processed in the dorsal spinal cord. Primary sensory neuronal axons from the periphery enter the dorsal spinal cord through the dorsal root where they synapse on projection neurons, local circuit interneurons, or even directly onto motor neurons, providing the first level of circuit integration and processing for somatosensory information. Broadly, the circuitry is spatially organized with nociceptive and thermosensitive afferents targeting the superficial dorsal laminae, cutaneous afferents targeting more ventral dorsal laminae, and proprioceptive afferents targeting cells more ventrally in the intermediate and ventral spinal cord (Fig. 1) (Todd, 2010). Spinal cord neurons use excitatory or inhibitory neurotransmitters, combined with multiple neuropeptides, to transmit and modulate these signals. How the diversity of neurons in the dorsal spinal cord configure somatosensory circuits and how these neurons function to integrate and relay somatosensory information is beginning to be uncovered.

The spinal cord is generated from the developing vertebrate neural tube (Fig. 1), which forms by invagination of the neuroepithelium followed by its closure into a tubular structure that will form the central nervous system. Rostral parts of the neural tube develop into the brain while caudal parts become the spinal cord. Over the past 20 years, the caudal neural tube has been used as a model system for understanding the spatial and temporal genetic principles that govern neuronal cell type specification. These studies have shown that cells within the caudal neural tube differentiate into diverse populations of neurons (Alaynick et al., 2011; Helms and Johnson, 2003; Jessell, 2000; Lee and Jessell, 1999; Lu et al., 2015). Although different cell types extend along the rostral-caudal axis, as demonstrated for motor neurons that reside in different columnar motor pools, dorsal-ventral patterning is a major determinant of cell identity in the developing spinal cord. Indeed, cross sections through the neural tube demonstrate the existence of discrete domains of combinatorial transcription factor (TF) expression that define particular cell types (Fig. 2).

Several dynamic processes have been shown to influence the number and type of neurons that form during the early stages of spinal cord neurogenesis and neuronal specification. These processes include interplay between signaling pathways and TF function, regulation of the timing of neurogenesis, mechanisms of cross-repression between TFs and the expression of TF-driven gene programs that are specific to neuronal identity. While these developmental mechanisms that generate specific cell types in the caudal neural tube are still under investigation, an open question is: how do the development and function of these neurons relate? With the advent of genetic techniques in mice to trace the lineage of various progenitor populations into adulthood, the field is now beginning to understand how neurons born in different progenitor domains give rise to the spinal interneurons that contribute to different aspects of somatosensation. The caudal neural tube is thus emerging as an important model system with which to understand not only how progenitor domains are established during development, but also if there is some logic tying the development of a neuron to its function.

In this review, we first provide an overview of the molecular mechanisms that specify cell fate and generate neuronal diversity in the developing spinal cord. We then explore how different developmental populations produce subsets of neurons with particular somatosensory functions. We do not cover ventral spinal cord development and diversity as this topic has been reviewed elsewhere (Alaynick et al., 2011; Arber, 2012; Goulding, 2009; Jessell, 2000; Lu et al., 2015; Matise, 2013); however, we will refer to ventral spinal cord populations when they lend insight into themes of neuronal migration and patterning.

Principles guiding the generation of neuronal diversity in the dorsal neural tube

Transcription factor codes define neuronal populations

As the caudal neural tube develops into the spinal cord, cells within progenitor domains in the ventricular zone (Fig. 2), defined mainly by TF expression, differentiate into diverse populations of postmitotic neurons. Examinations of the combinatorial expression of multiple families of TFs, largely homeodomain (HD) and basic helix-loop-helix (bHLH) factors, have led to the description of 11 early-born [embryonic day (E)10-E12.5] neuronal populations. Six of these (dorsal interneurons 1-6, dI1-6) are found

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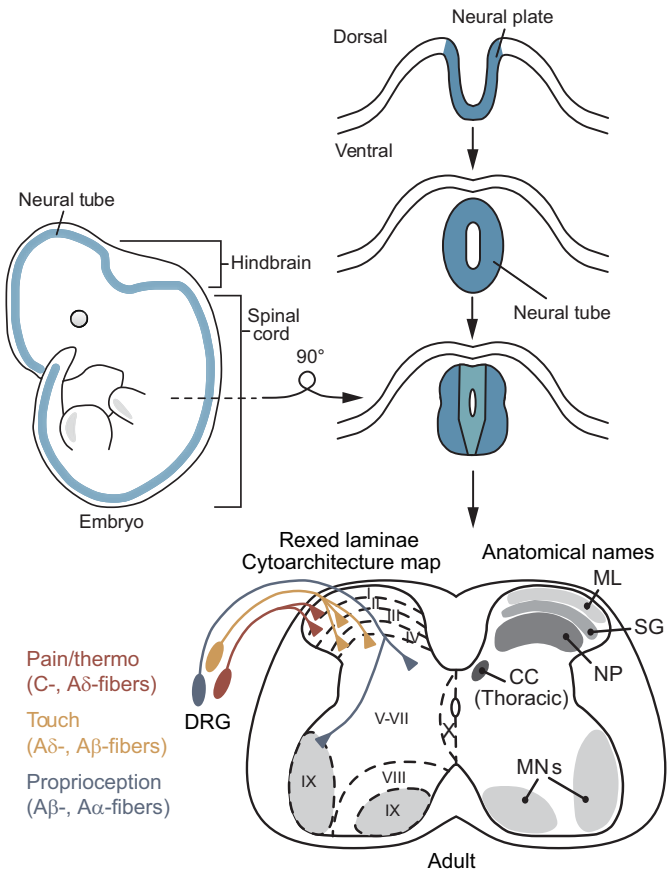

Fig. 1. Development of the spinal cord. During development, an invagination of the neural plate closes to form the neural tube, which will become the central nervous system. The most caudal parts of the neural tube will become the spinal cord. Rexed laminae I-X in the adult spinal cord are determined by cytoarchitectonic parameters. Broadly, pain and thermosensitive afferents (C-, Aδ-fibers) from the dorsal root ganglion (DRG) target laminae I-II, touch afferents (Aδ-, Aβ-fibers) target laminae II_{inner}-V and proprioceptive afferents (Aβ-, Aα-fibers) target more ventral laminae and MNs (see Boxes 1 and 2 for afferent fiber termination markers and definitions). Commonly used anatomical names for Rexed laminae regions are described: marginal layer (ML, lamina I), substantia gelatinosa (SG, lamina II), nucleus proprius (NP, laminae III-V), motor neurons (MNs, lamina IX). Clarke's column, or the dorsal nucleus of Clarke (CC), resides in the medial aspect of lamina VII mainly in the thoracic spinal cord.

in the dorsal neural tube, and the remaining five (V0-V3 and MN) are found in the ventral neural tube (Fig. 2). In addition, there are two late-born (E11-E13) dorsal domains (dIL_A and dIL_B). These defined populations can be further divided into subtypes using criteria such as axonal projections, resulting location in the spinal cord and neuropeptide expression. For example, the dI1 population can be split into two populations that are distinguished by their spatial location and axonal projections: dI1i (ipsilaterally projecting) and dI1c (contralaterally projecting) (Miesegaes et al., 2009; Wilson et al., 2008; Yuengert et al., 2015). These 13 main population designations are central to understanding how TF expression is patterned in response to morphogens and how TFs specify neuronal identity. Importantly, most of the TFs that mark these populations are required within the lineages where they are expressed. In particular, the bHLH factors, ATOH1, NEUROG1/2, ASCL1 and PTF1A are all necessary and sufficient to specify particular dorsal interneuron populations (Bermingham et al., 2001; Glasgow et al., 2005; Gowan et al., 2001; Helms et al., 2005; Mizuguchi et al., 2006; Wildner et al., 2006). This is in contrast to the ventral neural tube where HD TFs, rather than bHLH TFs, play the major specification function (Briscoe et al., 2000; Ericson et al., 1997; Pierani et al., 2001; Sander et al., 2000).

Although the TFs that define spinal cord neuronal populations are often depicted in a single static figure (as in Fig. 2), it should be noted that TF expression is dynamic and, in many cases, transient. Thus, just because a TF functions as a lineage marker at one stage does not mean that it serves that function throughout the development of the lineage. The bHLH factors ASCL1, ATOH1 and NEUROG1 are examples of TFs that are present in subsets of proliferating progenitors but that are rapidly lost as cells differentiate and become postmitotic (Fig. 3). In contrast, some of the HD TFs, such as PAX2 and TLX3, appear only when cells become postmitotic and are retained into postnatal stages (Fig. 3). HD factors are, therefore, particularly useful as markers for defining neuronal populations in the dorsal spinal cord (Fig. 2, blue text). Nevertheless, even the HD factors are not necessarily maintained into mature stages, and additional factors such as neurotransmitters and neuropeptides are needed to mark specific populations of neurons (Box 1).

Signaling pathways direct expression of transcription factors to pattern the neural tube

Multiple signaling pathways are active in the developing neural tube prior to the emergence of the TF-based patterning discussed above. These signals, such as fibroblast growth factor (FGF), act to maintain cells as progenitors, or they act during neuronal specification, as is the case for sonic hedgehog (SHH), bone morphogenetic proteins (BMPs), WNTs, retinoic acid (RA) and FGF (Fig. 4). As the role of morphogens and their signaling pathways have been recently reviewed (Briscoe and Small, 2015; Gouti et al., 2015; Le Dreau and Marti, 2012), we highlight here only some of the major concepts.

