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Spinal cord projection neurons: a superficial, and also deep analysis

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Today there are extensive maps of the molecular heterogeneity of primary afferents and dorsal horn interneurons, yet there is a dearth of molecular and functional information regarding the projection neurons that transmit pain and itch information to the brain. Additionally, most contemporary research into the spinal cord and medullary projection neurons focuses on neurons in the superficial dorsal horn; the contribution of deep dorsal horn and even ventral horn projection neurons to pain and itch processing is often overlooked. In the present review we integrate conclusions from classical as well as contemporary studies and provide a more balanced view of the diversity of projection neurons. A major question addressed is the extent to which labeled-lines are maintained in these different populations or whether the brain generates distinct pain and itch percepts by decoding complex convergent inputs that engage projection neurons.

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Introduction

Today, many studies describe a plethora of neurochemically distinct primary afferent and spinal cord interneuron populations that are tuned to discrete pain and itch stimulus modalities [1,2,3••,4–6]. However, whether and to what extent this specificity extends to the dorsal horn projection neurons is unclear. Are distinct modalities conveyed by labeled-lines, by convergence and coding of the signals, or a combination of both?

A labeled-line hypothesis necessitates distinct subpopulations of projections neurons. In fact, based on morphology, electrophysiology, projection targets, and neurochemistry, there is considerable evidence of projection neuron heterogeneity. As yet, however, there is no comprehensive atlas of the molecular profiles of discrete projection neuron populations. As a result, and despite evidence to the contrary, projection neurons are often regarded as a relatively homogeneous group. Here we summarize the literature on the spinal cord and medullary projection neurons that carry nociceptive and pruriceptive information to the brain, emphasizing the extent to which there is evidence for modality-specific subpopulations, and highlighting gaps in our knowledge.

Projection neurons: what do we know?

Projection neurons that transmit pain-relevant and itch-relevant information to the brain originate throughout the spinal cord and medullary trigeminal nucleus caudalis (TNC). There is a dense population in lamina I, and others are scattered throughout laminae III-VIII, X and in the lateral spinal nucleus (LSN) [1,2]. Together, these neurons give rise to several ascending pathways that terminate widely, in the nucleus of the solitary tract (NTS), the medial brainstem reticular formation, the caudal ventrolateral medulla (CVLM), the lateral parabrachial nucleus (LPb), the midbrain periaqueductal gray (PAG) and the thalamus and hypothalamus [2]. Importantly, many projection neurons have propriospinal collaterals that terminate intersegmentally in the spinal cord [7•,8]. Most projections are to contralateral loci; however, bilateral, and to a lesser extent, ipsilateral projections have been described [9].

Disappointingly, perhaps, recent studies are so superficial dorsal horn focused that the significant contribution of projection neurons in deep dorsal horn and even ventral horn (laminae VII and VIII) is largely ignored in contemporary pain research. Below, we attempt to redress this bias.

Lamina-specific evidence of projection neuron heterogeneity

Before reviewing the literature an important caveat must be emphasized. It is almost impossible to establish a consensus as to the functional heterogeneity of spinal and medullary projection neurons. Some studies were performed in decerebrate or spinalized preparations, and many under anesthesia, which can impact normal physiological function. Some studies characterized the properties of antidromically activated projection neurons; others did not. Many studies report on data obtained from very few cells, and unquestionably, there are species

differences. Finally, few studies examined the properties of projection neurons in chronic injury settings where, for example, injury-induced neurochemical changes in sensory neurons, including non-nociceptive Aβ afferents, can significantly alter the properties of dorsal horn neurons [10].

Lamina I

The classification of morphological subtypes of projection neurons in lamina I highlighted three major categories, defined by somatodendritic architecture: fusiform, pyramidal, and multipolar [11•,12,13]. Many studies probed whether these morphological subtypes correlate with specific physiological properties [14•], ascending projection [15–17], or receptor expression [18,19]. Unfortunately, the correlations are inconsistent [9,20•,21]. Lamina I projection neurons target the thalamus, LPb, CVLM and the PAG, and in the mouse, ~90% can be retrogradely labeled from the LPb, demonstrating a high degree of collateralization [9,22•]. Dendrites of the majority of these projection neurons arborize in lamina I where they receive direct input from nociresponsive C-fibers and Aδ-fibers [20•,23•,24–27]. Importantly, although some lamina I neurons respond to heat, pinch and noxious cold (HPC) [13], others, namely the nociceptive-specific (NS) neurons, are more selective, responding only to noxious pinch and/or heat and have different intrinsic properties [29]. Lamina I also contains wide dynamic range (WDR) neurons that respond in a graded manner to innocuous as well as noxious mechanical, thermal, and pruritic stimulation [25,30•,31–34], and some lamina I spinothalamic tract (STT) neurons in monkey [35] and cat [29] respond selectively to innocuous cooling. Recently, in the mouse, lamina I spinoparabrachial neuron subpopulations with varying degrees of modality selectivity were characterized, with one population responding exclusively to noxious cold [28••,36•,37•].

