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Review

The making of a proprioceptor: a tale of two identities

Joriene C. de Nooij[1,2,*] and Niccolò Zampieri[3,*]

Proprioception, the sense of body position in space, has a critical role in the control of posture and movement. Aside from skin and joint receptors, the main sources of proprioceptive information in tetrapods are mechanoreceptive end organs in skeletal muscle: muscle spindles (MSs) and Golgi tendon organs (GTOs). The sensory neurons that innervate these receptors are divided into subtypes that detect discrete aspects of sensory information from muscles with different biomechanical functions. Despite the importance of proprioceptive neurons in motor control, the developmental mechanisms that control the acquisition of their distinct functional properties and positional identity are not yet clear. In this review, we discuss recent findings on the development of mouse proprioceptor subtypes and challenges in defining them at the molecular and functional level.

Highlights

Recent single-cell transcriptome analyses have defined the molecular correlates of mouse proprioceptor subtypes innervating different muscles and end-organ receptors.

Proprioceptor muscle subtypes present distinct molecular profiles according to the identity of the muscle they innervate and express gene programs involved in the control of target selectivity.

Transcriptional profiling of proprioceptor receptor subtypes reveals up to seven molecularly distinct muscle spindle afferents, but only a single class of Golgi tendon organs, suggesting a higher need for precisely calibrated spindle feedback.

Proprioceptor muscle-type identity becomes apparent concomitantly with muscle innervation, while receptor-type appears to be defined after end-organ innervation, indicating a differential reliance on intrinsic and extrinsic signals.

The developmental dynamic of molecular identities suggests that proprioceptors undergo plastic changes in gene expression that reflect the needs of their different functional states.

Organization and function of proprioceptive neurons

Proprioceptive end organs in skeletal muscles are innervated by distinct sensory afferents – groups Ia and II for MSs and group Ib for GTOs – with specialized morphological, anatomical, and physiological properties [1]. Groups Ia and II afferents wrap around the intrafusal muscle fibers of the spindle, with Ia afferents located in the central part and group II afferents flanking them on one or both sides (Figure 1) [2,3]. Both afferents are sensitive to increases in muscle length such that their firing rates correlate with changes in limb/body position [4,5]. However, group Ia fibers possess a high dynamic sensitivity and can also relay information on the rate of change of muscle length, while group II afferents primarily report static muscle length [6,7]. Group Ib fibers innervate GTOs located at myotendinous junctions and chiefly respond to increases in tendon tension [5,8,9]. As such, group Ib firing rates are a measure of force.

The central collaterals of proprioceptive neurons innervate motor circuits in the spinal cord and brainstem. The specificity of these connectivity patterns is dictated by the afferent receptor type (groups Ia, Ib, or II) as well as the location and biomechanical function of the innervated muscle (Figure 1) [10–12]. At spinal levels, proprioceptive muscle afferents engage with several reflex pathways controlling alternation and coactivation of agonist and antagonist muscles [13–16]. Within supraspinal motor centers, proprioceptive feedback serves important roles in motor planning, learning, and perception [17–19]. Thus, in order to control movement and posture, the sensory information originating from Ia, Ib, and II afferents innervating individual muscles needs to be correctly integrated into the appropriate sensorimotor circuits controlling the body part where the feedback is generated. Here, we review current understanding of the molecular basis that underlies the remarkable diversity in proprioceptive sensory neurons, focusing on the mouse as a model system for vertebrate proprioception. We first discuss recent advances that led to the identification of molecularly distinct proprioceptor subtypes. We then consider the developmental logic and mechanisms that may control the acquisition of the defining features of individual proprioceptor subtypes.

[1]Department of Neurology, Division of Translational Neurobiology, Vagelos College of Physicians and Surgeons, 650 West 168th Street, New York, NY 10032, USA
[2]Columbia University Motor Neuron Center, Columbia University Medical Center, 630 West 168th Street, New York, NY 10032, USA

