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Review

Cell type-specific dissection of sensory pathways involved in descending modulation

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Decades of research have suggested that stimulation of supraspinal structures, such as the periaqueductal gray (PAG) and rostral ventromedial medulla (RVM), inhibits nocifensive responses to noxious stimulation through a process known as descending modulation. Electrical stimulation and pharmacologic manipulations of the PAG and RVM identified transmitters and neuronal firing patterns that represented distinct cell types. Advances in mouse genetics, *in vivo* imaging, and circuit tracing methods, in addition to chemogenetic and optogenetic approaches, allowed the characterization of the cells and circuits involved in descending modulation in further detail. Recent work has revealed the importance of PAG and RVM neuronal cell types in the descending modulation of pruriceptive as well as nociceptive behaviors, underscoring their roles in coordinating complex behavioral responses to sensory input. This review summarizes how new technical advances that enable cell type-specific manipulation and recording of neuronal activity have supported, as well as expanded, long-standing views on descending modulation.

Highlights

The periaqueductal gray (PAG) and rostral ventromedial medulla (RVM) are critically important hubs in the endogenous analgesia pathway.

Technological and conceptual advances have permitted the identification and targeting of neural ensembles in the PAG and RVM during complex behaviors, revealing that they divergently modulate distinct components of somatosensation.

In vivo imaging, viral tracing, and molecular genetic manipulations have afforded a new layer of insight by characterizing the function of genetically defined neuronal populations within the PAG and RVM.

How these identified pathways coordinate other autonomic, motivational, and defensive responses during ongoing nociception remains to be addressed in greater detail.

Further investigations into the PAG and RVM in the descending modulation of nociception, itch, and other complex behaviors are ongoing.

Recent progress in our understanding of the descending modulation of nociceptive responses

As early as the 1970s, initial forays to investigate endogenous modulation revealed that stimulation of the ventrolateral PAG (vlPAG) was sufficient to inhibit behavioral responses to noxious stimulation in rats [1,2]. This discovery was further translated and reproduced in humans, whereby neurosurgical electrical stimulation of the vlPAG produced patient-reported relief for pain [3,4]. Since then, a large body of work using a combination of pharmacology and electrical stimulation has identified the roles of key structures, particularly the PAG and a downstream projection field, the RVM, in the descending modulation of nociceptive behaviors [5–15]. These studies were instrumental in identifying the brain regions, neurotransmitters, and neuropeptides involved in descending modulation.

It has been challenging to identify specific cell populations and precise neural circuits that mediate descending modulation because previously available tools have broad effects on the regions targeted. For instance, electrical stimulation and microinjections of pharmacologic agents could act on multiple cell types and axons of passage in the vicinity of the site of stimulation or infusion. Furthermore, many studies were conducted in lightly anesthetized animals, which made it difficult to study neurons in the PAG and RVM in the context of natural behaviors.

Spurred by recent technological advances enabling cell type-specific recording and manipulation of neuronal activity, there has been a renewed interest in characterizing the mechanisms of descending modulation, with a focus on identifying the roles of specific cell types involved in descending modulation, and the organization of their connections (Figure 1). Recent efforts have used combinatorial genetic strategies, viral tools that enable improved connectivity tracing, *in vivo* imaging approaches, and targeted manipulation of identified cell types in awake, freely

