Cortical and subcortical modulation of pain

Milena De Felice\textsuperscript{1} & Michael H Ossipov\textsuperscript{\textasteriskcentered1,2}

Practice points

- Early clinical observations and animal studies on nociceptive reflexes strongly suggested the existence of endogenous pain modulatory mechanisms. Electrophysiologic, histochemical and pharmacologic studies in animals provided convincing evidence of bidirectional pain modulation arising from the periaqueductal gray (PAG) and activating the rostral ventromedial medulla (RVM) and noradrenergic nuclei to block ascending nociceptive inputs.

- Recent advances in neuroimaging studies allowing finer degrees of spatial and temporal resolution spurred intensive research that has greatly changed our perceptions of how pain is integrated and modulated in the CNS.

- From the linear system of pain modulation through the PAG–RVM system, we have progressed to envision a complex ‘pain matrix’ that includes important cortical regions, elements of the limbic system as well as midbrain and medullary sites. Moreover, imaging studies in humans have driven novel animal experiments in order to understand the neuroanatomical and biochemical implications of this pain matrix, especially at sites rostral to the PAG.

- Pharmacological and electrophysiological exploration of these regions are providing insights into the brain regions that are involved in many other neural functions, from autonomic regulation to sensory and emotional management and how they may interact to modify descending pain modulate.

- Ultimately, these rostral brain sites impact on the PAG–RVM system and modulate nociceptive inputs.

- A greater understanding of the components of these clinically validated pain modulatory circuits, and of the neurotransmitter systems that activate and inhibit these components, may offer approaches to develop improved pain therapy.

Pain is more than merely nociception and response, but rather it encompasses emotional, behavioral and cognitive components that make up the pain experience. With the recent advances in imaging techniques, we now understand that nociceptive inputs can result in the activation of complex interactions among central sites, including cortical regions that are active in cognitive, emotional and reward functions. These sites can have a bimodal influence on the serotonergic and noradrenergic descending pain modulatory systems via communications among the periaqueductal gray, rostral ventromedial medulla and pontine noradrenergic nuclei, ultimately either facilitating or inhibiting further nociceptive inputs. Understanding these systems can help explain the emotional and cognitive influences on pain perception and placebo/nocebo effects, and can help guide development of better pain therapeutics.

First draft submitted: 5 June 2015; Accepted for publication: 12 November 2015; Published online: 17 March 2016

\textsuperscript{1}The University of Sheffield, Academic Unit of Oral & Maxillofacial Medicine & Surgery, Sheffield, South Yorkshire, UK

\textsuperscript{2}Department of Pharmacology, University of Arizona College of Medicine, Tucson, AZ 85724-5050, USA

*Author for correspondence: michaelo@email.arizona.edu
Background
How pain is experienced by an individual can vary not only among different individuals but also within the same person. Nociception, due to the activation of selective receptors (i.e., nociceptors) that detect the presence of a potentially tissue-damaging stimulus, is only part of the total pain experience. The context of the pain experience is also a very important factor, and explains why individuals perceive pain differently even in the presence of similar injuries. This variability suggests that there are endogenous mechanisms that can be engaged to modulate a painful experience. Numerous preclinical and clinical investigations led to the development of the concept of a ‘top–down’ pain modulatory system. Pain modulation can arise from activation of brain regions that can modulate the flow of nociceptive inputs, thus enhancing or diminishing the pain experience. Numerous factors, including emotional state, attention, distraction and memories of past painful experiences can engage these ‘top–down’ modulatory circuits to profoundly change the sensory experience of pain. An understanding of the pathways and of the neurotransmitter systems that contribute to this top–down pain modulation can be exploited to develop more effective pain therapeutics.

• Pain matrix
Functional MRI (fMRI) studies have established the importance of several cortical and subcortical sites, including the rostral anterior cingulate cortex (rACC), insula, somatosensory cortex along with the amygdala, the periaqueductal gray (PAG) and the rostral ventromedial medulla (RVM) as contributing to a ‘pain matrix’ that can either enhance or inhibit pain [1,2]. The concept of a pain matrix is not to suggest a rigid regulatory pathway, but rather to suggest a collection of brain regions, many of which are not unique to pain processing but are involved in several neurological functions including cognition, emotion, motivation and sensation. These regions acting together in the context of modulation of nociception give rise to the experience of pain [3]. In fact, emotional context, expectation or attention all can change the interactions among the components participating in the pain experience and either inhibit or facilitate pain.

