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 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)10–12.5] neuronal populations. Six of these (dorsal interneurons 1-6, dI1-6) are found...
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 (dILA and dILB). 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 dIl1 population can be split into two populations that are distinguished by their spatial location and axonal projections: dIl1 (ipsilaterally projecting) and dIl1c (contralaterally projecting) (Mesegaes 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 Dreu 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., 2006; Wildner et al., 2006). This is in contrast to the major specification function (Briscoe et al., 2000; Ericson et al., 1997; Pierani et al., 2001; Sander et al., 2000).

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 dIl1-dI3 (termed class A) populations are dependent on these signals, whereas the more intermediate dIl4-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...
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) 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) 

### Oscillations in Notch signaling and transcription factor expression control neurogenesis

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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/dILA populations and ASCL1-expressing progenitors of dI5/dILB neurons demonstrate cross-repression; in the absence of PTF1A, dI4/dILA neurons are lost and unopposed ASCL1 activity results in excess dI5/dILB 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 7xs3 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
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 Rxed 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 (IIo, CGRP⁺ afferents), inner dorsal (IID, IB4⁺ afferents) and inner ventral (IIV, PKCγ⁺) laminae. 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., 2008), MRGPRD (Zylika 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; Hanman 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., 2011), TRKC, PV (Arber et al., 2000), VGLUT1, Vglut1 (Alvarez et al., 2004; Hanman 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).

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
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 LH2X and LH9X 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; Lair 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/dIIA-dI5/dIIA) 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; Crane 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 the cortex are born from distinct progenitor domains in the ventral telencephalon, as shown by lineage tracing (Jo et al., 2008). In the spinal cord, however, the laminar architecture is defined by TF expression in the ventricular zone, and the V3 domain generates neurons that extend into the dorsal horn.

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.

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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/dILA or dI5/dILb) 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/dILb lineage is involved in chemical itch sensation and a NPY+ subset of the dI4/dILA 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 spino-cerebellar 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/dILA populations form networks involved in proprioceptive and touch-activated or motor pathways involved in smooth movement, while the dI4/dILA and dI5/dILb 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; Oscarnsson, 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
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/dILA and dI5/dILB 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/dILA and dI5/dILB 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+.

### A Domain Proportion Transmitter Type Inputs Target(s) Function Citations

<table>
<thead>
<tr>
<th>Domain</th>
<th>Projection</th>
<th>Transmitter</th>
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<tr>
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<td>I, A</td>
<td>VGLUT2</td>
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<td>Hindbrain*</td>
<td>Prop/gross motor</td>
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<td></td>
<td>dI2</td>
<td>I, A/D</td>
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<td>dI4/dILA</td>
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<td>dI5/dLb</td>
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<td>C/I</td>
<td>GABA/GLY</td>
<td>Premotor</td>
<td>MNs</td>
<td>Motor/gait</td>
<td>Andersson et al. 2012, Goetz et al. 2015</td>
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### B Proprioceptive/motor

- **Cerebellum**
  - ALS target

- **Medulla**
  - Prop Aβ

- **Spinal cord**
  - MNs
  - LF, LA, SF, LMX1B

### C Pain/temperature/itch/touch

- **Thalamus**
  - STT

- **Spinal cord**
  - Aβ
  - V2a
  - VGLUT2
  - DYN
  - NPY
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) 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., 2015b). 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., 2012; 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-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/dILb 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, V0c 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 Islet1Cre+ 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/dILb lineages, which reside throughout the dorsal horn with some ventral expression (Szabo et al., 2015; Xu et al., 2008). Genetic manipulation of dI5/dILb 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/dILb 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/dILb 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 PKCy 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 feedforward 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 feedforward 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/dILb 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;
Jeffry et al., 2011; Ross, 2011), and identifying further subpopulations of the dI5/dILB lineage should help catalyze this discussion.

Lastly, while it is now known that many of the neurons relaying pain and itch sensations are dI5/dILB 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/dILB 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 dILA 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/dILA neurons to these different somatosensory behaviors, as has been done for the dI5/dILB 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 Lhx1/5 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/dILA 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 dILA, 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 alldynia. 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
Although overall motor function (as assayed by rotarod, grip strength and ladder rung behaviors) remains mostly intact in all of these manipulations of the dI4/dILA lineage (Duan et al., 2014; Fink et al., 2014; Foster et al., 2015; Kardon et al., 2014). This suggests that dI4/dILA 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 dILA 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 dILA neurons to the gait phenotype can be concluded. Furthermore, as analyses of subsets of Pf1a 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/dILA population can gate different somatosensory modalities. Uncovering how the dI4/dILA 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, DMR13, 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/dILA 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 et al., 2015).
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.

Acknowledgements

We apologize for not being able to cite the many researchers contributing to our understanding of dorsal spinal cord development. We thank Sarah E. Ross, H. Richard Koerber, Euiseok J. Kim, Tou Y. Vue, Bipasha Mona for valuable input.

Competing interests

The authors declare no competing or financial interests.

Funding

J.E.J. is funded by the National Institutes of Health [R01 HD037932 and R01 NS032817]. R.P.S. is funded by the Rita Allen Foundation and the American Pain Society. Deposited in PMC for release after 12 months.

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