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Review

# Descending control of nociception: Specificity, recruitment and plasticity

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ABSTRACT

The dorsal horn of the spinal cord is the location of the first synapse in pain pathways, and as such, offers a very powerful target for regulation of nociceptive transmission by both local segmental and supraspinal mechanisms. Descending control of spinal nociception originates from many brain regions and plays a critical role in determining the experience of both acute and chronic pain. The earlier concept of descending control as an "analgesia system" is now being replaced with a more nuanced model in which pain input is prioritized relative to other competing behavioral needs and homeostatic demands. Descending control arises from a number of supraspinal sites, including the midline periaqueductal gray-rostral ventromedial medulla (PAG-RVM) system, and the more lateral and caudal dorsal reticular nucleus (DRt) and ventrolateral medulla (VLM). Inhibitory control from the PAG-RVM system preferentially suppresses nociceptive inputs mediated by C-fibers, preserving sensory-discriminative information conveyed by more rapidly conducting A-fibers. Analysis of the circuitry within the RVM reveals that the neural basis for bidirectional control from the midline system is two populations of neurons, ON-cells and OFF-cells, that are differentially recruited by higher structures important in fear, illness and psychological stress to enhance or inhibit pain. Dynamic shifts in the balance between pain inhibiting and facilitating outflows from the brainstem play a role in setting the gain of nociceptive processing as dictated by behavioral priorities, but are also likely to contribute to pathological pain states.



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*Abbreviations:* COX, cyclo-oxygenase; C+ve, C-fiber input positive; C–ve, C-fiber input negative; DOR, δ opioid receptor; DRt, dorsal reticular nucleus of the caudal medulla; MOR, μ opioid receptor; PAG, periaqueductal gray; $PGE_2$, prostaglandin $E_2$; RVM, rostral ventromedial medulla; VLM, ventrolateral quadrant of the caudal medulla

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## Contents



## 1. Introduction

The dorsal horn of the spinal cord is the location of the first synapse in pain pathways, and as such, offers a very powerful target for the regulation of nociceptive transmission by both local segmental and supraspinal mechanisms. Supraspinal (or descending) control of spinal nociception originates from many brain regions and plays a critical role in determining the experience of both acute and chronic pain. Initial reports in the 1970's and 1980's were of inhibitory influences from sites in the midbrain periaqueductal gray (PAG) and from the midline nucleus raphe magnus and adjacent reticular regions in the pons and medulla, the rostral ventromedial medulla (RVM, see Fields et al., 2006 and Heinricher and Ingram, 2008 for recent reviews). For many decades attention focused on these areas as sources of descending *inhibitory* control, with a role in endogenous analgesia (antinociception) in states of extreme stress(Bolles and Fanselow, 1980; Terman et al., 1984) or in creating contrast in sensory signals that sharpened the signalling of pain by ascending pathways (Le Bars, 2002).

It is now evident that descending control can be *facilitatory* as well as inhibitory. Indeed, facilitatory and inhibitory influences on spinal events are often reported to emanate from a single brain region (e.g., Zhuo and Gebhart, 1997)]. Some descending influences are tonically active, but the balance between inhibition and facilitation is dynamic, and can be altered in different behavioral, emotional and pathological states. As already noted, it has long been recognized that intense stress and fear are associated with hypoalgesia (a decreased responsiveness to noxious stimuli) that reflects a shift towards descending inhibition. By contrast, inflammation and nerve injury, sickness, and chronic opioid administration are associated with hyperalgesia (an increased responsiveness to noxious stimuli) that in part reflects a shift towards descending facilitation. Of clinical importance, there is much evidence to suggest that descending facilitation of spinal nociception is a major contributor to central sensitization and the development of secondary hyperalgesia, indicating that the balance shifts in favor of facilitation in the transition from acute to chronic pain.

Descending control arises from a number of supraspinal sites, but the best studied is the PAG-RVM system mentioned above (Fig. 1). The PAG is heavily interconnected with the hypothalamus and limbic forebrain structures including the amygdala, and also receives direct spinomesencephalic input. The PAG projects to the RVM, which in turn sends its output to dorsal horn laminae important in nociceptive function. This system has a pivotal role in organizing strategies for coping with intrinsic and extrinsic stressors, and is also recognized as the central site of action of analgesic agents including opioids, cyclooxygenase inhibitors, and cannabinoids (Hohmann et al, 2005; Leith et al, 2007; Yaksh et al, 1976). Understanding the PAG-RVM system is thus of considerable importance from both a behavioral and therapeutic point of view. Spinal mechanisms that mediate descending control from the PAG are discussed in Section 2, and intrinsic organization of the RVM and recruitment of PAG-RVM system are considered in Section 3. Additional sources of descending modulation include pontine noradrenergic cell groups (Pertovaara, 2006) and two areas of the caudal medulla discussed in Section 4, the dorsal reticular nucleus (DRt) and ventrolateral medulla (VLM) (Tavares and Lima, 2007).