Perhaps the strongest claim for specificity comes from Craig and colleagues, who described lamina I STT populations in the cat that respond exclusively to discrete stimuli, for example, histamine [38•], innocuous cooling [29] or warming [39]. However, these conclusions were based on relatively few cells, and subsequent studies in monkey [30•,40] and rat [33] could not confirm these findings. Nevertheless, despite evidence for polymodal projection neurons [25,30•,32–34,40], some subpopulations may transmit differential, functionally relevant information. For example, in the monkey, among lamina I nociceptive neurons are pruriceptive populations that differentially respond to histamine and cowage [30•]. And interestingly, in the rat, trigeminothalamic (VTT) neurons that respond to both pruritogens and algogens are differentially influenced by morphine, compared to VTT neurons that respond exclusively to algogenic stimuli [41••]. Our own laboratory reported that morphine only suppresses noxious stimulus-evoked Fos expression in a subpopulation of rat spinoparabrachial neurons [42]. Many of these opioid effects are likely indirect, via superficial dorsal horn interneuron circuitry, which provides yet another mechanism for functionally segregating the properties of subpopulations of lamina I projection neurons.

Laminae III–V

Early, pain-relevant electrophysiological studies in the cat [43] and primate [44] focused on WDR neurons in deep dorsal horn (lamina V). Other studies described spinocervical tract cells in laminae III–IV, which likely constitute a parallel 'pain' transmission network [45]. Also of interest, but often ignored, are the postsynaptic dorsal column neurons (PSDC) that originate in laminae III/IV and X and transmit nociceptive information via axons that course in the dorsal columns and terminate in the dorsal column nuclei [46,47]. In fact, not until Christensen and Perl [23] described nociceptive specific neurons in lamina I did attention turn dramatically away from the deep dorsal horn.

Laminae III–V projection neurons are generally multipolar, with long, dorsally directed dendrites that can extend to lamina I. As a result, they receive input from primary afferents that terminate throughout the superficial dorsal horn, including a significant low threshold Aβ myelinated input [27,48,49]. Some of these projection neurons express the neurokinin 1 receptor (NK1R) [50] and receive a convergent nociceptive input from substance P (SP)-containing afferents [51], and likely also from local interneurons [52]. Importantly, many spinal and medullary projection neurons in laminae III-V respond robustly to pruritogens [30•,32,33]. Some WDR neurons in laminae III-V are somatotopically organized [44,53], which would provide a substrate for stimulus localization and intensity coding. Interestingly, in a translational study comparing rats and humans, the graded firing response of lamina V neurons to increasing stimulus intensity correlated with human intensity reports [54].

The PSDC neurons are of particular interest with respect to visceral pain. Not only do these neurons, particularly those located near the central canal, respond to noxious visceral stimulation [46,55] but noxious colorectal stimulation-induced activity of thalamic neurons can be blocked by dorsal column lesions [56]. And most interestingly, midline myelotomy, which severs PSDC axons, has successfully relieved pelvic cancer pain [43].

Laminae VII–VIII

Particularly disappointing to the present authors is the continuing lack of attention paid to the classical distinction between the neospinothalamic and paleospinothalamic systems. Granted this terminology may be outdated, but the reference of the latter to clinically relevant spinoreticular and spinoreticulothalamic (SRT) systems in pain processing is

perhaps where focus should be redirected. Importantly, these pathways carry 'pain' messages to midline thalamic structures long implicated in pain processing, regions that have even been targeted for ablation in patients, due to their contribution to the diffuse pain symptoms characteristic of many chronic pain conditions [57].

