# STATE-DEPENDENT OPIOID CONTROL OF PAIN

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Agonists for the μ-opioid receptor are powerful analgesics and are highly addictive; however, the contribution of the δ- and κ-opioid and opioid receptor-like receptors to motivational states is less clear. Agonists at each receptor modulate neurons in a circuit that selectively controls nociceptive transmission. This circuit can operate in both pain-inhibiting and pain-facilitating states, and the action of opioids contributes to and is determined by the state of the circuit. There is growing evidence that the state of the circuit is determined by aversive and appetitive motivational states, and that this contributes to adaptive behavioural choice.

Although the perception of pain is widely considered to be the invariant consequence of the activation of peripheral nociceptors by potentially tissue-damaging stimuli, such stimuli are just one of the many factors that are involved. There have been reports that, under conditions of great threat or strong emotion, people with severe injuries (including open wounds and bone fractures) report little or no pain[1]. Drug actions can also be highly variable; for example, opioid drugs that are selective for a single receptor can either relieve or worsen pain, depending on an animal's behavioural state. Such top–down variability of pain intensity and drug action highlights the importance of using a systems neuroscience approach to study pain modulation. This review focuses on the properties of an opioid-sensitive pain-modulating circuit: how the synapses of its component neurons are affected by opioids, how this action modulates nociceptive transmission, and how such modulation contributes to behavioural choice.

Opioids such as morphine and heroin are not only powerful analgesics; they also produce profound appetitive motivational actions. They can be addictive when used recreationally, and can enhance food and alcohol consumption. For these reasons, opioid receptors have received widespread interest from clinicians and basic scientists alike. Investigations into pain have focused largely on the μ-opioid receptor (MOR/OPRM), because its activation is necessary for the action of the most potent analgesics[2,3]. Other members of the opioid receptor family regulate pain, but their contribution has been more difficult to study because their pain-relieving actions are neither as robust nor as consistent as those of MOR ligands. So, the functional significance of opioid receptor diversity remains puzzling. However, experiments are beginning to shed light on the inconsistencies and paradoxes in the field, and a deeper understanding is emerging about how the members of this family of closely related receptors interact to provide a flexible choreography for pain control. Crucial to this improved understanding has been the systems neuroscience approach; the analysis of the firing properties and anatomical connectivity of neurons in defined opioid-sensitive pain-modulating circuits.

## Matching neural circuits to behaviour

Whether one's goal is to explain the pharmacology of an exogenously applied opioid or to understand the physiology of endogenous opioid peptide function, the systems neuroscience approach has contributed to the discovery process by producing mechanistic models of opioid function that are detailed and that have great predictive power. Because the biologically relevant output of the nervous system is behaviour, circuits are meaningfully defined in relation to a specific behaviour. Consequently, tracing a circuit requires the selection of the behaviour of interest.

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The first step in determining how a drug affects behaviour is to inject it directly into nuclei that are part of the behaviourally-defined circuit. The effect of the drug is then explained by determining how it changes the firing of neurons in that nucleus. Using innate nocifensive withdrawal reflexes as behavioural surrogates for pain, an opioid-sensitive circuit that selectively controls nociceptive transmission has been defined[4,5]. Locally acting opioids robustly alter the activity of neurons in several serially-connected relays within this circuit. These neurons modulate nociceptor-driven behaviours[6–8] and the synaptic mechanisms by which opioids directly regulate their activity have been determined[9–11]. Furthermore, through the use of selective opioid receptor antagonists, usefully constrained hypotheses of endogenous opioid function have been tested. Importantly, circuit models have been tested in human subjects through the use of functional imaging and opioid antagonists. Pain is a subjective experience and only humans can give 'direct' reports about what they feel, so the ability to conduct relevant experiments in humans provides a rare opportunity to validate hypotheses about human subjective experience based on animal research. Therefore, the circuit analytical approach allows us to establish a chain of causality from molecular events at the synapse to human perception and behaviour.

## Pain and pain-modulating circuits

The process that leads to pain perception is typically initiated by the activation of peripheral receptors, which selectively detect intense, potentially tissue-damaging stimuli. These primary afferent nociceptors have been studied extensively in animals and humans. We now know a great deal about the molecular mechanisms of transduction and the relationship of the firing of these neurons to stimulus intensity and to the psychophysics of perceived pain intensity[12–14]. Furthermore, although much remains to be learned about central processing, there is broad agreement on the general outlines of the afferent transmission pathways from primary afferent nociceptors through the dorsal horn and on to the thalamus and cortex[15] (FIG. 1).

At each of the identified nociceptive relay nuclei in rodents, cats and primates, neurons have been recorded with activity increasing as a function of stimulus intensity across the noxious range. Studies combining functional imaging and psychophysics have shown that activations in human thalamic and cortical nociceptive-receiving areas correlate with perceived pain intensity[13,16]. Furthermore, pain perception in humans is blocked by lesions in spinal cord and brain areas that are homologous to those identified as pain pathways in animals. The consistent and lawful relationships between stimulus intensity, neuronal firing and human reports of pain intensity, and the striking anatomical homologies across species have provided a simple, powerful and relatively complete explanation of the sensory processing that underlies pain perception. This robust and highly conserved afferent circuit provides a firm foundation for the study of pain modulation.