## 2. Descending control from the PAG distinguishes between the spinal processing of different sensory qualities, including different components of the pain signal

In the 40 years since Reynolds first described the phenomenon of stimulation-produced analgesia (Reynolds, 1969), the therapeutic potential of descending control has fuelled intense

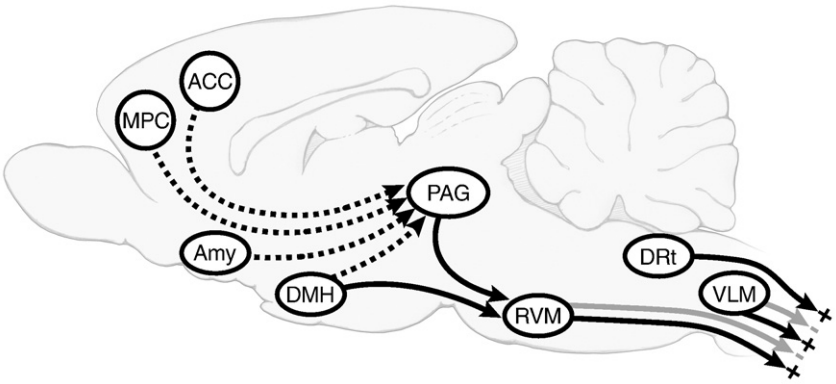


**Fig. 1 – Schematic illustrates main topics of this review: midline PAG-RVM system, which exerts bidirectional control over dorsal horn nociceptive processing, and the DRt and VLM in the caudal medulla. DRt is thought to be facilitating, and VLM primarily inhibitory, although it may, like the RVM, have both an inhibitory and facilitatory influence. The PAG especially, but also the RVM, DRt and VLM (not shown) receive important direct and indirect inputs from limbic forebrain areas including anterior cingulate cortex (ACC), amygdala (AMY), dorsomedial nucleus of the hypothalamus (DMH), and medial prefrontal cortex (MPC).**

investigation of how descending systems interface with nociceptive circuitry of the dorsal horn. There is nevertheless much conflicting information, and many unknowns: to what extent and under what conditions are descending controls mediated by presynaptic versus postsynaptic mechanisms; what neurotransmitters/neuromodulators prevail under different conditions and what are the interactions between them; and finally, do descending controls discriminate between different sensory qualities including different components of the pain signal and, if so, is this control dynamically regulated? Issues relating to the last question are the subject of this part of the review, which will consider descending control by the PAG of spinal processing of noxious versus non-noxious inputs, and of different components of the pain signal.

Initial reports of behavioral analgesia following stimulation in the PAG concluded that the effects of central stimulation were highly selective for behaviors evoked by noxious stimuli, and that animals continued to respond to non-noxious, tactile, stimuli and other non-aversive cues (Mayer et al, 1971). This finding was at odds with early electrophysiological studies in which activation of the PAG was often found to produce a non-selective inhibition of both non-nociceptive and nociceptive responses of dorsal horn neurons (Bennett and Mayer, 1979; Duggan and Morton, 1983; Gray and Dostrovsky, 1983; Kajander et al, 1984). It is likely that non-selective effects of electrical stimulation reflected activation of fibers of passage and/or antidromic activation of spinal neurons that project to the PAG. This is because activation of neuronal cell bodies in other studies revealed that PAG control of dorsal horn responses is highly selective for noxious inputs: comparison of electrical and chemical stimulation at the same sites in the PAG revealed non-selective and selective effects, respectively (Waters and Lumb, 1997). From a behavioral perspective it was concluded that selective descending control might operate as part of an integrated response to stressful or threatening stimuli. Selective suppression of nociception would allow an organism to respond in an appropriate manner to a life-threatening situation without the distraction or counterproductive motor responses that might be evoked by noxious input. The likelihood of survival would be further heightened as responses to potentially important non-noxious cues would be left intact.

The realization that descending control from the midline PAG-RVM system is specific for noxious relative to non-noxious input raises the question of whether selectivity in descending control extends further, to different aspects of the noxious signal. Information about actual or potential tissue damage in the periphery is conveyed to the spinal dorsal horn in A- and C-fiber nociceptors. These two classes of nociceptor have different electrophysiological properties, various chemical phenotypes (see Lawson, 2002 for review), signal different qualities of acute pain (Schady et al, 1983; Torebjork and Ochoa, 1990) and have distinct roles in the development and maintenance of chronic pain (Fuchs et al, 2000; Magerl et al., 2001; Pertovaara, 1998). Given the importance of descending control in defining the pain experience, together with the different roles of A- and C-fiber nociceptors in acute and chronic pain, it is important to determine how information flow in pathways activated by these distinct afferents is modulated from supraspinal sites.