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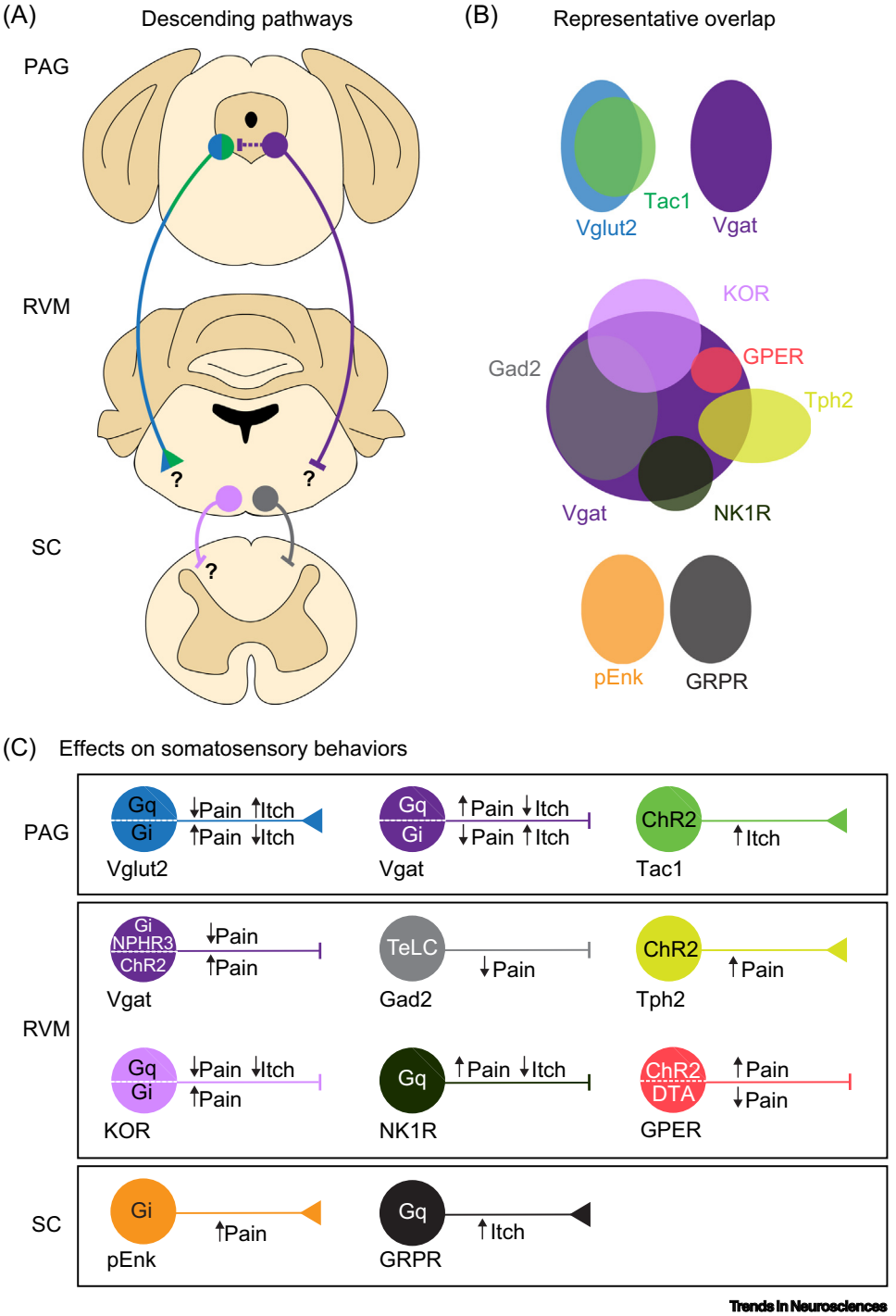

Figure 1. Identified circuits within the PAG, RVM, and SC. (A) An overview of the major components of descending modulatory pathways for nociception including the PAG, RVM, and SC. (B) Representative genetic overlap of PAG, RVM, and SC neuronal populations in the modulation of nociception and pruriception. Color codes in A are described in further detail. (C) Summary of effects of cell type-specific manipulations in the descending modulatory axis. Abbreviations: ChR2, channelrhodopsin-2; DTA, diphtheria toxin A; Gad2, glutamate decarboxylase 2; Gi, hM4Di, Gi-coupled human M4

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behaving mice. This review summarizes recent and foundational models for descending modulation and explores the roles of the descending modulatory systems in coordinating the responses to different types of sensory input, particularly nociception and pruriception.