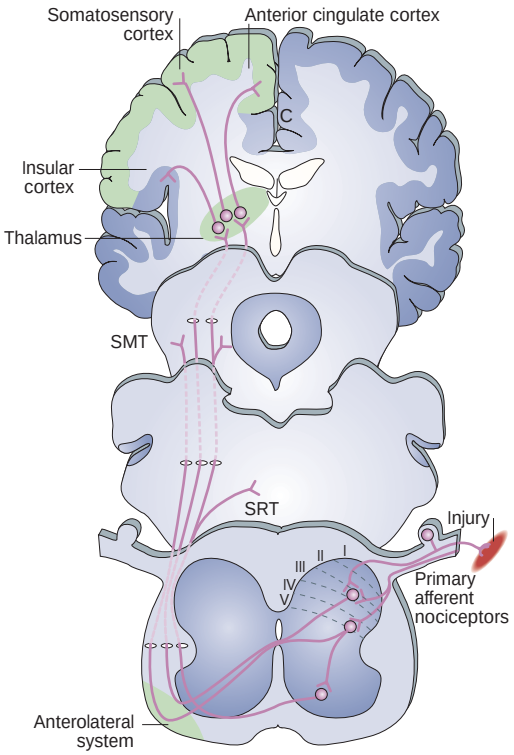


Figure 1 | **Schematic of afferent pathways underlying the sensation of pain.** Injury activates the primary afferent nociceptor (PAN), which transmits information to the dorsal horn of the spinal cord. The terminals of the PAN contact neurons in specific laminae of the dorsal horn where they release glutamate and peptides to activate the second order neurons. The axons of nociceptive dorsal horn neurons cross to the contralateral anterolateral quadrant to form an ascending tract, which terminates in the brainstem and several distinct areas of the thalamus, which contain higher order neurons that project to various cortical regions that mediate different aspects of the pain experience. These regions include somatosensory, anterior cingulate and insular cortices. SMT, spinomesencephalic tract; SRT, spinoreticular tract.

*Defining the pain-modulation circuit.* In the early 1970s, the circumstances were in place for explosive growth in our understanding of pain-modulation circuits. Recording from single neurons in the CNS led to the conceptual breakthrough that these neurons are feature detectors that are tuned to detect tissue-damaging stimuli. Building on this discovery, scientists were able to show that specific laminae (I, II and IV–VI) of the spinal cord dorsal horn were a vital relay for nociceptive signals[14]. A key advance in the modulatory field was Wall's discovery that nociceptive neurons in these laminae are subject to powerful control by supraspinal sites[17].

Stimulation-produced analgesia was the next crucial discovery. Working in rats and using simple withdrawal reflexes as the pain measure, Reynolds[18] and later Liebeskind and colleagues[19,20] showed that stimulation of a specific region of the midbrain — the periaqueductal grey (PAG) — inhibited behavioural responses to noxious stimulation. In a dramatic extension of this finding, electrical stimulation of the midbrain PAG in humans was reported by several neurosurgical groups to produce

clinically significant pain relief (for reviews, see REFS 21,22). Importantly, stimulation of this midbrain site inhibited nociceptive dorsal horn neurons, indicating that the behavioural changes were due to control of sensory transmission rather than motor responses[23,24].

Subsequent work using a combination of methods (brain mapping by electrical stimulation, anatomical tract tracing, inhibition of withdrawal reflexes and dorsal horn electrophysiology) rapidly led to detailed knowledge of the anatomy, physiology and pharmacology of this pathway[7]. The PAG receives direct inputs from the hypothalamus and from the LIMBIC forebrain, including several regions of the frontal neocortex and the central nucleus of the AMYGDALA (FIG. 2). The PAG controls nociceptive transmission indirectly by means of connections through neurons in the rostral ventromedial medulla (RVM) and the dorsolateral pontine tegmentum (DLPT). These two regions project through the spinal cord dorsolateral funiculus and selectively target the dorsal horn laminae that house the nociceptive relay neurons. So, the selective control of pain by this circuit is explained by its anatomical selectivity for primary afferent nociceptor terminals and somata of dorsal horn neurons that respond to noxious stimulation.

*Opioids in the pain-modulation circuit.* Morphine is the prototypical MOR agonist. MOR agonists produce analgesia through both pre- and postsynaptic mechanisms at multiple CNS sites (FIG. 3). MOR agonists can directly inhibit pain transmission at spinal levels through actions on primary afferents[25] and nociceptive relay neurons in the dorsal horn[26,27], but this review will focus on supraspinal modulatory circuits. The MOR is present in all of the known supraspinal components of the pain-modulation circuit including the insular cortex, amygdala, hypothalamus, PAG, DLPT, RVM and spinal cord dorsal horn[2,28–30]. Microinjection of MOR agonists into each of these sites inhibits behavioural responses to noxious stimulation[4,5,31,32]. Inactivating the RVM or cutting the axons of RVM neurons that project to the spinal cord dorsal horn reduces analgesia produced by morphine that has been given systemically or microinjected into supraspinal sites. This shows that MOR-agonist analgesia depends on activation of supraspinal neurons that project by way of the RVM to the spinal cord dorsal horn (FIG. 2).

Beyond its distributed MOR agonist sensitivity, another distinctive feature of the pain-modulation circuit is that the serial linkage of its component nuclei involves the release of endogenous opioids. The behavioural effect of activating the circuit at one site can be blocked by microinjection of opioid antagonists at a downstream site in the pathway. For example, analgesia produced by MOR agonists microinjected into the posterior hypothalamus or basolateral amygdala is reversed by a MOR antagonist in the PAG[33], and PAG-elicited analgesia is blocked by naloxone or selective MOR antagonists microinjected into the RVM[34,35]. Although the endogenous opioid that mediates these effects has not been identified, the fact that an enkephalinase inhibitor injected into the RVM produces analgesia implicates the enkephalins[36].

