Cannabis as pain relief: the role of the endocannabinoid system in the pathophysiology of chronic pain

Introduction

Ask a typical seven year-old who has just fallen over what the purpose of his or her pain is and you will likely be greeted with hostility or confusion. But despite its negative impact on the sufferer, acute pain confers a huge evolutionary advantage by minimising the extent of tissue injury (for example, by rapidly withdrawing one's hand from a hot radiator or resting a sprained ankle). Sufferers of a rare condition called 'congenital insensitivity to pain' will attest to the danger of their condition[1].

However, just as pain is important to survival, so is the body's ability to *modulate* pain. As hunter-gatherers, being able to temporarily numb the pain of a sprained ankle could mean escaping the lion that offers a far more imminent threat to survival. Resultantly, humans have evolved not only to experience pain, but also to control it in times of need.

Unfortunately, as with many aspects of our physiology, pain regulation is prone to misfiring. Chronic pain, which affects up to half of the UK adult population[2], imparts a huge burden on mental, social and physical wellbeing, and incurs an immense financial cost to society[3]. Although treatments are available for chronic pain, many of them, including opioid medications, are only effective for short periods[4]. A review of the clinical effectiveness of current treatments for chronic pain concluded that 'none of them appear capable of eliminating pain or significantly improving functional outcomes for all treated'[5].

Chronic pain may be broadly divided into: [1] nociceptive, which results from ongoing tissue damage such as arthritis in a joint; [2] neuropathic, which stems from the dysfunction of neurons in our peripheral and central nervous system; and [3] mixed, which results from a combination of the two. Our understanding of all three forms of chronic pain has come a long way in the last two decades, and one key area of ongoing research is on the endocannabinoid system, both its structure and how we can manipulate it therapeutically to treat sufferers of chronic pain.

Historically, the use of cannabis as pain relief can be traced back to China in 2737BC[6]. Its medical uses emerged much later in the West, and faded as a result of (then) novel treatments like aspirin and opioids. In 1928, cannabis became illegal in the UK because of its psychoactive properties; since then, regulations restricting cultivation have preoccupied governments not only here but worldwide[3].

In 1964, tetrahydrocannabinol (THC), the major psychoactive chemical found in *Cannabis sativa*, was isolated and identified from the plant's resin[7], but it was not until 1990 that the structure of the first cannabinoid receptor – CB1 – was described[8]. This receptor allows THC and other phytocannabinoids to exert an effect on the human body. Its discovery paved the way for research on endogenous cannabinoids, which have subsequently been elucidated[9]. A new field of research on the endocannabinoid system has since flourished, and offers important new opportunities for the challenges of pain relief.

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In the main section of this essay, I will begin by discussing the major pain pathways found in the human body, and then focus on the role of the endocannabinoid system in modulating these pathways. Finally, I will consider what the therapeutic implications are, and how these may shape the treatment of chronic neuropathic pain in future years.

In order to understand the processes that underpin chronic pain, we must distinguish between nociception and pain, as well as some of the symptoms that it produces (see *box 1*).

Box 1 – definitions of pain-related terms

- **Nociception** – detection of tissue damage by specialised transducers (nociceptors) attached to aδ and C fibres.
- **Pain** – an unpleasant sensory *and* emotional experience associated with actual or potential tissue damage: nociception with an affective component (e.g. unpleasantness and suffering).
- **Hyperalgesia** – increased pain sensation elicited by a noxious stimulus
- **Allodynia** – pain sensation elicited by a normally innocuous stimulus

Pain pathways

Signalling of pain can be classified, broadly speaking, into two pathways: ascending and descending. Ascending pathways carry the signals that relay information from noxious stimuli (e.g. touching a hot radiator) up to the brain, and descending pathways are the ways in which the brain can modify the flow of these signals.

Ascending pathways

The main ascending pain pathway in the human body is called the spinothalamic tract, a sequence of neurons that convey information from a painful stimulus up to the thalamus, a central hub of processing. From here, the thalamus projects to the somatosensory cortex of the brain, where we experience the location and character of the pain, as well as other areas like the limbic system, which control our emotive experience of it.