Dissection of neuronal populations in the PAG and RVM using transgenic approaches

Recent technological advances enable the targeting of distinct neuronal subsets expressing specific molecular markers. Expression of loci-specific gene recombinases (such as Cre, Flp, and Dre) in mice, via either targeted gene engineering or viral vectors, has led to the targeting of neuronal populations based on their anatomical location, their spatial projections, or genetic profile [16]. Combinatorial use of specific recombinase-dependent vectors further permits visualization, mapping, and direct manipulation of targeted neuronal circuits [17,18].

Overall, recent efforts using Cre-driven approaches have supported the conclusions of previous investigations. For example, activation of all neurons by delivery of glutamate or any agonist of ionotropic glutamate receptors to the vlPAG results in the elevation of sensory thresholds and/or reduced responses to noxious input [5,14,19]. By contrast, injection of GABA and GABA agonists into the vlPAG enhances behavioral response to noxious input [15,20,21], suggesting that pharmacological manipulations that increase PAG excitation facilitate antinociceptive behaviors. Consistent with prior studies, studies using Cre drivers that target Vgat (GABAergic) and Vglut2 (glutamatergic) neurons in the PAG have likewise revealed that these two classes of neurons reciprocally modulate nociceptive behaviors (Figure 1). Chemogenetic and optogenetic studies have shown that activation of Vgat neurons enhances behavioral responses to noxious input, whereas activation of Vglut2 neurons reduces behavioral responses to noxious input [22–24], consistent with previous models established using pharmacology and electrophysiology. However, a new insight was the discovery that GABAergic and glutamatergic PAG neurons exhibit modality specificity and are differentially engaged to modulate nociceptive and pruriceptive behaviors [22]. Targeting of the Vgat- and Vglut2-Cre populations in the PAG revealed that responses to nociceptive and pruriceptive input are divergently modulated: activation of Vglut2-Cre neurons reduces sensitivity to noxious heat but facilitates chloroquine-induced scratching, whereas activation of Vgat-Cre neurons does the reverse [22,23]. Thus, the ability to manipulate specific neuronal populations during complex behaviors has exposed apparent opposing roles of PAG neurons in the modulation of itch and pain behaviors.

The use of Cre drivers has led to the re-examination of prior models as well. For example, it was previously thought that glutamatergic PAG neurons comprised the majority of the descending projections to the RVM [5,25–29], while GABAergic neurons represented a tightly regulated inhibitory local microcircuit [5,11,30,31]. New evidence suggests that this idea is an oversimplification. Targeting of Cre-defined PAG populations has revealed that those projecting to the RVM can be glutamatergic [32–35] as well as GABAergic [36]. PAG GABAergic neurons have been observed to innervate spinally projecting RVM neurons [37]. Although most behavioral studies have tested the role of glutamatergic projections from the PAG to the RVM [32,33,35], the specific roles of local GABAergic interneurons versus neurons in the PAG that project to the RVM remain to be tested directly.

muscarinic DREADD; GPER; G protein-coupled estrogen receptor; Gq, hM3Dq, G_q-coupled human M3 muscarinic designer receptor exclusively activated by designer drug (DREADD); GRPR, gastrin-releasing peptide receptor; KOR, kappa-opioid receptor; NK1R, neurokinin 1 receptor; PAG, periaqueductal gray; pENK, proenkephalin; RVM, rostral ventromedial medulla; SC, spinal cord; Tac1, tachykinin 1; TeLC, tetanus toxin light chain; Tph2, tryptophan hydroxylase 2; Vgat, vesicular GABA transporter; Vglut2, vesicular glutamate transporter 2.

