

26. Bosking, W. H., Zhang, Y., Schofield, B. & Fitzpatrick, D. Orientation selectivity and the arrangement of horizontal connections in the low shrew striate cortex. *J. Neurosci.* **15**, 2112–2127 (1997).
27. Schmidt, K. E., Goebel, R., Löwel, S. & Singer, W. The perceptual grouping criterion of colinearity is reflected by anisotropies of connections in the primary visual cortex. *Eur. J. Neurosci.* **9**, 1083–1089 (1997).
28. Bour, L. J., Van Gisbergen, J. A. M., Bruijns, J. & Ottes, F. P. The double magnetic induction method for measuring eye movement—results in monkey and man. *IEEE Trans. Biomed. Eng.* **31**, 419–427 (1984).
29. Snodderly, D. M. & Gur, M. Organization of striate cortex of alert, trained monkeys (*Macaca fascicularis*): ongoing activity, stimulus selectivity, and widths of receptive field activating regions. *J. Neurophysiol.* **74**, 2100–2125 (1995).
30. Gattass, R., Gross, C. G. & Sandell, J. H. Visual topography of V2 in the macaque. *J. Comp. Neurol.* **201**, 519–539 (1981).

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An analgesia circuit activated by cannabinoids

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Although many anecdotal reports indicate that marijuana and its active constituent, delta-9-tetrahydrocannabinol (delta-9-THC), may reduce pain sensation^{1,2}, studies of humans have produced inconsistent results^{3–6}. In animal studies, the apparent pain-suppressing effects of delta-9-THC and other cannabinoid drugs^{7–12} are confounded by motor deficits^{13,14}. Here we show that a brainstem circuit that contributes to the pain-suppressing effects of morphine¹⁵ is also required for the analgesic effects of cannabinoids. Inactivation of the rostral ventromedial medulla (RVM) prevents the analgesia but not the motor deficits produced

by systemically administered cannabinoids. Furthermore, cannabinoids produce analgesia by modulating RVM neuronal activity in a manner similar to, but pharmacologically dissociable from, that of morphine. We also show that endogenous cannabinoids tonically regulate pain thresholds in part through the modulation of RVM neuronal activity. These results show that analgesia produced by cannabinoids and opioids involves similar brainstem circuitry and that cannabinoids are indeed centrally acting analgesics with a new mechanism of action.

The discoveries of the CB1 and CB2 cannabinoid receptors^{16,17} and of two putative endogenous ligands for the CB1 receptor^{18,19} and the development of a specific CB1-receptor antagonist (SR141716A) (ref. 20) have intensified interest in the function of endogenous cannabinoid systems. The CB2 receptor is located in peripheral tissues, whereas the CB1 receptor is present on neurons throughout the brain, including in several pain-modulating centres²¹. Projections from the RVM to the dorsal horn of the spinal cord are important for the production of analgesia originating from supraspinal sites²². The RVM, which includes the nucleus raphe magnus, the nucleus gigantocellularis pars alpha and the adjacent reticular formation, is essential for systemic opioid-induced analgesia¹⁵, and may also be involved in cannabinoid analgesia, as microinjection of cannabinoid agonists into the RVM suppresses pain-related behaviours²³.

To determine the contribution of the RVM to analgesia produced by a systemically administered cannabinoid, we inactivated the RVM by microinjection of the GABA_A (γ-aminobutyric acid subtype A) receptor agonist muscimol. Rats with physiological saline microinjected into the RVM showed significant increases in tail-flick latencies after intravenous administration of 0.125 mg kg⁻¹ and 0.25 mg kg⁻¹ doses of WIN55,212-2, a cannabinoid receptor agonist (Fig. 1a). In contrast, animals microinjected with muscimol (50 ng) in the RVM before the administration of WIN55,212-2 showed no increase in tail-flick latencies (Fig. 1b). Muscimol microinjection also produced significant hyperalgesia in animals treated with systemic vehicle as compared with control rats microinjected with saline (latency 3.29 ± 0.21 versus 4.73 ± 0.09 s; *P* < 0.05). These results indicate that the activity of neurons in the RVM is necessary