• Endogenous pain inhibitory system
One of the most vivid descriptions of endogenous pain modulation comes from the experiences of the physician and anesthesiologist, HK Beecher, during the Second World War [4]. He observed that many severely wounded soldiers reported moderate or no pain, and were reluctant to accept pain relief medication, suggesting that pain can be blocked by ‘strong emotions’ [4].

Role of the anterior cingulate cortex in descending pain modulation
Since Beecher’s observations, numerous studies employing diverse strategies have led to the identification of endogenous mechanisms that both positively and negatively alter the pain experience. Higher brain centers, including the hypothalamus, the amygdala and the ACC converge onto to the PAG, which in turn communicates with medullary sites to form a descending pain modulatory circuit. Neurons within the nucleus raphe magnus (NRM) and nucleus reticularis gigantocellularis, pars alpha, comprise the RVM and send bidirectional projections to the spinal and medullary dorsal horns to either enhance or diminish nociceptive traffic [5]. This descending pain modulatory circuit is relevant to the pain experience in different contexts, including chronic dysfunctional pain. It is also relevant to the actions of pain-relieving drugs including opioids, cannabinoids and serotonin/norepinephrine reuptake blockers. Descending pain modulatory pathways underlie the robust and clinically important phenomenon of placebo analgesia. Importantly, placebo analgesia can be blocked by naloxone, indicating the activation of endogenous opioid-mediated inhibition [6]. This concept is supported by neuroimaging studies showing marked overlaps between brain regions activated by opioids and activated during placebo analgesia [7,8]. Studies employing fMRI showed that the rACC and the PAG mediate endogenous opioidergic signaling essential to both opioid-induced and placebo-mediated analgesia [9]. Importantly, naloxone administration abolished the placebo response as well as the activation of the ACC and PAG [9].

Nocebo, the converse of the placebo response, refers to the expectation of a worsening outcome in response to a treatment [10]. Subjects that expected an enhancement of pain reported that the application of a nonpainful stimulus was perceived as painful, and that noxious stimuli produced enhanced pain [11]. Hypnotic suggestions given to patients to expect increased unpleasantness of nociceptive stimuli resulted in increased activity of the ACC, but not in...
the somatosensory cortex [12]. Activity of the ACC was associated with increased perceived pain, even though the stimulus intensity was unaltered [12].

A major role of the ACC in pain modulation has been observed also in animal studies. Injections of formalin into the hindpaw of rats produced conditioned place aversion to the formalin-associated context [13]. Neurochemical lesion of the rACC [13] or blockade of glutaminergic NMDA receptors of this region prior to conditioning [14], abolished formalin-induced conditioned place aversion without altering evoked behavioral responses. Additionally, more recent studies highlighted the importance of the rACC in mediating the aversive quality of ongoing pain, but not of the somatosensory component [15].

Role of the amygdala in descending pain modulation

The importance of the amygdala in emotional responses, stress and anxiety suggests that this may be a site where these emotions are integrated with pain processing. Imaging studies show responses of the amygdala during the application of noxious stimuli, as well as interactions among the amygdala, cortical sites and the PAG [1,2]. Microinjection of opioids into the amygdala elicits antinociception that is blocked by lidocaine in either the PAG or RVM [16]. These studies are consistent with the conclusion that opioid-sensitive stress-induced antinociception is likely to involve interactions between the amygdala and elements of descending pain inhibitory systems, including the PAG and RVM in particular [17].

The interplay of complex connections between the prefrontal cortex and the amygdala make up the emotional/affective modulation of cognitive functions in persistent pain states, and impact cognitive functions such as decision-making, assessment of risk/reward versus pain or punishment avoidance [18].