The use of Cre alleles to explore the function of the RVM in descending modulation has also helped to reframe the traditional classification of RVM neurons. In the RVM, single-unit recordings in lightly anesthetized rats have classified neurons into three types: ON, OFF, and NEUTRAL cells, based on their firing activity and responses to noxious stimulation [25,38,39]. ON cells exhibit increased discharge during noxious stimulation, are inhibited by morphine, and have been proposed to facilitate nociceptive responses. By contrast, OFF cells exhibit decreased discharge during noxious stimulation, are excited by morphine, and are thought to inhibit nociceptive responses [25,40–42]. NEUTRAL cells are unaffected by noxious cutaneous stimuli or by exogenous opioids, and their role in nociception is unclear, although they are generally thought to be largely serotonergic [43] and are believed to participate in autonomic and homeostatic functions [39,44–47].

The role of inhibitory neurons in the RVM, as a collective population, has been hotly contested. GABAergic RVM neurons have historically been identified as pro-nociceptive, or ON cells, based on pharmacology and electrophysiology studies [48–50]. However, it has been shown that inhibitory neurons in the RVM also function to suppress the activity of ON cells, suggesting they serve mixed functions [48,49,51]. When GABAergic (Vgat-Cre) RVM neurons were activated using optogenetic or chemogenetic actuators, they were found to facilitate mechanical, but not thermal responses [52]. Facilitation of thermal and mechanical responses was also observed when a subpopulation of GABAergic RVM neurons, marked by G protein-coupled estrogen receptor (GPER-Cre) was activated [53]. However, another study found that chemogenetic activation of Gad2 RVM neurons (which comprise a subpopulation of Vgat neurons) produced inhibition of both thermal and mechanical responses [54]. Activation of descending KOR neurons in the RVM, an exclusively GABAergic population, robustly inhibited nociceptive and pruriceptive behaviors [33,55], a finding consistent with previous literature indicating that KOR neurons correspond to OFF cells [48,49,51]. RVM neurons containing the neurokinin-1 receptor (NK1R), which are also GABAergic, were recently shown to be ON cells that facilitated nociceptive responses yet suppressed pruritogen-induced scratching behavior [56]. Together, recent efforts further refine the ON/OFF model by reinforcing the idea that GABAergic neurons in the RVM likely comprise several discrete cell types, including both ON and OFF cells, which have opposing functions in the behavioral responses related to itch and pain. Furthermore, the manipulations of defined cell types have now delineated the specific roles of the GABAergic subpopulations in specific nociceptive behaviors.

Lastly, the role of serotonergic neurons, classically thought of as NEUTRAL cells [57], in nociception is also controversial. For example, *in vivo* recordings of serotonergic neurons have shown that they are predominantly NEUTRAL cells [43], but pharmacological blockade of serotonergic signaling between the RVM and spinal cord has also revealed that they can have both pro- and antinociceptive roles [58–60]. A cell type-specific approach has been used to define the role of 5HT neurons in the modulation of nociceptive behaviors in awake mice using targeting of Tph2 expression for transgenic approaches. Tph2 neurons were found to facilitate mechanical and thermal sensitivity upon optogenetic activation [61]. Fiber photometry recordings of RVM Tph2 neurons also demonstrated that they are activated in the presence of noxious mechanical and thermal stimulation [62]. It is interesting to note that a subset of serotonergic neurons has been shown to co-express *Oprm1* [63,64], an indication that serotonergic neurons may comprise subsets of ON and NEUTRAL cells. Reconciling recent findings, it is likely that activation of serotonergic neurons gives rise to a pronociceptive phenotype due to the specific excitation of 5HT ON cells [61].

Cell type-specific manipulations represent an efficient means to characterize subpopulations of RVM neurons, which exhibit complex molecular identities. New Cre manipulations reveal the

challenges in segregating neuronal subpopulations in the RVM across the original ON/OFF/NEUTRAL schema. To harmonize recent findings with past recordings of lightly anesthetized rats, it is clear that while activation of some GABAergic RVM neurons facilitates nociceptive responses (i.e., consistent with ON cells) [52], not all inhibitory neurons in the RVM are pro-nociceptive [54]. The serotonergic neurons that do not contain the mu-opioid receptor may indeed correspond to NEUTRAL cells based on the classical schema.