The question of descending control of A- versus C-nociceptor-evoked responses in the spinal dorsal horn has been the subject of a number of studies (for example, Jurna, 1980). However, most of these studies have employed electrical stimulation to activate afferents, which could confound interpretation of the data. Electrical stimulation of peripheral nerves evokes un-physiological, synchronous inputs to the spinal cord, which may be resistant to modulation. It also simultaneously activates afferents innervating excitatory and inhibitory receptive fields of spinal neurons. One approach to overcoming these limitations is to establish the profile of A- and C-fiber input to an individual neuron using electrical stimulation, which enables assumptions about the fiber types mediating the naturally evoked responses of that cell. Thus, dorsal horn neurons can be

classified as "C-positive" (C+ve, those showing a response at C-fiber latency, in addition to A-fiber responses) or "C-negative" (C–ve, those in which there is no evidence of C-fiber-evoked activity) on the basis of their responses to percutaneous electrical stimulation. If it is then assumed that pinch-evoked responses of C+ve neurons are mediated, at least in part, by C-fibers and that those of C–ve cells are mediated by A-fiber nociceptors alone, it is possible to gain insights into any selectivity in descending control of dorsal horn activity evoked by A- or C-fiber nociceptors. This approach revealed that pinch-evoked responses of C+ve cells are generally depressed, whilst those of C–ve cells show a net facilitation following activation of the PAG (Waters and Lumb, 2008), indicating that descending control from the PAG distinguishes between neurons with and without C-fiber inputs. Although post-synaptic excitation of dorsal horn neurons from medullary pain control centers has been reported (Light et al, 1986; Yezierski, 1990), there is reason to think that the facilitatory effects on C–ve neurons reflect removal of segmental inhibition normally exerted by the C+ve neurons that were suppressed by the PAG activation (Waters and Lumb, 2008).

A limitation of the approach described above is that it relies on the assumption that pinch-evoked responses of C+ve are mediated by C- and/or A-fiber nociceptors, and C–ve neurons solely by A-fibers. It would be desirable to identify a more direct approach in which A- or C-fiber nociceptors were activated differentially using natural stimulation of the cutaneous receptive field. One way to do this is to use different rates of skin heating to preferentially activate A- or C-heat nociceptors, as first described by Yeomans et al. (1996) and Yeomans and Proudfit (1996). This technique has been further refined and, in a number of experimental paradigms, has been shown reliably to activate these distinct groups of nociceptors (Leith et al, 2007; McMullan et al, 2004). Fast rates of heating ($7.5 \pm 1$ °C $s^{-1}$) are used to preferentially activate A-fiber (myelinated, capsaicin-insensitive) heat nociceptors, whereas slow rates of heating ($2.5 \pm 1$ °C $s^{-1}$) activate C-fiber (unmyelinated, capsaicin-sensitive) heat nociceptors.

Taking this approach, Lumb and colleagues examined whether withdrawal reflexes evoked by fast and slow rates of skin heating were differentially modulated by PAG activation. C-fiber mediated withdrawals were found to be inhibited, and A-fiber evoked reflexes unaffected (Lu et al, 2004; McMullan and Lumb, 2006b; Simpson et al, 2008). Similar differential effects on A- versus C-fiber evoked spinal reflexes were also described following inhibition of cyclo-oxygenase (COX) in the PAG (Leith et al, 2007). Suppression of C-fiber mediated reflexes was consistent with the inhibition of C+ve dorsal horn neurons. However, the lack of effect on A-nociceptor-evoked withdrawal reflexes was unexpected, given the strong facilitation of C–ve neurons described above. The explanation may be that reflexes evoked by A-heat nociceptors are presumably mediated by both C+ve and C–ve neurons. Since the former would have been inhibited and the latter facilitated by PAG stimulation, the net effect on the withdrawal reflex would be null.

To show that the differential PAG modulation of reflexes evoked by A- versus C-fibers reflected an action at the dorsal horn, Lumb and colleagues next performed parallel experiments recording activity of deep dorsal horn C+ve neurons evoked by fast and slow rates of heating. Interestingly, although the thresholds for activation of the neurons by A- and C-heat nociceptors were raised to a similar extent by PAG stimulation, coding of suprathreshold stimuli was differentially affected. Stimulus-response functions of C+ve neurons to fast and slow rates of skin heating are remarkably similar to those of peripheral A- and C-heat nociceptors respectively (McMullan and Lumb, 2006a; Yeomans and Proudfit, 1996) in that fast rates of skin heating are encoded faithfully well into the tissue-damaging range whereas slow rates of skin heating are only poorly encoded. PAG activation causes a rightward shift in the stimulus–response relationship for A-evoked activity, but the linear relationship between skin temperature and firing rate is maintained. Such a relationship between stimulus temperature and cell activity as existed with the slow ramp was disrupted by PAG activation. Differential regulation of A- versus C-fiber inputs is further supported by the observation that the degree to which the heat-evoked activity of a given C+ve dorsal horn neuron is depressed by PAG activation is correlated with the strength of the C-fiber input to that neuron. Neurons with robust C-fiber inputs ("strong" C+ves) are more strongly inhibited by PAG stimulation than neurons that are less responsive to C-fiber stimulation ("weak" C+ves) (Leith et al., 2008). It remains to be determined whether A-heat nociceptor-evoked responses of C–ve neurons are facilitated by descending control from the PAG (Waters and Lumb, 2008).

### 2.1. *Mechanisms of differential descending control of C- versus A-fiber-evoked spinal nociception*

The following model (Fig. 2) builds on what is known of C- and A-nociceptive input to the dorsal horn in order to explain how the magnitude of descending control of deep dorsal horn neurons could be directly proportional to the degree of their C-fiber input.