Role of the PAG in descending pain modulation

Several early, historically important observations established that activation of the PAG either electrically or by opioid administration produced robust antinociception and analgesia in animal models and humans, respectively (for reviews see [19–21]). The discovery of opioid-mediated endogenous pain modulation spurred numerous efforts to delineate this pain modulatory system. Descending projections from the amygdala [16,22] and from the ACC can modulate nociception by direct and indirect interactions with the PAG to ultimately activate descending opioidergic pain inhibitory mechanisms [7,9]. The ACC has direct projections to the PAG [23,24] and reciprocal connections with medial thalamic nuclei that communicate with the PAG [25]. In addition, the PAG receives ascending nociceptive inputs routed from spinal dorsal horns to the parabrachial nuclei [22]. The PAG, through its reciprocal connections with the RVM, contributes to descending pain modulation [5]. Studies in rats have shown that increased activation of PAG neurons elicits excitation of RVM neurons and inhibits nocifensive reflexes [26]. Consequently, the PAG is ideally localized to modulate both nociceptive inputs as well as pain perception by interacting with ascending and descending projections from numerous sites.

Neurotransmitters that modulate PAG activity

The PAG is rich in the expression of μ-opiate receptors and in neurons expressing enkephalin and β-endorphin [5], which is consistent with its prominent antinociceptive effects in response to exogenous opioids. In addition to endogenous opioids, other neurotransmitter systems also modulate PAG activity. The PAG also contains neurotensin-expressing nerve terminals and neuropeptide receptors, and neurotensin is released in response to morphine or DAMGO microinjected into the PAG [27]. Neurons expressing neurotensin project from the PAG to the RVM and appear to have a bidirectional modulation of RVM activity [28]. Electrophysiologic studies have also shown that neurotensin can have antinociceptive and pronociceptive activities in the PAG [29]. While the role of spinal substance P is pronociceptive, this neuropeptide exerts an antinociceptive effect by acting at NK1 receptors in the PAG [30]. It is likely that the substance P-expressing neurons are not tonically active [30], and that substance P is released by opioids. It is believed that substance P contributes to antinociception by activation of descending inhibition [30,31]. The peptide cholecystokinin (CCK) is also found in nerve terminals and cell bodies in the PAG, as is the CCK1 receptor [32,33]. Early studies in rats found that increased levels of CCK in the PAG were associated with diminished antinociceptive responses [34]. Knock-down of PAG CCK by antisense mRNA to the pro-CCK gene enhanced antinociception induced
by electroacupuncture or morphine [34]. Other studies showed that microinjection of CCK into the PAG attenuated morphine antinociception, whereas CCK2 antagonists in the PAG enhanced morphine’s effects [35]. The molecular and cellular mechanisms providing for the antiopioid effect of CCK remains unclear; however, evidence suggests that CCK attenuates the inhibition of GABA release by opioids [36,37].

**Role of the RVM in descending pain modulation: on & off cells**

The region of the RVM includes the serotonergic NRM, the nucleus reticularis gigantocellularis pars alpha and the nucleus paragigantocellularis lateralis [38]. While this region receives inputs from the thalamus, the parabrachial region and the noradrenergic locus coeruleus (LC), the large part of its inputs come from the PAG and it is often described in terms of the PAG–RVM system [39]. The RVM is also likely the final common relay in descending modulation of pain, as this region sends descending projections through the dorsolateral funiculus to the spinal dorsal horns as well as projections to the dorsal horns of the trigeminal nucleus caudalis [39]. Early neuroanatomical studies showed that the descending projections from the RVM form synaptic connections with primary afferent terminals, projection neurons and interneurons and thus are well situated to modulate nociceptive inputs [40–42].

Studies in lightly anesthetized rats, where neuronal activity in the RVM was correlated to a spinal reflex to noxious heat (i.e., the tail-flick response), revealed important insights into the nature of descending pain modulation from this region [39,43]. Different populations of RVM neurons were identified, based on their response characteristics to noxious heat. The ‘on-cells’ increase firing just prior to the initiation of the nociceptive reflex, and the ‘off-cells’ decrease or cease firing immediately prior to the tail-flick [44–46]. The firing rates of the ‘neutral’ cells did not correlate with nociceptive stimuli. In addition, the on-cells are inhibited by systemic or intra-RVM morphine whereas the off-cells are markedly excited by morphine [5,47]. Large subpopulations of each of these three populations project to the spinal and trigeminal dorsal horns and exert modulatory influences on nociceptive inputs [5,39].

