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Review article

The dorsal spinal cord and hindbrain: From developmental mechanisms to functional circuits

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A B S T R A C T

Neurons of the dorsal hindbrain and spinal cord are central in receiving, processing and relaying sensory perception and participate in the coordination of sensory-motor output. Numerous cellular and molecular mechanisms that underlie neuronal development in both regions of the nervous system are shared. We discuss here the mechanisms that generate neuronal diversity in the dorsal spinal cord and hindbrain, and emphasize similarities in patterning and neuronal specification. Insight into the developmental mechanisms has provided tools that can help to assign functions to small subpopulations of neurons. Hence, novel information on how mechanosensory or pain sensation is encoded under normal and neuropathic conditions has already emerged. Such studies show that the complex neuronal circuits that control perception of somatosensory and viscerosensory stimuli are becoming amenable to investigations.

1. Introduction

An astonishing trait of the mature nervous system is the enormous diversity of neurons that vary in anatomical, chemical and electrophysiological properties. This diversity is critical for the correct elaboration of functional circuits that shape the adult brain. It is generated during development, and how specification of the multitude of cell types is coordinated has fascinated developmental and molecular biologists for many years (Tanabe and Jessell, 1996). The basic molecular mechanisms have now been defined. Already early work showed that an important step in the generation of neuronal diversity is the patterning of the embryo (Lumsden and Krumlauf, 1996). Subsequent work demonstrated that morphogens 'pattern' neural progenitors, i.e. assign a spatial and molecular identity to them. Thus, patterning signals drive the expression of specific sets of transcription factors and subdivide the developing nervous system into discrete progenitor domains (reviewed in Cohen et al., 2013; Gavalas and Krumlauf, 2000; Helms and Johnson, 2003). The assigned identity depends on the location of the progenitors in the neural tube, i.e. their exact position along the anterior-posterior and dorsal-ventral axes. The transcription factors expressed in response to patterning signals in turn control the fates of neurons that the progenitors will generate. We will concentrate in our review on a discussion of the mechanisms that create neuronal diversity in the dorsal spinal cord and hindbrain, but it should be noted that similar principles operate in other parts of the central nervous system. We will particularly emphasize similarities in molecular mechanisms employed in development of these distinct parts of the nervous system.

2. Acquisition of hindbrain and spinal cord identity: anterior posterior patterning

Soon after the onset of neural induction, the anterior-posterior identity of progenitors is specified, and hindbrain and spinal cord adopt their posterior identity. This is achieved by signaling centers located in the neural tube and surrounding tissues that produce fibroblast growth factors (FGFs), bone morphogenetic proteins (BMPs), retinoids and Wnt proteins (Doniach, 1995; Lumsden and Krumlauf, 1996; Stern, 2005). The result of this early patterning is a structure in which distinct anterior-posterior segments are defined by the expression of combinations of different transcription factors, mainly members of the Hox family (Philippidou and Dasen, 2013). In the hindbrain, morphologically discrete segmental units called rhombomeres are thus defined (rhombomere 1–7 in mice). In the spinal cord, four major units are distinguished, cervical, thoracic, lumbar and sacral, of which each again contains several segments. Each rhombomere and spinal segment is then patterned along the dorso-ventral axis. The final result of patterning is the formation of a two-dimensional grid of molecularly distinct progenitor regions, each of which expresses specific transcription factors and is able to generate particular neural cell types that will emerge at a stereotypical position at defined developmental stages.

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3. Dorso-ventral patterning

During development of the posterior neural tube, two sets of specialized cells behave as organizers to establish its dorso-ventral axis, the roof and floor plate. Both organizers were first identified on basis of their distinctive cellular morphology. Subsequent transplantation experiments indicate that the floor plate imposes particular fates on neighboring progenitor cells (Roelink et al., 1995). Roof and floor plate act antagonistically and exert their function via the secretion of morphogenic cues like Sonic hedgehog (Shh) by the floor plate or BMPs and Wnt proteins by the roof plate (Liem et al., 1997; Roelink et al., 1995; Ulloa and Marti, 2010) Morphogenic signals diffuse from the site of their synthesis, and are thought to set up concentration gradients. Thus, progenitor cells are exposed to variable concentrations of the cue, which depends on their distance from the source. They respond in a dose-dependent manner to the cue, for instance by expressing a particular transcription factor.

4. The ventricular zone of the dorsal neural tube

The patterning cues acting along the dorso-ventral axis establish 6 and 8 progenitor domains in the dorsal spinal cord and hindbrain, respectively (Fig. 1 and Fig. 2). The dorsal-most progenitor cells (class A progenitors) express the bHLH factor Olig3 that acts together with other bHLH factors to define three or four subdomains (Muller et al., 2005; Storm et al., 2009). These dorsal domains are specified by signals provided by members of the BMP (e.g. GDF7, BMP6/7) and Wnt (Wnt1, Wnt3a) families, which first emanate from the roof plate, and subsequently also from dorsal progenitors (Lee et al., 1998; Liem et al., 1997; Muroyama et al., 2002). Ventrally abutting, progenitor domains (class B progenitors) generate neurons that express Lbx1, and at least one Lbx1+ neuronal type arises independently of roof and floor plate signals. Lbx1+ neurons represent thus a 'default' fate of spinal cord neurons (Gross et al., 2002; Muller et al., 2002; Pagliardini et al., 2008; Sieber et al., 2007). It should be noted however that no single transcription factor defines class B progenitors in a manner as Olig3 does for class A progenitors.