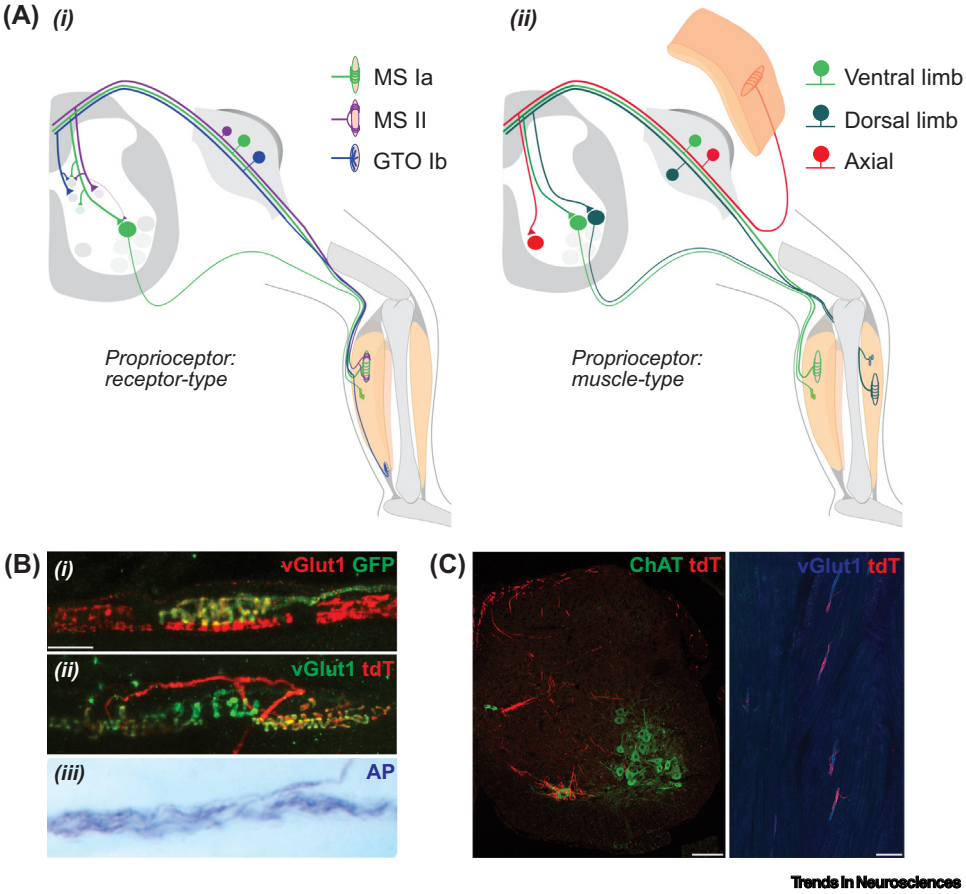

Figure 1. Anatomical organization of spinal proprioceptive circuits. (A) Schematic representing the anatomical organization of proprioceptor subtypes. Proprioceptors can be distinguished based on their receptor-type identity (left panel) and their muscle-type identity (right panel). Receptor types include groups Ia and II muscle spindle (MS) afferents, as well as group Ib Golgi tendon organ (GTO) afferents. Each of these afferents contacts both distinct and overlapping targets in the spinal cord [86–89]. Muscle-type identity is best described for group Ia MS afferents and is characterized by central connectivity to homonymous motor neurons that control the activity of the same muscle target. (B) Genetic labeling of mouse MS and GTO afferents on the basis of their distinct transcriptional profiles [26]. vGlut1^{+}GFP^{+} group Ia and vGlut1^{+}tdTomato^{+} (tdT) II sensory endings in muscles of *Calb2:Cre; Mapt:lxp-STOP-lxp:mGFP-iNLZ* mice (top panel) and *Tac1:Cre; PvFlpO; Ai65* mice (middle panel), respectively. Group Ib sensory endings labelled through alkaline phosphatase (AP) activity in muscle of *PV:Cre;Brn3c-AP* mice (bottom panel). Scale bar 20 μm. Reproduced, with permission, from [26]. (C) Genetic labeling of mouse proprioceptors innervating back muscles on the basis of their transcriptional profile [32]. tdTomato^{+} central afferents targeting motor neurons (ChAT^{+}) in the medial motor column (left panel) and vGlut1^{+}tdTomato^{+} sensory endings in back muscles (right panel) of *Trpv1-Cre;PV-FlpO; Ai65* mice. Scale bars: 100 μm. Reproduced, with permission, from [32].

Identification of proprioceptor muscle and receptor subtypes

The past century has seen major strides in understanding proprioceptive system function, yet the relative contribution of feedback from individual receptors and muscles has remained poorly characterized. New advances in omics, genetics, and tracing technologies have spurred the functional investigation of selected neuronal populations. In particular, transcriptome analysis at the single cell level can provide insights in the molecular makeup of neurons at an unprecedented resolution, allowing unbiased cell type classification based on differential gene expression profiles [20]. This knowledge is now being leveraged to unlock access to different proprioceptor subtypes with high efficiency and specificity to study their development, connectivity, and function.

[3]Max-Delbrück-Center for Molecular Medicine in the Helmholtz Association (MDC), Robert-Rössle-Strasse 10, 13125 Berlin, Germany

*Correspondence:
sd382@cumc.columbia.edu
(J.C. de Nooij) and
niccolo.zampieri@mdc-berlin.de
(N. Zampieri).