The first neuron in the spinothalamic pathway is known as a nociceptor, which fires an action potential when a painful stimulus is strong enough to depolarise it via the various receptors shown in *figrue 1a*[10]. The cell body of this nociceptor is located in the dorsal root ganglion (DRG), and synapses with a second-order neuron in the dorsal horn of the spinal cord (*figure 1b*)[10]. From here, the neuron decussates (crosses over) the spinal cord and travels up through the brainstem where it synapses with a third-order neuron in the thalamus (*figure 1c*)[10].

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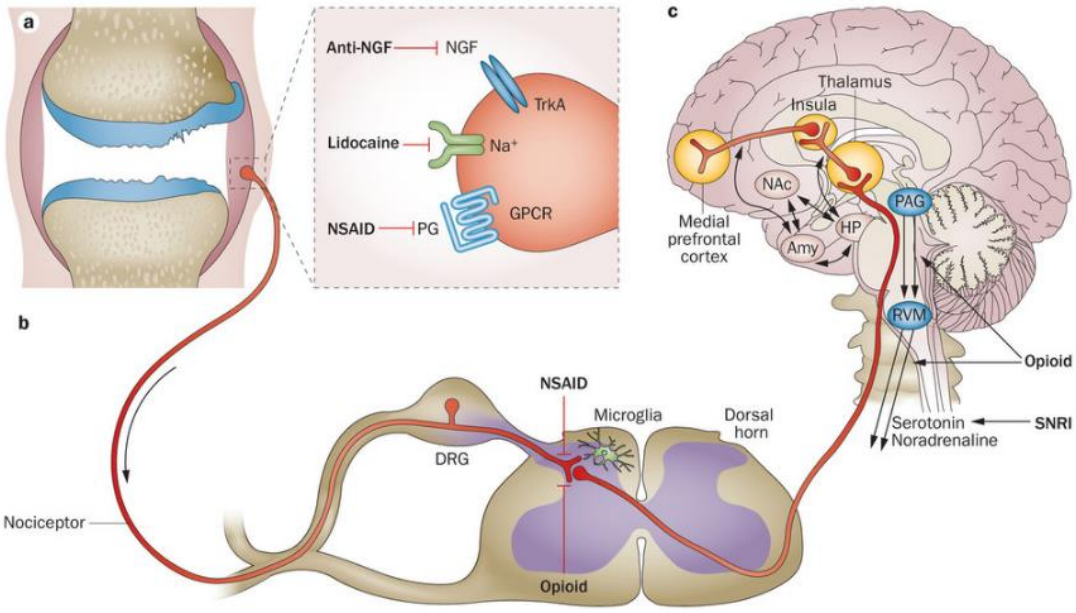
Figure 1 – overview of the pathways that relay pain signals, and the modulation of these signals (reproduced with permission from *Malfait and Schnitzer*[10]).



a) Pain signals (in this case from the knee) trigger nociceptors which project to the dorsal horn of the spinal cord. b) These first-order neurons synapse with projection neurons which decussate and ascend up the lateral spinothalamic tract, terminating at the thalamus. c) From the thalamus, pain signals project to different areas of the brain, including the cortex, hypothalamus and amygdala. Black arrows indicate the role of descending pain signals, which may modulate the effect of subsequent incoming pain signals. The diagram also shows the areas in which current analgesic medications have effect.

Abbreviations: Amy – amygdala, DRG – dorsal root ganglion, GPCR – G-protein coupled receptor, HP – hippocampus, NAc – nucleus accumbens, NSAID – non-steroidal anti-inflammatory drug, NGF – nerve growth factor, PAG – periaqueductal gray, PG – prostaglandin, RVM – rostral ventromedial medulla, SNRI – serotonin-noradrenaline reuptake inhibitor.