Overall, recent studies that use cell type-specific manipulations of neuronal activity in the PAG and RVM have generally supported previous models for descending modulation. However, in the case of RVM ON/OFF/NEUTRAL cells, it is clear that manipulation of distinct cellular subtypes does not always produce results in agreement with classifications based on physiology. Furthermore, although recent efforts have not significantly challenged previous frameworks for descending modulation, cell type-specific approaches have contributed new perspectives on the cells and circuits involved in the descending modulation of nociception. In particular, the ability to directly manipulate distinct neuronal populations in rodents has permitted the study of the role of specific populations in complex behaviors in awake animals, revealing that descending modulatory systems are differentially engaged in pruriceptive and nociceptive behaviors.

Neurons in descending modulatory pathways participate in distinct aspects of somatosensation

Exploration of cell types in the PAG and RVM using cell type-specific manipulations has yielded surprising insights into the modulation of pruriception and nociception. Although most work examining the role of endogenous sensory modulation has emphasized reflexive responses to nociceptive assays, recent areas of focus have been broadened to include other sensory modalities, particularly pruriception.

Previously, electric stimulation of the PAG had been shown to reduce the histamine-evoked activity of neurons in the spinal cord dorsal horn in lightly-anesthetized rats [65]. Much like noxious stimuli, pruritogens were thought to facilitate the activity of ON cells and inhibit OFF cells in the RVM [66], suggesting that descending modulatory systems regulate pruriception and nociception similarly. However, more recent cell type-specific manipulation studies of PAG pathways have shown that both glutamatergic and GABAergic neurons differentially modulate pruriceptive and nociceptive behaviors [22]. Activation of PAG GABAergic neurons inhibits scratching but facilitates nociceptive behavior, whereas activation of glutamatergic neurons inhibits responses to noxious input yet facilitates scratching behavior [23]. This divergence illustrates that there are at least two classes of PAG neurons that exert dichotomous effects on nociceptive and pruriceptive signaling pathways.

Thus, a major benefit of manipulating cell types in awake mice is the ability to examine a diverse repertoire of responses to sensory testing. Chloroquine induced conditioned place aversion (CPA) in control animals, but CPA was not observed with activation of GABAergic neurons in the PAG [22], suggesting that the PAG is important for both the expression of the affective component of itch as well as the motor response to pruriception. While the PAG and RVM are important for the reflexive responses to pain and itch assays, additional work is necessary to examine their contributions to the modulation of affective responses, and whether affective responses could be due entirely to descending modulation, or whether ascending projections from the PAG and RVM to other supraspinal structures may also be involved.

Advances in protein engineering permit imaging of neuronal activity during complex behaviors based on changes in intracellular calcium. For example, using fiber photometry recordings, it

was found that Tac1-Cre neurons in the PAG are active during pruritogen-induced scratching [32]. Chemogenetic or optogenetic manipulation of Tac1 neurons further demonstrated that they are both sufficient and necessary for eliciting scratching behavior and that the behavioral effects of activating Tac1-Cre neurons in the PAG are proposed to be mediated through glutamatergic signaling in the RVM. Interestingly, Tac1-Cre neurons were not found to be involved in nociception [32], further illustrating a role in modality specificity among PAG neurons.