During patterning of the dorsal-ventral axis of the spinal cord, SHH produced at the floor plate is instrumental for the formation of ventral cell type identities and it acts by activating or repressing the expression of TFs (largely HD TFs) in a concentration-dependent manner (Briscoe et al., 2000). Thus, the gradient of SHH from the floor plate sets up the initial pattern of TF expression that is later refined through cross-regulatory mechanisms between TFs (Ericson et al., 1997; Novitch et al., 2001; Sander et al., 2000). In contrast, BMPs and WNTs comprise the predominant signaling pathways that pattern the TFs that set up dorsal cell type identity. These signals are produced largely in the roof plate, involve multiple family members and regulate proliferation as well as specification of the progenitors (Chesnutt et al., 2004; Chizhikov and Millen, 2005; Hazen et al., 2012; Ikeya et al., 1997; Liem et al., 1997; Muroyama et al., 2002; Nguyen et al., 2000; Tozer et al., 2013; Wine-Lee et al., 2004). In particular, BMPs and WNTs are crucial for generating the dorsal interneuron populations shown in Fig. 2. Alterations to BMP levels, for example through mutations or ablation of the roof plate, demonstrate that specification of the dorsal dI1-dI3 (termed class A) populations are dependent on these signals, whereas the more intermediate dI4-dI6 (class B) populations form independent of BMP signaling (Fig. 4) (Lee et al., 2000; Müller et al., 2002).

Patterning of the rostral-caudal axis, by contrast, involves the graded expression of FGF, RA and the TGFβ family factor GDF11, all of which provide positional identity along this axis (reviewed in Philippidou and Dasen, 2013). The transcriptional output from these signals results in different combinations of HD-containing homeobox (HOX) TFs being expressed in progenitors and postmitotic neurons. For example, *Hox4-Hox8* are expressed at the cervical and brachial

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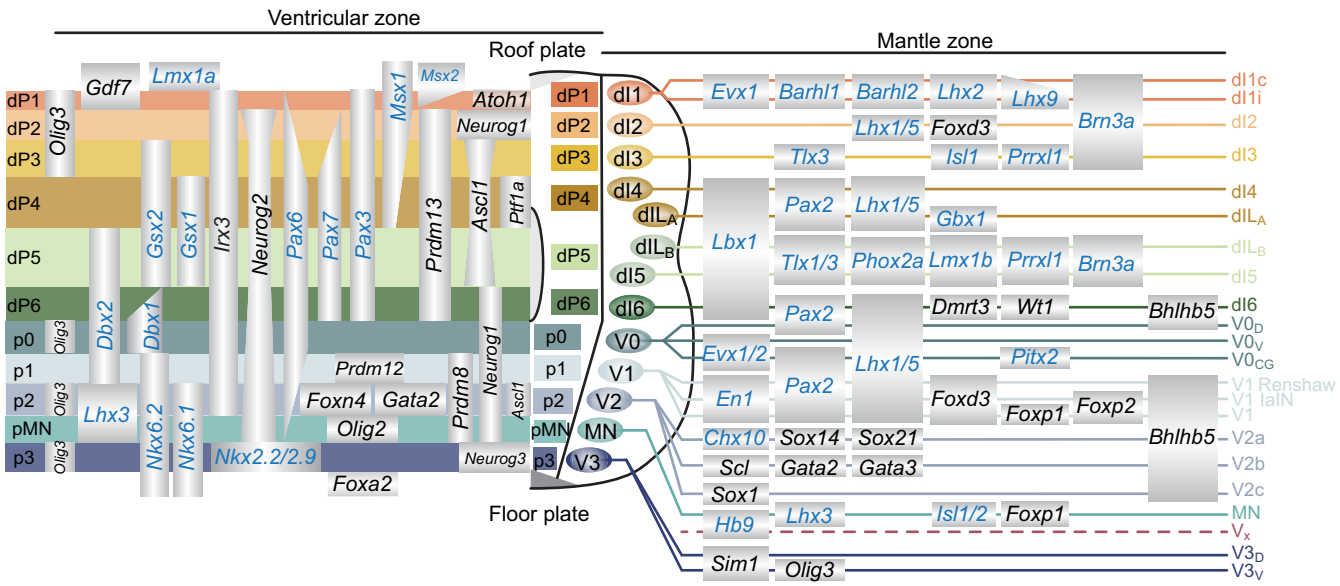

Fig. 2. Summary of the transcription factors that set up spinal cord neuronal diversity. The key transcription factors (TFs) that coordinate neuronal diversity in the developing spinal cord are shown, highlighting those that are expressed in the various progenitor domains (dP1-dP6, p0-p3 and pMN) in the proliferating ventricular zone of the developing spinal cord and those that define mature neuronal populations (dI1-6, V0-3 and MN) and their subsets in the differentiating mantle zone. TFs containing a homeodomain are indicated in blue text. Old gene symbols *Hb9* (*Mnx1*), *Chx10* (*Vsx2*), *Brn3a* (*Pou4f1*) are shown. Dorsal progenitor (dP), dorsal interneuron 1 contralaterally and ipsilaterally-projecting ($dI1_C$, $dI1_I$), dorsal interneuron late born populations (dIL_A, dIL_B), V0 or V3 dorsal, ventral, or cholinergic and glutamatergic ($V0_D$, $V0_V$, $V0_{CG}$, $V3_D$, $V3_V$), Ia interneuron (IaIN), V_x is an $HB9^+$ population of cells of unknown developmental origin. *Msx1*, *Msx2* (Timmer et al., 2002), *Gdf7* (Lee et al., 2000), *Atoh1*, *Neurog1/2*, *Ascl1*, *Ptf1a*, *Pax2*, *Pax3*, *Pax6*, *Pax7*, *Lbx1*, *Foxd3*, *Brn3a*, *Lhx1/5*, *Lhx2/9*, *Barhl1*, *Barhl2*, *Isl1*, *Lmx1b*, *Phox2a* (Bermingham et al., 2001; Ding et al., 2004; Glasgow et al., 2005; Gowan et al., 2001; Gross et al., 2002; Liem et al., 1997; Müller et al., 2002; Saba et al., 2005; Wilson et al., 2008), *Dbx1/2*, *Evx1/2*, *En1* (Burrill et al., 1997; Moran-Rivard et al., 2001; Pierani et al., 1999, 2001), *Olig2/3* (Mizuguchi et al., 2001; Müller et al., 2005; Novitch et al., 2001; Takebayashi et al., 2002), *Neurog3* (Sommer et al., 1996), *Gsx1/2* (Kriks et al., 2005; Mizuguchi et al., 2006), *Lmx1a* (Millonig et al., 2000), *Nkx6.1/6.2*, *Nkx2.2/2.9*, *Irx3*, *Lhx3*, *Chx10*, *Sim1* (Briscoe et al., 2000; Ericson et al., 1997; Fan et al., 1996; Persson et al., 2002), *Prdm13* (Chang et al., 2013), *Prdm12* (Thelie et al., 2015), *Prdm8* (Komai et al., 2009), *Gata2/3*, *Foxn4*, *Bhlhb5*, *Pitx2*, *Foxp1/2*, *Olig3* (Francius et al., 2013, 2015; Li et al., 2005; Morikawa et al., 2009; Nardelli et al., 1999; Rousso et al., 2008; Skaggs et al., 2011; Zagoraiou et al., 2009), *Foxa2* (Ruiz i Altaba et al., 1993), *Tlx1/3* (Qian et al., 2002), *Prrxl1* (Rebelo et al., 2010), *Gbx1* (John et al., 2005), *Dmrt3*, *Wt1* (Andersson et al., 2012; Dyck et al., 2012), *Sox1*, *Sox14*, *Sox21* (Hargrave et al., 2000; Panayi et al., 2010; Sandberg et al., 2005), *Scl* (Smith et al., 2002), *Hb9*, *Isl1/2* (Pfaff et al., 1996).

levels, while *Hox8-Hox9* are expressed in thoracic regions and *Hox10-Hox13* in lumbar regions. Graded expression of RA induces HOX gene expression in cervical and brachial regions, whereas GDF11 functions at the most caudal regions (Bel-Vialar et al., 2002; Dasen et al., 2003, 2005; Liu et al., 2001). The combinations of HOX genes induced have also been shown to pattern motor columns in the ventral spinal cord, such that motor neurons at limb levels are different from those at intercostal or abdominal levels. The mechanisms that regulate rostral-caudal identity in the dorsal spinal cord projection neurons and interneurons are less well understood, although HOX genes are likely players there as well.

Although the signaling molecules mentioned above are the primary ones influencing the patterning of neurons generated along the dorsal-ventral and rostral-caudal axes, they are not the only players. Notably, the responsiveness of progenitors to these patterning signals changes over time, probably as a result of the TFs themselves altering components in the signaling pathways to enhance or attenuate the signals (Nishi et al., 2015). Furthermore, it should be pointed out that although SHH, BMPs, WNTs, FGF and RA are essential for patterning TFs in the neural tube, they have additional functions at later stages, such as providing axon guidance signals (Butler and Dodd, 2003; Lyuksyutova et al., 2003; Yamauchi et al., 2013).

Oscillations in Notch signaling and transcription factor expression control neurogenesis

What are the mechanisms that signal progenitor cells to exit the cell cycle and begin the process of neurogenesis? Recent studies suggest that this is controlled by the balance between Notch signaling molecules and bHLH factors such as ASCL1 and NEUROG2 (called proneural bHLH factors). Together, these factors are key for influencing the number of neurons generated. In general, a high level of Notch signaling maintains cell proliferation, whereas high proneural bHLH levels drive differentiation of that cell.