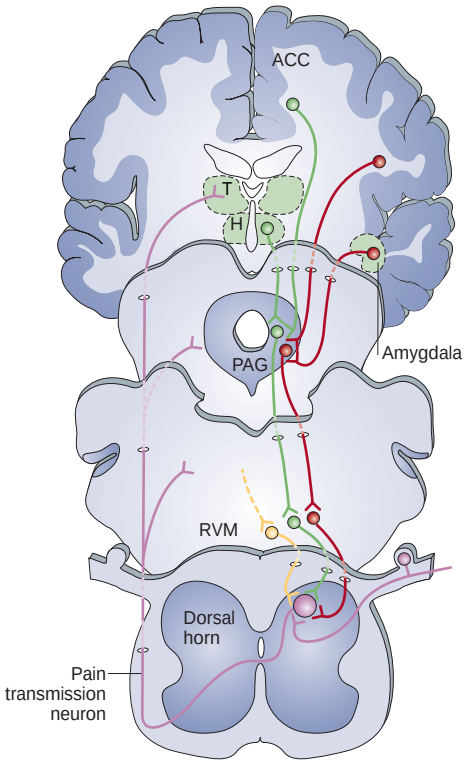


Figure 2 | **Outline of opioid-sensitive pain-modulating circuit.** This is a top–down pathway that can be activated by both exteroceptive stimuli and certain motivational states. Limbic forebrain areas, including the anterior cingulate cortex (ACC), other frontal cortical areas, the hypothalamus (H) and central nucleus of the amygdala project to the midbrain periaqueductal grey (PAG), which can be thought of as a main output pathway of the limbic system. The PAG, in turn, indirectly controls pain transmission in the dorsal horn through the rostral ventromedial medulla (RVM). This pathway can exert both inhibitory (green) and facilitatory (red) control. A separate control channel through serotonergic neurons in the RVM (yellow) can also modulate pain in a state-dependent manner. T, thalamus.

## Physiological activation of opioid circuits

So far, we have described the connectivity of the pain-modulation circuit and some of its pharmacological features. Clearly, the circuit can operate in a serial, opioid-linked fashion; but how is it normally activated? Noxious stimuli that are prolonged and inescapable are particularly effective for activation of the PAG–RVM network. For example, forepaw shock in rats produces an acute anti-nociceptive effect that is blocked by the opioid antagonist naloxone and by RVM lesions[37,38]. There is evidence that similar mechanisms operate in humans (see REF. 7 for a review). First, rodent and human opioid receptors are almost pharmacologically identical. Second, endogenous opioid peptides and opioid receptors are present in human brain areas that are homologous to brainstem pain-modulating nuclei. Third, analgesia has been produced in people by stimulating the PAG. Last, naloxone enhances experimental and clinical postoperative pain in human subjects who have not received exogenous opioids[39,40].

LIMBIC
A term that refers to a collection of cortical and subcortical structures that are important for processing memory and emotional information. Prominent structures include the hippocampus and amygdala.

AMYGDALA
A small almond-shaped structure, comprising 13 nuclei, buried in the anterior medial section of each temporal lobe.

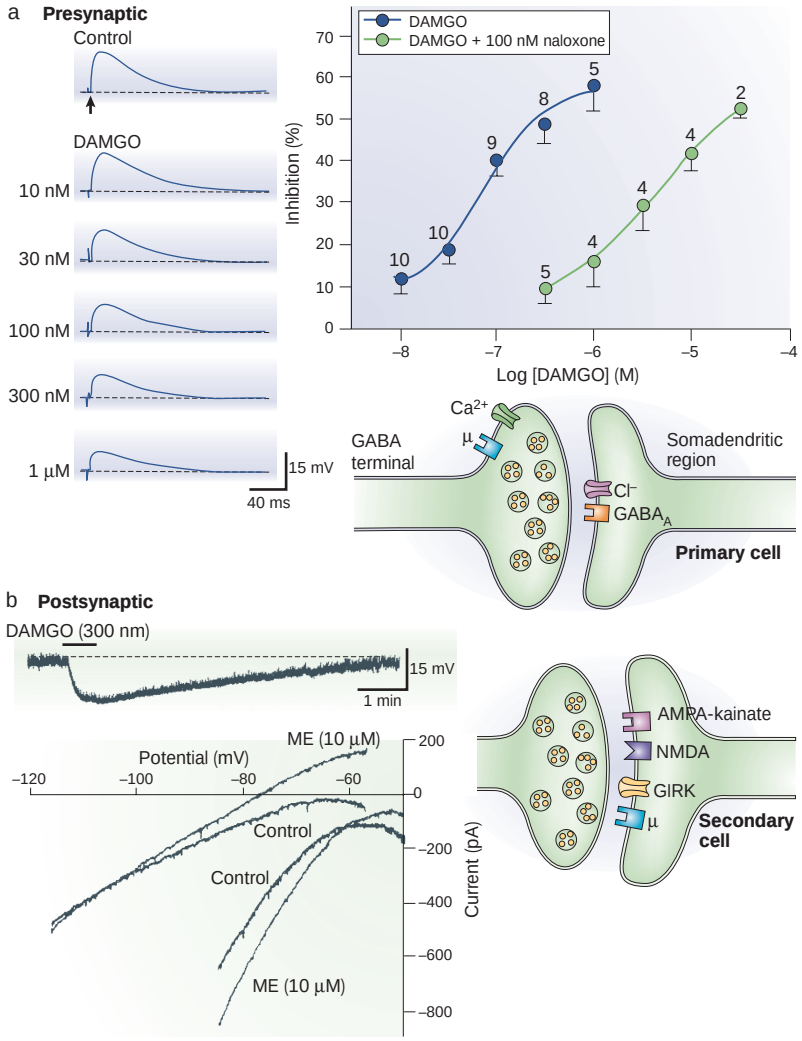


Figure 3 | **Synaptic actions of μ-opioid receptor (MOR) agonists in the rostral ventromedial medulla (RVM).** Reproduced, with permission, from REF. 66 © (1990) The Physiological Society. **a** | MOR agonists reduce release of GABA (γ-aminobutyric acid) through a dose-dependent presynaptic action. Reduced GABA-mediated inhibition accounts for the activation (disinhibition) of off cells by MOR agonists. **b** | By contrast, MOR agonists directly inhibit on cells (secondary cells *in vitro*) through activation of a potassium conductance. AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; DAMGO, [D-Ala$^2$, *N*-Me-Phe$^4$, Gly-ol]enkephalin; GIRK, G-protein-coupled inwardly rectifying potassium channel; ME, [met$^5$]enkephalin; NMDA, *N*-methyl-D-aspartate.