Molecular and functional diversity at the receptor level

Several laboratories have used transcriptomic approaches to profile dorsal root ganglia (DRG) somatosensory neurons, providing a clearer picture of the molecular basis of the ability to detect a wide variety of chemical, thermal, and mechanical stimuli [21–25]. However, distinctions amongst proprioceptive sensory neurons were not detectable in these analyses. Proprioceptors represent a discrete but small population of neurons (5–8%) when compared with other sensory modalities [21]. Thus, it is perhaps not surprising that global profiling of all somatosensory neurons exposed shared proprioceptor features rather than molecular distinctions that define individual subtypes. To overcome this problem, two recent studies used mouse genetic strategies to enrich for proprioceptors prior to performing transcriptome analysis [26,27]. These efforts managed to reveal the molecular basis of the anatomical and physiological features that distinguish Ia, Ib, and II proprioceptor groups. Surprisingly, rather than uncovering three molecularly distinct cell types, bioinformatic analyses suggested the existence of up to eight different subtypes in adult mice (Figure 2B). Validation of these transcriptomic data *in vivo* indicated that the majority of these subtypes can be assigned to groups Ia and II afferents, while group Ib afferents are represented by a single molecular class.

The high sensitivity and resolution that come with transcriptome studies offer opportunities but also challenges [28]: are all the observed molecular distinctions indicative of different cell type

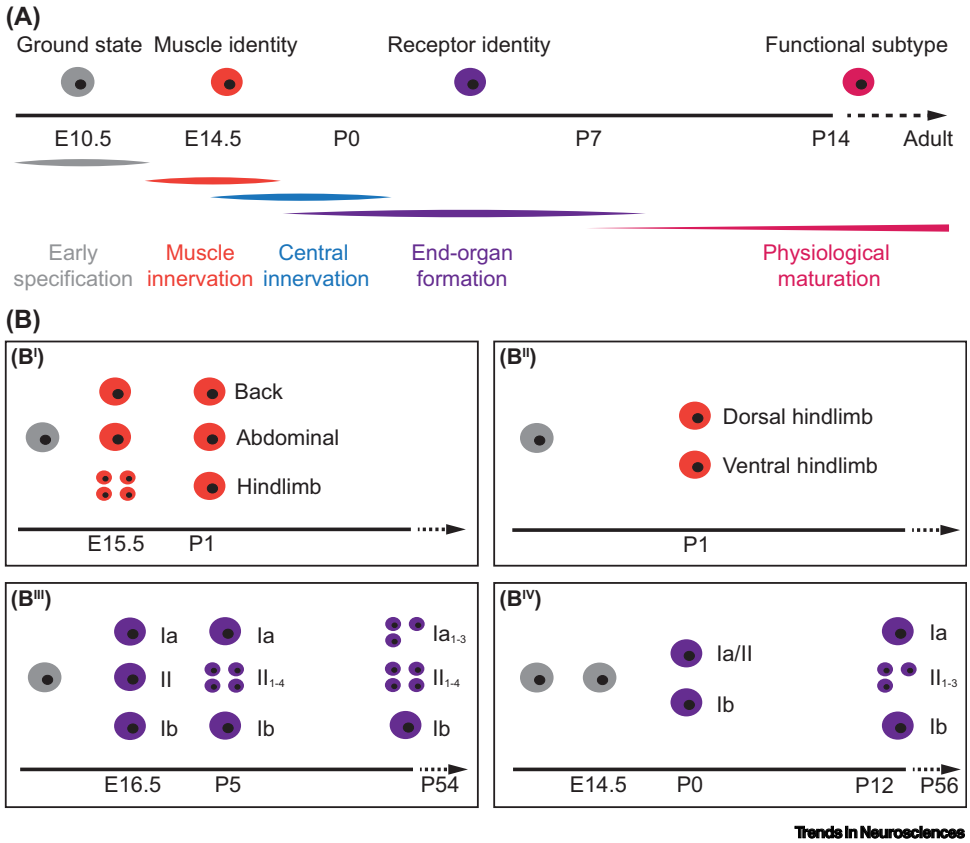

Figure 2. Proprioceptive neurons molecular identities. (A) Timeline of mouse proprioceptive sensory neurons embryonic and postnatal development. (B) Schematics representing the molecular identities identified in published proprioceptor transcriptome studies in mice. (B^{I}) Dietrich *et al.* [32]; (B^{II}) Poliak *et al.* [30] and Norovich *et al.* [31]; (B^{III}) Wu *et al.*[27]; (B^{IV}) Oliver *et al* [26]. Each cell represents a distinct molecular identity. Gray: ground state; orange: muscle identity; purple: receptor identity. Abbreviations: E, embryonic day; P, postnatal day.

identities? If so, what is the meaning of such diversity? Potential clues can come from developmental analyses in mice. At embryonic day (E)14.5, when sensory end-organ formation has just started, molecular distinctions between the two observed clusters primarily appear to reflect differences in developmental maturation [26]. At E16.5 and postnatal day (P)0, three molecularly distinct clusters are apparent, and only at P5 – >10 days after the afferents establish their peripheral connections – the number of molecular subtypes (six) approaches the number observed in the adult (eight) [26,27]. While these analyses require further validation with larger cell numbers, smaller developmental intervals, and direct correlations of molecular subtypes with peripheral end organs, these data suggest that the diversity observed in adult mice may in part correspond to gene expression programs involved in the maturation of specific functional properties. Single afferent recordings in the rat have demonstrated that groups Ia and II MS afferents exhibit a diverse range of firing properties [11], which could be related to the differential expression of ion channels observed in individual Ia and II subtypes [26,27]. With respect to the many groups Ia and II subtypes, it thus far remains largely unclear how their molecular signatures correlate with specific anatomical, physiological, or circuit features. However, some of the groups Ia and II subtype markers show biased expression in either limb or thoracic DRG [27]. As such, it will be relevant to test the possibility that select combinations of individual groups Ia or II subtypes may be responsible for providing feedback information from muscle targets with different physiological roles.