There are many complexities in the Notch pathway, including extensive post-translational modifications, localization of components in the endoplasmic reticulum (ER) versus the cell surface and crucial protease cleavage steps (see review by Kopan and Ilagan, 2009), but the core of the canonical signaling pathway is as follows. Activation of Notch signaling through binding one of its ligands, such as DLL1, in trans with a NOTCH receptor on another cell results in release of the NOTCH intracellular domain (NICD) and its translocation to the nucleus (Fig. 3). NICD forms a transcriptional activator complex that, among other things, activates transcription of the HES1 transcriptional repressor. An important HES1 function is to repress the expression of proneural bHLH factors such as ASCL1 and NEUROG2, which have specific functions in neuronal subtype fate specification, as mentioned above (Fig. 2). Because high levels of the proneural bHLH factors drive neuronal differentiation, repression of these factors biases cells to the progenitor stage. Importantly, in a feedback mechanism, the proneural bHLH factors activate the expression of Notch ligands such as DLL1. Thus, one might expect that some proneural bHLH activity in surrounding cells is needed to keep Notch signaling active in the progenitor cell. A model emerges whereby low levels of

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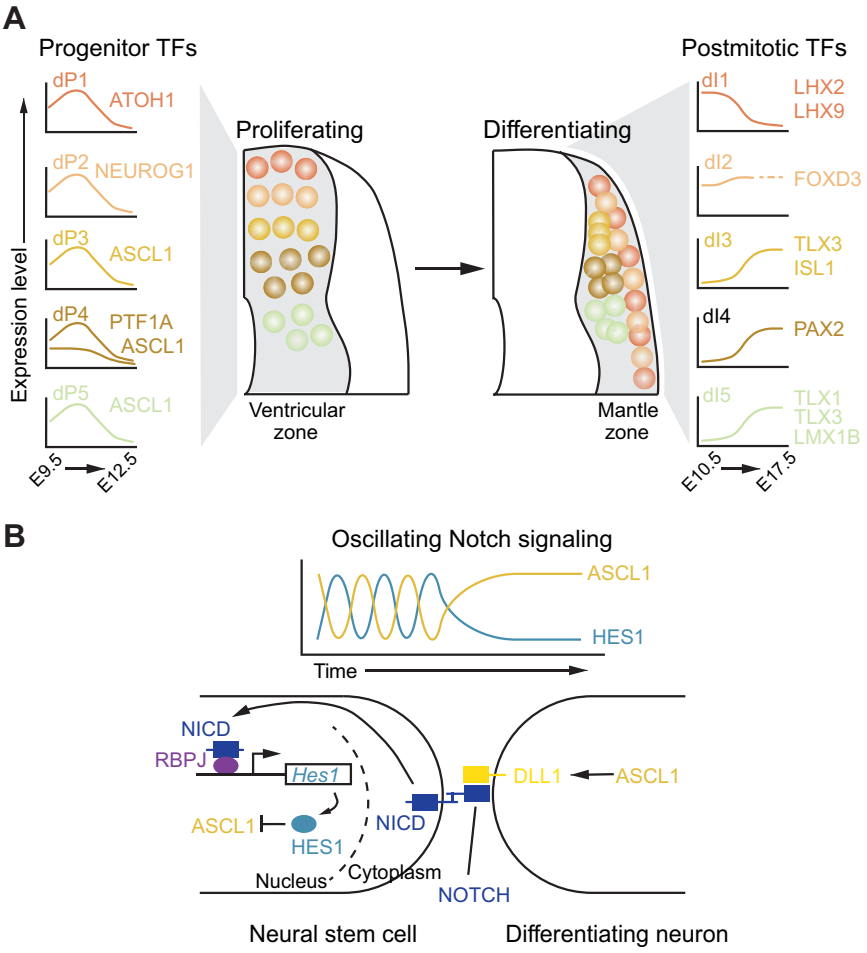

Fig. 3. Dynamic expression of transcription factors in the developing spinal cord. The expression of transcription factors (TFs) in the developing neural tube is highly dynamic. (A) Peak expression of the basic helix-loop-helix (bHLH) transcription factors ATOH1, NEUROG1, ASCL1 and PTF1A within various progenitor domains (dP1-5) in the proliferating ventricular zone occurs at E10.5 and then declines. As these neuronal populations become postmitotic and migrate into the mantle zone, they begin expressing transcription factors that either decline (LHX2/9) or increase (TLX1/3, ISL1, PAX2, LMX1B) over developmental time. It is unknown how FOXD3 expression changes at later development time points (dashed line) (Gross et al., 2002). (B) The interplay between activating bHLH TFs such as ASCL1 and repressive TFs such as HES1, mediated through Notch signaling, results in oscillatory expression of these TFs in neural stem cells; these oscillations control the timing of neurogenesis. Eventually, sustained expression of ASCL1 leads to neuronal differentiation.

proneural bHLH activity are in a balance with active Notch signaling to maintain progenitor cells (Castro et al., 2011). When an imbalance allows elevated levels of proneural bHLH expression, the progenitor differentiates. Because of feedback regulation of HES1, cross-regulatory relationships as stated above and instability of the factors involved, the levels of the TFs and Notch ligands oscillate. Indeed, an emerging model is the oscillation model for maintaining progenitors (Kageyama et al., 2008; Shimojo et al., 2016, 2008). In this model, progenitors are maintained in a proliferative state. When expression of the neural bHLH factors is elevated and sustained, the progenitors undergo cell cycle exit and neuronal differentiation. For details on this Notch signaling oscillation-based model and a description of the live cell imaging experiments that support the model, see recent reviews by Imayoshi et al. (2015) and Isomura and Kageyama (2014).

Cross-repression between transcription factors specifies distinct neuronal identities

Repressing inappropriate gene expression programs in a lineage is just as crucial to specifying appropriate cell fate as inducing the proper cell type-specific genes. Indeed, cross-repression between TFs has emerged as a major principle in setting up boundaries that delineate either progenitor domains or their resulting neurons (Fig. 4). This concept was first described in the ventral neural tube where neighboring progenitors repressed each others' expression of class I or class II HD TFs to generate discrete progenitor boundaries (Briscoe et al., 2000; Ericson et al., 1997). In the dorsal neural tube, cross-repression is also evident and has been shown to occur between bHLH factors. For example, ATOH1- and NEUROG1-expressing progenitors give rise to dI1 and dI2 neurons, respectively (Fig. 4). Cross-repression is evidenced by the fact that dI1 neurons are lost in *Atoh1* mouse mutants while NEUROG1 expression is expanded and excess dI2 neurons are generated (Gowan et al., 2001). Similarly, PTF1A-dependent dI4/dIL$_A$ populations and ASCL1-expressing progenitors of dI5/dIL$_B$ neurons demonstrate cross-repression; in the absence of PTF1A, dI4/dIL$_A$ neurons are lost and unopposed ASCL1 activity results in excess dI5/dIL$_B$ neurons (Glasgow et al., 2005; Mizuguchi et al., 2006; Wildner et al., 2006).

How can these bHLH factors, which are activators of transcription, repress fate in neighboring cells? Recent studies of PTF1A-dependent populations show that repression of dI5 fate is mediated through a member of the PRDM family of TFs. PRDM TFs contain zinc finger domains and a domain with similarity to the SET domain that has histone methyltransferase activity (Hohenauer and Moore, 2012). A recent study demonstrated that PRDM13 is a PTF1A target that represses expression of the dI5-specific HD factor TLX3 in dI4 neurons (Chang et al., 2013). Furthermore, PRDM13 may function through switching ASCL1 from an activator of *Tlx3* expression to a repressor as a means to shut down gene programs for alternative fates within a differentiating neuron. As another example, PRDM12 was shown to be a factor that supports the V1 lineage by repressing V0 genes in the progenitors of these neurons (Thelie et al., 2015).

Until recently, the cross-repressive mechanisms elucidated in the developing neural tube have been limited to gene programs in

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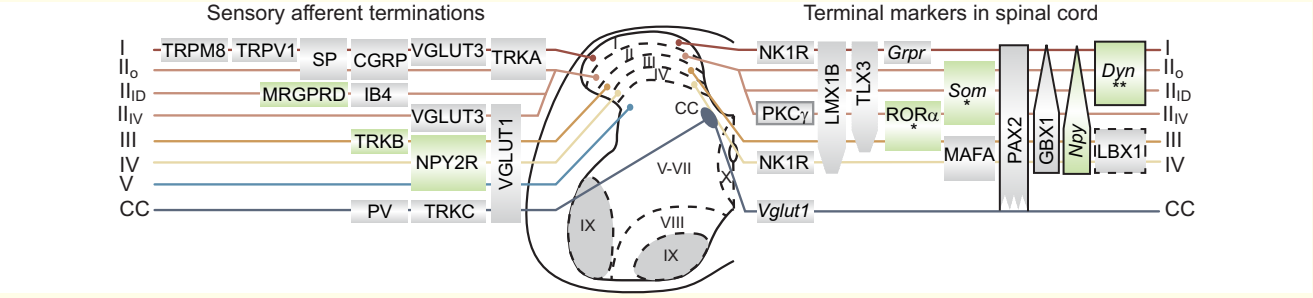
Box 1. Expression of terminal markers in the spinal cord