## Bidirectional control of pain transmission

One of the more striking and informative discoveries about the pain-modulating circuit is that it can facilitate as well as inhibit nociceptive transmission[41–43]. In addition to the inhibition discussed above, stimulation of the RVM can enhance behavioural and dorsal horn neuronal responses to noxious stimulation[44–46]. Furthermore, prolonged nociceptor inputs, including thermal and chemical stimulation, inflammation or nerve injury produce a state of generalized hyperalgesia that is reversed by lesions or reversible inactivation of the RVM[42,47,48]. So, the activation of RVM neurons can generate either facilitation or inhibition of pain transmission under different conditions. How are we to understand this apparent paradox?

It is now clear that this dual control results from the activity of two neuronal subpopulations. The two cell classes, which are present in the PAG, DLPT and RVM, exhibit phasic reciprocal changes in firing that precede nociceptor-elicited withdrawal reflexes[4,49,50]. One class, termed 'off cells', shows a pause in firing that begins before the withdrawal reflex. The other class — 'on cells' — shows a burst of activity that begins before the reflex (FIG. 4a). Consistent with their role in pain modulation, RVM on- and off-cell axons project directly and selectively to dorsal horn laminae that relay nociceptive signals[51].

When MOR agonists are administered systemically, either into the PAG or locally in RVM, off-cell firing accelerates and becomes continuous (FIG. 4c). Withdrawal reflexes are inhibited and no off-cell pause is seen. Selective blockade of off-cell activation prevents morphine's anti-nociceptive effect[52]. Therefore, off-cell activation is necessary for the pain-inhibitory effects of MOR ligands given systemically or supraspinally[53,54].

*On cells and pain facilitation.* The correlation of on-cell discharge with withdrawal reflexes indicates that their action is to facilitate such responses and their dorsal horn projection target indicates that this effect is achieved through control of nociceptive transmission. Several independent lines of evidence support this idea. Analgesic doses of MOR agonists silence on cells. On cells in the RVM contribute to the enhanced nociception that accompanies various manipulations, including nerve injury, inflammation, tonic activation of nociceptors and systemic cytokine administration (see table 1 in REF. 42). Tonic activation of nociceptors results in prolonged on-cell activity, which enhances certain withdrawal reflexes[48,55]. Furthermore, destruction of RVM on cells by a MOR-selective neurotoxin blocks the hyperalgesic state elicited by nerve injury[56,57]. Finally, selective activation of on cells enhances responses to noxious stimulation[8].

*Reciprocal and state-dependent neuronal activity.* Recordings of pairs of neurons in the RVM of lightly anaesthetized rats demonstrate that on- and off-cell populations are active at different times[6,58,59]. In fact, these cells show reciprocal patterns of activity under various conditions (FIG. 4a–c, TABLE 1). Despite the tendency towards reciprocal firing, each population is capable of independent action. For example, one can block the reflex-related on-cell burst without affecting the off-cell pause[60]. Conversely, the activation of off cells by opioids can be blocked without affecting the on-cell burst. These findings indicate that the reciprocity of activity in the two RVM populations depends on shared upstream connectivity rather than direct inhibitory connectivity within the RVM.

One of the more striking features of the pattern of activity for both on- and off-cell populations is that it consistently varies with the animal's state of arousal[61]. For example, in awake unrestrained rats, the off-cell population fires intermittently, only to accelerate and