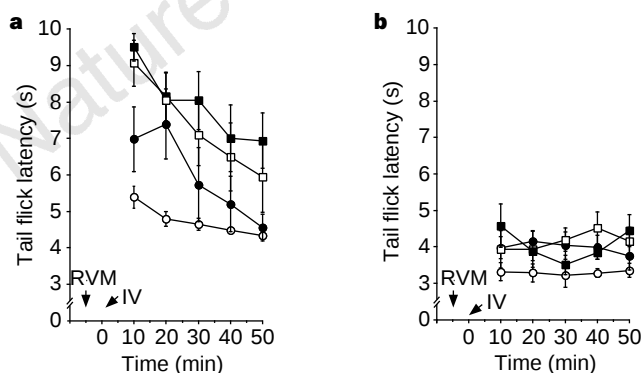


Figure 1 Inactivation of the RVM with microinjection of muscimol prevents the antinociception produced by the cannabinoid agonist WIN55,212-2. We measured tail-flick latencies in groups that received **a**, saline, or **b**, muscimol microinjections into the RVM before intravenous administration of vehicle (open circles); 0.0625 mg kg⁻¹ WIN55,212-2 (filled circles); 0.125 mg kg⁻¹ WIN55,212-2 (open squares); or 0.25 mg kg⁻¹ WIN55,212-2 (filled squares). In saline-injected animals, consistent antinociception was achieved at the 0.125 and 0.25 mg kg⁻¹ doses of WIN55,212-2 (*P* < 0.01; analysis of variance (ANOVA) followed by Fisher's least-significant difference (LSD) test). Muscimol-injected animals showed no difference in tail-flick latencies at any of the WIN55,212-2 doses (*P* > 0.05, ANOVA and Fisher's LSD test). Each value is the mean ± s.e.m.; tail-flick latencies represent the average of three trials; arrows indicate RVM and intravenous (IV) drug administration; *n* = 6 rats per group.

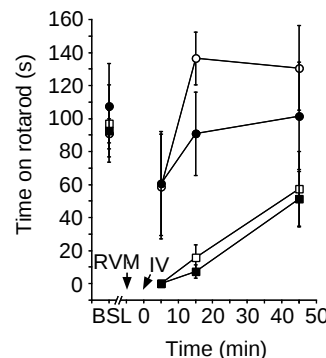


Figure 2 Cannabinoid-induced motor impairments are not affected by inactivation of the RVM with muscimol. Time on the rotarod treadmill (mean ± s.e.m.) was recorded from each of four groups (RVM microinjection/intravenous injection): open circles, saline/vehicle; filled circles, muscimol/vehicle; open squares, saline/WIN55,212-2; filled squares, muscimol/WIN55,212-2. Groups that received WIN55,212-2 (0.25 mg kg⁻¹) spent significantly less time on the rotarod as compared with vehicle-treated groups (*P* < 0.01; ANOVA and Fisher's LSD test). Injection of saline or muscimol into the RVM did not affect rotarod scores (*P* > 0.05; ANOVA). Baseline times (BSL) on the rotarod for each group before treatments were similar. Arrows indicate times of RVM and intravenous (IV) drug administration; *n* = 5 rats per group.

for systemic cannabinoid-induced antinociception, and that the activity of neurons in the RVM contributes to basal nociceptive thresholds.

At antinociceptive doses, cannabinoids produce motor deficits in rats, including hypomotility and catalepsy^{13,14}. Thus, it is necessary to differentiate between the sensory and motor effects of cannabinoids, especially when using a movement such as the tail flick as an index of antinociception^{10,11}. We quantified the effect of RVM inactivation on cannabinoid-induced motor impairments using a rotarod treadmill. Intravenous administration of WIN55,212-2 (0.25 mg kg⁻¹) severely impaired the ability of animals to stay on the rotarod throughout the 45-min test period (Fig. 2). Microinjection of muscimol (50 ng) into the RVM did not prevent the WIN55,212-2-induced motor impairments (Fig. 2). There was no difference between groups given vehicle that were microinjected with saline or muscimol, and baseline times were similar for all groups (Fig. 2, $P > 0.05$). These results show that the prevention of cannabinoid-induced antinociception observed following RVM inactivation is not due to a reversal of cannabinoid-induced motor deficits.