While transcription factors have been shown to define discrete domains during spinal cord development, the molecular markers that define a particular Rexed lamina are less well-described, in part because particular laminae may have different sensory afferent terminations with several different neuronal cell types. Nonetheless, recent studies have been able to molecularly refine subpopulations within a given laminae. For example, lamina II is subdivided into an outer (II$_o$, CGRP$^+$ afferents), inner dorsal (II$_{ID}$, IB4$^+$ afferents) and inner ventral (II$_{IV}$, PKCγ$^+$) lamina. These molecular designations are summarized in the above image. Expression patterns were determined using antibody staining (capitalized protein symbol), mRNA detection (italicized gene symbol) or genetically modified mice (green boxes). Terminal markers for excitatory (no outline), inhibitory (black outline), mixed excitatory/inhibitory (dashed outline), unknown excitatory/inhibitory (gray outline), mostly excitatory (*), and mostly inhibitory (**) neurons are shown. TRPM8 (Bautista et al., 2007), TRPV1 (Villeda et al., 2006), MRGPRD (Zylka et al., 2005), SP, CGRP, IB4, TRKA (Snider and McMahon, 1998), VGLUT3 (Seal et al., 2009), TRKB, NPY2R (Li et al., 2011), TRKC, PV (Arber et al., 2000), VGLUT1, *Vglut1* (Alvarez et al., 2004; Hantman and Jessell, 2010; Llewellyn-Smith et al., 2007), NK1R, PKCγ (Todd, 2010), *Grpr* (Sun and Chen, 2007), *Som*, *Dyn* (Duan et al., 2014; Xu et al., 2008), LMX1B, RORα, MAFA, LBX1, TLX3, PAX2, GBX1 (Bourane et al., 2015b; Del Barrio et al., 2013; Szabo et al., 2015), *Npy* (Bourane et al., 2015a).

neighboring progenitor populations. However, unbiased approaches for identifying specific targets of TFs, such as RNA-seq coupled with ChIP-seq, are beginning to uncover broader programs of repression than previously appreciated. This emphasizes the concept that there is broad transcriptional activation throughout the neural tube, possibly involving SOXB1 factors (Bylund et al., 2003; Kutejova et al., 2016), that requires progenitor-specific active repression of genes for alternative cell identities. In particular, two recent studies of three ventral neural tube TFs – NKX2.2, NKX6.1 and OLIG2 – revealed that they directly repress all alternative fates, including dorsal cell fate programs, in the ventral neural tube (Kutejova et al., 2016; Nishi et al., 2015). Additionally, these repressor networks target multiple SHH signaling components, providing negative feedback to ongoing SHH signaling, emphasizing the dynamic relationship between TFs and signaling pathways (Nishi et al., 2015) (Fig. 4).

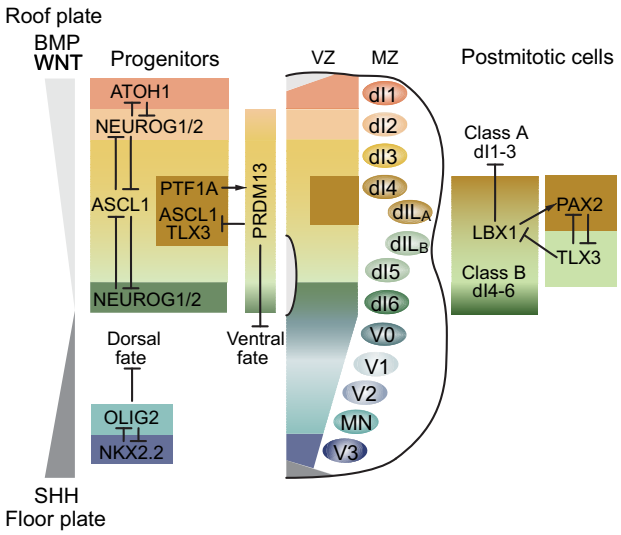


Fig. 4. Cross-repression between TFs in the developing neural tube. Morphogens released from the roof plate (BMP, WNT) and floor plate (SHH) set up gradients that impact the expression of TFs in the developing neural tube. For example, dI1-3 (class A) neurons are influenced by BMP signaling, while dI4-6 (class B) neurons are not. Furthermore, cross-repressive activities between individual TFs, both direct and indirect, play an important role in setting up boundaries between interneuron domains.

Lastly, cross-repression between TFs is not just seen in setting up progenitor domain boundaries, but is also a mechanism used in early postmitotic populations. An example is seen in the case of the HD factor network that includes LBX1, TLX3 and PAX2, and defines dI4-dI6 populations (Gross et al., 2002; Müller et al., 2002). LBX1 marks all three of these populations and is involved in regulating PAX2 expression. However, PAX2 is only expressed in dI4 and dI6 inhibitory neurons, while the excitatory neuronal dI5 population expresses TLX3. It turns out that TLX3 inhibits LBX1 activity, resulting in a decrease in PAX2. Thus, TLX3 provides a switch that specifies the excitatory neuronal phenotype while repressing inhibitory neuronal programs in these postmitotic populations (Cheng et al., 2004, 2005). Extrinsic signaling can also influence the levels of these TFs. For example, altering spontaneous Ca^{2+} currents in the developing *Xenopus* neural tube was shown to influence the generation of inhibitory versus excitatory neurons and this process involved regulation of *Tlx3* expression by phosphorylated JUN (Marek et al., 2010; Spitzer, 2012). Thus, cross-repression between TFs that specify neuronal subtypes in progenitors and postmitotic neurons, which can be influenced by activity-dependent processes, is a key mechanism in generating neuronal diversity and ensuring definitive cell identities in the spinal cord.

Transcription factors drive genetic pathways important for terminal neuronal phenotypes

As mentioned above, bHLH and HD TFs have been used extensively to define and couple progenitor populations to their terminal neuronal populations, but less is known about the identity

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of the direct downstream targets of these TFs that could connect them to terminal differentiation processes such as axon guidance and neurotransmitter or neuropeptide fate (Avraham et al., 2009; Brohl et al., 2008; Cheng et al., 2004, 2005; Hobert, 2011; Pillai et al., 2007). However, for both bHLH and HD TFs there are a few examples of how these TFs direct terminal gene programs to specify cell identity. For example, the HD TFs LHX2 and LHX9 in the dI1 population regulate the expression of *Robo3* (previously known as *Rig1*), a gene that is important for axon guidance and determining whether axons project ipsilaterally or contralaterally (Wilson et al., 2008). In addition, hexameric complexes containing the HD factors ISL1 and LHX3 in the ventral neural tube have been shown to directly regulate a battery of cholinergic pathway genes, such as those encoding acetylcholine synthesizing enzymes and transporters in developing motor neurons (Cho et al., 2014). Thus, terminal neuronal phenotypes can be directly regulated by sustained expression of HD factors in mature neurons. Furthermore, transiently expressed TFs, such as the bHLH TFs ATOH1, PTF1A and ASCL1, have been shown to directly regulate genes that control terminal neuronal phenotypes in addition to their role in regulating the expression of HD TFs (Borromeo et al., 2014; Lai et al., 2011; Russ et al., 2015; Wildner et al., 2013). For example, PTF1A directly regulates genes encoding GABA synthesizing enzymes and GABA and glycine transporters required for inhibitory neuronal functions, but it also regulates the expression of PAX2 (Borromeo et al., 2014). Given the transient nature of expression of the bHLH regulators, as opposed to the more sustained expression of some HD TFs, it is possible that bHLH TFs act to set up chromatin accessibility for later persistently expressed TFs that maintain the expression of cell type-specific genes (Borromeo et al., 2014).

In summary, the past two decades of research have yielded multiple fundamental principles that guide the development of neuronal diversity in the neural tube. The use of TFs as markers to define progenitor and neuronal populations has been essential for uncovering strategies that direct neuronal diversity in the developing neural tube. The combined roles of extrinsic signaling gradients to set up patterned TF expression and oscillations in TF expression provide instructions for generating the correct number and composition of neurons needed for neural circuit formation. Finally, current unbiased approaches for identifying transcriptional targets for these TFs are extending our understanding of the importance of repressing gene programs for all alternative fates to eliminate ambiguities in neuronal identity. Together, these studies have fueled our understanding of how neuronal diversity is established in the developing spinal cord. As we move on to discuss below, some recent and exciting studies are now beginning to reveal how these diverse neuronal populations mature and migrate to their final position in the spinal cord, and how their generation is linked to their ultimate function within spinal cord somatosensory circuits.

The migration of neurons during spinal cord circuit formation

Given the discrete molecularly defined domains that originate in the developing neural tube during neurogenesis, one might expect that this patterning defines the spinal cord laminar designations described by Bror Rexed (1954). However, lineage-tracing experiments have revealed that during development spinal cord neurons in fact migrate long distances along the dorsal-ventral axis from their original progenitor positions in the ventricular zone. The mechanisms regulating this migration remain largely underexplored. Overall, while dorsal-born neurons stay mostly in the dorsal horn and ventral-born neurons stay mostly in the ventral horn, the laminar structure defined by specific TF expression in the ventricular zone during development (dI1-V3) is not maintained into maturity and does not necessarily correspond one-to-one with the Rexed laminae I-X defined by cytoarchitecture (Rexed, 1954) (Fig. 5). Indeed, *Atoh1* lineage neurons (dI1), which are born from the dorsal-most progenitor domain, migrate ventrally to the intermediate gray area of the spinal cord (laminae V-VII) with a smattering of neurons even reaching the ventral horn (Miesegaes et al., 2009; Wilson et al., 2008; Yuengert et al., 2015). In addition, dI2 and dI3 neurons settle in the intermediate to ventral parts of the spinal cord (Bui et al., 2013; Hadas et al., 2014; Quinones et al., 2010). In contrast, *Sim1* lineage neurons (V3), which mark the ventral-most derived neurons, reside mainly in laminae VIII but can migrate dorsally as far as laminae IV (Borowska et al., 2013). Meanwhile, interneurons born from dorso-intermediate regions of the neural tube (dI4/dIL$_A$-dI5/dIL$_B$) migrate both dorsally and laterally (Glasgow et al., 2005; Gross et al., 2002; Müller et al., 2002; Xu et al., 2008) and interneurons born from ventro-intermediate regions (V0-V2) migrate ventrally and laterally (Bikoff et al., 2016; Crone et al., 2008; Gosgnach et al., 2006; Lanuza et al., 2004; Zagoraiou et al., 2009; Zhang et al., 2014).