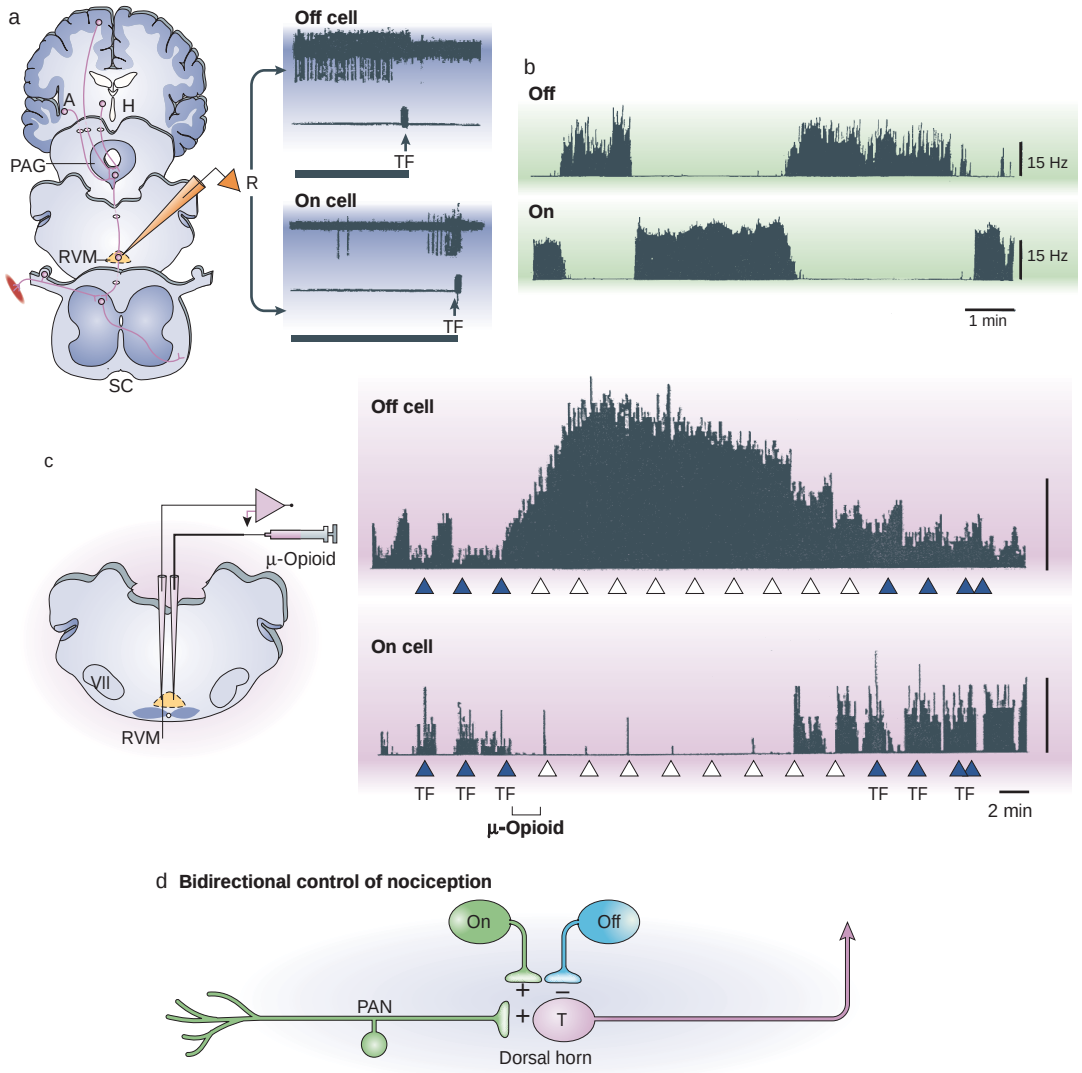


Figure 4 | **Two populations of neurons exert opposing modulatory actions. a** | Recordings from neurons in the rostral ventromedial medulla (RVM) during application of noxious heat to the tail reveals one class that pauses (off cells) and one that bursts (on cells) just prior to the withdrawal tail flick (TF). A, amygdala; H, hypothalamus; PAG, periaqueductal grey; R, recording electrode; SC, spinal cord. **b** | In the absence of imposed stimuli, RVM cycles between periods of off- and on-cell activity. Reproduced, with permission, from REF. 59 © (1989) Taylor and Francis. **c** | Reciprocal firing is also seen when μ-receptor agonists are microinjected directly into the RVM; off-cell discharge accelerates and on cells shut down with concomitant inhibition of the TF (open triangles). Reproduced, with permission, from REF. 70 © (1994) Elsevier Science. **d** | On cells facilitate and off cells inhibit nociceptive transmission at the level of the dorsal horn. PAN, primary afferent nociceptor; T, pain transmission neuron.

become continuously active during slow-wave sleep[62]. Conversely, on cells show markedly reduced activity during slow-wave sleep. Taken together, these findings indicate that the modulatory circuit can operate in one of two opposing states: an on-cell state that enhances nociceptive transmission and an off-cell state that inhibits nociceptive transmission. Administration of MOR ligands changes the circuit into the off-cell state, whereas the presence of a prolonged somatic noxious stimulus changes it into an on-cell state. The concept that the modulatory circuit can operate in two distinct modes is crucial for understanding how a given opioid ligand can have different behavioural effects when given at different times.

G PROTEIN
A heterotrimeric GTP-binding and -hydrolysing protein that interacts with cell-surface receptors, often stimulating or inhibiting the activity of a downstream enzyme. G proteins consist of three subunits: the α-subunit, which contains the guanine-nucleotide-binding site; and the β- and γ-subunits, which function as a heterodimer.

### The opioid receptor family and pain modulation

The known opioid receptors are members of the large G-PROTEIN-coupled receptor family. There are currently four well-established members of the opioid receptor family — μ, δ (DOR/OPRD), κ (KOR/OPRK1) and opioid receptor-like (ORL1/OPRL) (for reviews, see REFS 63,64). The first three were defined on the basis of ligand-binding studies, and subsequent cloning of their genes revealed high sequence homology. The ORL1 receptor was identified on the basis of its high sequence homology with the other three receptors. The anatomical distributions of DOR, KOR and ORL1 receptors parallel that of the MOR; they are present in the component nuclei of the pain-modulating circuit[2,29,30,63,65]. Ligands

Table 1 | Rostral ventromedial medulla (RVM) neurons and behavioural state

| | On cell | Off cell | Nociceptive response | Blocked by MOR antagonist | Blocked by ORL or KOR agonists |
|---|---|---|---|---|---|
| MOR agonist | – | + | – | Y | Y |
| Acute MOR abstinence | + | – | + | N/A | Y |
| Tonic noxious stimulus | + | – | + | N/A | N/A |
| Low dose neurotensin (In RVM) | + | 0 | + | N/A | N/A |
| Threat or appetitive motivational state | – | + | – | Y | Y |

+, increases; –, decreases; 0, no effect. KOR, κ-opioid receptor; MOR, μ-opioid receptor; N/A, not applicable; ORL, opioid receptor-like; Y, yes.

that are selective for each opioid receptor regulate various motivated behaviours including feeding, alcohol and psychostimulant consumption and pain.

*Actions of MOR ligands on pain-modulating neurons.* Morphine is the prototypical opioid ligand, and its actions require the MOR[3]. *In vitro* studies of neurons in pain-modulating nuclei have revealed several types of synaptic actions by MOR ligands (FIG. 3). Direct post- and presynaptic inhibition of GABA (γ-aminobutyric acid) release have been related to the nociception-modulating function of the PAG and RVM (FIG. 3). Direct postsynaptic inhibition of subsets of PAG and RVM neurons is produced by MOR agonists through activation of an INWARDLY RECTIFYING POTASSIUM CHANNEL[10,66]. *In vivo* iontophoresis of morphine in the RVM selectively inhibits on-cells, and blocks their excitation by applied glutamate. This shows that neurons in this region that are postsynaptically inhibited by MOR agonists are on cells[67].