The existence of neuronal populations with opposing responses to nociception is consistent with the numerous electrophysiologic and behavioral studies that demonstrate a ‘bidirectional’ pain modulation from the RVM, such that nociception can be inhibited or enhanced [19,48–49]. RVM lidocaine inhibited responses of dorsal horn neurons to electrical and natural stimulation in normal and nerve-injured rats, suggesting that the predominant influence from the RVM is facilitatory [50]. It was found that descending facilitation of nociception was enhanced after peripheral nerve injury [50]. Lidocaine micro-injected into the RVM of rats abolished hyperalgesia in several animal models with enhanced pain [51–54].

The descending pain facilitatory and inhibitory systems function in parallel to maintain a baseline homeopathic state [55–56]. Injury, inflammation or illness can destabilize this balance [57]. The role of descending inhibition in counterbalancing an enhanced pain state was demonstrated in an animal model of neuropathic pain. RVM lidocaine as well as the selective inhibition of pain inhibitory neurons of the RVM with the κ-opioid agonist U69593, unmasked behavioral signs of enhanced abnormal pain in rats with nerve injury but without signs of neuropathic pain [52]. These results suggest that the presence of descending inhibition of nociception protects against development of enhanced pain states [52], and, together with other studies, that dysfunctional pain states may occur because of a deficit in endogenous descending pain inhibitory systems [1–2,39,58–59].

The function of the RVM with regard to descending pain inhibition and facilitation has led to the examination of the nature of these RVM neurons. The administration of opioids either systemically, into the PAG or into the RVM enhances ongoing off-cell activity and maintains the off-cell activity that normally shuts off in response to a noxious stimulus [39,47]. The increased firing of off-cells is mediated by activation of μ-opiate receptors, resulting in inhibition of release of GABA and thus disinhibition of the off-cell [39,47].

In contrast to the off-cells, the on-cells are the only population of RVM neurons directly inhibited by opioids and that express the μ-opioid receptors [39,47,60]. The on-cells also express the CCK, receptor which is highly colocalized with μ-opioid receptors [61,62]. Microinjection of CCK into the RVM markedly increased on-cell activity [61] and enhanced nociception, [63,64]. Based on the concept that enhanced
abnormal pain in nerve injury is maintained by a preponderance of descending facilitation over inhibition, as described above, inhibition of neurons with a profile suggestive of on-cells is shown to block behavioral signs of enhanced abnormal pain [62–63, 65–68]. Additionally, selective ablation of on-cells suggested the idea that increased net descending facilitation is essential to maintain chronic pain, while the early stage is presumably driven by enhanced excitability of nociceptors [62, 65, 69].

Serotonergic & nonserotonergic descending pain modulation from the RVM
Since the RVM includes nuclei that are a major source of spinopetal serotonergic neurons, the role of serotonin with regard to pain modulation from the RVM has been the subject of considerable interest. Studies that employed retrograde labeling techniques indicated the presence of spinopetal serotonergic projections to arising from the NRM, the nucleus paragigantocellularis and the ventral portion of the nucleus gigantocellularis [70]. Spinal administration of 5-HT agonists elicited antinociception [71], and electrical stimulation of the PAG or the RVM elicited release of serotonin in the spinal cord [72]. Additionally, antinociception from RVM stimulation was blocked by systemic or intrathecal serotonergic antagonists [73, 74].

Approximately 20% of neurons that project from the RVM to the spinal dorsal horn are serotonergic, while the remainder are likely to be GABAergic or glycineric [75, 76]. Electrophysiological studies showed that the 5-HT neurons were all neutral cells, and not on-cells or off-cells [77]. However, all classes of RVM neurons had serotonergic apposition [46] and it is suggested that the on-cells and off-cells of the RVM can be modulated by serotonergic neutral cells [78, 79]. Selective depletion of 5-HT in serotoninergic RVM neurons in rats together with a substantial reduction of spinal 5-HT resulted in reduced nociception in several animal models of chronic, but not acute, pain [80]. Electrical stimulation of the RVM and administration of μ-opiate and κ-opiate agonists led to the conclusion that at least some on-cells projecting to the spinal cord are serotonergic, and that serotoninergic neurons were not required for opioid-mediated antinociception [80].