Several pieces of evidence suggest that Wnts and BMPs contribute to the acquisition of the dorsal identity of class A progenitor (reviewed in Ulloa and Marti, 2010). First, loss of GDF7 results in reduced numbers of dI1 progenitors and their derivatives (Lee et al., 1998; Millonig et al., 2000). Ablation of the roof plate eliminates class A neurons, and ablation of BMP type I receptors reduces class A neuron production; class B neurons are generated at their expense (Lee et al., 2000; Wine-Lee et al., 2004). Loss of the Wnt signal transducer β-catenin prevents expression of the bHLH factor Olig3 and eliminates dI1-3 neurons, whereas over-expression of Wnt/β-catenin expands the pool of Olig3+ dorsal progenitors at the expense of more ventral progenitors (Alvarez-Medina et al., 2008; Zechner et al., 2007). Furthermore, ablation of Wnt1/Wnt3a in mice results in a severe decrease of derivatives of dl1-3 progenitors accompanied by generation of supernumerary class B neurons (Muroyama et al., 2002). In keeping with this, inhibition of Wnt signaling by electroporation of dominant-negative forms of Tcfs in chicks represses development of dorsal progenitors and expands ventral markers (Alvarez-Medina et al., 2008, 2009). In addition, Wnt signaling in zebrafish is required for patterning dorsal progenitor domains (Bonner et al., 2008). Thus, BMP and Wnt signals cooperate during dorsal patterning. An important aspect of Wnt in dorsal patterning is its ability to repress Gli3, a transcriptional mediator of Shh (Alvarez-Medina et al., 2009; Lei et al., 2006; Wang et al., 2011). Wnts thus restrict the range of cells that respond to Shh signals. It should be noted that in addition to its patterning function, Wnt also provides proliferative cues for neuronal progenitors (Alvarez-Medina et al., 2009; Bonner et al., 2008; Muroyama et al., 2002; Zechner et al., 2003). Progenitors in all parts of the central nervous system and in many species respond to this proliferative signal.

Whereas dorso-ventral patterning of the spinal cord was extensively studied, reports on mechanisms of hindbrain patterning are scarce. Many of the patterning signals are expressed dorsally and ventrally along the entire spinal cord and hindbrain. Moreover, BMPs and Shh were demonstrated to act during hindbrain patterning (Arkell and Beddington, 1997; Echelard et al., 1993). In addition, many progenitor 'stripes' defined by expression of particular transcription factors extend from the spinal cord into the hindbrain, indicating that general patterning mechanisms are conserved (Fig. 3). For instance, a stripe of Olig3+/Atoh1+ progenitors extends along the entire spinal cord and hindbrain. However, some differences also exist. For instance, Olig3/

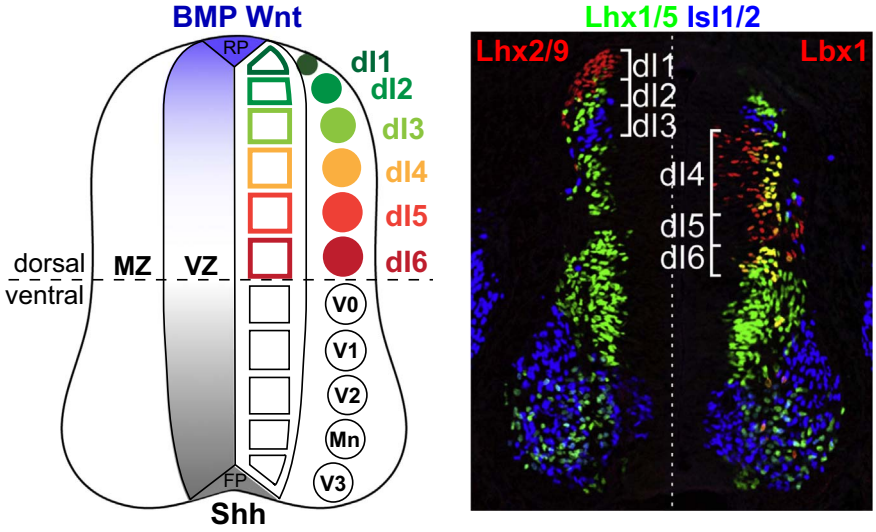

Fig. 1. Development of neuronal cell types in the dorsal spinal cord. (**Left**) Schematic drawing of the developing spinal cord at E11. Graded signals from the roof plate (RP; BMPs and Wnts) and floor plate (FP; Shh) pattern the dorso-ventral axis of the spinal cord. Progenitor cells located in the ventricular zone (VZ) differentially respond to these graded signals, acquire distinct identities and segregate into progenitor domains (illustrated as boxes). Subsequently, each progenitor domain generates a specific neuronal cell type (illustrated by circles) that migrates laterally into the marginal zone (MZ). Six progenitor domains exist in the dorsal spinal cord, which produce six distinct neuronal cell types known as dI1-6. We refer to the three dorsal-most progenitor domains as class A progenitors (illustrated as green boxes), whereas we call the three ventrally abutting progenitor domains class B progenitors (illustrated as red boxes). (**Right**) Composed image of two hemi-sections of the spinal cord immunostained against distinct transcription factors. On the left, the combinatory expression of Lhx1/5, Isl1/2 and Lhx2/9 distinguishes dI1-3 neuronal cell types. On the right, the combinatory expression of Lhx1/5, Isl1/2 and Lbx1 demarcates dI4-6 neural cell types.