The acquisition of the diverse molecular identities of groups Ia and II subtypes in mice mostly appears to occur after the afferents connect to their nascent muscle spindle receptor organ. (Figure 2A). In addition, it was shown that the expression of certain group Ia markers can change in adult mice undergoing exercise, possibly indicating that mature proprioceptor molecular identities may not be fixed but can adapt to different activity levels [27]. The functional implications of such plasticity remain to be elucidated, but we speculate that the ability to dynamically change the response properties of MS afferents, potentially along with their central wiring pattern or synaptic efficacy, may tailor the proprioceptive system to specific requirements of different motor tasks. Under this lens, some of the molecular distinctions in receptor types captured by transcriptomic analysis may not be indicative of different cell identities but could rather represent plastic changes in gene expression related to the needs of different functional states.

Proprioceptor character defined by muscle connectivity

The exquisite selectivity with which proprioceptive neurons form connections with their peripheral and central targets is a defining feature of their function [29]. A striking example is found in the stretch reflex circuit, where the connections of group Ia sensory afferents with motor neurons follow stringent rules of specificity [11]. Strong monosynaptic contacts are made with motor neurons controlling the same muscle (homonymous connections), weaker contacts with motor neurons controlling agonist muscles, and essentially none with motor neurons controlling antagonist muscles (heteronymous connections) [10,14]. This basic plan of connectivity is largely conserved in all vertebrates that have been examined and its assembly occurs at late embryonic stages. Mature groups Ib and II afferents have few (II) or no (Ib) direct contacts with motor neurons and predominantly terminate in the intermediate spinal cord along with collaterals of group Ia afferents [12]. However, the identity of the specific populations of interneurons contacted by individual subtypes remains poorly resolved (see Outstanding questions).

The anatomical organization of the stretch reflex circuit implies that during development proprioceptive neurons should possess distinct molecular characteristics reflecting the identity of their peripheral muscle target (Figure 1). Published work as well as a preprint study reporting the transcriptional profiling of ankle flexor or extensor proprioceptors at early postnatal stages in mice

revealed molecular distinctions between the neurons innervating discrete dorsoventral and proximodistal compartments of the hindlimb (Figure 2B) [30,31]. Expression analysis of these markers in limb-projecting proprioceptors show that neurons innervating functionally related muscles, such as synergists operating on the same joint, share common molecular features [30,31]. These data indicate the existence of molecular distinctions that may capture features of the organization of the musculoskeletal system such as biomechanical function (i.e., flexion, extension, adduction, and abduction) and anatomical location (i.e., epaxial, hypaxial, and limb). This notion is supported by the differential expression of some group Ia and II subtype markers in limb or thoracic DRG [27]. Building on the idea that positional organization may be reflected at a molecular level, a recent single cell transcriptome analysis profiled proprioceptors obtained from DRG at different segmental levels [32]. The results show that neurons residing in thoracic DRG possess molecular identities that reflect their innervation of either abdominal or back muscle compartments, while neurons in lumbar DRG mostly exhibit hindlimb muscle character. From here on, we refer to the positional character of proprioceptors as proprioceptor muscle-type identity. Regional (abdominal, back, and hindlimb) muscle types were observed at E15.5 when proprioceptors have already invaded their muscle targets and project a central collateral into the spinal cord. This suggests that distinctions in muscle types may reflect differences in genetic programs coordinating peripheral and central connectivity (Figure 2). Several members of the Eph/ephrin family of axon guidance molecules were found to distinguish limb- versus axial-projecting proprioceptors, and elimination of *Efna5*, a limb-specific proprioceptor marker, perturbs innervation of limb muscles [32]. Whether the same programs coordinate the connectivity of central projections remains to be determined, but it is an intriguing possibility given the well-known roles of ephrins in controlling the wiring of spinal circuits [33,34]. The expression of muscle type (abdominal, back, or hindlimb) proprioceptor markers persists into early postnatal stages but preliminary data indicate that it is, at least in part, lost in adulthood (unpublished data, Poghosyan and Zampieri). This raises the question whether molecular signatures that define regional muscle connectivity may represent a transient state rather than constitute fixed cell-type identities. In line with this notion, when the expression of muscle-type molecules subsides, markers related to the maturation of receptor-type character may become predominant [26,27,32] (Figure 2). Thus, transcriptomic profiling conducted at different timepoints can report dynamic temporal changes in gene expression that highlight distinct phases of proprioceptor development (Figure 3). Together, the genetic programs that regulate muscle and receptor properties orchestrate the acquisition of the positional and functional features of individual proprioceptor subtypes.