Because C-fibers terminate primarily in the superficial dorsal horn, the C-fiber evoked responses of neurons in the deep dorsal horn must be received on superficially directed dendrites or relayed via superficial interneurons (Morris et al., 2004). A-fiber nociceptors also terminate heavily in the superficial dorsal horn, although some also provide direct input to the deep dorsal horn. Individual neurons in the superficial dorsal horn may be dominated by C-fiber inputs, A-nociceptive inputs, or by a mix of A- and C-fiber inputs (Andrew and Craig, 2002; Craig and Andrew, 2002). All deep dorsal horn neurons receive indirect and/or direct inputs from A-nociceptors. Like the C-fiber primary afferents, descending modulatory pathways terminate heavily, although not exclusively, within the superficial dorsal horn (Basbaum et al., 1978, 1986; Fritschy et al., 1987; Ruda et al., 1981). Hence, although the activity of deep dorsal horn cells may be influenced directly by descending pathways, much of the descending influence is likely to be secondary to modulation in the superficial dorsal horn.

The model developed by Lumb and coworkers focuses on the interneurons as both the source of C-fiber input to the deep dorsal horn and the target for descending control from the PAG. It postulates that "strong" C+ve neurons in the deep

dorsal horn receive C-fiber input relayed by numerous superficial C-receptive neurons, whilst "weak" C+ve neurons are targeted by relatively few C-receptive interneurons, and that C–ve neurons in the deep dorsal horn receive no projections from superficial C-receptive interneurons. In addition, evidence has been provided for reciprocal segmental inhibition of deep dorsal horn neurons, whereby activity in C+ve neurons inhibits activity in C–ve neurons and vice versa (Waters and Lumb, 2008). Descending inhibition of C+ve neurons will thus disinhibit weak C+ve and C–ve neurons. Given that descending control spares ongoing and non-noxious-evoked activity of class 2 deep dorsal horn neurons (Waters and Lumb, 2008), the inhibition of noxious-evoked activity is unlikely to result entirely from postsynaptic inhibition in the *deep* dorsal horn. However unlike in the deep dorsal horn, inhibition exerted in the *superficial* dorsal horn from the PAG is *not* selective for C-fiber input (Koutsikou et al., 2007), although it is likely to be at least in part post-synaptic (see review in Fields et al., 2006).

Taken together, these lines of evidence suggest that the differential effect of activating descending inhibition from the PAG on C-evoked activity in the deep dorsal horn is mediated by a direct post-synaptic action in the superficial dorsal horn. The strength of inhibitory control of the C-evoked activity in a given deep neuron reflects the degree to which that neuron receives C-fiber input relayed through the superficial layers.

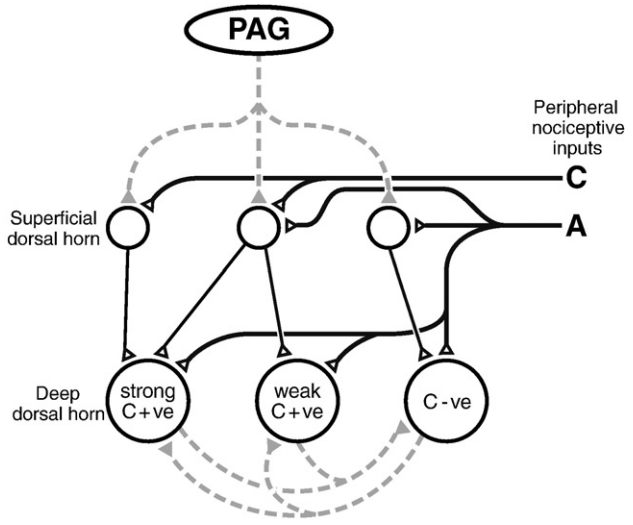


**Fig. 2 – A simplified model to explain how descending control from the PAG, which targets different populations of superficial dorsal horn neurons, could produce an inhibition of deep dorsal horn neurons that is proportional to their C-fiber input, but a facilitation of other neurons with weak or no C-fiber input. Solid lines represent direct (monosynaptic) connections, dotted lines represent indirect (polysynaptic) connections between neurons; open triangles/black lines are excitatory synapses and gray lines/filled triangles are inhibitory connections. The PAG inhibits superficial dorsal horn neurons that relay information carried by C-fibers to the deep dorsal horn. The net inhibitory or facilitatory effect of PAG stimulation is also a function of reciprocal inhibition between C+ve and C–ve neurons at the segmental level. C+ve: C-fiber input positive, C–ve: C-fiber input negative.**

Because the strong C+ve deep dorsal horn neurons receive multiple inputs from the superficial dorsal horn, descending control produces a large net inhibition of nociceptive activity (both A- and C-fiber-evoked). By the same argument, activity of weak C+ve neurons, which receive a small input from the superficial dorsal horn, is only modestly depressed by PAG stimulation. Strong inhibitory influences on C+ve neurons will lift weak C+ve cells and C–ve cells from segmental inhibition, resulting in a net facilitatory effect. Importantly, direct A-nociceptor inputs to the deep dorsal horn that are not subject to descending control may act to further protect A-nociceptor-evoked responses. Together these mechanisms could account for the range in descending modulation of deep dorsal horn neurons, from strong inhibition to significant facilitation.