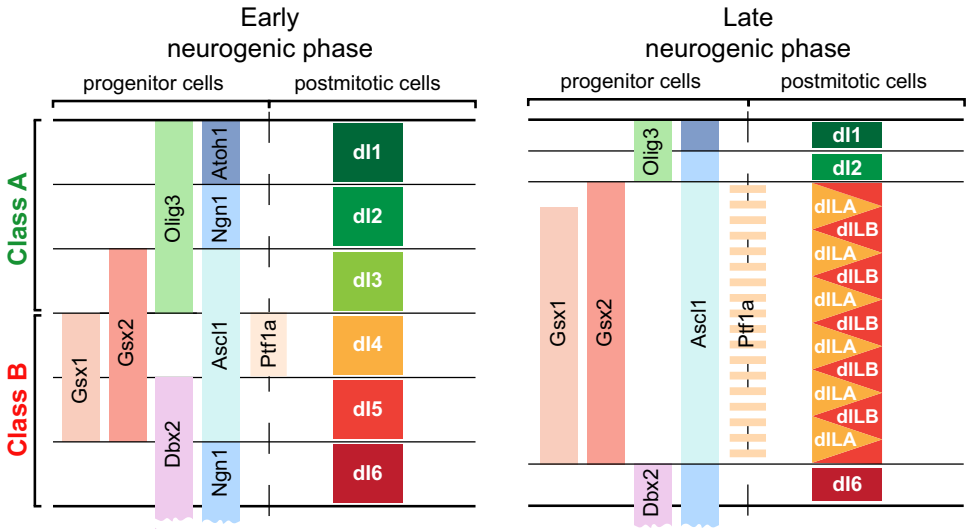

Fig. 2. A transcriptional code specifies progenitor domains and neuronal cell types in the dorsal spinal cord. Neuronal cell types in the dorsal spinal cord are specified in two temporal distinct phases. (**Left**) During the early neurogenic phase (in mice between E9.5-E11.5), two classes of progenitor cells are observed. Class A progenitors express Olig3, and the expression of three additional factors, Atoh1, Ngn1 and Ascl1, further subdivide class A progenitors into three distinct domains. Similarly, the combinatorial expression of Ascl1, Ptf1a, Gsx1, Dbx2 and Ngn1 subdivides class B progenitors into three domains (see Table 1 for additional transcription factors expressed by class A and B progenitors). Each neuronal cell type specified from the class A and B progenitors possesses a distinctive molecular identity (see Table 2). (**Right**) In the late neurogenic phase (in mice between E11.5-E13.5), the class B domain expands, and generates late born neurons known as dILA and dILB. Both dILA and dILB neurons emerge in a salt and pepper manner (see Table 2).

Ngn1 progenitors exist only in the spinal cord and rhombomere 7, but not in other rhombomeres. Further, an additional progenitor domain exists in rhombomeres 2–6 that expresses Phox2b, and this domain is not present in rhombomere 7 or in the spinal cord. We summarize similarities and differences of dorsal progenitor domains present in the hindbrain and spinal cord and the characteristic transcription factors they express in Fig. 2 and Tables 1, 2.

Experimental tools like ablation or ectopic expression provide insights into the function of transcription factors that act downstream of patterning signals. The general outcome of such changes in expression is that the fate of neuronal derivatives shifts, i.e. neurons are still produced from the altered progenitor domain but adopt an 'incorrect' fate, typically the fate of a neighboring population. Thus the patterning factors have a dual function, they specify a particular identity and suppress neighboring fates.

5. Temporal changes in patterning and neuronal specification

The molecularly defined progenitor domains, once established, are not rigid since domain boundaries can shift and new transcription factors can appear. Such changes occur in a temporarily controlled manner and further contribute to the diversity of neural cell types generated. A well-known example is the ventral Olig2+ pMN progenitor domain that gives rise early to motoneurons, subsequently to oligodendrocytes and finally astrocytes (Lu et al., 2002; Masahira et al., 2006; Takebayashi et al., 2002; Zhou and Anderson, 2002). The analogous domain in the hindbrain is known to generate first motoneurons, subsequently serotonergic neurons, and then oligodendrocytes (Jacob et al., 2007; Lu et al., 2002; Pattyn et al., 2000, 2003; Zhou and Anderson, 2002).

	Spinal cord	sc	r7	r6	r5	r4	r3	r2	Hindbrain
Class A	dI1	dI1	dA1	dA1	dA1	dA1	dA1	dA1	dA1
	dI2	dI2	dA2						dA2
	dI3	dI3	dA3	dA3	dA3	dA3			dA3
			dA4	dA4	dA4	dA4	dA4	dA4	dA4
Class B	dI4 dILA dILB	dI4 dILA dILB	dB1 dBLa dBLb	dB1	dB1	dB1	dB1	dB1	dB1 dBLa dBLb
				dB2	dB2	dB2	dB2	dB2	dB2
	dI5	dI5	dB3	dB3	dB3	dB3	dB3	dB3	dB3
	dI6	dI6	dB4	dB4	dB4	dB4	dB4	dB4	dB4

Fig. 3. Neuronal cell types emerging from the dorsal spinal cord and hindbrain. Comparison of neuronal cell types emerging from the dorsal spinal cord (sc) and different rhombomeres of the hindbrain (r2-r7), between E9.5-E11.5 in mice. A number of class A and class B neuronal types are present in the dorsal spinal cord and hindbrain, for instance dI1/dA1 that emerge from progenitors that expresses Olig3 and Atoh1. Other neuronal subtypes are present in the spinal cord but only found in a few rhombomeres, for example the dI2/dA2, which emerge from Olig3+/Ngn1+ progenitor cells, that is only present in the rhombomere 7. The developing hindbrain contains two unique neuronal types not observed in the spinal cord, i.e. dA4 and dB2. Similar to the spinal cord, a class B progenitor domain expands during late neurogenesis (E11.5-E13.5) in the dorsal hindbrain and generates dBLa and dBLb that exhibit similar molecular identities as dILA and dILB neurons of the spinal cord.

Table 1
Transcription factors expressed in progenitor domains of the dorsal hindbrain and spinal cord.

Early progenitor domains	bHLH transcription factors	Homeodomain transcription factors
pdI1/pdA1	Olig3, Atoh1	Pax3, Msx1
pdI2/pdA2	Olig3, Ngn1, Ngn2	Pax3, Pax7^{low}, Msx1
pdI3/pdA3	Olig3, Ascl1, Ngn2	Pax3, Pax6, Pax7, Gsx2
pdA4	Olig3, Ascl1, Ngn2, Ptf1a	
pdI4/pdB1	Ascl1, Ngn2, Ptf1a	Pax3, Pax6, Pax7, Gsx1/2
pdB2		Phox2b
pdI5/pdB3	Ascl1	Pax3, Pax6, Pax7, Gsx1/2, Dbx2
pdI6/pdB4	Ngn1, Ngn2	Pax3, Pax6, Pax7, Dbx2
Late progenitor domains	**bHLH transcription factors**	**Homeodomain transcription factors**
pdILA/pdBLa	Ascl1, Ptf1a	Pax3, Pax6, Pax7, Gsx1/2
pdILB/pdBLb	Ascl1	Pax3, Pax6, Pax7, Gsx1/2

Transcription factors reported to be expressed in progenitor cells of the dorsal hindbrain and spinal cord are indicated in blue, those only observed in the hindbrain are indicated in orange. The expression patterns of most homeodomain transcription factors have been characterized primarily in the spinal cord, which is indicated in black.