We used extracellular single-unit recording to determine which type of RVM neuron contributes to cannabinoid-induced antinociception. Three physiological classes of RVM neurons have been described²⁴. 'Off' cells inhibit spinal nociceptive transmission and show a pause in activity just before withdrawal reflexes. Conversely, 'on' cells show a burst of activity just before withdrawal reflexes and have a facilitating effect on pain transmission. The activity of a third group of neurons—neutral cells—is not correlated with withdrawal reflexes. While recording from RVM neurons, we intravenously administered anaesthetized rats with, successively, WIN55,212-2 (0.125 mg kg⁻¹), the cannabinoid receptor antagonist SR141716A

(0.5 mg kg⁻¹), morphine sulphate (2.0 mg kg⁻¹) and naloxone (0.2 mg kg⁻¹). The effect of WIN55,212-2 on 'on' and 'off' cell activity was similar to that of morphine (Fig. 3a, b). Administration of either WIN55,212-2 or morphine eliminated the 'off' cell pause, the 'on' cell burst, and the tail-flick reflex in all animals tested (six 'off' cells and five 'on' cells). Quantitative analysis of 'off' cell activity during the tail-flick period showed an elimination of the 'off' cell pause (that is, an increase in firing to baseline levels) after WIN55,212-2 administration, with activity increasing from an average rate of 4.4 ± 1.8 spikes s⁻¹ to 24.8 ± 9.7 spikes s⁻¹ ($P < 0.01$). The 'off' cell pause returned (7.3 ± 5.3 spikes s⁻¹) after subsequent administration of SR141716A. Morphine given after SR141716A eliminated the 'off' cell pause in a naloxone-reversible manner (25 ± 12.1 and 3.0 ± 2.3 spikes s⁻¹, respectively). Overall, there was no change in baseline activity of 'off' cells after WIN55,212-2 administration (from 19.1 ± 10.7 to 25.6 ± 9.6 spikes s⁻¹).

The 'on' cell burst during the tail-flick period was reduced to baseline firing levels after the administration of WIN55,212-2 (18.2 ± 6.6 to 1.7 ± 1.7 spikes s⁻¹), and returned to normal following administration of SR141716A (19.5 ± 9.7 spikes s⁻¹; $P < 0.01$). Administration of morphine after SR141716A reduced the 'on' cell burst to levels seen after WIN55,212-2 and was naloxone-reversible (2.0 ± 2.0 and 17.3 ± 9.8 spikes s⁻¹, respectively). There was no change in the baseline activity of 'on' cells after administration of WIN55,212-2 (from 2.6 ± 1.7 to 1.6 ± 1.5 spikes s⁻¹). Of the five neutral cells tested, the activity of three was unaffected, the activity of one decreased, and the activity of one increased after WIN55,212-2 was administered ($>30\%$ change). As 'off' cells project to the spinal cord dorsal horn²⁵ and are the only neurons activated by WIN55,212-2, activation of 'off' cells probably contributes to cannabinoid analgesia by inhibiting dorsal horn nociceptive neurons.

Direct inhibition of 'on' cells by μ -opioids disinhibits 'off' cells²⁶. Furthermore the release of endogenous opioids in the RVM mediates both the inhibition of 'on' cells and the antinociception seen after activation of neurons in the midbrain periaqueductal grey^{27,28}. Systemically administered cannabinoids could inhibit 'on' cells and activate 'off' cells through a similar circuit to that involving endogenous opioids. We tested the involvement of a direct opioid link in the cannabinoid-induced changes in RVM neuronal activity

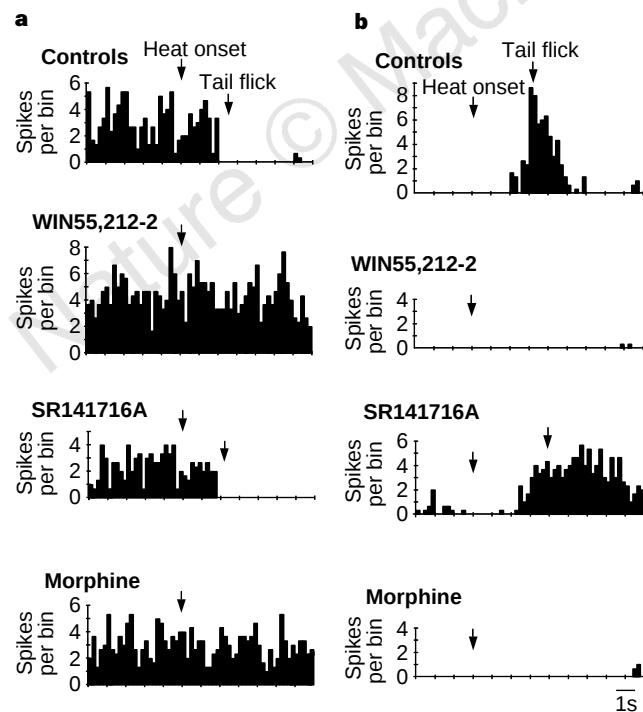


Figure 3 Systemic cannabinoids reduce the 'off' cell pause and 'on' cell burst of RVM neurons, and eliminate the tail-flick reflex in a manner similar to morphine. **a**, An example of 'off' cell activity during control tail flicks and after successive intravenous administrations of WIN55,212-2, SR141716A, and morphine sulphate. **b**, Activity of an 'on' cell during control tail flicks and after successive administrations of WIN55,212-2, SR141716A, and morphine sulphate. Arrows represent the initiation of the thermal stimulus and tail flick. Each histogram represents the average of three trials; bin width, 200 ms.