This non-radial migration of developing spinal cord neurons is different from the migration observed during cortical neurogenesis, where a laminar structure forms from radial migration, with neuronal specification of excitatory projection neurons resulting from a combination of birth date and the expression of cell fate determinants (Franco and Muller, 2013). In the cortex, inhibitory neurons migrate from distant sites in the ventral telencephalon, far away from those giving rise to excitatory cortical neurons (Kepecs and Fishell, 2014). By contrast, excitatory and inhibitory neurons in

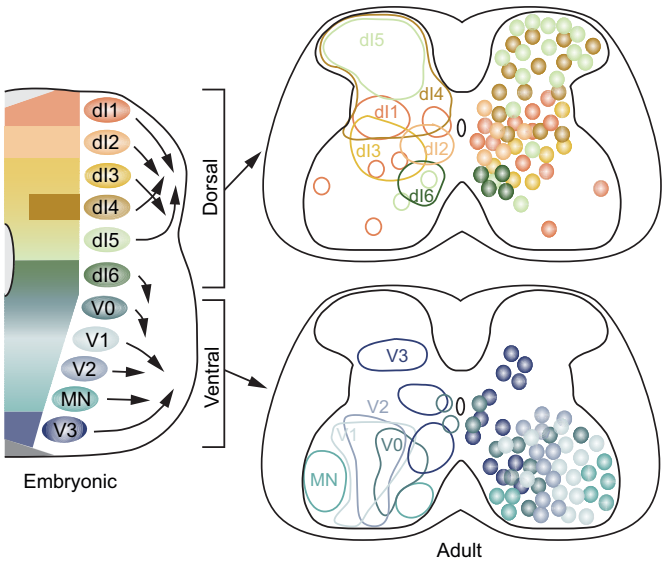

Fig. 5. Migration of neurons during spinal cord development. Neurons derived from discrete progenitor domains in the developing neural tube migrate quite extensively from their original birth location and do not follow a one-to-one correspondence with Rexed laminae (Fig. 1). For example, while dI1-dI5 neurons remain largely in the dorsal and intermediate spinal cord, dI1-dI3 neurons travel ventrally to the intermediate spinal cord, while dI4/dIL$_A$ and dI5/dIL$_B$ neurons migrate dorsally and laterally. V0-V3 populations remain largely ventral, but the V3 domain generates neurons that extend into the dorsal horn. Although dI6 is considered to be dorsally derived, neurons from this domain migrate ventrally (Andersson et al., 2012). Molecular maps represent the current known state of the field.

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the spinal cord are born from neighboring and interspersed progenitor domains in the ventricular zone. Indeed, the Rexed laminae of the spinal cord (I-X) do not follow any known logical birth dating pattern like that seen in cortical lamination (Altman and Bayer, 1984). However, the date of birth of a particular progenitor pool has been shown to correlate with the functional properties of that set of neurons. For example, excitatory and inhibitory neurons derived from the *Lbx1* lineage (dI3, dI4/dIL$_A$ or dI5/dIL$_B$) form neurons presynaptic to motor neurons. Those innervating a flexor muscle group are mostly born at E10.5 while those innervating an extensor muscle group are mostly born at E12.5. Therefore, function can partially be separated by birth date, but again the neurons reside scattered across lamina V-VII following no particular laminar distribution (Tripodi et al., 2011). Similarly, birth date can distinguish the formation of Renshaw cells and Ia inhibitory interneurons that derive from the V1 progenitor domain in the ventral spinal cord (Benito-Gonzalez and Alvarez, 2012; Stam et al., 2012). Altogether, although the organization of the progenitors does not prefigure the organization of the spinal cord with regards to lamina distribution, they do predict where particular developmental lineages settle in the adult spinal cord and dictate some functional properties of these neurons. Based on this, we outline a molecular-lineage map of the spinal cord (Fig. 5), which provides a useful framework for describing functional populations in the spinal cord. Such a map explains why functional sets of neurons in any particular laminae are difficult to distinguish, since several developmental lineages can be co-mingled in a given area. While these maps are focused on dorsal-ventral and medio-lateral distribution, it should be noted that there are significant rostral-caudal differences in the expression of particular neuronal subsets.

Connecting developmental identity to functional identity within somatosensory circuits

Current molecular genetic tracing techniques in mice allow researchers to classify neurons based on anatomical connectivity, electrophysiological signature, neurotransmitter/neuropeptide expression and developmental lineage. Indeed, much of the progress in the last several years has shown that any given developmental lineage in the dorsal spinal cord appears to be partly unified by its association with a particular sensory modality, even though it may give rise to neurons with different axonal projections, firing types and neuropeptide expression. These studies suggest, therefore, that developmental lineage is roughly tied to sensory function. In particular, such studies have demonstrated that molecular markers can define specific subsets of neurons of a particular sensory modality and that neurons that were previously thought to be similar based on anatomical connectivity can develop from different progenitor domains. For example, a GRPR$^+$ subset of the dI5/dIL$_B$ lineage is involved in chemical itch sensation and a NPY$^+$ subset of the dI4/dIL$_A$ lineage is involved in mechanical itch pathways, giving credence to the idea that there are distinct somatosensory submodalities that are integrated via distinct spinal microcircuits (Bourane et al., 2015a; Ma, 2012; Sun et al., 2009). However, neurons that have been defined by anatomical characteristics may arise from more than one developmental population. For example, dorsal spinocerebellar tract (DSCT) neurons derive from at least two developmental sources: dI1i and as yet unknown sources (Yuengert et al., 2015). Similarly, Ia inhibitory interneurons in the ventral spinal cord derive from both V1 and V2b (Zhang et al., 2014), and propriospinal neurons that target motor neurons and the lateral reticular nucleus have been shown to derive from several developmental populations (dI3, V1, V2, V3) (Pivetta et al., 2014). These examples suggest either evolutionary convergence of different developmental populations to a common function or as yet unidentified divergent functions of anatomically similar neurons. How particular developmental populations relate to different functional sets of neurons in the mature spinal cord is still under active investigation and the principles behind the developmental progression of these functional units is still emerging.

Below, we review the connectivity and function of these different sets of neurons (summarized in Fig. 6), organized by sensory modality. In general, dorsal developmental populations (dI1-3) and some of the dI4/dIL$_A$ populations form networks involved in proprioceptive and touch-activated or motor pathways involved in smooth movement, while the dI4/dIL$_A$ and dI5/dIL$_B$ populations form much of the circuits and gate control pathways involved in pain, thermosensation, itch and touch. The dI6 population appears to be more ventral-motor related as it is involved in rhythmicity of gait.

Proprioception

Proprioception, the sense of limb and body position, is important for the timing of rhythmic movements such as walking and swimming as well as coordination of muscle activity across joints (Akay et al., 2014). This sense is detected by sensory neurons (see Box 2) such as group Ia, Ib, and II fibers that detect changes in muscle length and tension. Spinal targets of these sensory neurons, largely labeled by parvalbumin (PV) (Arber et al., 2000; de Nooij et al., 2013), include secondary neurons in spinal cord that send this information up to the cerebellum (via spinocerebellar tracts, SCTs) (Brown, 1981; Oscarsson, 1965; Yuengert et al., 2015) and motor neurons for monosynaptic reflex arcs (Arber et al., 2000). SCTs consist of an ipsilateral-projecting population (the dorsal SCT, DSCT) and a contralateral-projecting population (the ventral SCT, VSCT). Studies have shown that dI1i and dI1c neurons contribute to both the DSCT and VSCT, respectively (Bermingham et al., 2001; Miesegaes et al., 2009; Wilson et al., 2008; Yuengert et al., 2015). However, recent work using *Atoh1* lineage tracing shows that the dI1 population only makes a subset of the DSCT and VSCT, suggesting that there are other developmental sources for these tracts (Yuengert et al., 2015). In addition, the conditional knockout of *Atoh1* caudal to the lower medulla results in mice that can walk relatively normally, but have a loss of coordinated motor function, consistent with the idea that only a subset of proprioceptive relay neurons have been lost (Yuengert et al., 2015). The dI2 population, which is mostly contralateral-projecting but has some ipsilateral-projecting neurons, is a potential candidate for the other developmental source (Avraham et al., 2009; Sakai et al., 2012). Analysis of dI2 axonal projections using dI2 enhancers driving fluorescent reporters in chick shows that they can project rostrally to the cerebellum (Avraham et al., 2009; Sakai et al., 2012) via the lateral funiculus. In addition, dI1 and dI2 neurons have been suggested to also contribute to the spino-olivary or anterolateral system since their axons can project past the isthmus of the hindbrain-midbrain border via the ventral funiculus (Gross et al., 2002; Sakai et al., 2012); however, a more detailed analysis is necessary to pinpoint their precise synaptic targets.