A subset of RVM neurons, which must include off cells, is not hyperpolarized by MOR agonists but does have GABA-releasing inputs that are presynaptically inhibited by MOR ligands[67]. Presynaptic inhibition of GABA-releasing inputs by MOR agonists has also been demonstrated in PAG neurons of rats and mice[68,69]. RVM off cells are activated by local infusion of either MOR selective ligands or the $GABA_A$ receptor antagonist bicuculline[70,71], and this leads to an anti-nociceptive effect. Because *in vitro* studies show no direct excitatory effect of MOR ligands on any cell class, RVM off-cell excitation by local microinfusion of MOR agonists is at least partly due to inhibition of GABA-releasing inputs. Although presynaptic inhibition of glutamatergic transmission to on cells in both the RVM and PAG could contribute under some circumstances[72,73], the analgesic effect of MOR agonists acting in both PAG and RVM is probably due to disinhibition of off cells.

*Contribution of the δ-opioid receptor.* The role of the DOR in pain modulation is puzzling (reviewed by REF. 74). DOR agonists microinjected into the PAG produce little or no anti-nociceptive effect in the rat[75,76]. Consistent with this observation, *in vitro* experiments have failed to show either hyperpolarization of neurons or inhibition of transmitter release by DOR agonists in rat PAG[10,73,77]. By contrast, in the C57B16/J mouse, DOR agonists hyperpolarize a small subset of PAG neurons (24% compared with 72% for MOR agonists)[69,70]. In the RVM, DOR is present on axon terminals, and DOR selective antagonists can block the analgesia that is produced by PAG activation[78,79]. Furthermore, in the RVM, DOR agonists produce weak to moderate analgesia[80] and changes in RVM on and off cells that are similar to but weaker than those produced by MOR agonists[81]. There are no published studies of DOR synaptic actions in the RVM.

The modest cellular and behavioural effects of selective agonists imply a relatively minor contribution of the DOR to pain modulation. However, another possibility is that synthesis or cellular trafficking of the DOR is variable, and more potent DOR effects could be demonstrated under the right conditions. For example, inflammation increases the targeting of the DOR to the plasma membrane in the spinal cord dorsal horn[82]. Furthermore, prolonged inflammation is associated with an enhanced anti-nociceptive effect for a DOR-selective ligand (deltorphin) in the RVM[83]. These results indicate that DOR function might be more robust if the receptor is studied under appropriate conditions.

*State-dependent effects of KOR and ORL1 ligands.* KOR-selective agonists have at least two synaptic actions in the RVM. They directly hyperpolarize neurons that are not hyperpolarized by MOR agonists (off and/or neutral cells), and they inhibit excitatory glutamatergic inputs to RVM neurons, including those that are hyperpolarized by MOR agonists (on cells). Nociceptin, a ligand that is selective for the ORL1 receptor, strongly hyperpolarizes all classes of neurons in the RVM and PAG through activation of an inwardly rectifying potassium channel[85,86]. In addition, nociceptin inhibits GABA release by a presynaptic action[84,86]. *In vivo*, RVM microinjection of the same ORL1 ligand strongly inhibits all on and off cells[53]. In summary, KOR agonists act presynaptically and ORL1 agonists act postsynaptically to inhibit both on and off cells in the RVM. FIGURE 5b summarizes the synaptic distribution of opioid receptors in the RVM on and off cell circuits. The cells of origin of the opioid-regulated afferent terminals of RVM on and off cells have not been definitively identified.

In contrast to the robust cellular and synaptic actions of KOR and ORL1 *in vitro*, their behavioural effects on pain transmission are highly variable. Various effects of supraspinal injection of ORL1 agonists have been reported; no effect, enhancement of nociceptive responsiveness or enhancement followed by inhibition (reviewed by REF. 87). Similarly, in comparison to MOR agonists, KOR agonists produce analgesia that is weaker and more dependent on the type of noxious stimulus

INWARDLY RECTIFYING POTASSIUM CHANNELS
Potassium channels that allow long depolarizing responses, as they close during depolarizing pulses and open with steep voltage dependence on hyperpolarization. They are called inward rectifiers because current flows through them more easily into than out of the cell.

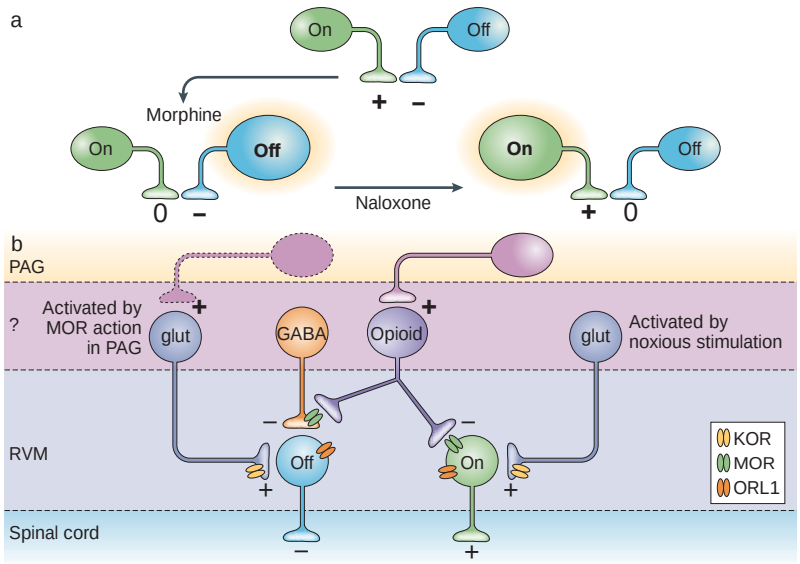


Figure 5 | **Shifts in nociceptive modulatory state during morphine analgesia and acute naloxone-induced abstinence. a** | Morphine activates off cells to inhibit pain (lower left), whereas when naloxone is used to precipitate acute abstinence, on cells are activated and produce a hyperalgesic state (lower right). **b** | Synaptic distribution of opioid receptors within the rostral ventromedial medulla (RVM). μ-Opioid receptor (MOR) is located on GABA (γ-aminobutyric acid)-releasing terminals at off cells and the somadendritic region of on cells. Both cell classes have somadendritic opioid receptor-like (ORL1) receptors and both are excited by κ-opioid receptor (KOR)-bearing glutamatergic terminals (glut) that arise from different input neurons. Whereas MOR agonists produce anti-nociceptive effects by inhibiting on cells and disinhibiting off cells, ORL1 and KOR agonists acting in the RVM can block analgesia by inhibiting off cells or block hyperalgesia by inhibiting on cells. PAG, periaqueductal grey.

that is used[9,88,89]. In addition, KOR agonists can functionally antagonize MOR-mediated analgesia[9,11,88–90].