In the RVM, Cre-dependent manipulations of neuronal populations have also revealed that they have different effects on behaviors that are mediated by distinct nociceptive circuits. Manipulations of RVM GABAergic neurons drive opposing behavioral phenotypes in distinct nociceptive assays. Whereas Vgat-Cre neurons facilitate mechanical withdrawal responses, Gad2-Cre neurons inhibit thermal sensitivity to hotplate testing [52,54]. GABAergic RVM neurons containing NK1R were recently shown to attenuate scratching behaviors yet drive different effects in mechanical sensitivity depending on the Cre allele used [56]. Activation of NK1R neurons in the RVM using the NK1R-CreER allele reduced mechanical thresholds in the von Frey assay, but when NK1R neurons were activated with the NK1R-Cre line, no effect on mechanical thresholds was observed [56], possibly due to the differences in the efficiency of capturing the NK1R population in the different Cre alleles. It is also important to note that it is generally difficult to compare the functions of different neuronal populations across different papers because not all studies included the same assays. Nevertheless, recent observations contrast with those from single-unit recordings of RVM neurons, which previously highlighted that RVM neurons respond to different sensory stimuli – including innocuous and noxious mechanical, thermal, and itch stimuli – similarly [66–68]. The variety of stimuli that can elicit similar responses from individual RVM neurons previously suggested that these cells may not be tuned to the processing of distinct modalities of sensory input. Recent evidence challenges this view, though testing with consistent behavioral assays is necessary to understand the extent to which RVM populations modulate responsivity to distinct types of somatosensation or distinct types of behavioral responses. Additional work using recordings and imaging in freely behaving animals will also help to address these important questions.

The evidence that manipulations of PAG and RVM neurons drive diverse responses to somatosensory input supports the idea that these structures contribute to the state dependence of somatosensation, including nociception [39,45,69–72]. Although the PAG and RVM have been classically thought to be important for the descending modulation of nociception, a view extended more recently to pruriception, new research has also elucidated their roles beyond somatosensation. These other functions include the modulation of anxiety, the activation of defensive responses, and the regulation of the autonomic nervous system [24,37,73–82]. The PAG and RVM are extensively connected with several brain regions involved in other complex functions, including the parabrachial nucleus (PBN), central amygdala (CeA), zona incerta, prefrontal cortex (PFC), locus coeruleus (LC), hypothalamus, and spinal dorsal horn [25,36,83–95]. Recent studies have begun to identify how some complex behaviors are modulated by distinct neuron populations in the PAG and RVM that are defined by projection target as well as neurochemical phenotype.

As an example, dopaminergic (DA) neurons in the PAG are critically important for the modulation of nociception by opioids [96,97]. The antinociceptive effects of PAG DA neurons have been attributed to projections to the RVM [98] as well as to the bed nucleus of the stria terminalis (BSNT) [78]. Recent chemogenetic and optogenetic characterizations of PAG dopaminergic projections to the BNST have unveiled their novel roles in modulating defensive responses in a sex-dependent manner [99]. Activation of PAG dopaminergic projection to the BNST inhibits thermal and mechanical nociception and responses to inflammatory injury in male, but not female,

rodents. By contrast, activation of this pathway promotes locomotion in female, but not male, mice [99]. These observations hint at the possibility that the PAG concurrently organizes complex motor responses (including increased locomotion and reflexive withdrawal to noxious testing) in the modulation of nociception.

Extensive neural networks may also explain the mechanisms by which the endogenous modulatory system could coordinate responses to nociception in addition to its other roles in homeostasis and survival. Recordings of neurons in modulatory circuits revealed that these neurons are tuned for diverse functions including sleep, micturition, respiration, sexual arousal, and thermoregulation [39,69,71,100,101]. Optogenetic stimulation of excitatory PAG neurons projecting to the magnocellular nucleus of the medulla induces freezing [35] and a group of excitatory neurons within the gigantocellular nucleus have also been shown to be involved in the modulation of ongoing locomotion through a direct pathway to the ventral horn [102]. Although behavioral results following manipulations of cell types in the PAG and RVM are often attributed to a descending pathway, the connectivity of the PAG and RVM with other brain regions could also account for the observed changes in pruriception and nociception with global manipulation of cell types in these areas. Thus, the diverse sensory, autonomic, and defensive roles of the pain-modulatory pathways underscore their various contributions to integrating and coordinating ascending and descending pathways that are crucial for survival.