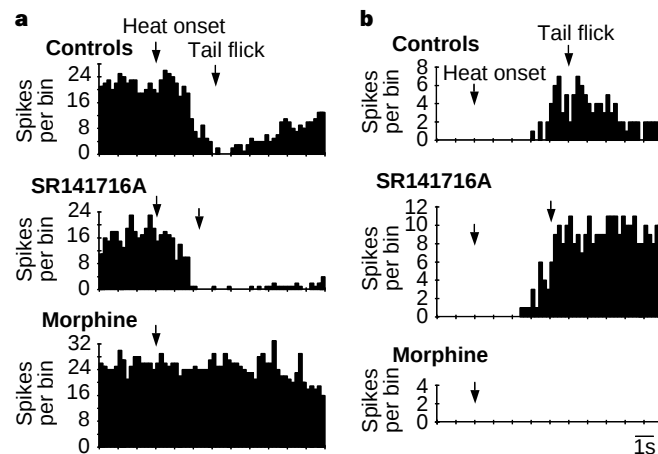


Figure 4 The cannabinoid receptor antagonist SR141716A decreases 'off' cell activity, increases 'on' cell activity and produces hyperalgesia. **a**, Activity of an 'off' cell during control tail flicks, and following successive administrations of SR141716A and morphine sulphate. **b**, 'On' cell activity during control tail flicks, and following successive administrations of SR141716A and morphine sulphate. Arrows represent the initiation of the thermal stimulus and tail flick. Each histogram represents the average of three trials; bin width, 200 ms.

by administering naloxone (0.2 mg kg^{-1} , i.v.) after WIN55,212-2 (0.125 mg kg^{-1} , i.v.) while recording from RVM neurons. Naloxone did not affect the elimination of the 'on' cell burst ($n = 5$) or the 'off' cell pause ($n = 5$) produced by WIN55,212-2 (data not shown), indicating that endogenous opioids are not required for the cannabinoid-induced changes in RVM neuronal activity.

In the mouse, intrathecal administration of SR141716A results in hyperalgesia in the hot-plate test²⁹, indicating that tonically released endogenous cannabinoids modulate nociceptive thresholds. Activity of a precursor enzyme for the synthesis of the endogenous cannabinoid ligand anandamide is highest in the brainstem³⁰. To examine whether a tonically released endogenous cannabinoid controls RVM neurons, we injected SR141716A (0.5 mg kg^{-1}) intravenously, followed by morphine (2.0 mg kg^{-1}) and naloxone (0.2 mg kg^{-1}), while recording from 'on' cells ($n = 5$) and 'off' cells ($n = 5$). Following SR141716A administration, tail-flick latencies decreased from 4.41 ± 0.30 to $3.35 \pm 0.31 \text{ s}$ ($P < 0.01$, $n = 10$). Furthermore, reduction in the 'off' cell tail-flick-related activity was enhanced by more than 30% in all five neurons (for example, Fig. 4a). Baseline activity of 'off' cells also decreased in four out of five neurons ($>30\%$). 'On' cell baseline activity increased in one out of five neurons after administration of SR141716A, and two out of five 'on' cells showed an increase in tail-flick-related activity ($>30\%$) after SR141716A was given (Fig. 4b). Although it is possible that SR141716A acts as an inverse agonist at the CB1 receptor, these results indicate that tonic release of endogenous cannabinoids increases 'off' cell and decreases 'on' cell activity, thereby elevating nociceptive thresholds.