### 2.2. *Functional significance of differential descending control of spinal nociception mediated by C-fibers and A-fibers*

Our recent data have demonstrated clear differences in the descending control of C- versus A-fiber mediated spinal nociception from the PAG. C-fiber evoked activity is powerfully suppressed, whereas A-fiber nociception is unaffected or even enhanced. Such differential control is of considerable behavioral significance given the central role of the PAG in coordinating survival strategies. Potentially distracting input arising from C-fiber activation would be suppressed, preserving rapidly conducted sensory-discriminative information carried by A-fiber nociceptors.

These findings may also be relevant to development and maintenance of chronic pain states, since C-fiber inputs play a significant role in the sensitization of dorsal horn neurons (Fuchs et al., 2000; Magerl et al., 2001). Descending inhibitory control of those inputs may in many cases limit development of a central sensitized state, and failure of this inhibition may permit recruitment of descending facilitation (see Sections 3 and 4 below).

## 3. Organization and recruitment of pain modulating circuitry of the RVM

The PAG does not project directly to the spinal cord. Instead, its principle descending projection is to the RVM, which can be considered the output of the midline pain-modulation system. The RVM is defined functionally, as the midline pontomedullary area in which electrical stimulation or opioid microinjection produces behavioral antinociception. It includes the nucleus raphe magnus and adjacent reticular formation, and projects diffusely to dorsal horn laminae important in nociceptive processing, including superficial layers and deep dorsal horn (Fields and Heinricher, 1985).

### 3.1. *ON-cells and OFF-cells as the neural basis for bidirectional control from the RVM*

The ability of the PAG-RVM system to suppress nociception is well documented (Fields et al., 2006), but as noted above (Section 1), descending control can be facilitatory as well as inhibitory. Functionally opposing facilitation and inhibition may in some instances arise from distinct brain regions, as

occurs in the caudal medulla (Section 4, below). However, in the case of the RVM, facilitatory and inhibitory influences have been found to overlap. Non-selective stimulation or inactivation of RVM neurons can suppress or enhance nociception, depending on the functional context. Electrical stimulation can produce facilitation or inhibition with different thresholds, or over the course of a developing inflammatory response (Ren and Dubner, 2002, Zhuo and Gebhart, 1990, 1992, 1997). Focal application of opioids in the RVM evokes analgesia, whereas the neuropeptide cholecystokinin produces behavioral hyperalgesia (Heinricher and Morgan, 1999; Heinricher and Neubert, 2004; Kovelowski et al., 2000). Whether neurotensin microinjection in the RVM produces analgesia or hyperalgesia varies with dose, and presumably, the receptor type activated (Buhler et al, 2005; Neubert et al., 2004; Smith et al., 1997; Urban and Smith, 1994). The effects of RVM inactivation are similarly complex. Lesion or general inactivation of RVM neurons may produce modest hyperalgesia or have no effect under basal conditions, but raise the nociceptive threshold in acute and chronic hyperalgesic states (Heinricher and Kaplan, 1991; Kaplan and Fields, 1991; Porreca et al., 2002).

It is difficult to understand how the RVM could produce both analgesia and hyperalgesia when approaching the problem at the level of the region as a whole, using *c-fos* expression, bulk labelling, or non-specific pharmacological manipulations. However, the increasing appreciation of the RVM as mediating bidirectional control of nociception has been paralleled by a growing understanding of the functional physiology of RVM neurons. In 1983, Fields and colleagues described RVM neurons that exhibited abrupt state changes associated with nocifensive withdrawal and named these "ON-cells" and "OFF-cells". ON-cells entered a period of activity, and OFF-cells a period of silence (Fields et al., 1983a). (The remaining neurons were classified by exclusion, and referred to as NEUTRAL-cells.) The validity of this categorization for classifying RVM neurons has been repeatedly confirmed by the distinct pharmacological profiles exhibited by the different cell classes (Heinricher et al., 1988, 1992; Heinricher and Neubert, 2004; Meng et al., 1998; Meng and Johansen, 2004; Neubert et al., 2004; Selden et al., 2007). At least some cells of each class project to the spinal cord, and specifically to the dorsal horn (Fields et al., 1995; Vanegas et al., 1984). Within 15 years of describing the ON/OFF/NEUTRAL cell classes, it became evident that it is the OFF-cells that function as the antinociceptive output from the RVM (see Heinricher and Ingram, 2008 for comprehensive review).

Determining a role for the ON-cells proved more challenging. ON-cells were at first relegated to a role as inhibitory interneurons mediating the reflex-related pause in firing that characterizes OFF-cells. In the absence of functional evidence for descending facilitation from the RVM, the suggestion that these neurons could have a permissive or possibly facilitatory influence received little attention (Fields and Heinricher, 1985). However, it subsequently became clear that ON-cells are not inhibitory interneurons in the RVM (Cleary et al., 2008; Heinricher and McGaraughty, 1998). Moreover, growing evidence pointed to a pain facilitatory role for the RVM under a variety of conditions in which behavioral hyperalgesia was correlated with increased activity of ON-cells and suppression of OFF-cell firing (Barbaro et al., 1986; Bederson et al., 1990; Fields et al., 1983b; Heinricher et al., 1989; McGaraughty et al., 2003; Morgan and Fields, 1994; Morgan et al., 1994; Pan et al., 2000). However, reports that noxious-evoked activation of apparent ON-cells is correlated with analgesia rather than hyperalgesia (Azami et al., 2001; Li et al., 1998; Thurston and Randich, 1995), and claims that ON- and OFF-cells had no role in pain modulation (Mason, 2001), underscored the limitations of correlative methods for defining the function of these neurons.