The cells of origin of the SRT pathways are scattered throughout laminae VII and VIII [58,59,60•,61]. Most of these cells send ipsilateral or bilateral projections through the anterolateral funiculus and either terminate in the medial brainstem reticular formation or emit collaterals as they course to the medial thalamus (including the intralaminar nuclei and nucleus parafascicularis) [59,61,62]. As the consequences of activity at a particular collateral will reflect the different circuits engaged by the collateral, it follows that activity of these projection neurons will have incredibly diverse influences in pain processing. Electrophysiologically, some of these projection neurons are nociceptive-specific and respond to both cutaneous and visceral inputs. Others have WDR properties, and surprisingly, some respond only to innocuous stimuli [59,61]. What distinguishes the laminae VII and VIII nociresponsive neurons from those located dorsally is that many have very large, bilateral receptive fields, with complex excitatory and inhibitory inputs, and are extremely anesthetic sensitive [60•]. Conceivably, it is those properties that discourage their analysis by contemporary pain researchers; however, their significant contribution to pain processing argues for reinvestigation.

Lateral spinal nucleus (LSN)

Located in the white matter of the rodent dorsal horn, lateral to lamina I, the LSN extends along the rostrocaudal length of the spinal cord, and is replaced at the most rostral cervical levels by the neurochemically distinct lateral cervical nucleus [63]. Many LSN neurons express the NK1R [64], but in contrast to those in laminae I and III–IV, they do not receive a direct primary afferent input. Rather, they receive a polysynaptic, neurochemically rich peptidergic input, including interneuronal and propriospinal-derived SP [7•,65]. The LSN neurons project to brainstem, hypothalamus, and thalamus [2], and collateralize within the spinal dorsal horn, and they convey nociceptive information mainly from deep somatic and visceral structures [66•].

Neurochemistry of the projection neurons

Modern approaches to map complex neural circuits require knowledge of the molecular language that defines cell type specificity. However, with few exceptions (see below), NK1R remains the marker consistently used to define projection neurons and even to interrogate their contribution to pain and itch [67•,68•]. In rat and mouse, ~80 and 90% of lamina I projection neurons, respectively, express the receptor [64,69–71], as does the majority (70%) of spinoparabrachial neurons in the LSN [64]. Fewer projection neurons in laminae III–V express the NK1R (~33% of STT neurons in the rat [69], and 44% of spinoparabrachial neurons in the mouse [71]). The relatively small number of NK1R-negative projection neurons in lamina I, which have large, multipolar cell bodies, are defined molecularly only by gephyrin puncta and expression of the glycine α1 (GlyRα1) and GluR4 AMPA receptor subunits [72•,73]. As yet, however, the functional significance of this differential molecular make-up is not clear.

There is some progress in defining molecular subsets of the NK1R-expressing neurons. For example, some lamina I neurons that project to the NTS in the rat [74], and about 10% of NK1R-expressing spinoparabrachial neurons in the mouse [71] double label for the sst_{2A} somatostatin receptor. Whether these represent functionally distinct subtypes is not known. Interestingly, some NK1R-expressing lamina I spinoparabrachial neurons in the cat [75] and mouse [71] express SP, which could, in an autoreceptor fashion, presynaptically modulate neurotransmission at terminal targets. Most recently, Huang *et al.* [76••] found SP-expressing NK1R-positive neurons in lamina I that project to the superior LPb and medial thalamic nuclei. The authors proposed that these neurons contribute selectively to a thalamocortical circuit that underlies complex behavioral responses to sustained pain, rather than reflex responses to acute noxious stimuli.

Towards molecular signatures of the projection neurons: RNA-sequencing

As per their expression of VGLUT2 and lack of expression of glycine or GABA, NK1R-expressing projection neurons are excitatory [71,77]. Other studies documented neurochemical heterogeneity of the projection neurons, with some expressing calbindin [78,79], dynorphin or enkephalin [80,81], bombesin [82], galanin [83], cholecystokinin [82,83] or vasoactive intestinal peptide [81], but again there are inconsistencies among studies [71,84], and there are no confirmed functional correlates of these markers.

More recently, RNA-sequencing has systematically and with great sensitivity defined molecularly diverse populations of dorsal horn neurons, including projection neurons. For example, using unbiased single cell transcriptomics, Häring *et al.* [85••] delineated 15 excitatory and 15 inhibitory categories of dorsal horn neurons, and by combining retrograde tracing with *in situ* hybridization, confirmed that spinoparabrachial neurons are concentrated in the Glut 15 excitatory cluster. This cluster included many other molecular markers, for example, LYPD1, a forebrain protein previously implicated in anxiety disorders, but as LYPD1 labels ~95% of spinoparabrachial projection neurons [85••], it likely does not define a functionally distinct subset.