Spinal neurons targeted by descending modulation

The spinal cord represents the final target of the descending modulatory system. The necessity of spinal transmission within the context of descending modulation has been well characterized [13,103–106]. Recent efforts have begun to elucidate the precise identities of spinal neurons that receive input from the RVM in the context of somatosensation (Figure 2). Optogenetic and chemogenetic manipulations of Vgat-Cre RVM neurons have suggested that GABAergic RVM neurons descend to pre-synaptically inhibit mechanosensory input onto spinal inhibitory enkephalinergic (Penk) interneurons [52]. Thus, descending GABAergic neurons could facilitate mechanical nociception through the process of disinhibition in the dorsal horn. Alternatively, combined optogenetic and electrophysiological experiments in spinal cord slices have suggested that descending GABAergic RVM neurons inhibit GRPR-expressing neurons in the spinal cord [107],

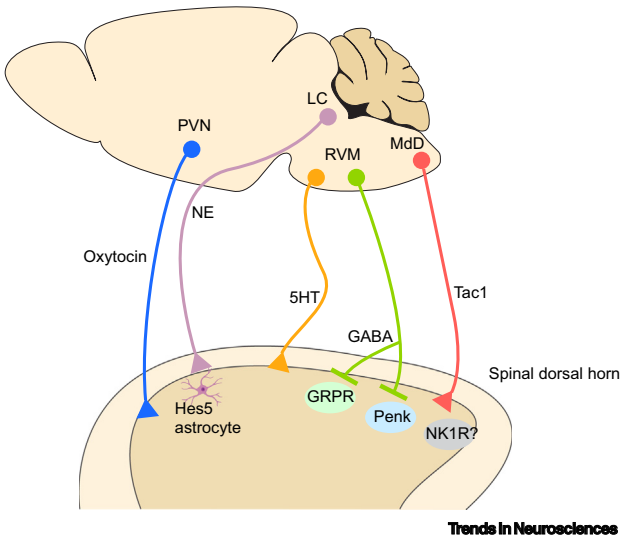

Figure 2. Genetically defined spinally projecting pathways for nociception and pruriception. Schematic demonstrating various spinal projection pathways involved in the modulation of nociception and pruriception that have been revealed using cell type-specific measurements and manipulations. Abbreviations: 5HT, 5-hydroxytryptamine; GABA, glutamate decarboxylase; GRPR, gastrin-releasing peptide receptor; LC, locus coeruleus; MdD, medullary reticular nucleus; NE, norepinephrine; NK1R, neurokinin 1 receptor; Penk, proenkephalin; PVN, paraventricular nucleus of hypothalamus; RVM, rostral ventromedial medulla; Tac1, tachykinin 1.

a population that has been implicated in driving scratching behavior [108,109]. Together, these reports suggest that GABAergic RVM neurons could facilitate nociceptive behaviors through the inhibition of Penk spinal neurons and inhibit pruriceptive behaviors through the inhibition of excitatory GRPR neurons (Figure 2). Using similar approaches, studies have also identified cell types in other brain regions including the LC [110], medullary dorsal reticular nucleus [111], and hypothalamus [112] that directly modulate spinal nociceptive circuits (Figure 2). It remains unclear whether these other hubs for descending modulation of nociception intersect with PAG and RVM pathways or represent independent and parallel circuits for the descending modulation of nociception. Together, these different pathways likely represent partially overlapping components of descending modulation.

Concluding remarks and future perspectives

Technological advances have allowed for the precise spatial and temporal manipulations of molecularly identified neuronal populations. New investigations into descending modulatory circuits expand upon decades of work involving *in vivo* pharmacology, electrical stimulation, lesion studies, as well as single-unit recordings conducted in lightly anesthetized animals. Assessment of neuronal activity in awake and freely behaving animals has confirmed and extended the roles of the PAG and RVM in the descending modulation of nociception. Both past and present investigations shed light on the complexity of the heterogeneous populations of neurons involved in descending modulation. The application of novel tools and techniques has largely confirmed previous models, and, by extension, permitted the anatomical and behavioral characterization of discrete neurons in awake, freely behaving rodents.