A much stronger approach is to determine the net behavioral effect (facilitatory or inhibitory) of targeted activation or inactivation of each population. To achieve this, a combined single-cell recording/microinjection approach in which neuronal activity within the RVM and nocifensor reflex threshold are recorded before, during and after focal application of a drug within the RVM was developed (Heinricher and Tortorici, 1994). When appropriate pharmacological tools are available, this approach permits differential manipulation of the activity of the different cell classes, and allows the determination of the net behavioral effect of altering the firing of each class in the intact animal. Using this approach, it was possible to show that ON-cells are the facilitating output from the RVM (Heinricher and Neubert, 2004; Heinricher and Ingram, 2008; Kincaid et al., 2006; Neubert et al., 2004; Xu et al., 2007).

### 3.2. *NEUTRAL-cells, serotonin and descending control*

Whether NEUTRAL-cells have any role in modulation of pain has been an important unresolved question ever since the term was first applied to all cells that were neither ON-cells nor OFF-cells. NEUTRAL-cells do not respond during nocifensor withdrawals or during acute inflammation (Kincaid et al., 2006; Xu et al., 2007). NEUTRAL-cell firing is also unchanged following focal microinjection of μ-opioids, cannabinoids, $\alpha_2$-agonists, and the neuropeptides cholecystokinin and neurotensin, all at doses that have unambiguous effects on activity of ON-cells and/or OFF-cells as well as on behavioral threshold (Heinricher et al., 2001a; Heinricher and Neubert, 2004; Meng and Johansen, 2004; Neubert et al., 2004). The failure of NEUTRAL-cells to respond in a way that can be linked to nociceptive behavior provides no obvious hypothesis as to how these neurons might contribute to descending control, and their distinct pharmacology further corroborates their segregation into a class distinct from ON- and OFF-cells. However, one possibility is that NEUTRAL-cells are recruited to become ON- or OFF-cells during development of chronic pain states (Miki et al., 2002). Although apparently inconsistent with the distinct pharmacology of NEUTRAL-cells, this latter idea may be related to the wide variation in excitability of ON- and OFF-cells under basal conditions (Heinricher et al., 1989). In addition, at least some NEUTRAL-cells, but apparently no ON-cells or OFF-cells are serotonergic (Gao and Mason, 2000; 2001; Potrebic et al., 1994; Winkler et al., 2006). Given the widely accepted importance of serotonin in nociceptive modulation (Suzuki et al., 2004), the role(s) of NEUTRAL-cells in pain modulation remains an open question of significant interest.

### 3.3. *Recruitment of RVM ON-cells and OFF-cells in positive feedback loops*

ON- and OFF-cells appear to exert a "mass-action" regulation of dorsal horn function, and nociceptive threshold varies with the balance between the two populations. Cells within each class fire in phase, with the two classes out of phase. In lightly anesthetized animals, the two populations alternate spontaneously, with active periods in each class lasting seconds to many minutes. Nocifensive reflexes such as the tail flick or paw withdrawal to noxious heat are also marked by a shift in the balance between the populations such that ON-cells more or less synchronously enter an active phase, whereas OFF-cells show the opposite response, and become silent (Barbaro et al., 1989; Heinricher et al., 1989). Nociceptive threshold is lowest when the ON-cell population is active and OFF-cells are silent (Heinricher et al., 1989, 1991).

The equilibrium between the ON- and OFF-cell populations under basal conditions likely reflects a role for the RVM in mediating subtle shifts in the priority of nociceptive responding relative to other behavioral tasks. Thus, for example, it has long been recognized that the pain threshold is elevated when hungry animals are given access to food (Casey and Morrow, 1988, 1989). Moreover, there appears to be an equilibrium between responding to noxious inputs and the need to maintain energy balance. Feeding is suppressed in favor of pain behaviors during the first phase of the formalin response, generally thought to represent a relatively intense sensation. By contrast, pain behaviors are reduced in favor of feeding during the second, less intense, phase of the formalin response (LaGraize et al., 2004). There is anecdotal evidence that this is mediated by OFF-cells in the RVM (Foo and Mason, 2005). Similar elevations in nociceptive threshold are observed during micturition, and this presumably allows the bladder to be emptied without disturbance by reflexive movements evoked by noxious stimulation (Baez et al., 2005). Analgesic drugs such as opioids and the novel agent improgan take advantage of this system, and produce their effects by causing OFF-cells to become continuously active (Heinricher et al., 1994, 1999, 2001a,b; Nalwalk et al., 2004), an action that may be pharmacological rather than physiological. Conversely, an encounter with a noxious stimulus increases nociceptive vigilance, at least temporarily, and activation of ON-cells and inhibition of OFF-cells triggered by a discrete noxious experience lowers the behavioral threshold for subsequent noxious inputs (Ramirez and Vanegas, 1989). Reflex-related ON-cell activity apparently plays a role in the magnitude or intensity of the behavioral response to noxious input (Jinks et al., 2007).