How can we resolve the discrepancy between the robust and consistent synaptic actions of KOR and ORL1 agonists and their weak and variable behavioural effects? The key is that the pain-modulating circuit has two opposing states, and that the behavioural effect of an opioid depends on whether the circuit is in the on-cell or off-cell state. These points are clearly illustrated by recent studies of the effects of KOR and ORL1 agonists on nociceptor-elicited behaviours, and on the activity of RVM neurons during morphine analgesia (off-cell state) and during naloxone-precipitated morphine abstinence (on-cell state).

When morphine is administered (systemically or into the PAG), the on cells become silent and the off cells fire continuously (FIG. 4c and FIG. 5a). In the off-cell state, dorsal horn neurons and withdrawal reflexes are inhibited. This inhibition is reversed by inactivation of the RVM or selective inhibition of off-cell firing. In the off-cell state, microinjection of either an ORL1 or KOR agonist will inhibit off cells and will have an anti-analgesic (pain-promoting) action[11,85]. Conversely, if naloxone is given following systemic administration of an analgesic dose of morphine, off-cell firing is shut down, on-cell firing increases and becomes continuous, and withdrawal reflexes are enhanced[91,92] (FIG. 5). In this on-cell state, microinjection of an ORL1 or KOR agonist will inhibit on cells and will have an anti-hyperalgesic (pain-reducing) action[11,85].

These experiments illustrate the power (and the necessity) of using neural circuit analysis to explain behavioural pharmacology. In addition to the descending control that is exerted by on and off cells, serotonergic neurons in the RVM that project to the dorsal horn provide a third, state-dependent element that controls nociceptive transmission (BOX 1).

## The biological imperative for pain modulation

Although the concept of state dependence is helpful for understanding the role of different opioid receptors in pain control, it raises an intriguing question: what is the biological meaning of a 'behavioural state'? The available evidence indicates that the most promising framework for approaching this question is to conceptualize pain as primarily a motivational state that has a powerful influence on decision making.

Noxious stimuli produce distinctly unpleasant sensations and elicit various innate behaviours that are appropriate to a continuing physical threat such as escape, defence and vocalization[93]. Following injury, nociceptive inputs elicit recuperative behaviours such as quiescence, licking and guarding[94]. The nociceptive input that activates and maintains these behaviours can be conceptualized as inducing a drive state with powerful motivational effects[95]. Because they induce a motivational state, noxious stimuli serve as teaching signals, allowing animals to avoid situations that have either caused or threatened tissue injury in the past[96,97]. The powerful behavioural demand that is produced by noxious stimuli presents the animal with a biological problem. There will be circumstances — for instance, the presence of a predator — in which choosing to respond overtly to a noxious stimulus, such as with vocalization or sudden movement, places the animal at risk of even greater injury or death[94]. Exhibiting certain 'pain' behaviours in the presence of a competing dominant male conspecific might significantly reduce reproductive efficiency. So, when the motivational demand for a behaviour that is typically elicited by a noxious stimulus occurs in the presence of a biological cost for its execution, a mechanism that can block the behaviour confers a potential evolutionary advantage.

Opioid-mediated inhibition of pain has been demonstrated in these situations. For example, naloxone-reversible analgesia is induced in male rodents by the presence of a predator[98], or an aggressive male conspecific[99]. Through classical conditioning, initially neutral contextual cues can acquire the motivational power to elicit opioid-mediated analgesia. In the conditioned fear model, after contingent pairing with an inescapable foot shock, an initially neutral light or tone can elicit an anti-nociceptive effect. This conditioned analgesia is blocked by microinjection of MOR- but not KOR- or DOR-selective antagonists into the basolateral amygdala, PAG and RVM[100–102]. Similar to the analgesia that is elicited by PAG MOR agonists, the analgesia that accompanies conditioned fear is inhibited by the microinjection of a KOR agonist in the RVM[103]. Activation of the opioid-mediated anti-nociceptive network is part of the process of deciding to respond to the anticipated threat rather

**Box 1 | Serotonergic neurons and pain modulation**

There is little doubt that serotonin (5-hydroxytryptamine, 5-HT) contributes to brainstem control of nociceptive transmission at spinal levels[61,126,127]. However, the weight of evidence indicates that rostral ventromedial medulla (RVM) serotonergic neurons are part of a pathway that is anatomically coextensive and that interacts with, but is functionally distinct from, the opioid-mediated pain-modulatory circuit. In adult rodents, about 20% of RVM neurons are serotonergic, and most project through the dorsolateral funiculus to innervate the dorsal horn. However, cytochemical studies of physiologically identified RVM neurons have clearly shown that serotonergic neurons in the RVM are neither on nor off cells; the response of serotonergic RVM neurons to noxious stimulation is weak and variable[128]. Furthermore, *in vivo* studies in adult animals indicate that serotonergic RVM neurons are not robustly affected by either PAG stimulation or morphine at doses that are sufficient to inhibit nociceptive transmission[61]. By contrast, *in vitro* studies of spinally projecting serotonergic RVM neurons in young rats have shown postsynaptic inhibition by μ-opioid receptor (MOR)- and κ-opioid receptor (KOR)-selective agonists[129], and MOR-mediated presynaptic inhibition of both glutamate and GABA (γ-aminobutyric acid) transmission[72]. Serotonin has both excitatory and inhibitory synaptic actions on nociceptive dorsal horn neurons[130,131], and spinally administered 5-HT receptor antagonists reduce both the pain-facilitating and inhibiting-effects of RVM stimulation[132–135]. The discharge patterns of serotonergic RVM neurons have been studied in anaesthetized cats. Their activity closely tracks periodic shifts in the sleep–wake cycle, being highest during waking, lower during slow-wave sleep and lowest during paradoxical sleep. In summary, serotonergic RVM neurons provide a state-dependent and potentially bidirectional modulatory channel that is parallel to, but operationally distinct from, the controls exerted by on and off cells. *In vitro* studies indicate that the serotonergic channel might be subject to opioid control under certain, as yet unspecified, conditions. The three channels converge at the level of the dorsal horn. The dynamic interplay between serotonin and the action of on and off cells represents an important field for future research.

than the ongoing tissue-damaging stimulus. The decision is the outcome of a computation of the relative cost and probability of the threat compared with that of the noxious stimulus[94].