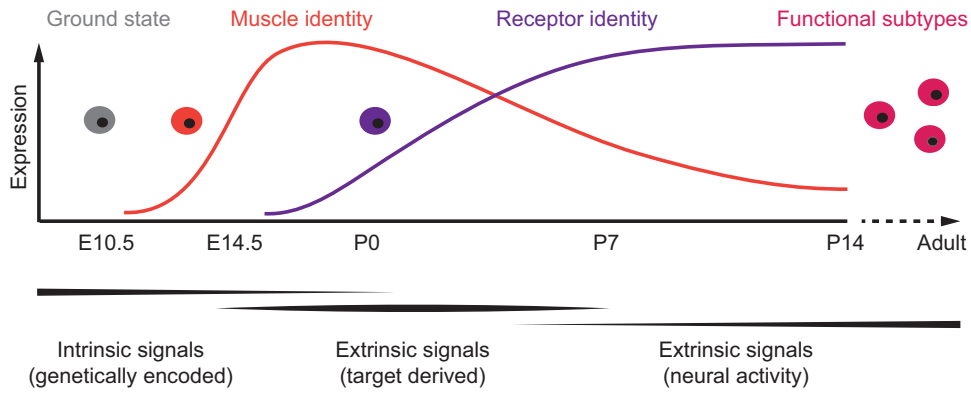

Figure 3. Development of proprioceptive neuron functional subtypes. Schematic representing the expression dynamic of muscle (orange) and receptor (purple) identities in relation to the presence of potential intrinsic and extrinsic factors controlling their acquisition during mouse development. Gray: ground state; orange: muscle identity; purple: receptor identity. Abbreviations: E, embryonic day; P, postnatal day.

Developmental strategies involved in the emergence of proprioceptor identities

Functional identities of proprioceptive neurons, as described earlier, are multilayered, with unique anatomical and physiological attributes. Intuitively, the need for such a composite identity system is easy to comprehend; for example, dynamic Ia feedback is useless if the central nervous system does not receive information regarding the muscle this feedback derives from. As described below, recent studies have begun to offer insights into how muscle and receptor character may emerge, but it is not clear yet to what extent, or how, these developmental trajectories are coordinated with each other (see Outstanding questions).

Acquisition of proprioceptor muscle-type identity

Transcriptome analyses offer unequivocal evidence that developing proprioceptors exhibit distinct molecular characters that predict the regional location or specific identity of the muscle they innervate [27,30–32,35]. In mice, these markers are present at least as early as E15.5, shortly after afferents innervate their muscle targets (Figure 2A) [32]. When afferents first acquire their muscle-type identity remains uncertain but it could potentially already be specified at the neural crest stage. Transcriptional profiling of neural crest cells between E8.5 and E10.5, reveals the rostrocaudal expression pattern of several *Hox* family transcription factors. This initial patterning appears to affect the developmental potential of cranial, vagal, and trunk crest cells and their derivatives [35]. *Hox* genes are well known to serve prominent roles in body patterning, including in the developing spinal cord [36–38]. Thus, possibly, differential or graded expression of posterior *Hox* genes may not only bias trunk crest cells toward a sensory fate but may also endow them with rostrocaudal segmental identities [35]. Consistent with this idea, developing DRG neurons, including proprioceptors, express posterior *Hox* genes in regionally restricted patterns along the rostrocaudal body axis [34]. This *Hox* expression pattern was found to be maintained following limb ablation at an early embryonic stage, prior to afferent innervation. These findings suggest that regional sensory identities could derive from intrinsic genetic mechanisms that are independent of muscle target-derived signals, but sources of extrinsic factors other than the limb may also play a role [35,39]. The *Hox*-mediated partitioning of proprioceptors along the rostrocaudal axis may differentially influence general properties of their phenotype such as the capacity to extend axons into specific peripheral targets [40]. Later in development, proprioceptors that innervate different forelimb muscles express distinct *Hox* genes [35]. This could indicate that *Hox*-based mechanisms may also account for the finer grained muscle-to-muscle selectivity. However, initially all forelimb innervating proprioceptors express overlapping sets of *Hox* genes, suggesting that muscle-derived signals, motor axon-derived signals, or activity-dependent mechanisms may be required to selectively maintain specific *Hox* expression profiles.