Table 2
Transcription factors expressed in neuronal cell types emerging from the dorsal hindbrain and spinal cord.

Early born neuron types	Transcription factors
dI1/dA1	Pou4f1, Barh1, Lhx2, Lhx9, Evx1
dI2/dA2	Pou4f1, Lhx1, Lhx5, Foxd3, Foxp2
dI3/dA3	Pou4f1, Tlx3, Prrxl1, Isl1, Phox2b, Lmx1b
dA4	Foxd3, Foxp2
dI4/dB1	Lbx1, Pax2, Lhx1, Lhx5
dB2	Lbx1, Phox2b, Atoh1
dI5/dB3	Lbx1, Tlx3, Lmx1b, Prrxl1, Pou4f1, Prrxl1
dI6/dB4	Lbx1, Pax2, Lhx1, Lhx5, Wt1, bHLHb5, Dmrt3
Late born neuron types	**Transcription factors**
dILA/dBLa	Lbx1, Pax2, Lhx1, Lhx5, bHLHb5
dILB/dBLb	Lbx1, Tlx3, Lmx1b, Prrxl1

Transcription factors reported to be expressed in neuronal cell types born in the dorsal spinal cord and hindbrain are indicated in blue, those only observed in the hindbrain and spinal cord are indicated in orange and black, respectively.

In the dorsal spinal cord and hindbrain, several progenitor domains are known to generate distinct neurons in early and late development (Fig. 2). Examples are the Ascl1 domain present in the spinal cord and hindbrain that gives rise early to a single Lbx1+ neuronal type but later generates two other Lbx1+ types that arise in a salt and pepper pattern (Gross et al., 2002; Muller et al., 2002). A second example, the Olig3+/Ascl1+ progenitor domain for dA3 neurons in the hindbrain gives first rise to adrenergic neurons (that depend on the expression of both factors), and generates later the nucleus of the solitary tract (that depends on Olig3) (Pattyn et al., 2006; Storm et al., 2009). Many other domains generate more than a single neuronal derivative, and these frequently arise during distinct developmental stages.

6. Neuron types produced in the dorsal spinal cord and hindbrain

Even if we restrict ourselves to the development of the dorsal spinal cord and hindbrain, a stunning diversity of neuronal types is generated. From the various progenitor domains described below, neuronal types are produced that each express distinct sets of transcription factors. Many of these factors define specific neuronal characteristics like neurotransmitter identity, migratory pattern and thus the site where the neuron eventually settles, or its axonal projection pattern that determines the integration into functional circuits. Because of the neuronal heterogeneity, specific combinations of transcription factors provide good tools to define a particular neuronal type. Moreover, the analyses of animals that carry mutations in such transcription factor genes have provided useful insights into the function of circuits in which the neurons are integrated.

7. Major neuronal classes in dorsal spinal cord and hindbrain

Olig3 is the major determinant that specifies class A neuronal identity. Ablation of Olig3 interferes with the normal generation of all class A neurons, and instead supernumerary Lbx1+ class B neurons are specified in the entire dorsal spinal cord and hindbrain. Conversely, overexpression of Olig3 suppresses the emergence of class B neurons (Liu et al., 2008; Muller et al., 2005; Storm et al., 2009). Lbx1 is produced by all class B neurons and is acting in an apparently antagonistic manner to Olig3. When Lbx1 is ablated, all class B neurons assume class A fates, and ectopic expression of Lbx1 suppresses class A fates (Gross et al., 2002; Muller et al., 2002; Pagliardini et al., 2008; Sieber et al., 2007). However, no single transcription factor expressed by class B progenitors is known to eliminate production of all class B neurons. We will go on to shortly describe the different neuronal types that emerge from the dorsal spinal cord and hindbrain, emphasizing transcription factors whose expression can be used for their identification and that are often also essential for their development (Fig. 2 and Fig. 3). Additional markers for these progenitor domains and neurons are shown in Table 1 and Table 2.

8. Class A neurons

8.1. dI1 and dA1 neurons

The identity of the most dorsal progenitor domain in the hindbrain and spinal cord is defined by co-expression of Olig3 and Atoh1, and these generate dI1 and dA1 neurons in the spinal cord and hindbrain, respectively (Ben-Arie et al., 1997; Bermingham et al., 2001; Helms and Johnson, 1998; Muller et al., 2005; Storm et al., 2009). Both, dI1 and dA1 neurons acquire an excitatory phenotype and express the same molecular markers (Pou4f1, Barh1/2, Lhx2/9) (Bermingham et al., 2001; Ding et al., 2012; Saba et al., 2003). In the spinal cord, dl1 neurons settle in the intermediary spinal cord where they are innervated by proprioceptive neurons. They project anteriorly, in an ipsi- or contralateral manner, through the spinocerebellar tracts (Bermingham et al., 2001; Kohl et al., 2012). Early reports already indicated that the population is heterogeneous and that the derivatives possess distinct characteristics, which depend on the time of their birth and might correlate with projection patterns (Lee et al., 1998). In the hindbrain, dA1 derivatives have been carefully mapped by lineage-tracing experiments (Landsberg et al., 2005; Liu et al., 2008; Machold and Fishell, 2005; Machold et al., 2011; Miesegaes et al., 2009; Rose et al., 2009a; Wang et al., 2005). They function in sensory information processing (proprioception and interoception). In particular, they generate all precerebellar nuclei that form mossy fibers, i.e. the pontine gray, reticulotegmental, lateral reticular and external cuneate nuclei. In summary, the most dorsal progenitors in the hindbrain and spinal cord express similar sets of transcription factors and generate neuronal populations that share many aspects of their molecular identity; subpopulations take over functions as proprioceptive relay neurons.