Touch

The sensation of touch plays important roles in motor control, social interaction, and distinguishing different textures (Abraira and Ginty, 2013). This information is relayed from the skin through low threshold mechanoreceptor (LTMR) primary sensory afferents (see

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A

	Domain	Projection	Transmitter	Type	Inputs	Target(s)	Function	Citations
Proprioceptive/motor	dI1c	C, A	VGLUT2	VSCT	Group I and/or II	Hindbrain*	Prop/gross motor	Bermingham et al. 2001, Miesegaes et al. 2009, Wilson et al. 2008, Yuengert et al. 2015
	dI1i	I, A	VGLUT2	DSCT	Group I and/or II	Hindbrain*	Prop/gross motor	
	dI2	C/I, A/D	?	SCT and/ or ALS?	?	Hindbrain and/ or Thalamus?*	?	Avraham et al. 2009, Sakai et al. 2012
	dI3	I, A/D	VGLUT2	Propriospinal	Group I and Aβ (no Merkel)	MN and LRt	Grip/gross motor	Bui et al. 2013, Pivetta et al. 2014, Goetz et al. 2015, Avraham et al. 2010
Pain/temperature/itch/touch	dI4/dIL$_A$ GABApre	I	GABA	Interneuron	?	Prop afferents	Prop, reach Smooth motor	Betley et al. 2009, Fink et al. 2014
	dI4/dIL$_A$	I	GLY	Interneuron	Myelinated Sensory	Cut afferents and ?	Gating pain, thermo, itch	Foster et al. 2015
	dI4/dIL$_A$ DYN	I	GABA/GLY	Interneuron	Aβ	SOM+ dI5/dIL$_B$	Gating mechanical pain	Duan et al. 2014
					C	GRPR+ dI5/dIL$_B$	Gating chemical itch	Kardon et al. 2015
	dI4/dIL$_A$ NPY	?	GABA/GLY	Interneuron	Aβ, Aδ, C	?	Gating mechanical itch	Bourane et al. 2015a
	TLX3$^+$ or LMX1B$^+$	I	VGLUT2	ALS or Interneuron			Pain, thermo or itch	Xu et al. 2013, Szabo et al. 2015
	dI5/dIL$_B$ RORα		VGLUT2	Interneuron	Aβ, Aδ, C no Pacinian	MN, V0c, V2a, neurons of PSDC in laminae III/IV	Light touch Fine motor	Bourane et al. 2015b
	dI5/dIL$_B$ SOM		VGLUT2	Interneuron	Aβ, Aδ, C	STT neurons	Mechanical pain	Duan et al. 2014
	dI5/dIL$_B$ VGLUT3		VGLUT2/3	Interneuron	Aβ, Aδ, C	Dorsal horn neurons	Mechanical pain	Peirs et al. 2015
	dI5/dIL$_B$ GRPR				C		Chemical itch	Sun et al. 2007, 2009
Motor	dI6	C/I	GABA/GLY	Premotor	?	MNs	Motor/gait	Andersson et al. 2012 Goetz et al., 2015

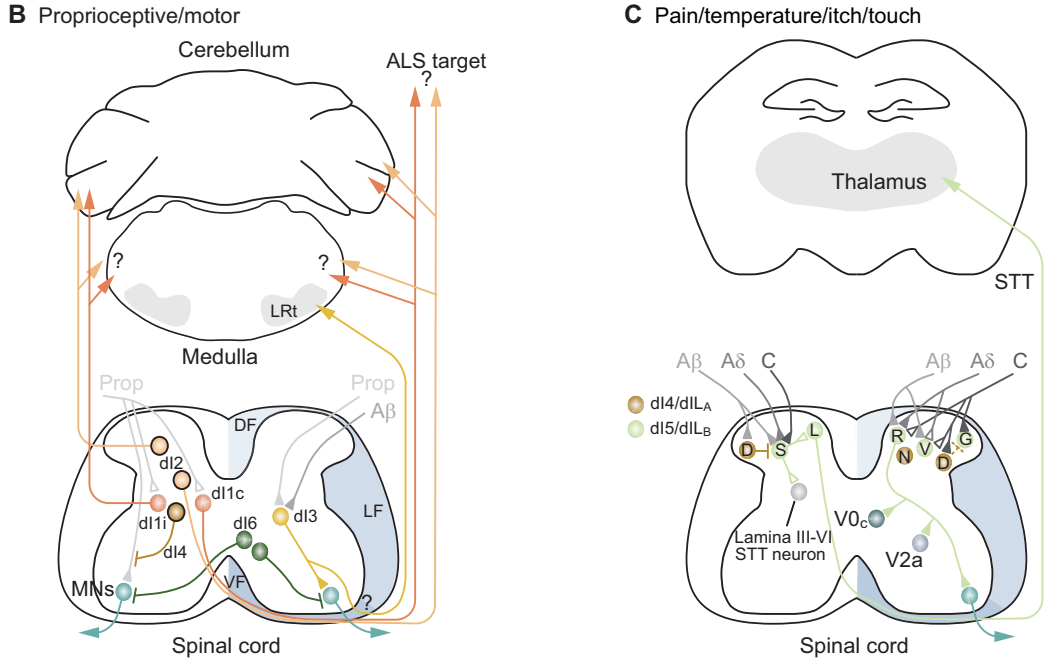

Fig. 6. Function of neurons arising from dorsal progenitor cells. Neurons derived from a common progenitor source tend to form neurons involved in circuits associated with a particular somatosensory function. Details of these circuits are still under active investigation. (A) Neurons from dI1, dI3 and some of the dI4 domain form networks involved in proprioception, touch-related gross motor and smooth motor control. It is unknown which circuits dI2 lineage neurons produce (dashed line), although some groups suggest they may form SCTs or components of the ALS. By contrast, dI4/dIL$_A$ and dI5/dIL$_B$ lineage neurons form circuits involved in pain, temperature, itch and touch. Although dI6 lineage neurons are associated with the developing dorsal neural tube, their known function is in gait motor control in the ventral spinal cord. (B) Summary of the circuits formed by dI1, dI2, dI3, dI4 and dI6 lineage neurons. It is unknown how dI1 and dI2 neurons might project to the medulla, pons, thalamus or other targets of the ALS (?, see text for details). It is also unknown how the axons of dI3 propriospinal neurons travel to the LRt (?, see text for details). (C) Summary of networks formed by dI4/dIL$_A$ and dI5/dIL$_B$ neurons. A putative STT in lamina III-VI is of unknown developmental origin (gray circle). Circles outlined in black represent neurons whose soma location is unknown. Excitatory synapses are indicated by solid triangles for monosynaptic connections and open triangles for polysynaptic or unknown monosynaptic connections. Inhibitory synapses are indicated by perpendicular lines at the end of axons. A dashed line indicates the inhibition is indirect. C, contralateral; I, ipsilateral; A, ascending; D, descending; DSCT, VSCT, dorsal/ventral spinocerebellar tract (SCT); ALS, anterolateral system; STT, spinothalamic tract; Prop, proprioceptive; Cut, cutaneous; MN, motor neuron; LRt, lateral reticular nucleus; PSDC, postsynaptic dorsal column; DF, dorsal funiculus; LF, lateral funiculus; VF, ventral funiculus; L, LMX1B$^+$ in lamina I; S, SOM$^+$; R, RORα$^+$; G, GRPR$^+$; V, VGLUT3$^+$; D, DYN$^+$; N, NPY$^+$.

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Box 2) of varying size and conduction velocities (Abraira and Ginty, 2013). The central terminals of cutaneous low threshold sensory afferents ascend ipsilaterally through the dorsal funiculus (dorsal column-medial lemniscus pathway), but also send out branches that terminate in inner laminae II (II_i) to V (Li et al., 2011). While our understanding of how these cutaneous afferents are processed within the dorsal horn is still incomplete, recent studies have provided insight into the developmental origins of the neuronal populations involved.

Two populations of dorsal interneurons have been implicated in receiving touch information in the spinal cord. The first – dI3 neurons – mediate touch-activated grasping behavior (Bui et al., 2013). These neurons, which are located in laminae V-VII, receive both proprioceptive and Aβ-LTMR inputs and send axonal projections ipsilaterally to motor neurons and the lateral reticular nucleus (LRt) (Bui et al., 2013; Goetz et al., 2015; Pivetta et al., 2014; Stepien et al., 2010). However, it is unclear if the dI3 axons projecting to motor neurons and the LRt are the same cell with axon collaterals traveling ipsilaterally in the dorsal and ventrolateral funiculus, or if there are two subtypes of dI3 neurons whose axons travel in the different funiculi (Alstermark and Ekerot, 2013; Avraham et al., 2010; Pivetta et al., 2014). Consistent with their role in grasping behavior, dI3 neurons synapse preferentially on motor neurons that innervate limb muscles over those that innervate axial muscles (Goetz et al., 2015).

A second population of neurons defined by RORα expression is reported to be involved in detecting cutaneous inputs necessary for light touch and corrective foot movements (Bourane et al., 2015b). These RORα$^+$ cells are located in lamina II_{iv}/III and are innervated by primary sensory neurons that terminate in Meissner corpuscles, Ruffini corpuscles and Merkel cells as well as D-hair afferents and Aβ and Aδ afferents that terminate as transverse lanceolate endings in hairy skin. The RORα neurons are also indirectly activated by C-fibers. Since these neurons are mostly LMX1B$^+$ and PAX2$^-$, they are probably a subset of dI5/dIL$_B$ VGLUT2$^+$ neurons (Bourane et al., 2015b; Del Barrio et al., 2013). Consistent with the function of their sensory inputs, the ablation of RORα$^+$ neurons in the mouse spinal cord causes deficiencies in dynamic and static light touch, but not pain, thermosensation or itch. In addition, even though RORα$^+$ neurons synapse on limb MNs, $V0_c$ cholinergic neurons and V2a interneurons, the ablation of these neurons has no effect on locomotion, although impaired corrective foot movements on raised beam tests are observed, suggesting that cutaneous information is needed for fine motor control.