The role of neural activity in controlling proprioceptor muscle-type identity has been extensively investigated using, as a read-out, the anatomical or physiological connectivity patterns of afferent collaterals in the spinal cord. Using such assays, it has been shown that coordinated activity between homonymous sensory and motor neurons is not a determining factor in the specification of their connections and by extension, their muscle-type identity [41,42]. What other mechanisms may play a role in defining muscle-type identity? An alternate strategy is represented by target-derived instructive signals. Developing limbs first partition into molecularly defined dorsoventral and proximodistal segments under the influence of morphogens that control limb development [43,44], and subsequently differentiate into molecularly defined individual muscles [45]. Thus, mesenchymal- or muscle-derived signaling molecules could differentially influence the development of distinct proprioceptive afferents. Studies in the chick and in mice provide compelling evidence for such target-derived signals in regulating proprioceptor muscle-type identity [30,31,46]. In addition, classic work in the frog showed that proprioceptors that are rerouted to ectopic muscle targets in the periphery centrally rewire to connect to motor neurons that innervate

these muscles [47]. More recently it was shown that molecular markers that define proprioceptors innervating the distal hind limb presented altered distributions under conditions in which the dorsoventral identity of the limb was reversed, or in mouse mutants that lacked muscles altogether [30,31]. The identity of the target-derived signaling molecules remains largely unclear (see Outstanding questions). However, studies in mice demonstrated that *neurotrophin 3* (*NT3*) is differentially expressed in developing muscles between E15.5 and P0 [39,48,49]. NT3 is an essential trophic factor for early embryonic proprioceptors and is implicated in regulating the dorsoventral termination of their afferents in the spinal cord [50,51]. Its role in controlling muscle specific features of late embryonic proprioceptors remains unclear but could involve the modulation of *Runx3* or *Etv1* expression levels [39]. These two transcription factors regulate various aspects of proprioceptive identity and are well positioned to drive distinctions in the expression of cell surface recognition molecules through their transcriptional activities [49,52,53]. The mechanisms that are responsible for controlling muscle *NT3* levels remain unclear, but could similarly regulate the expression of other, yet to be identified, signaling molecules (see Outstanding questions).

In addition to target-derived signals, transplantation experiments in frog and chick embryos showed an important role for motor axons in controlling guidance of sensory projections [54–56]. More recent work in mice demonstrated that this may rely on transaxonal recognition mediated by members of the Eph/ephrin family of axon guidance molecules which can control sensory axon tracking along pre-existing motor projections to epaxial and hypaxial compartments [57,58]. Embryonic muscle-type proprioceptors are also characterized by differential expression of Eph/ephrin molecules and their elimination results in muscle targeting defects [32]. This suggests that a similar mechanism may control proprioceptive neuron coupling with motor axons to direct them to the correct muscle targets [30,32].

The extent to which aspects of muscle-type identity rely on intrinsic or extrinsic signals is not clear. It is possible that early gene expression programs (i.e., *Hox*, *Eph/ephrin*) that control general features of muscle-type identity, for instance innervation of axial or limb compartments, may be intrinsically defined, while target-derived signals may promote the continued expression of muscle-specific character (Figure 3) [35]. It is also possible that the different features of muscle-type identity such as central and peripheral connectivity could each be regulated through different developmental mechanisms. Perhaps peripheral target selection depends on intrinsic genetic programs, while establishment and refinement of central target selectivity may depend on target-dependent signals. In this scenario, proprioceptor subtype identity may first emerge through intrinsic determinants that control the competence to differentiate in regional appropriate subtypes. Individual proprioceptor muscle-type identities may subsequently be refined by various extrinsic target-associated signals. Furthermore, the transition point between intrinsic control and regulation through extrinsic signals may differ for distinct features of muscle-type identity (Figure 3). Additional experiments will be required to provide further clarity on these issues.

Acquisition of proprioceptor receptor-type identity

Group Ia, Ib, or II afferents are defined by specialized morphological sensory endings (MS or GTO), physiological properties, and target selection in the spinal cord and brainstem (Figure 1). Transcriptomic analyses of developing sensory neurons have begun to shed light on the molecules that distinguish between these afferents, as well as their ontogeny. At early stages of mouse development (E10.5–11.5) proprioceptors can first be distinguished from other somatosensory neurons by virtue of the expression of the NT3 receptor *TrkC* and the transcription factor *Runx3* [59]. At E11.5, $TrkC^{+}Runx3^{+}$ neurons appear transcriptionally uniform [21,52]. Surprisingly, when proprioceptors contact their nascent sensory end organs (~E14.5), transcriptome analyses still fail to cluster neurons into MS or GTO classes [24]. Only starting from E16.5

molecularly distinct proprioceptor subtypes can be detected (Figure 2) [25]. However, it is important to reiterate that only few developmental time points have been sampled, and that the number of neurons analyzed at E14.5 was relatively low. Thus, the existence of molecular markers of receptor-type identities before E16.5 cannot yet be excluded. Nevertheless, these initial observations may suggest that during early development, proprioceptors have equal competence to develop into MS or GTO subtypes and only acquire distinct molecular characters after the afferents connect with their receptor targets.