To achieve a more focused analysis of the molecular profiles of NK1R-positive and NK1R-negative dorsal horn projection neurons, our laboratory has taken a projection neuron-centric approach to RNA-sequencing. Our

preliminary findings, presented at the 2018 IASP World Congress on Pain, uncovered marker genes of projection neurons, including cholecystokinin (CCK) and neuronal pentraxin 2 (NPTX2). CCK is also expressed in excitatory interneurons [86] and has long been implicated in pain processing as an anti-opioid [87], but whether the CCK anti-opioid action involves projection neurons is not known. NPTX2 is of particular interest. Although Miskimon *et al.* reported its expression in sensory neurons and found that its knockout in mice did not affect pain processing [88], our observation of NPTX2 expression in nociceptive projection neurons (retrogradely labeled and noxious stimulus-evoked, Fos-expressing) suggests that spinal cord-derived NPTX2 is functionally relevant to pain processing. To develop a comprehensive molecular and correlated functional database of projection neurons we are expanding the analysis. This database will enable development of subtype-specific tools for cell ablation and manipulation and allow more precise dissection of ascending pain and itch circuits.

Modality coding of the projection neurons

There is a remarkable diversity of projection neuron populations in the spinal cord and TNC. However, the organizational principles that define this diversity, or as Piers and Seal (4) write, 'the logic of the projection neuron populations', remain unknown. We conclude that the evidence for convergence of multiple modalities onto the projection neurons is overwhelming. Most projection neurons that respond to pruritogens also respond to algogens, most projection neurons that respond to noxious heat also respond to noxious mechanical stimulation, and many neurons that respond to pain-provoking inputs also respond to innocuous stimulation. Perhaps with the exception of cold-specific projection neurons [29,36•,37•], we do not find compelling the claims that modality-specific percepts arise from spinal cord or medullary projection neurons that strictly respond to and convey information of a single modality, particularly since the latter usually constitute no more than 5% of the population [37•,38•,39,89]. Thus, the question remains: how does the brain interpret convergent information so as to generate percepts that are clearly different? One suggestion is that the brain interprets a 'population code' generated by activity across polymodal projection neurons [90]. In this formulation, although there is limited specificity as to the modality to which an individual projection neuron responds, there is specificity in the modality information that a population of neurons can convey. This broader interpretation of labeled-lines has been demonstrated with the TRPV1-positive sensory neurons that co-express MRGPRA3 [91]. When selectively activated by capsaicin, this TRPV1 subpopulation of afferents convey itch rather than noxious heat, despite their polymodal responsiveness to heat, capsaicin and pruritogens. This is a particularly interesting example, but the fact remains that there is a dearth of molecular markers that define distinct subsets of projection neurons, which pose a barrier to detect potential modality-specific subpopulations within a population code system. Ultimately, a comprehensive atlas of dorsal *and* ventral spinal cord and TNC projection neurons must be delineated if we are to understand how different pain and itch modalities are processed, and how the information that the projection neurons transmit results in distinct percepts.

Summary and conclusions

Pain and itch relevant projection neurons are remarkably diverse with respect to their morphology, projection targets, electrophysiological properties and location of their cell bodies in the dorsal horn and TNC. Importantly, regarding location, despite an overemphasis in the literature on the lamina I projection neurons, 'pain' and 'itch' relevant projection neurons are distributed throughout the superficial and deep dorsal horn, ventral horn, and even white matter (LSN). What is not clear is the extent to which this heterogeneity is paralleled by modality specificity in the transmission of heat, cold and chemical pain and itch messages. In our opinion, there is limited evidence for strict labeled-line properties at the level of the projection neurons. However, modality-specific information could be transmitted to the brain via a population code. An important advance, we suggest, will come from the identification of molecular markers that define functional subpopulations. Unfortunately, the NK1R remains the prominent projection neuron marker, even though its widespread expression, particularly in lamina I, precludes it from being a useful marker of subsets of projection neurons. Additionally, there are 'painfully' few specific markers of the non-NK1R-expressing projection neurons in deep dorsal horn. For this reason, we are optimistic that RNA-sequencing characterization of the molecular profiles of projection neurons, combined with functional, for example, Fos induction analyses, will advance the field by elucidating molecularly defined populations of functionally distinct projection neurons.

Conflict of interest statement

Nothing declared.

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