A more detailed understanding of descending modulatory systems could provide insights into the basis for interesting observations such as the finding that chronic pain disproportionately affects women compared with men [113]. For example, sex differences have been observed in the rat PAG with respect to opioid receptor expression and intrinsic GABA signaling [21,114], which could explain differences in response to opioid medications observed in humans [113]. Furthermore, it was recently shown that dopaminergic activity in the PAG differentially engages motor and sensory behaviors in male and female mice [99]. Additional work in this area is important because the existing models for descending modulation are based on work conducted primarily in male rodents.

It is important to note that most recent studies have been conducted in mice whereas many original discoveries on descending modulation were performed in rats. Despite the use of different models, work from both species has generally yielded congruent results. For example, the functional similarities observed in the PAG across both species are reflected in antinociceptive responses to local opioid administration within the PAG [115,116], the electrophysiological responses of PAG neurons to noxious stimuli [117,118], and the anatomical connection between the PAG and the RVM [37,77,119,120]. However, differences between species have also been observed with respect to opioid receptor expression and signaling, highlighting the limitations of extrapolating findings from single-species studies [119,121–124]. Thus, the lack of parallel comparison obscures the potential and fundamental differences between the two models. Translation of research conducted in both mice and rats to humans is an important consideration for future studies.

Another interesting question surrounds the contributions of the descending modulatory system to chronic pain states. The structural and molecular changes that could occur in identified PAG and RVM circuits during chronic injury states may underlie the development of chronic pain conditions. Plasticity, such as increases in GABA tone within the PAG, has been observed in models

Outstanding questions

The PAG and the RVM are involved in the integration of homeostatic processes such as arousal, motor, and defensive behaviors. What are the responsible neuronal ensembles that coordinate these behaviors with somatosensation?

What are the genetic cell types involved in descending modulation? A comprehensive genetic atlas of cell types in the PAG and RVM is presently lacking. To date, the characterization of cell types using Cre drivers has been conducted based on the availability of mouse lines. Single-cell sequencing of these structures would provide a more detailed understanding of the diverse cell types, which could then be targeted through specific Cre alleles.

Sex differences in rodents, as well as humans, have been reported in descending modulatory pathways. How do sex differences affect the endogenous pain modulatory system in both uninjured and injured states?

Acute and chronic stress can elicit analgesia and hyperalgesia, respectively. Structures such as the PAG and its descending circuitry are thought to be involved in mediating these responses to stress. What is the neural basis for stress-induced modulation of pain?

As discussed in this review, the descending modulation of pain, and more recently, itch has been studied in detail. What is the role for descending modulation in the context of other sensory modalities, such as touch, cold, and heat?

of persistent inflammation [21,125]. The use of cell type-specific manipulations, combined with electrophysiological techniques could further facilitate investigations into alterations in specific pathways in the context of chronic pain.

While cell type-specific manipulations represent relatively new developments in the field of neurobiology, one limitation is that behavioral responses elicited by chemogenetic and optogenetic manipulations may not represent physiological states. Furthermore, artificial activation may not necessarily reflect responses to natural settings. Another major limitation of these approaches is that conclusions made from the targeting of individual populations often ignore or oversimplify the heterogeneity within the nervous system. Although it is possible to discern unique properties among neurons within a subset, these neurons may not represent truly unique populations *per se* because the diversity of cell types within descending modulatory hubs, such as the PAG and RVM, remains only partially characterized. The testing of specific cell types has been limited by the lack of a full understanding of the diversity of cell types in these areas as well as the availability of related mouse lines. A detailed atlas of cell types in the PAG and RVM would tremendously advance research in the field of descending modulation by identifying additional cell types that could then be validated and targeted, for instance, using Cre alleles (see Outstanding questions). The validation of the precise neuronal subpopulations and pathways involved in descending modulation holds promise for the discovery of targets and therapies for disorders of somatosensation including pain and itch.

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Declaration of interests

The authors declare no conflicts of interest.

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