The apparent initial lack of definitive transcriptional distinctions between MS and GTO afferents contrasts with the anatomical observations on end-organ development. MS intrafusal muscle fibers can be distinguished as early as E15.5 by virtue of their innervation by $TrkC^+$ or PV^+ afferents [49,60]. In most muscles, contact by proprioceptive afferents is in fact necessary for MS development [61]. The *Ig-Neuregulin 1* isoform (*Ig-NRG1*), expressed on or released from proprioceptive terminals, signals through ErbB receptors on nascent intrafusal fibers to initiate MS differentiation, possibly by inducing expression of the *Egr3* transcription factor within the intrafusal muscle fibers [60,62]. The absence of Ig-NRG1 in proprioceptors not only blocks spindle development but also arrests the normal morphological development of the afferent terminals, suggesting that intrafusal fibers also provide a retrograde signal to the afferents [60]. Consistent with this observation, molecular markers known to segregate with a MS afferent identity are vastly diminished or absent in DRG of *Egr3* mutant mice [44]. In contrast to MSs, the development and innervation of GTOs appears unperturbed in *Ig-NRG1* mutant mice [60]. These observations may indicate that differential Neuregulin expression in proprioceptors, or their differential responses to NRG-ErbB2/Egr3 evoked end-organ-derived retrograde signals, could constitute the earliest molecular hallmarks of their specific receptor-type identity. NRG1 exists in multiple isoforms [63], yet recent analyses did not investigate distinctions in isoform expression levels. Thus, future work should offer more clarity on these possibilities. Altogether, while definitive insights into the relevance of intrinsic or extrinsic signaling mechanisms in driving proprioceptor end-organ identity remain lacking, spindle-derived retrograde signals appear to be required to help shape the molecular specialization of MS afferent identities (see Outstanding questions).

Diversification of MS afferent subtypes

Molecular profiling of adult proprioceptive afferents in mice highlighted the existence of a single cluster of GTO afferents and up to seven classes of MS afferents, three for group Ia and four for group II (Figure 2B) [26,27]. The two transcriptomic studies published to date are not completely aligned with regards to the number of subtypes and identified markers (Figure 2B), but many of the key molecular signatures are shared across the two studies. There are, however, three additional receptor subtypes, two group Ia and one type II, observed by Wu *et al.* [27]. This difference most likely originates from the higher number of cells that was profiled in this study, which provided the computational power to segregate groups Ia and II afferents into additional molecular subtypes. Discrepancies are also found in the selectivity of some markers. For instance, in the work by Wu *et al.* [27], *Lmcd1* defines all three classes of group Ia afferents, *Runx1* marks the group Ia_1 population, and *Fxyd7* defines all group II afferents. In the study by Oliver *et al.* [26], instead, these molecules appear to label most proprioceptors (*Lmcd1*), two subsets of group II afferents (*Runx1*), and one of the group II afferent subtypes and GTO afferents (*Fxyd7*). The causes of these inconsistencies are not fully clear but may relate to differences in bioinformatics analyses, the developmental time points at which sequencing was performed, the segmental levels from which neurons were derived, or the genetic background of the animals. Nevertheless, both studies show that groups Ia and II subtypes emerge through a gradual developmental process in which mature MS identities slowly become apparent. For instance, expression of Calretinin (encoded by *Calb2*), a molecular marker for group Ia afferents is first detected

after P6 (Figure 1) [26]. Lineage tracing of *Calb2* expressing neurons demonstrate its restricted onset within group Ia afferents, indicating that it is selectively induced, rather than gradually lost from other spindle innervating afferents [26].

How does the timing of the molecular specification of groups Ia and II afferents align with their morphological and physiological specialization? Similar to *Calb2*, several other molecules whose expression correlates with mature group Ia afferents (e.g., *Colq* and *Hpse*) emerge during postnatal development [26,27]. The postnatal molecular maturation of groups Ia and II afferent identities is consistent with the gradual remodeling of spindle sensory endings [61,64,65]. Yet, the relatively slow molecular specification of Ia afferents may seem at odds with other features of their identity. For instance, vibration sensitivity is believed to be a specific feature of group Ia afferents, and MS afferents in various species were shown to respond to vibration stimuli two days after birth [61,66]. Similarly, Ia afferents, but not or to a lesser extent group IIs, establish selective connections to homonymous and synergistic motor neurons in the spinal cord (Figure 1). In mice these connections can be detected shortly after birth [9,12]. Data from Wu *et al.* [27] suggest that groups Ia and II afferents are molecularly distinct by P5, and as such, it is possible that many of the molecular drivers of the aforementioned physiological and circuit features of Ia and II afferents may already be in place shortly after birth, even while these afferents may require further molecular maturation (e.g., expression of *Calb2*) with respect to other physiological or morphological properties.