Altogether, these data suggest that there are layers of touch-responsive networks that feed into gross and fine motor behavior that ultimately connect to limb motor neurons for appropriate motor control. Notably, eliminating *Vglut2* (*Slc17a6*) neurotransmission in dI3 neurons and other neurons marked by *Islet1*$^{Cre/+}$ in mice, impaired their ability to cross a horizontal ladder, decreased time hanging from a wire grid and decreased grip strength (Bui et al., 2013). These behavioral defects are similar to those seen in caudal *Atoh1* conditional knockouts (Yuengert et al., 2015), indicating that both dI1 and dI3 neurons may feed into similar proprioceptive and cutaneous networks that execute proper gross motor control. By contrast, Merkel cells (light touch sensory inputs) and RORα interneurons, which relay light touch inputs, are not required for gross motor behavior (Bourane et al., 2015b; Maricich et al., 2012), but RORα interneurons have been shown to play a role in fine motor control. Therefore, it will be interesting to see how dI1 and dI3 neurons may receive different sensory inputs compared with RORα neurons and how they might differentially send this information to motor neurons, potentially providing insights into circuits that direct gross versus fine motor control.

Pain, temperature and itch

Pain, temperature and itch are first detected in the periphery by primary sensory neurons that project primarily to laminae I/II of the dorsal horn (Todd, 2010). The information is then relayed to supraspinal locations by projection neurons of the anterolateral system (ALS) whose soma reside in laminae I or III-V. Importantly, excitatory and inhibitory interneurons located throughout the dorsal horn (laminae I-V) are also required for local processing of these sensory modalities. These excitatory interneurons are derived mainly from the dI5/dIL$_B$ lineages, which reside throughout the dorsal horn with some ventral expression (Szabo et al., 2015; Xu et al., 2008). Genetic manipulation of dI5/dIL$_B$ neurons as a whole (via elimination of spinal cord TLX3) leads to defects in dynamic light touch, noxious thermosensation, mechanical and chemical pain, and itch, but not in motor control (Xu et al., 2013). Further dissection of dI5/dIL$_B$ lineages has shown that the RORα$^+$ subset is in part responsible for dynamic light touch, as discussed above (Bourane et al., 2015b), while noxious thermosensation appears to derive from a LMX1B$^+$ population – potentially the neurons in lamina I that contribute to the spinothalamic tract (STT) division of the ALS (Szabo et al., 2015; Todd, 2010). Meanwhile, at least three subpopulations (positive for somatostatin, SOM, in laminae II-III, calretinin in the inner part of lamina II and the transient vesicular glutamate transporter 3, VGLUT3, in laminae II-III) are important for mechanical allodynia, a condition in which touch becomes painful after injury (Duan et al., 2014; Peirs et al., 2015). Assignment of the SOM$^+$ and transient VGLUT3 populations to the dI5/dIL$_B$ lineage is based on their excitatory nature and their expression of *Lbx1* during development. The origin of the excitatory calretinin population is mixed because most, but not all cells are derived from the *Lbx1* lineage (Duan et al., 2014; Peirs et al., 2015). The SOM$^+$ population makes up a large proportion (~59%) of the excitatory interneurons in lamina II (Gutierrez-Mecinas et al., 2016). Those residing at the lamina II/III border overlap with PKCγ neurons, a population also implicated in mechanical allodynia (Malmberg et al., 1997; Petitjean et al., 2015). SOM$^+$ neurons in the outer part of lamina II and at the II/III border are not normally activated by Aβ low threshold mechanosensory input (touch) because of a feed-forward inhibitory mechanism (discussed below). However, in the context of mechanical allodynia and in accordance with the gate control theory, it is predicted that injury diminishes the feed-forward inhibition (Fig. 6), thus allowing Aβ activation of SOM$^+$ neurons to turn touch into pain (Duan et al., 2014). Transient VGLUT3 cells, which reside predominantly in lamina III, an area of the dorsal horn associated with touch, have been suggested to reside at an entry point to the mechanical allodynia pathway (Peirs et al., 2015).

The neurons that relay chemical itch signals (histaminergic and nonhistaminergic) are GRPR$^+$ and are likely dI5/dIL$_B$ derived since they reside in the superficial laminae and since conditional knockout of TLX3 causes complete elimination of GRPR in the spinal cord (Xu et al., 2013). The GRPR$^+$ neurons receive inputs from unmyelinated C-fiber sensory neurons and are selectively required for itch, as pain sensation is normal in the GRPR knockout mouse and when GRPR$^+$ neurons are ablated (Sun and Chen, 2007; Sun et al., 2009). Although GRPR$^+$ neurons reside in lamina I, they appear to be distinct from STT neurons. Further work is necessary to understand how itch and pain sensations relate (Braz et al., 2014;

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Box 2. Major classes of primary sensory neurons

Myelination / Fiber diameter

Sensory fiber	End organ	Stimulus	Molecular markers
Muscle			
Aα-fibers (> 40 m/s)			
Ia	Muscle spindle	Dynamic stretch	PV, TRKC VGLUT1
Ib	Golgi tendon organ	Tension	PV, TRKC VGLUT1
Aβ-fibers (? m/s)			
II	Muscle spindle	Static stretch	PV, TRKC VGLUT1
Cutaneous			
Aβ-fibers (13.8-40 m/s)			
Aβ-RA1	Meisner's corpuscles Longitudinal laneolate ending	Light stroking Slow vibration	NPY2R
Aβ-RA2	Pacinian corpuscles	Fast vibration	NPY2R
Aβ-SA1	Merkel cells	Sustained indentation	TRKC
Aβ-SA2	Ruffini endings	Stretch	
Aβ-field	Circumferential ending (transverse lanecolate)	Light stroking	TRKC
Aδ-fibers (1.3-13.6 m/s)			
Aδ-HTMR	Free nerve ending hairy and glabrous	Noxious Heat Mechanical	Peptidergic CGRP
Aδ-LTMR (D-hair)	Longitudinal lanceolate ending hairy skin	Light stroking Cooling	TRKB
C-fibers (0.2-1.3 m/s)			
C-C	Free nerve ending hairy and glabrous	Cooling	Non-peptidergic (TRPM8)
C-H	Free nerve ending hairy and glabrous	Heat	Peptidergic (CGRP, TRKA, SP, TRPV1)
C-polymodal	Free nerve ending hairy and glabrous	Noxious Polymodal	Non-peptidergic (MRGPRD, IB4, RET)
C-LTMR	Longitudinal lanceolate ending hairy skin	Light slow stroking Indentation Cooling	Non-peptidergic (TH, VGLUT3, TAFA4)

Several different types of primary sensory neurons transmit somatosensory information from the skin and deep tissues centrally to the spinal cord and/or dorsal column nuclei of the dorsal column-medial lemniscus pathway. General classification is based on size and degree of myelination, varying from the large and heavily myelinated Aα neurons that innervate muscle and transmit proprioception, to the small unmyelinated C-fibers that transmit temperature, pain, itch and some forms of touch. These classes are further divided into groups based on their response to innocuous and noxious mechanical, chemical and thermal stimuli *in vivo*, whether they express neuropeptides or bind IB4, and their pattern of peripheral innervation (Cain et al., 2001; Koltzenburg et al., 1997; Li et al., 2011; Molliver et al., 1997). Most Aβ-LTMRs innervate end organs such as Meissner's corpuscles, Pacinian corpuscles, Ruffini endings and Merkel cells. Others surround hair follicles as longitudinal lanceolate or circumferential endings. With the exception of C-LTMRs and Aδ-LTM (D-hair), which also form longitudinal lanceolate endings, most C- and Aδ-fibers innervate skin as free nerve endings. RA, rapidly adapting; SA, slowly adapting; HTMR, high-threshold mechanoreceptor; LTMR, low-threshold mechanoreceptor.

Jeffry et al., 2011; Ross, 2011), and identifying further subpopulations of the dI5/dIL$_B$ lineage should help catalyze this discussion.

Lastly, while it is now known that many of the neurons relaying pain and itch sensations are dI5/dIL$_B$ derived, the origin of STT neurons from deeper laminae (III-V) are still unknown (Szabo et al., 2015). Additionally, although our discussion has focused on neuronal populations whose developmental lineage is most evident, the developmental source of neurons relaying major pathways for pain and thermosensation is still not completely understood. Teasing out the functional contributions of additional subsets of dI5/dIL$_B$ lineage neurons will require a careful molecular and temporal (early versus late born) analysis to fully understand the developmental origins of functional circuit units as has been done for some of the neurons contributing to mechanical pain and itch.