8.2. dI2 and dA2 neurons

The ventrally abutting progenitor domain is defined by co-expression of Olig3, Ngn1 and Ngn2, and exists in rhombomere 7 and the

spinal cord (Gowan et al., 2001; Kriks et al., 2005; Landsberg et al., 2005; Muller et al., 2005; Storm et al., 2009). Neuronal populations derived from these progenitors have an excitatory fate, and co-express Pou4f1, Lhx1, Lhx5. A distinguishing feature of these neurons in the spinal cord (dl2) and hindbrain (dA2), is the fact that they co-express Foxd3 and Foxp2 respectively (Pagliardini et al., 2008; Storm et al., 2009). In the spinal cord, dI2 some neurons migrate to the intermediate layers (VI, VIII, and X) of the spinal cord and they might project through the spinothalamic tract. A second subpopulation migrates to the ventral horn, and projects ascending commissural axons (Gross et al., 2002). The fate of hindbrain dA2 neurons has not been defined yet.

8.3. dI3 and dA3 neurons

The identity of these progenitors is defined by co-expression of Olig3, Ascl1, Ngn2 and Gsx2 (Helms et al., 2005; Kriks et al., 2005; Muller et al., 2005; Pattyn et al., 2006; Storm et al., 2009). Neurons that derive from this domain are excitatory, express Pou4f1, Tlx3 and Prrxl1. They are called dI3 in the spinal cord and dA3 in the hindbrain and differ by expression of Isl1 and Phox2b, respectively (Chen et al., 2001; Cheng et al., 2004; D'Autreaux et al., 2011; Liu et al., 2008; Muller et al., 2005; Qian et al., 2001, 2002; Storm et al., 2009). dI3 neurons locate to the deep and intermediate dorsal horn of the spinal cord and relay low threshold cutaneous information to motoneurons (Bui et al., 2013; Stepien et al., 2010). In rhombomere 7, dA3 neurons mature into several derivatives that might differ in their birth date: (i) area postrema, a chemosensory nucleus in the dorsal hindbrain that controls vomiting; (ii) the neighboring nucleus of the solitary tract that relays viscerosensory information; (iii) caudal adrenergic neurons that settle in the dorsal and ventral hindbrain and to which baroreflex functions have been assigned (Dauger et al., 2003; Pattyn et al., 2006).

8.4. dA4 neurons- a unique population generated in the hindbrain

The further ventrally abutting class A progenitor domain exists in rhombomere 2–7 and is defined by co-expression of Olig3, Ngn2, Ascl1 and Ptf1a (Kim et al., 2008; Liu et al., 2008; Storm et al., 2009; Yamada et al., 2007). A single neuronal population emanates from this domain, which is called dA4. dA4 neurons co-express Foxd3, Lhx1, Lhx5 and FoxP2 and form the inferior olive, a pre-cerebellar structure located in the ventral hindbrain that projects climbing fibers (Iskusnykh et al., 2016; Liu et al., 2008; Storm et al., 2009).

9. Class B progenitors

Class B progenitors represent a heterogeneous population that can be subdivided. Their neuronal derivatives share expression of Lbx1, an important determinant of their fate.

9.1. dl4 and dB1 neurons

The most dorsal class B progenitor domain, is defined by co-expression Ascl1, Ngn2, Ptf1a and Gsx1/2 (Glasgow et al., 2005; Gross et al., 2002; Helms et al., 2005; Muller et al., 2002; Pagliardini et al., 2008; Sieber et al., 2007). This domain generates dl4 and dB1 neurons (Lbx1+, Pax2+, Lhx1+, Lhx5+) in the spinal cord and hindbrain, respectively, that assume an inhibitory fate. The spinal dI4 neurons migrate ventrally into the lateral deep dorsal horn and project ipsilaterally to superficial spinal cord laminae containing sensory interneurons. In the hindbrain, dB1 neurons appear to migrate to various ventral locations where they contribute a large proportion of inhibitory interneurons and are thought to modulate local circuits (Gross et al., 2002; Iskusnykh et al., 2016; Muller et al., 2002; Pagliardini et al., 2008; Pillai et al., 2007; Sieber et al., 2007).

9.2. dB2 neurons are unique for the hindbrain

This progenitor domain exists only in rhombomeres 2–6. Its identity is defined by Phox2b expression, and it generates dB2 neurons. dB2 neurons become excitatory, co-express Phox2b and Lbx1 and a small subpopulation turns on Atoh1 during maturation (Dubreuil et al., 2008; Pagliardini et al., 2008; Rose et al., 2009b; Sieber et al., 2007). This subpopulation of dB2 neurons settles around the trigeminal and facial motor nuclei. The best-characterized dB2 derivative is the retrotrapezoid nucleus that is located below the facial motor nucleus, and comprises a small group of rhythmically active neurons, which increase their firing rate when the pH of the blood becomes acidic due to high CO_2 levels. The retrotrapezoid nucleus thus senses CO_2 and accordingly adjusts the breathing rate by entraining the activity of the respiratory rhythm generator, the preBötzinger complex (Dubreuil et al., 2008, 2009; Huang et al., 2012; Ruffault et al., 2015).

9.3. dI5/dB3 neuron types

The identity of the progenitor domain is defined by co-expression of Ascl1, Gsx1 and Gsx2. Neuronal populations emerging from this spinal and hindbrain domain are termed dl5 and dB3, respectively. The neurons become excitatory and co-express Lbx1, Pou4f1, Prrxl1, Tlx3 and Lmx1b (Chen et al., 2001; Cheng et al., 2004; Gross et al., 2002; Muller et al., 2002; Pagliardini et al., 2008; Sieber et al., 2007). Their derivatives are not yet clearly defined.