The implications of descending serotonergic projections are further complicated by the nature of serotonergic receptors. Activation of spinal serotonergic receptors can be either inhibitory or facilitatory, depending on the receptor subtype that is activated. The activation of serotonergic 5-HT₁A, 5-HT₁B, 5-HT₁D, and 5-HT₇ receptors is inhibitory whereas activation of the 5-HT₂A and 5-HT₃ receptors is facilitatory [81–86]. However, how the RVM neurons modulate spinal serotonin, and the source of serotonergic transmission in the spinal cord remains unclear and await further investigation.

Studies aimed at determining which nonserotonergic systems could mediate actions of the RVM showed that GABAergic neurons from the PAG synapse with spinopetal GABAergic and non-GABAergic neurons [87]. Moreover, these studies provided evidence that GABAergic as well as non-GABAergic PAG neurons synapse onto on-cells and off-cells that project to the spinal cord [87]. In addition, the RVM on-cells and off-cells may be either GABAergic or non-GABAergic [87], implying that the classification of RVM neurons may be more complex than neutral, on- and off-cells [87]. Anatomical and electrophysiological studies showed thatpain-likely projecting neurons from the RVM express GABA, glycine or both [88], and that descending GABAergic or glycineric projections inhibit nocuous inputs into the dorsal horn of the spinal cord [78]. The implications of these heterogeneous inputs to the RVM from the PAG deserve further investigation, as do the descending GABAergic projections from the RVM.

Noradrenergic descending pain modulation
Early studies showed that stimulation of RVM and PAG releases norepinephrine into the spinal cerebrospinal fluid along with producing antinociception [72, 89]. The spinal administration of noradrenergic antagonists attenuated antinociception from electrical stimulation of the RVM or the PAG as well as opioid microinjection into these regions [90–94]. Spinally administered α₁ adrenergic agonists produce a strong antinociceptive effect [95–97], and show a strong antinociceptive synergy with opioids [95–98]. In contrast, activation of spinal α₂ adrenoceptors is excitatory and enhances neuronal responses to nociceptive inputs [91]. Some of the neurons excited by α₂ adrenoceptors are inhibitory GABAergic interneurons and may contribute to antinociception [99].

Bruinstroop et al. showed that spinopetal noradrenergic projections from the LC and to a lesser extent, from the Kolliker-Fuse (A7) region,
are positioned to modulate somatosensory transmission [100].

The RVM receives noradrenergic inputs from the LC and A7 regions, and can thus modulate descending modulation through this pathway [101]. The A7 receives projections from RVM neurons that express substance P [102] and from enkephalinergic RVM neurons [103]. Interestingly, activation of the enkephalinergic projections from A7 produces a bidirectional modulation of spinal nociceptive inputs, with inhibition of nociception mediated by the spinal α2 adrenoceptors and a pronociceptive effect driven by spinal α1 adrenoceptors [103]. The PAG projects to both the A7 and LC [104]. In addition to inputs from the PAG, the LC also receives projections from the RVM and the amygdala as well as thalamic nuclei and the insular cortex [105]. The interaction between the descending noradrenergic system and the PAG–RVM pathway is demonstrated by a potent antinociceptive synergy of morphine administered into the LC and the RVM [106]. Electrical or chemical stimulation of the RVM produced antinociception that was abolished by spinal administration of a nonselective α-adrenergic antagonist [107,108]. A noradrenergic descending projections, like those from the RVM, can exert a bidirectional control of nociceptive inputs [109].