Inhibitory neurons

Inhibitory neurons are necessary to gate the flow of excitatory information coming in from the different somatosensory modalities (pain, thermosensation, itch, touch and proprioception). The entire set of inhibitory neurons in the dorsal spinal cord is derived from a *Ptf1a*-expressing population that makes dI4 and late-born dIL$_A$ neurons (Glasgow et al., 2005). These *Ptf1a* lineage neurons are a mixture of GABAergic and glycinergic neurons. Ablation of a subset of GABAergic neurons leads to defects in goal-directed reaching behavior and increased scratching behavior (Fink et al., 2014), while ablation or inhibition of glycinergic neurons (many of which also release GABA) leads to increased sensitivity to mechanical pain, thermal sensation and itch (Foster et al., 2015). While these studies have provided important insights, it should be noted that the manipulations could affect a large number of inhibitory neurons that comprise numerous subpopulations. As such, researchers have begun to dissect out the different contributions of subsets of dI4/dIL$_A$ neurons to these different somatosensory behaviors, as has been done for the dI5/dIL$_B$ population. For example, the defect in goal-directed reaching behavior has been attributed to a set of GABApre, GlyT2$^-$ neurons that control the gain of proprioceptive sensory neurons through presynaptic inhibition (Betley et al., 2009; Fink et al., 2014) (Fig. 6B). Furthermore, combinatorial transcription factor expression within the *Ptf1a* lineage directs the expression of distinct neuropeptide fates. Expression of *Lhx*1/5 is required for the NPY$^+$ fate, while expression of *Neurod1*/2/6 is required for the dynorphin-expressing (DYN$^+$) fate (Brohl et al., 2008). The NPY$^+$ dI4/dIL$_A$ lineage mainly in laminae III-IV has recently been shown to gate itch behaviors, specifically mechanical itch as opposed to chemical-evoked itch (histaminergic and non-histaminergic) (Bourane et al., 2015a) whereas the DYN$^+$ fate has been implicated in gating mechanical pain and chemical itch (discussed in the next section).

Manipulations of the dIL$_A$, dynorphin-expressing (DYN$^+$) subset of inhibitory neurons in laminae I-III by two different groups suggest two potential roles for these neurons (Duan et al., 2014; Kardon et al., 2014; Liu et al., 2007; Ross et al., 2010; Xu et al., 2008). Genetic ablation of all developmental and adult DYN$^+$ inhibitory interneurons in the dorsal horn produced a selective and marked increase in mechanical pain sensitivity (Duan et al., 2014) consistent with a role for the cells in gating mechanical allodynia. In contrast, deletion of the *Bhlhb5* transcription factor in the dorsal horn of mice resulted in the developmental apoptosis of mainly the DYN$^+$ inhibitory population [~90% reduction in DYN$^+$ cells when assessed by immunohistochemistry (Kardon et al., 2014) and ~50% reduction when assessed by *in situ* hybridization (Duan et al., 2014)]. Interestingly, the most striking somatosensory phenotype of the *Bhlhb5* knockout mice was an increase in spontaneous

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scratching and histamine-dependent and independent itch (Ross et al., 2010). In terms of pain, the second phase (central sensitization) of the formalin test was increased, which may also reflect an increase in itch (Ross et al., 2010). A role for the DYN^+ inhibitory interneurons in suppressing itch was suggested by the observation that intrathecal delivery of kappa opioid agonists and antagonists inhibit and activate chemical-induced itch, respectively (Kardon et al., 2014). The connectivity of DYN^+ neurons with peripheral sensory neurons was also examined in each study. Duan et al. reported that DYN^+ neurons receive Aβ low threshold input and likely form a feed-forward inhibitory gate onto the dI5/dIL_A SOM^+ pain neurons, consistent with the emergence of mechanical allodynia with DYN^+ cell ablation. In contrast, Kardon et al. (2014) reported that DYN^+ neurons receive input from many types of C-fibers including those activated by heat, pain, chemical and cooling (i.e. afferents that express $TRPV1^+$, $TRPA1^+$ and $TRPM8^+$), suggesting that these neurons form a gate for the inhibition of itch by chemical and thermal counter-stimuli. Indeed, menthol failed to inhibit itch in the *Bhlhb5* knockout mice (Kardon et al., 2014). Results from these studies raise the question as to whether DYN^+ neurons have a role in mechanical pain, chemical itch, or both. Differences in the methods used to manipulate the neurons (i.e. adult ablation versus pharmacological or genetic knockout) or in the number or type of neurons manipulated, may account for the different behaviors observed. Selective and reversible activation or inhibition of the inhibitory DYN^+ population by designer receptors or optogenetics may help to further define the precise role of the neurons in somatosensation.

Notably, overall motor function (as assayed by rotarod, grip strength and ladder rung behaviors) remains mostly intact in all of these manipulations of the dI4/dIL_A lineage (Duan et al., 2014; Fink et al., 2014; Foster et al., 2015; Kardon et al., 2014). This suggests that dI4/dIL_A lineage inhibitory neurons are not necessary for gross motor function and, therefore, that inhibitory neurons in the ventral spinal cord are primarily responsible for gross motor behavior (Arber, 2012; Goulding et al., 2014). However, it has been shown that mice null for *Gbx1*, which marks a subset of dIL_A neurons (John et al., 2005), show no aversive behaviors but do have abnormal hindlimb gait (Buckley et al., 2013; Meziane et al., 2013 preprint). Given that this was a complete *Gbx1* knockout, and knowing that *Gbx1* is expressed more broadly in the ventricular zone of the caudal neural tube and regions that will develop into the hindbrain and inhibitory cortical interneurons (Buckley et al., 2013; John et al., 2005; Rhinn et al., 2004), the manipulation of *Gbx1* lineage neurons specifically in the spinal cord is necessary before a definitive contribution of dIL_A neurons to the gait phenotype can be concluded. Furthermore, as analyses of subsets of *Ptf1a* lineage neurons become more refined, the full extent to which inhibitory neurons gate or attenuate somatosensory inputs will be revealed. Altogether, these findings argue that different molecularly defined subsets of inhibitory neurons derived from the dI4/dIL_A population can gate different somatosensory modalities. Uncovering how the dI4/dIL_A lineage is subdivided could provide further insights into how specific inhibitory sensory microcircuits in the spinal cord develop.

Lastly, a set of inhibitory neurons coming from the dI6 population migrates ventrally and is involved in coordinating gait (Andersson et al., 2012). A natural mutation of the dI6 marker, DMRT3, in horses appears to affect the synchrony of gait types a horse can perform. It is likely that these neurons form a contralateral and ipsilateral set of premotor neurons that have preferences in targeting different subsets of motor neurons and are rhythmically active to coordinate gait (Andersson et al., 2012; Dyck et al., 2012; Goetz et al., 2015).

Conclusions

The developing dorsal spinal cord has been an important model system for understanding the molecular mechanisms that direct cell type specification and differentiation. Seminal work by numerous groups has uncovered the roles of combinatorial TF expression, morphogen gradients, oscillatory expression, repressive mechanisms and TF target genes in setting up discrete progenitor domains that define distinct neuronal cell types. The use of these molecular markers to identify how the lineage of a particular progenitor domain is incorporated into neuronal networks is proving to be a valuable tool for understanding how somatosensory and motor circuits develop, organize and function. Overall, these studies have shown that the dorsal progenitor domains (dI1-6) define neurons generally in the dorsal horn, but that some neurons from these lineages migrate to more ventral regions. Furthermore, the neurons that stem from these domains do not maintain their original dorsal-ventral positioning, but travel quite extensively throughout the dorsal horn with no obvious spatial logic. Lastly, in general, there is both convergence and divergence of both somatosensory modality and developmental lineage. Indeed, a particular progenitor domain can generate neurons belonging to several somatosensory submodalities and neurons that serve in the same somatosensory modality may come from different developmental lineages, although there are some general trends (see Fig. 6), implying that developmental lineage is roughly tied to sensory function.

Future work is needed to understand how different developmental populations set up the neuronal networks in the dorsal spinal cord and confer unique functions for the neurons they generate. Such work could help illuminate how much crosstalk there is between different sensory modalities such as pain, touch and itch that shape our sensory perception. In addition, how different networks in the dorsal spinal cord feed into the motor networks of the ventral spinal cord is still an open question. For example, both V2a neurons and GABApre dI4/dIL_A lineage neurons have been implicated in reaching behavior (Azim et al., 2014; Fink et al., 2014). However, differences in the reaching phenotype suggest that these neurons may be involved in different microcircuits that guide this behavior. As the field moves forward, such careful phenotypic analyses are necessary to allow for accurate functional interpretation of spinal cord neurons in somatosensory behavior.

In the next 10 years, we anticipate that great progress will be made in understanding how somatosensory circuits develop and function. The spinal cord is somatotopically organized, with hindlimb information being processed at lumbar levels and forelimb information at cervical levels. What is the developmental logic that coordinates populations of neurons along the dorsal-ventral and rostral-caudal axes? Furthermore, how does a progenitor population specify a particular function for a set of neurons? How many different subtypes exist within a given developmental population? While progress has been made on all these fronts, we are just at the tip of the iceberg. Indeed, extensive molecular analysis of the V1 population in the ventral spinal cord has identified up to 50 transcriptionally defined subsets that distinguish neuronal populations with unique physiological properties and connectivity (Bikoff et al., 2016; Gabitto et al., 2016). Similarly, identification of molecularly and developmentally defined populations in the dorsal horn is beginning to distinguish microcircuits that mediate particular somatosensory behaviors, such as mechanical allodynia and proprioception (Duan et al., 2014; Peirs et al., 2015; Yuengert

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et al., 2015). Altogether, identifying these circuits will establish the foundation for developing new therapies to treat neuropathic conditions and spinal cord injury. For example, understanding the circuits that underlie pain or itch could lead to targeted therapies that reduce activation of these pathways. Furthermore, knowing how these circuits are built and wired will serve as the basis for directed regeneration of specific pathways for either spinal cord injury or neurodegenerative diseases. Basic understanding of how various tissues develop has already influenced the fields of regenerative medicine and cancer. Likewise, seminal discoveries are anticipated from the new insights gained by studying the development of somatosensory circuits.

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Competing interests

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