These results show that cannabinoids produce analgesia through activation of a brainstem circuit that is required for opioid analgesia. As with the opioids, activation of 'off' cells in the RVM contributes to cannabinoid-induced antinociception. However, the modulation of 'on' and 'off' cell activity by cannabinoids does not require an endogenous opioid receptor ligand. Furthermore, endogenous cannabinoids seem to be tonically released and to control basal nociceptive thresholds through the modulation of RVM neuronal activity. Given their unique side-effect profile (for example, cannabinoids increase appetite, whereas opioids can cause nausea and respiratory depression), cannabinoids may be useful in improving the treatment of pain. □

Methods

Microinjections. Male Sprague–Dawley rats (275–350 g) were implanted with chronic jugular catheters, and 26-g guide cannulae were positioned 2 mm above the RVM (AP -2.1 (interaural), ML 0, DV 8.5)³¹ and cemented to the skull with dental acrylic. Internal cannulae (33-gauge) that protruded 2 mm beyond the tip of the guide cannula were inserted into the guides for injections. Injector cannulae were connected to 100- μl Hamilton syringes through PE10 tubing backfilled with water. Microinjections of drugs into the RVM (500 nl) were made with an infusion pump over a period of 5 min. All injection sites were histologically verified to be within the RVM.

Rats were handled and adapted to a plexiglas chamber with a glass bottom for 3 days before testing. Tail-flick latencies were measured in awake, unrestrained rats using radiant heat from a modified Hargreaves thermal stimulator. On the test day, intravenous injection of drugs was given 5 min following RVM microinjections. Tail-flick measurements were taken every 2 min for 60 min after administration of intravenous drugs. In rats tested on the rotarod treadmill, initial training determined the ability of each rat to walk for at least 60 s at 10 rotations per min. In all other trials, the rotarod started at 6 rotations per min and accelerated by 2 rotations every min. A pretreatment score was recorded, and was followed by RVM microinjection and, 5 min later, by intravenous drug administration. Rats were tested for their ability to stay on the rotarod at 5, 15 and 45 min following intravenous drugs.

Electrophysiology. Male Sprague–Dawley rats (300–475 g) were anaesthetized with sodium pentobarbital. A jugular vein was catheterized for constant infusion of anaesthetic (sodium methohexital, $15\text{--}30 \text{ mg kg}^{-1} \text{ h}^{-1}$) and administration of drugs. Single RVM neurons were recorded extracellularly

with tungsten electrodes ($3\text{--}4 \text{ M}\Omega$). 'On' cells showed a burst of activity and 'off' cells a pause just before a tail flick. Neutral cells showed no consistent change in activity related to the tail flick. Tail-flick-related activity was examined every 3 min. After 3 stable control tail flicks, rats were given drugs in successive order every 12–18 min. Baseline neuronal activity for 10 s before the heat onset was compared before and after drug administration. Tail-flick-related neuronal activity was defined as the frequency of firing during a 4-s window around the average control tail-flick latency calculated over all experiments. Only one neuron was tested per animal.

Materials. WIN 55,212-2 was purchased from Research Biochemicals International. SR141716A was a gift from the US National Institute of Drug Abuse. Vehicle consisted of ethanol, Alkamuls EL-620 (Rhône-Poulenc) and 0.9% saline at 1:1:18.