Several recent studies lead to the suggestion that nerve injury and neuropathic disease states result in enhanced activity of descending noradrenergic systems in an effort to modulate the enhanced pain state. Noradrenergic neurons of the LC, express the NK1 receptor [110] and administration of substance P into this region produces a marked reversal of tactile allodynia, but not thermal hyperalgesia, in rats with peripheral nerve injury, without altering behavioral responses in uninjured rats [110]. Moreover, the effects of substance P were blocked by the NK1 antagonist WIN 51708 in the LC or the α2 adrenergic antagonist yohimbine given spinally [110]. Electrical stimulation of the LC releases norepinephrine in the spinal dorsal horns [111] and attenuates behavioral responses to thermal nociceptive stimuli [112]. It was reasoned that the levels of norepinephrine released in the spinal cord by LC in the normal condition are insufficient to modulate normal baseline responses, but are sufficient in the nerve-injured state because of the enhanced efficacy of spinal α1 adrenergic receptors that occurs with injury [110]. Activation of noradrenergic activity likely contributes to the analgesic effects of recent novel therapies effective against chronic pain states. Tramadol and tapentadol both show weak activity at the α-opioid receptor, but they also are effective inhibitors of norepinephrine reuptake [113–115]. Numerous animal and clinical studies strongly indicate that the clinical efficacy of these drugs as analgesics is due to a synergistic interaction between opioid activity and increased activation of spinal α1 adrenergic receptors resulting from elevated levels of norepinephrine [113–115]. The clinical efficacy of the noradrenergic reuptake inhibitor duloxetine against inflammatory, neuropathic and dysfunctional pain states is attributed to enhanced noradrenergic activity [116–118]. The gabapentinoids, gabapentin and pregabalin are among the more efficacious agents used for the treatment of neuropathic and fibromyalgia pain [119,120]. In addition to modulating the α2δ subunit of voltage-gated calcium channels, gabapentin also elevated spinal cerebrospinal fluid levels of norepinephrine in surgical patients and reduced the opioid requirement for postoperative pain relief [121].

**Conclusion**

The early discoveries that stimulation of specific regions of the brain could produce analgesia, along with the discoveries of endogenous opioids and of the opioid receptors led to an explosion of investigations into pain modulatory systems. From the initial concepts invoking a linear system that can bidirectionally modulate pain and gate sensory inputs at the level of the spinal cord, we have, over the years, progressed to thinking of pain and its control in terms of complex interactions among diverse regions of the brain that involves both ascending and descending components. While the role of descending spinopetal systems from pontine-medullary sites in the inhibition of ascending signals remains an important component of current pain models, there is also a growing appreciation for the role that cortical sites that control concentration, cognition, emotions and memory can play in pain perception. This interaction provides a neurophysiologic basis for pain reduction due to placebo, hypnosis or suggestion, distraction and emotional state. Likewise, a rational basis for pain augmentation, also from suggestion, expectation and mood is provided by consideration of a cortical pain matrix. In addition, our current understanding of these regulatory systems provide a theoretical basis for understanding...
seemingly idiopathic pain states that arise in the absence of any known injury or precipitating factor, such as irritable bowel syndrome, fibromyalgia and migraine headache.

**Future perspective**

Imaging technologies are constantly improving both in temporal and spatial resolution. As the resolution of fMRI increases, it should be possible to detect changes occurring in increasingly smaller, localized brain regions. Associations among changes in the activity of discrete brain regions will help further delineate pathways that are activated in response to nociceptive signaling, and also reveal whether there are changes in associations among sites that can distinguish chronic pain states versus pain-free states. Enhancement of imaging with PET scans and radiolabeled ligands selective for various receptor subtypes will provide a quantification of changes in released neurotransmitters in the brain, such that we would be able to better understand the role of neurotransmitters and their associated receptors at distinct brain regions in acute and chronic pain states. The adaptation of novel techniques, such as fast-scan voltometry, will allow near-instantaneous real-time recording of the release of neurotransmitters in discrete brain regions in animal models. These studies would shed light on neuromodulatory changes that occur with the development of enhanced pain in animal models of neuropathy, inflammation and cancer pain.

**Financial & competing interests disclosure**

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

**References**

24. Floyd NS, Price JL, Ferry AT, Keay KA, Bandler R. Orbitomedial prefrontal cortical projections to distinct longitudinal columns


Cortical & subcortical modulation of pain


92 Camarata PJ, Yaksh TL. Characterization of the spinal adrenergic receptors mediating the spinal effects produced by the microinjection of morphine into the periaqueductal gray. *Brain Res.* 336(1), 133–142 (1985).


94 Yaksh TL. Pharmacology of spinal adrenergic systems which modulate spinal nociceptive