A shift in the balance ON- and OFF-cell populations such that ON-cells predominate for extended periods likely underlies the pro-nociceptive influence that this region develops during chronic inflammatory and nerve injury states (Heinricher et al., 2003; Porreca et al., 2002). ON-cells enter periods of prolonged activation during acute inflammation (Kincaid et al., 2006), and the increase in *c-fos* expression in the RVM following acute inflammation of the ankle joint (Pinto et al., 2007) likely reflects activation of these pro-nociceptive neurons. Interestingly, the pattern is quite different in chronic arthritis (kaolin and carrageenan in the knee joint) or following nerve injury (spinal nerve ligation, spared nerve injury). With chronic arthritis, both ON- and OFF-cell classes display a modest increase in spontaneous activity, and there is no change in the threshold for withdrawal to noxious heat (Pinto-Ribeiro et al., 2008). The lack of behavioral change may reflect co-activation of the pro-nociceptive and anti-nociceptive outflow from the RVM. The finding that chronic ankle joint inflammation (Complete Freund's adjuvant) increased *c-fos* expression in the RVM with no change at the level of the dorsal horn (Pinto et al., 2007) could be considered consistent with the increase in ongoing activity of both ON- and OFF-cell classes reported by Pinto-Ribeiro et al. (2008). Interestingly, responses of both ON- and OFF-cells to noxious pinch are reported to be *reduced* during chronic inflammation (Pinto-Ribeiro et al., 2008), although pinch-evoked *c-fos* expression in RVM neurons is enhanced, and associated with decreased expression at the level of the dorsal horn (Pinto et al., 2007). Further work will clearly be required to link findings obtained using the *c-fos* method with functionally distinct cell populations in the RVM.

In neuropathic models, mechanical allodynia and thermal hyperalgesia are maintained by the RVM (Porreca et al., 2002). Surprisingly, the ongoing activity of ON- and OFF-cells does not predict behavioral hypersensitivity in this situation. However, both ON- and OFF-cells developed novel responses to innocuous mechanical stimuli, and enhanced responses to noxious heat and mechanical stimulation of the nerve-injured limb (Carlson et al., 2007; Gonçalves et al., 2007). This suggests that there are important differences in dynamic reorganization of the RVM in inflammation as compared to nerve injury, or that compensatory processes attempt to realign this system as a chronic pain state progresses.

### 3.4. *Top-down recruitment of the RVM in illness and stress*

Changes in the dynamics of the RVM during inflammation or following nerve injury doubtless represent important components of central positive feedback loops engaged by noxious input. However, the PAG particularly, but also the RVM, receive substantial afferent input from higher centers, particularly the hypothalamus, amygdala and prefrontal cortex (Fig. 1). The amygdala is known to be a critical relay to the PAG-RVM system in the analgesic states associated with intense fear (Helmstetter, 1992; Helmstetter and Tershner, 1994), and opioid action in the basolateral nucleus of the amygdala recruits OFF-cells in the RVM (McGaraughty and Heinricher, 2002). Higher centers may also be important in hyperalgesia, as well as analgesia. For example, stimulation in the ventrolateral orbital cortex activates RVM ON-cells, and hyperalgesia produced by stimulation in the anterior cingulate requires the RVM (Calejesan et al., 2000; Hutchison et al., 1996). Prostaglandin $E_2$ ($PGE_2$) in the medial preoptic area similarly activates ON-cells and produces hyperalgesia (Heinricher et al., 2004). This is of interest because the medial preoptic area is a primary site at which $PGE_2$ acts to organize autonomic, neuroendocrine and behavioral elements of the sickness response (Elmquist et al., 1997; Ivanov and Romanovsky, 2004; Kluger, 1991). Activation of the dorsomedial nucleus of the hypothalamus, a region implicated in autonomic aspects of the response to psychological stress, also evokes behavioral

hyperalgesia mediated by ON-cells (Martenson et al., in press). Importantly, associated fever and tachycardia are not mediated by ON-cells, arguing against the proposal that autonomic and pain-modulating function are conflated in the RVM (Mason, 2001), and instead pointing to segregation of these functions of the RVM at the level of individual neurons.

Evidence for top-down control of the RVM provides a possible neural substrate for the influence of cognitive and emotional factors on pain. Just as *suppression* of pain could be advantageous in highly stressful or dangerous situations where other behaviors must pre-empt pain responses and recuperative behaviors in order to ensure survival, *facilitation* of pain could promote recuperative behaviors during illness, and enhance vigilance in situations where threat is possible, but not imminent.