9.4. dI6/dB4 neuron types

The identity of the progenitor domain is defined by the co-expression of Ngn1, Ngn2 and Dbx2 (Gross et al., 2002; Muller et al., 2002; Pagliardini et al., 2008; Pierani et al., 2001; Sieber et al., 2007). Neuronal populations emerging are termed dl6 and dB4 neurons in the spinal cord and hindbrain, respectively. These neurons become inhibitory, express bHLHb5, Lbx1, Pax2, Lhx1, Lhx5, Dmrt3 and a subpopulation expresses Wt1 (Andersson et al., 2012; Gross et al., 2002; Muller et al., 2002; Pagliardini et al., 2008; Sieber et al., 2007). In the spinal cord, dI6 neurons are diverse and some subpopulations also use glycine as inhibitory neurotransmitter. They project contralaterally and function in coordinating locomotor activity of the left and right limbs (Andersson et al., 2012; Lanuza et al., 2004). In the hindbrain, dB4 neurons become glycinergic and GABAergic inhibitory interneurons, and a fraction contributes to the Bötzinger nucleus that functions in respiration (Pagliardini et al., 2008).

9.5. dILA/dBLa neuronal types

The identity of these progenitors is defined by the expression of Ascl1 and Gsx1/2 and they appear to arise from the early dI4/dB1 progenitor domain that expands dorsally in later development. A subpopulation of the differentiating progenitors expresses Ptf1a and generates dlLA and dBLa neurons in the spinal cord and hindbrain, respectively. Like dI4/dB1 neurons, they assume an inhibitory fate and co-express Lbx1, Pax2, Lhx1 and Lhx5 (Borromeo et al., 2014; Cheng et al., 2004; Glasgow et al., 2005; Gross et al., 2002; Muller et al., 2002; Pagliardini et al., 2008; Pillai et al., 2007; Sieber et al., 2007). dILA neurons settle in superficial laminae (i.e I-III) of the dorsal horn and function in gating of somatosensory information. dILA are heterogeneous and subpopulations express Neurod1/2/6, Lhx1/5 and bHLH5b (Brohl et al., 2008; Huang et al., 2008). In the hindbrain, dBLa neurons account for many inhibitory neurons, and a large subpopulation settles in the spinal trigeminal nucleus (Hori et al., 2008; Pagliardini et al., 2008; Sieber et al., 2007), which has a similar function in sensory information processing as the dorsal horn of the spinal cord.

9.6. dILB/dBLb neuron types

The same late progenitor domain that expresses Ascl1 and Gsx1/2 generates a second neuronal type, dILB/dBLb that emerges from Ptf1a-negative progenitors (Borromeo et al., 2014; Gross et al., 2002; Muller et al., 2002; Pagliardini et al., 2008; Sieber et al., 2007). Thus, dILB/dBLb and dlLa/dBLa emerge in a salt and pepper pattern. The neurons emerging in the spinal cord (dlLB) and hindbrain (dBLb) become excitatory and co-express Lbx1, Prrxl1, Pou4f1, Tlx3 and Lmx1b. In the spinal cord, dILB neurons migrate dorsally, occupy laminae I-V of the dorsal horn and receive information on temperature, mechanosensory and noxious stimuli from the skin (Chen et al., 2001; Cheng et al., 2004, 2005; Ding et al., 2004; Gross et al., 2002; Muller et al., 2002; Qian et al., 2002; Rebelo et al., 2010; Szabo et al., 2015; Xu et al., 2013). In the hindbrain, dBLb neurons generate the spinal trigeminal nucleus that receives sensory information from facial skin (Pagliardini et al., 2008; Qian et al., 2001; Sieber et al., 2007). Thus, spinal cord and hindbrain derivatives of these progenitors have similar molecular identity and function.

10. Postmitotic maturation and its contribution to neuronal diversity

As outlined above, general mechanisms of generation of neuronal diversity in the hindbrain and spinal cord are now known. Once specified, neurons mature and express particular neurotransmitters, neurotransmitter receptors, extend dendrites and axons and assemble into circuits. Many of these features are determined by the transcription factors they express when they reach a postmitotic state. However, during postmitotic maturation additional mechanisms are active that refine neuronal properties, and that are less well understood. One clear example of this phenomenon is the postmitotic development of dILB neurons in mice, an excitatory population that settles in the dorsal horn. At the time of their birth (around E12) they co-express Lbx1, Pou4f1, Tlx3 and Lmx1b. These neurons subsequently move to the dorsal spinal cord and arrange at E13.5 in layers in which the most superficial layer maintains expression of Tlx3/Lmx1b but extinguishes Pou4f1 and Lbx1, the ventrally abutting layer maintains Lbx1/Pou4f1 and the most ventral layer maintains only Pou4f1 (Fig. 4; Gross et al., 2002; Muller et al., 2002). In addition, a multitude of neuropeptides becomes subsequently expressed in distinct subpopulations, frequently but not always in a layer-specific manner (Xu et al., 2008, 2013). Which signals are responsible for layering has remained an open question. In particular, sensory innervation commences after the early layering of the dorsal horn has happened and can therefore not account for it. Indeed, independent analyses indicate that cues provided by spinal neurons guide sensory neurons to specific layers in the spinal cord.

Also inhibitory neurons diversify during postmitotic maturation. Previously all inhibitory neurons were thought to possess similar local projection patterns, and to be relatively homogenous. Their molecular and functional diversity is only beginning to be defined. For instance, all dILA neurons born late in the dorsal spinal cord dependent on Ptf1a and Rbpj for their specification (Borromeo et al., 2014; Gowan et al., 2001; Hori et al., 2008). The Ptf1a and Rbpj interaction represents a non-canonical Rbpj function, as Rbpj mainly acts as transcriptional mediator of Notch. The postmitotic inhibitory dILA cells co-express Lbx1, Pax2, and Lhx1/5, but during maturation these factors are not uniformly maintained in all inhibitory neurons (Pillai et al., 2007). In addition, Neurod1/2/6 and bHLHb5 appear in subpopulations of these neurons. bHLHb5-dependent interneurons are required to suppress the sensation of itch (Ross et al., 2010). The transcription factors drive expression of neuropeptides and other functionally important receptors, for instance Neurod1/2/6 specify dynorphin+ and galanin+ inhibitory subpopulations, whereas Lhx1/5 instruct the NPY inhibitory fate (Fig. 5; Brohl et al., 2008). Interestingly, some inhibitory neuronal