What may drive the molecular segregation of MS afferents? Possibly, groups Ia and II may be exposed to different concentrations of known (e.g., NT3) or yet to be identified signaling factors when first innervating the intrafusal myofibers [49,60]. Alternatively, Ia and II afferents may initially share similar molecular identities and over time become distinct through activity-dependent mechanisms. In this regard, an interesting parallel can be made with type I spiral ganglion neurons (SGNs) in the cochlea. During late embryonic/early postnatal development, type I SGNs segregate into three distinct classes – types Ia, Ib, and Ic – that mature different physiological and anatomical properties [67,68]. In absence of synaptic activity from inner hair cells, many SGNs coexpress molecular markers normally segregated across the three SGN subtypes [69,70]. Distinctions between groups Ia and II proprioceptors could similarly rely on activity-dependent mechanisms, such that in absence of activity, groups Ia and II afferents could remain in a common physiological state and may both form contacts with motor neurons. At present there is no evidence for a role of activity in regulating group Ia/II identity. However, in mice lacking synaptic activity, MS afferent central collaterals form excess numbers of synaptic contacts on motor neuron targets while preserving their muscle target specificity [42].

Merging proprioceptor muscle and receptor identities

How muscle and receptor characters are combined to give rise to functional proprioceptor identities remains unclear. It appears that muscle-type character is established first, given that molecules specifying this aspect of identity are observed at an earlier embryonic stage while molecular distinctions that correlate with receptor type only appear to emerge later and consolidate over postnatal maturation [26,27,32]. In addition, muscle-type markers are shared by all MS and GTO afferents that innervate the same muscle [31,32]. It nevertheless remains possible that during early developmental stages molecules associated with muscle identity are highly expressed and dominate the bioinformatics analyses even while transcripts related to end-organ identity are present. For example, expression of *Pou4f3* becomes restricted to presumptive GTO afferents between E14.5 and P0, but at the molecular level Pou4f3^{+} neurons are only clearly segregated from other proprioceptors by P0 [26]. Thus, possibly, the acquisition of muscle and receptor identities is more simultaneously organized than currently appreciated.

Neural identities that incorporate positional information and physiological function are of course a recurrent theme in differentiating neural systems. Similar to proprioceptors, the aforementioned SGNs are organized in a positional tonotopic map, with Ia, Ib, and Ic subtypes distributed relatively evenly throughout [69]. It appears that the apical–basal tonotopic map is already established by E16.5, before SGNs subtypes are fully defined [71,72] Likewise, mouse spinal motor neurons, which establish their anatomical topographic organization shortly after they become postmitotic, are transcriptionally homogeneous until E17.5 (well after target innervation) and only then mature into physiologically distinct gamma and alpha motor neuron subtypes [73–75]. These observations suggest that establishing a positional identity may be the first need in differentiating neural systems.

To what extent are proprioceptor identities plastic? Specifically, can proprioceptor subtypes adjust their molecular character when faced with a changing environment during development or upon injury (see Outstanding questions)? The notion that proprioceptor muscle-type character may emerge from a combination of intrinsic determinants and extrinsic signals that derive from the developing mesenchyme/muscle/spinal cord suggests that this aspect of identity may not be as malleable at postnatal stages, given that the developmental signals may no longer be present (Figure 3). Receptor identity, however, was shown to be relatively plastic. Studies in cats demonstrated that, following muscle nerve section and re-ligation, all afferents, including group Ib, randomly innervated the vacant end organs, in particular muscle spindles [76,77]. Some afferents were found to adjust their central synaptic efficacy according to their new targets [77]. In line with these observations, it appears that the molecular identities of proprioceptive afferents can be modulated by activity-dependent processes [27,78]. In particular, in mice, the expression of molecular markers that identify group Ia subtypes 1, 2, and 3 was shown to change following exercise training [27]. Thus, the relative abundance of different MS afferent subtypes could be fine-tuned in adult stages, possibly through an activity dependent mechanism. The ability to switch subtype composition would offer a mechanism to permit dynamic adjustments in sensorimotor circuits to fine tune the system to specific motor function needs.

Outstanding questions

Are proprioceptor muscle-type and receptor-type identities specified in sequence or in parallel?

Are the developmental pathways controlling muscle and receptor identities interdependent or independent?

What are the sources and nature of the signaling cues that instruct proprioceptor muscle-type and receptor-type identities?

What is the identity of the spinal interneuron targets of individual afferent subtypes?

How are different proprioceptive neuron subtypes wired into functional circuits appropriate for both muscle and receptor identity?

To what extent do muscle or receptor type molecular identities remain, or become obsolete, at later developmental stages?

Are muscle and receptor identities equally plastic?

Concluding remarks

Recent advances aimed at uncovering the molecular diversity of proprioceptors represent an important step toward a comprehensive understanding of the development and function of proprioceptive neurons. These studies, by allowing selective access to molecularly defined subtypes in genetically tractable models, open the way to dissect the mechanisms controlling the acquisition of muscle and receptor characters and to define how different subtypes wire into central circuits and function in the control of movement. The importance of proprioceptive information is exemplified by the dramatic effects on motor control and musculoskeletal integrity observed in people with impaired proprioceptive function [79–81]. In addition, other physiological and pathological conditions, including aging, inflammation, and neurodevelopmental disorders, are also associated with impairments in proprioception [82–85]. Future work bears the exciting promise of addressing outstanding questions in the field with important implications for proprioceptive function in health and disease.

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Declaration of interests

The authors declare no competing interests.

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