## 4. Descending control from the caudal medulla

In addition to the PAG-RVM system, two areas of the caudal medulla, the dorsal reticular nucleus (DRt) and caudal lateral ventrolateral medulla (VLM), have also been implicated in descending control of dorsal horn nociceptive processing. The DRt is reciprocally connected with dorsal horn laminae important in nociception, and experimental stimulation of the DRt facilitates behavioral measures of nociception, implicating this region in a positive feedback loop that is closely tied to processing of nociceptive information (Lima and Almeida, 2002). The VLM also participates in a closed loop with dorsal horn nociceptive processing laminae (Tavares and Lima, 2002). Based on the importance of spinobulbospinal loops in central sensitization (Suzuki and Dickenson, 2005), the reciprocal connections of the DRt and VLM with the spinal cord provide an important anatomical background for the participation of those areas in central sensitization during chronic pain. Additionally, the VLM shows close parallels with the more rostral areas, namely the RVM and the $A_5$ noradrenergic cell group, the latter being relevant for pain modulation (Tavares et al., 1997). Stimulation in the VLM potently inhibits behavioral and dorsal horn nociceptive responses, whilst lesions result in apparent disinhibition. This suggests that the VLM exerts a tonic inhibitory control of dorsal horn nociception (Foong and Duggan, 1986; Gebhart and Ossipov, 1986; Janss and Gebhart, 1988). Nevertheless, the VLM may, like the RVM, exert a facilitatory influence, as neurons with features of ON and OFF cells have been identified in this region (Pinto-Ribeiro et al., 2006).

Studies using *c-fos* detection as a functional anatomical method point to dynamic changes in both DRt and VLM in acute and chronic inflammatory models. Acute inflammation induced by intra-articular injection of a solution of $PGE_2$ and bradykinin induces a strong neuronal activation both at the VLM and the spinal cord. This suggests that at the initial phases of inflammation, descending inhibition from the VLM fails to inhibit the strong nociceptive transmission arising from the spinal cord (Pinto et al., 2007). With chronic inflammation (Complete Freund's adjuvant in the knee joint), innocuous stimulation of the affected paw gives rise to an inverse correlation between *c-fos* expression in the VLM and dorsal horn, suggesting that descending inhibition is sufficient to suppress spinal activation. However, when intense pinch is applied to the same limb, strong *c-fos* expression is seen at both levels, implying either that the *c-fos* expression represents activated pain-facilitating neurons or, if expression is in pain-inhibiting neurons, that descending inhibition is insufficient to suppress activation of the dorsal horn neurons (Pinto et al., 2006a, 2007).

The imbalance between inhibition and facilitation during chronic pain is likely to be due to complex changes at the pain control centers. At the RVM, the activity of ON- and OFF-cells is affected during the course of chronic pain installation (Kincaid et al., 2006). Recent studies suggest that in other areas besides the RVM, the neuronal activity is strongly affected, as recently shown by increased activity of ON-cells and OFF-cells at the VLM of monoarthritic rats (Pinto-Ribeiro et al., 2008). At the DRt, neurochemical changes have been described during chronic inflammatory pain. The pronociceptive effects of the nucleus are maintained during chronic pain (Sotgiu et al., 2008), which is probably associated with a decreased inhibitory tone. The expression of μ-opioid receptors (MOR) and δ-opioid receptors (DOR) is decreased at the DRt during chronic pain without changes in local levels of opioids (Neto et al., 2008; Pinto et al., 2006b). This is likely to directly affect descending modulation from the nucleus, since spinally-projecting DRt neurons express MOR receptors (Pinto et al., 2008) and the instillation of the MOR agonist DAMGO ([d-Ala (Azami et al., 2001), *N*-MePhe(Barbaro et al., 1986)Gly-ol (Barbaro et al., 1989)]-enkephalin) at the DRt has opposite effects in non-inflamed and monoarthritic rats (Pinto et al., 2006b). The changes described at the DRt are opposite from those described in other pain control centers, namely at the RVM, where the local efficacy of opioids increases without changes in opioid receptor expression (Hurley and Hammond, 2001). This shows that different components of the supraspinal pain-control system are differentially affected by the installation of chronic pain. Strategies for manipulation of the supraspinal pain control system should take into account the regional changes induced by chronic pain and could be based on vector delivery of the suitable transgenes using gene therapy approaches (Tavares and Lima, 2007).

## 5. Concluding remarks

Our understanding of pain mechanisms and pain control has in large part focused on the properties of primary afferent and dorsal horn nociceptive neurons and ascending pathways. The role of supraspinal processing has undergone a recent renaissance with the advent of functional imaging techniques, which have pointed to an interacting cortical "matrix", rather than a "pain center" as underlying the pain experience. Nevertheless, a complete understanding of the neural basis of pain requires recognition that the brain is not a passive receiver of a "pain message". Rather, there is an active regulation of sensory transmission at the level of the dorsal horn by means of descending projections from the brainstem. Although brain control of sensory input is by no means unique to pain, it seems to have a particularly important role in this system. The need to regulate nociceptive input likely reflects the imperative of responding to stimuli that harm or threaten

to harm the body. Depending on the behavioral context, signals related to noxious or potentially noxious input could receive enhanced attention or be subordinated to other bodily needs of higher priority (Bolles and Fanselow, 1980; Koyama et al., 2005; Quevedo and Coghill, 2007). Understanding how the descending control systems interface with dorsal horn nociceptive processes, how they are recruited to effect changes in the priority of pain relative to other behaviors, and how the dynamics of these systems are altered and contribute to pathological pain states, are important questions that are only now starting to be fully addressed.

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