Consistent with a crucial role in decision making, opioid-mediated pain-modulatory circuits can be engaged during appetitive as well as aversive motivational states. Feeding sucrose to animals[104,105] or human infants[106] produces a naloxone-reversible analgesic effect. Sucrose-induced analgesia in rodents is blocked by lesions of the ventromedial hypothalamus[107], which projects to both the PAG and RVM. Interestingly, the RVM on-cell burst and off-cell pause are reduced during food or water consumption[62]. Furthermore, the anticipation of a food reward can have the same effect. For example, placing animals in an environment where they have previously received a desired food raises their withdrawal threshold, and this effect is blocked by naloxone[108]. This demonstrates that food consumption or food predictive sensory cues activate an opioid-mediated pain-modulatory circuit, increasing the probability that the animal will consume the food despite conflicting drives. Consistent with a direct link between opioid analgesia and appetitive choice, microinjection of MOR (or MOR and DOR) agonists into the nucleus accumbens (a region of the BASAL GANGLIA that is crucial for linking motivation to action) induces both anti-nociception[109] and consumption of sweet and rich foods and ethanol[110]. So, instinctive as well as learned motivational states with either appetitive or aversive valence are associated with activation of opioid-mediated anti-nociceptive mechanisms.

BASAL GANGLIA
A group of interconnected subcortical nuclei in the forebrain and midbrain that includes the striatum (putamen and caudate nucleus), globus pallidus, subthalamic nucleus, ventral tegmental area and substantia nigra.

### Reward expectancy, opioids and placebo

The concept that activation of opioid-mediated pain-modulatory circuits is driven primarily by motivational state provides a heuristic basis for interpreting the literature on neural mechanisms of placebo analgesia. Shortly after the discovery of endogenous opioids in the mid-1970s, placebo analgesia was shown to be blocked by naloxone[111] — a result that has been replicated several times[112,113]. An important mediating process that underlies placebo analgesia is expectancy[114].

Expectancy can be induced verbally by telling an individual that they are about to receive a powerful analgesic, or by conditioning[115]; for example, by giving an individual a treatment (pill, ointment or intravenous infusion) that produces a powerful analgesic effect. After conditioning, giving the subject a physiologically inert treatment that closely resembles the appearance of the actual analgesic produces a powerful, naloxone-reversible analgesic effect[116]. By contrast, informing the subject that they are getting an inert placebo will 'reverse' the effect of conditioning and prevent the placebo-induced analgesic response[117]. From the standpoint of motivational processes, an effective placebo manipulation can be considered to be a reward-predictive cue because pain relief is 'rewarding' (negative reinforcement). So, by virtue of leading to the expectation of pain relief, placebo analgesics have appetitive motivational power. Just as the rodent anticipating a desired food reward engages its opioid-mediated analgesia circuit, so might a person anticipating pain relief engage a homologous opioid-mediated circuit (see REF. 118). A sugar pill that resembles a previously administered analgesic would have the advantage of directly engaging opioid-mediated circuits through its sweet taste as well as the cognitive expectation of pain relief.

Functional imaging studies support the idea that expectancy, and expectation of pain relief in particular, can engage opioid-mediated pain-modulating circuitry. Using positron emission tomography and an experimental pain model, Petrovic and colleagues[119] studied brain areas that are activated by the powerful MOR agonist remifentanyl. The same subjects were then given a saline infusion with the instruction that it was a powerful analgesic. Subjects who experienced significant relief with the placebo infusion showed activation in areas that were largely coextensive with those activated by the MOR agonist. These areas included the rostral anterior cingulate, and brainstem areas that overlap with nuclei that have been implicated in pain modulation. Wager and colleagues took the story further by showing that activation of the anterior cingulate cortex (ACC) and midbrain PAG correlated with placebo analgesia in human subjects[120]. Through its connection to the PAG, the ACC is anatomically linked to the opioid-mediated pain-modulatory circuit[121].

It is noteworthy that the increase in activity in these areas occurred prior to noxious stimulation. This probably represents anticipation or expectation of pain relief. Placebo administration activates areas of the ACC that are also activated by reward expectancy in humans[122] and primates[123]. The ACC also projects to the nucleus accumbens[124], which, as noted above, is crucial for linking motivation to action. It is noteworthy that activity in the human nucleus accumbens occurs rapidly following noxious stimulation and precedes activation of cortical areas that have been implicated in pain perception[125]. This activity might reflect neural processing that underlies the 'decision' to either respond to or suppress ascending nociceptive pathways.

## Conclusions

As outlined above, our knowledge of the biological significance of opioid receptors and endogenous opioid peptides has leaned heavily on the use of opioid antagonists. Although the pain inhibition that is associated with conditioned fear is primarily a MOR-mediated effect, most published work has used non-selective opioid antagonists such as naloxone and naltrexone, so the function of endogenous agonists for the other opioid receptor classes is currently obscure. Addressing their contribution to motivational control will require the use of selective antagonists and some ingenuity to determine the behavioural conditions that are required to activate circuits that release endogenous ligands for DOR, KOR and ORL1.


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Competing interests statement
The author declares that he has no competing financial interests.

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