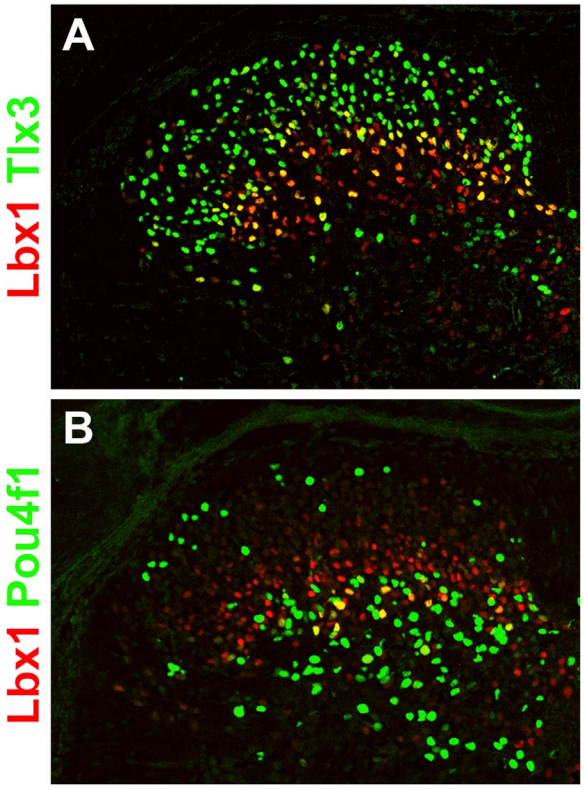

Fig. 4. Postmitotic maturation of late born excitatory and inhibitory class B neuronal types. Transverse sections of the lumbar spinal cord immunostained for Lbx1 shown in red, Tlx3 (**A**) and Pou4f1 (**B**) shown in green at E18.5. During postmitotic development of dILB neurons, the three transcription factors that were initially co-expressed become differentially expressed in the subpopulations.

subtypes are again arranged in layers of the dorsal horn (Wildner et al., 2013). Currently, nine different inhibitory neuronal subtypes were distinguished in the dorsal horn, but whether this is the final number remains open (Del Barrio et al., 2013). New technologies like single-cell sequencing can help to analyze this diversity. A recent estimate of the number of inhibitory neuronal types in the ventral spinal cord indicates that nineteen transcription factors are expressed in one single lineage (V1) during maturation, and their combinatorial expression distinguishes as many as fifty inhibitory neuronal types (Bikoff et al., 2016). Thus, the molecular heterogeneity of interneuron types in the spinal cord is as stunning as the one of excitatory neurons. Moreover, evidence for functional diversity starts to emerge.

11. Processing of cutaneous sensory information in the hindbrain and spinal cord

As discussed, excitatory and inhibitory second-order sensory neurons in the dorsal horn and the spinal trigeminal nucleus derive from dILB/dBLb and dILA/dBLa neurons. Excitatory and inhibitory neurons are distinguished by transient expression of Pou4f1/Tlx3/Lmx1b and Ptf1a/Pax2/Lhx1/5 that impose excitatory and inhibitory fates, respectively. Second-order sensory neurons are innervated by primary sensory neurons that convey information about different modalities like touch, temperature or pain. In the spinal cord, afferents from first-order nociceptive neurons synapse on interneurons in laminae I-II (marginal zone and substantia gelatinosa), while afferents from low-threshold mechanosensory neurons target interneurons located in laminae III-V (the nucleus proprius) (Abraira and Ginty, 2013; Snider and McMahon, 1998; Todd, 2010). Moreover, a somatotopic map exists, and cutaneous afferents with distal peripheral targets in the limbs project to the medial dorsal horn, while those with more proximal peripheral targets project laterally (Levinsson et al., 2002;

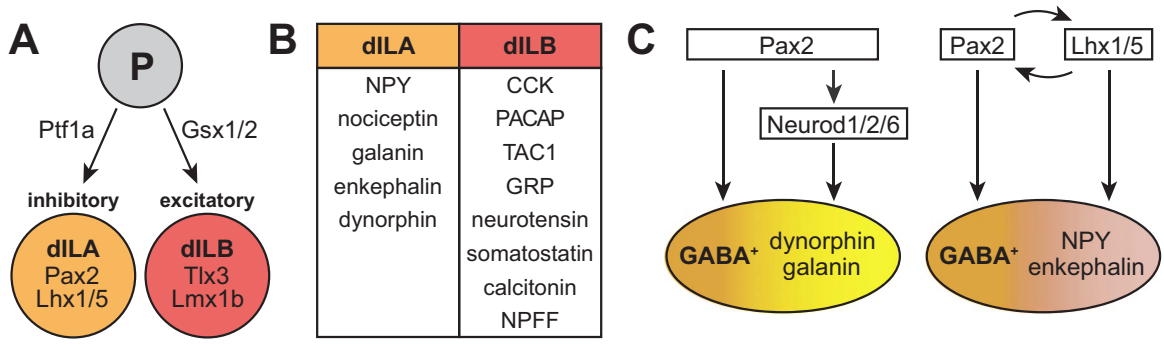

Fig. 5. Postmitotic maturation and neuropeptide expression in dorsal horn neurons. (**A**) Class B progenitor cells (P) specify inhibitory (dILA) and excitatory (dILB) neuronal fates that depend on Ptf1a and Gsx1/2, respectively. At the time of birth, dILA neurons co-express Pax2 and Lhx1/5, while dILB express Tlx3, Lmx1b and Brn3a. (**B**) During postmitotic maturation, inhibitory and excitatory neurons begin to express distinct neuropeptide and neuropeptide receptors. **(C)** Molecular mechanisms that control neurotransmitter and neuropeptide phenotypes in inhibitory neurons. Pax2 directly controls the expression of Gad1 and thus imposes an inhibitory fate. Pax2 together with downstream factors like Neurod1/2/6 controls the expression of neuropeptides like dynorphin and galanin, whereas Lhx1/5 controls neuropeptides NPY and enkephalin.