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- Mikuriya, T. H. *Marijuana: Medical Papers 1839–1972*. (Medi-Comp Press, Oakland, California, 1973).
- Dunn, M. & Davis, R. The perceived effects of marijuana on spinal cord injured males. *Paraplegia* **12**, 175 (1974).
- Noyes, R., Brunk, S. F., Avery, D. H. & Canter, A. The analgesic properties of delta-9-tetrahydrocannabinol and codeine. *Clin. Pharmacol. Ther.* **18**, 84–89 (1975).
- Clark, W. C., Janal, M. N., Zeidenberg, P. & Nahas, G. G. Effects of moderate and high doses of marihuana on thermal pain: a sensory decision theory analysis. *J. Clin. Pharmacol.* **21** (Suppl.), 299–310 (1981).
- Libman, E. & Stern, M. H. The effects of delta-9-THC on cutaneous sensitivity and its relation to personality. *Person. Individ. Diff.* **6**, 169–174 (1985).
- Holdcroft, A. et al. Pain relief with oral cannabinoids in familial Mediterranean fever. *Anaesthesia* **52**, 483–488 (1997).
- Buxbaum, D., Sanders-Bush, E. & Efron, D. Analgesic activity of tetrahydrocannabinol (THC) in the rat and mouse. *Fed. Proc.* **28**, 735 (1969).
- Sofia, R. D., Nalepa, S. D., Harakel, J. J. & Vassar, H. B. Anti-edema and analgesic properties of delta-9-tetrahydrocannabinol (THC). *J. Pharmacol. Exp. Ther.* **186**, 646–655 (1973).
- Moss, D. E. & Johnson, R. L. Tonic analgesic effects of delta-9-tetrahydrocannabinol as measured with the formalin test. *Eur. J. Pharmacol.* **61**, 313–315 (1980).
- Lichtman, A. H. & Martin, B. R. Cannabinoid-induced antinociception is mediated by a spinal α -noradrenergic mechanism. *Brain Res.* **559**, 309–314 (1991).
- Martin, W. J., Hohmann, A. G. & Walker, J. M. Suppression of noxious stimulus-evoked activity in the ventral posterolateral nucleus of the thalamus by a cannabinoid agonist: correlation between electrophysiological and antinociceptive effects. *J. Neurosci.* **16**, 6601–6611 (1996).
- Herzberg, U., Eliav, E., Bennett, G. J. & Kopin, I. J. The analgesic effects of R(+)-WIN 55,212-2 mesylate, a high affinity cannabinoid agonist, in a rat model of neuropathic pain. *Neurosci. Lett.* **221**, 157–160 (1997).
- Gough, A. L. & Olley, J. E. Delta-9-tetrahydrocannabinol and the extrapyramidal system. *Psychopharmacol.* **54**, 87–99 (1977).
- Ueki, S. Abnormal behavior induced by delta-9-tetrahydrocannabinol. *Trends Pharmacol. Sci.* **1**, 126–129 (1980).
- Mitchell, J. M., Lowe, D. & Fields, H. L. The contribution of the rostral ventromedial medulla to the antinociceptive effects of systemic morphine in restrained and unrestrained rats. *Neuroscience* **87**, 123–133 (1998).
- Matsuda, L. A., Lolait, S. J., Brownstein, M. J., Young, A. C. & Bonner, T. I. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* **346**, 561–564 (1990).
- Munro, S., Thomas, K. L. & Abu-Shaar, M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* **365**, 61–65 (1993).
- Devane, W. A. et al. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258**, 1946–1949 (1992).
- Stella, N., Schweitzer, P. & Piomelli, D. A second endogenous cannabinoid that modulates long-term potentiation. *Nature* **388**, 773–778 (1997).
- Rinaldi-Carmona, M. et al. SR 14176A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett.* **350**, 240–244 (1994).
- Tsou, K., Brown, S., Sanudo-Pena, M. C., Mackie, K. & Walker, J. M. Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. *Neuroscience* **83**, 393–411 (1998).
- Basbaum, A. I. & Fields, H. L. Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry. *Annu. Rev. Neurosci.* **7**, 309–338 (1984).
- Martin, W. J., Tsou, K. & Walker, J. M. Cannabinoid receptor-mediated inhibition of the rat tail-flick reflex after microinjection into the rostral ventromedial medulla. *Neurosci. Lett.* **242**, 33–36 (1998).
- Fields, H. L., Vanegas, H., Hentall, I. D. & Zorman, G. Evidence that disinhibition of brain stem neurones contributes to morphine analgesia. *Nature* **306**, 684–686 (1983).
- Fields, H. L., Malik, A. & Burstein, R. Dorsal horn projection targets of ON and OFF cells in the rostral ventromedial medulla. *J. Neurophys.* **74**, 1742–1759 (1995).
- Heinricher, M. M., Morgan, M. M. & Fields, H. L. Direct and indirect actions of morphine on medullary neurons that modulate nociception. *Neuroscience* **48**, 533–543 (1992).
- Pan, Z. Z. & Fields, H. L. Endogenous opioid-mediated inhibition of putative pain-modulating neurons in rat rostral ventromedial medulla. *Neuroscience* **74**, 855–862 (1996).
- Roychowdhury, S. M. & Fields, H. L. Endogenous opioids acting at a medullary μ -opioid receptor contribute to the behavioral antinociception produced by GABA antagonism in the midbrain periaqueductal gray. *Neuroscience* **74**, 863–872 (1996).
- Richardson, J. D., Aanonsen, L. & Hargreaves, K. M. Hypoactivity of the spinal cannabinoid system results in NMDA-dependent hyperalgesia. *J. Neurosci.* **18** (1998).
- Cadas, H., di Tomaso, E. & Piomelli, D. Occurrence and biosynthesis of endogenous cannabinoid precursor, N-arachidonoyl phosphatidylethanolamine, in rat brain. *J. Neurosci.* **17**, 1226–1242 (1997).
- Paxinos, G. & Watson, C. *The Rat Brain in Stereotaxic Coordinates* (Academic Press, New York, 1986).

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