Wilson and Kitchener, 1996).

In addition, a somatotopic map exists in the second-order sensory neurons of the spinal trigeminal nucleus that is comprised of three subdivisions located along the anterior posterior axis, oralis, interpolaris and caudalis subnuclei. For instance, nociceptive and mechanoreceptive input from the orofacial region are processed in the oralis and caudalis subnuclei (Ren and Dubner, 2011). Further, somatosensory information from the whiskers is represented in modular units known as barreletes that are arranged in rows and each barrelete receives mechanosensory information from a single whisker. Barreletes are restricted to interpolaris and caudalis subnuclei (Erzurumlu and Jhaveri, 1992; Erzurumlu et al., 2010; Ma, 1991).

In addition to the molecular and functional similarities between second-order sensory neurons in the spinal cord and hindbrain, they also share a number of common brain targets. Anterograde and retrograde axonal tracing experiments illustrated that neurons of the dorsal horn and the spinal trigeminal nucleus project to hindbrain nuclei like the viscerosensory nucleus of the solitary tract and parabrachial complex, which might function in somato-visceral reflexes (Cechetto et al., 1985; Menetrey and Basbaum, 1987; Sato, 1995).

12. Ontogenetic relationship between somatosensory and visceroscensory neurons in the hindbrain

In addition to somatosensory neurons that perceive exteroceptive stimuli, the hindbrain contains neurons that process and relay viscerosensory information necessary for regulating body homeostasis. These second order viscerosensory neurons express Phox2b, and are located in the dorsal hindbrain and in the nucleus of the solitary tract, a dA3 neuronal derivative (Dauger et al., 2003). It is interesting to note that Phox2b expression is an evolutionarily conserved feature of the viscerosensory system (Nomaksteinsky et al., 2013). In the absence of Phox2b, the neurons retain a sensory fate but generate somatosensory instead of viscerosensory neurons. Thus, Phox2b acts as a selector gene and imposes a viscerosensory fate on dA3 neurons (D'Autreaux et al., 2011). A reverse mechanism is observed when Lbx1 is mutated: then neurons that normally possess the fate of somatosensory relay neurons instead assume a fate of viscerosensory relay neurons. Thus, Lbx1 suppresses the viscerosensory and imposes a somatosensory fate (Sieber et al., 2007). In conclusion, the loss (or gain) of a single transcription factor can decide on development of second order viscero- and somatosensory neuronal fate.

These findings raise interesting questions about the evolution of the somatic-visceral dualism. Did the somatic and visceral sensory nervous system evolve independently, or by duplication of a single generic sensory precursor circuit? In this connection it is interesting to note that the hypothetical ancestor of all bilateral animals (the ur-bilaterian) was a marine animal. In the marine world, the canonical viscerosensory stimuli that provide information about the interior milieu (e.g. osmolarity, pH, O_2 and CO_2 concentration) reflect the quality of the surrounding water and are thus exterior cues. This would imply that 'visceral' and 'somatic' circuits in the ur-bilaterian organisms were in fact identical in the sense that they convey information about the environment, and thus might have been sorted into divergent circuits during subsequent evolution (cf. Bertucci and Arendt, 2013).

13. Functional circuits of dorsal horn neurons

The perception of pain is an important defense mechanism, but in many clinical settings pain is perceived even if appropriate stimuli are lacking. Dorsal horn neurons process somatosensory information, convey it to higher brain centers and are therefore essential for the perception of pain. How sensory information is processed, and how information processing goes wrong when pain is felt without an appropriate stimulus can be due to a dysfunction of dorsal horn neurons (Prescott et al., 2014). It has been very difficult to assign specific functions to the heterogeneous neuron types, but information about their development and molecular diversity has provided tools that allowed investigating and defining their specific functions in sensory-related behaviors. By using intersectional genetics, the groups of Martyn Goulding and Qiufu Ma recently defined discrete class B neuronal types that have specific roles in transmission and gating of mechanical pain. Lbx1+/somatostatin+ neurons are enriched in lamina II of the dorsal horn and required to sense mechanical but not thermal pain. They transmit mechanosensory information from incoming Aβ • fibers to pain output neurons located in laminae I and V. Ablation of these excitatory neurons eliminated mechanical allodynia, a mechanism that sensitizes to pain and constitutes a central response to peripheral inflammation (Duan et al., 2014). An additional second population, Lbx1+/dynorphin+ inhibitory neurons, also receives input from Aβ•fibers and locates to lamina II. Ablation of Lbx1+/dynorphin+ neurons results in spontaneous allodynia, which occurs since the neurons normally gate Aβ•fibers and thus suppress the activation of Lbx1+/somatostatin+ neurons (Duan et al., 2014). A third subtype of class B neurons is directly involved in mechanical itch, a particular itch sensation induced by light mechanical stimuli that is normally suppressed by mechanoreceptive inputs. Specifically, elimination or silencing of Lbx1+/NPY+ inhibitory neurons enriched in laminae III and IV results in spontaneous and continuous scratching after light mechanical stimulation, which suggests that Lbx1+/NPY+ neurons suppress mechanical itch perception without altering the sensitivity to chemical itch or pain (Bourane et al., 2015). Thus, the role of the dorsal spinal cord in encoding painful sensations and neuropathic conditions is becoming clearer.

14. Summary and outlook

The enormous diversity of neuronal types that arise in the dorsal spinal cord and hindbrain has for many years fascinated developmental and molecular neurobiologists. Intense research on the developmental mechanisms that underlie this diversity has provided new genetic tools that define neuronal subpopulations with greater precision. The long-lasting obstacles associated with the analysis of neurons in specific circuits that control animal behavior are thus becoming amenable for neuroscientist. The knowledge on molecular mechanisms that impose particular neuronal fates and contribute to neural heterogeneity are beginning to be used together with genetic tools that ablate, silence or activate specific neuronal populations. Such approaches allow the exploration of functional circuits with greater detail, and already provided exciting insights into the physiological function of specific neuronal subtypes. Such strategies are opening also new avenues for the study of animal behavior.

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