Descending pathways

Two important structures in the brain associated with descending pathways are the rostral ventromedial medulla (RVM)[11], and the peri-aqueductal gray (PAG)[12], which are shown in *figure 1c*[10]. One role they play is to send inhibitory signals to the afferent nerve fibres conveying pain in the spinothalamic pathway by altering synaptic transmission between ascending nerves, which prevents propagation of an impulse up the spinothalamic tract[13].

The transmission of pain in the body relies on functioning interaction between ascending and descending pathways. Endogenous opioids are a well-documented modulator of these pathways, but another level of control is independently mediated through the endocannabinoid system[14].

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Endocannabinoid system (ECS)

The ECS comprises a group of receptors, endogenous neurotransmitters and enzymes involved in biosynthesis and degradation, located in the peripheral and central nervous system and involved in a variety of physiological functions, including appetite, mood, memory and pain[15]. Two main receptors make up the ECS: CB1, which is located in the central nervous system and CB2, which mainly acts peripherally[3]. These receptors are acted on by the fatty acid neurotransmitters anandamide and 2-AG, which are the endogenous equivalents of the phytocannabinoids THC and CBD, respectively. Breakdown of anandamide and 2-AG relies on uptake by surrounding glial cells, and degradation by the enzymes fatty acid amide hydrolase (FAAH), and monoacylglycerol lipase (MAGL).

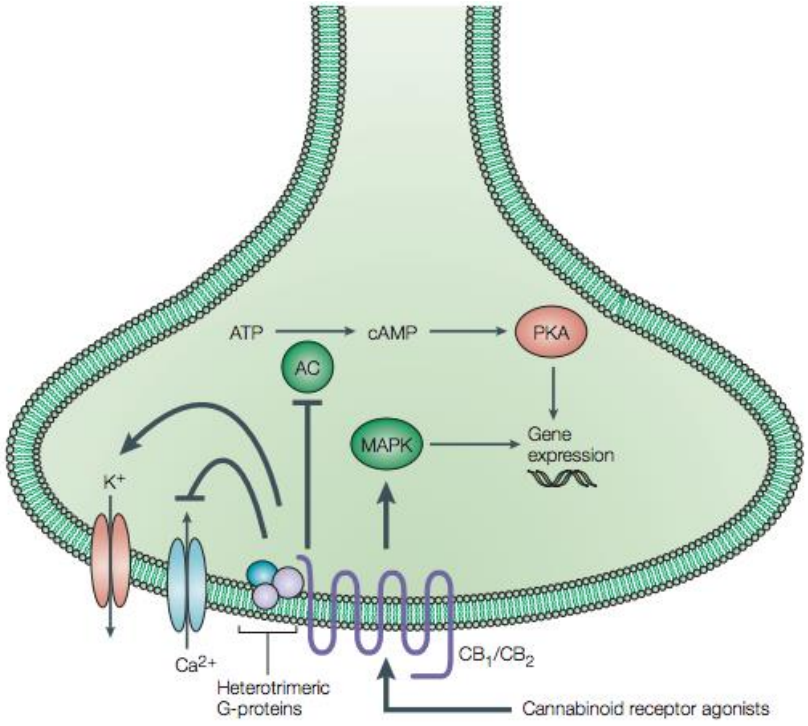
The role of the ECS in analgesia is well demonstrated through animal models. Mice given the substance SR141716A, an endocannabinoid antagonist, develop hyperalgesia[16], as do those with the CB1 gene knocked out[17]. In contrast, mice lacking the degrading enzymes FAAH exhibit higher levels of anandamide and experience hypoalgesia[18].

Although our understanding of the ECS is far from comprehensive, we are beginning to appreciate that its effects on pain relief are not just mediated through CB1/CB2 receptors, but may involve other receptors like VR1, interaction with pre-existing pathways, and behavioural modulation.

CB1/CB2 receptors

The intracellular signalling pathways associated with CB1/CB2 activation are shown in *figure 2*[19]. CB1 receptors are highly expressed in forebrain areas associated with higher cognitive function[20], whereas CB2 receptors tend to be more peripherally expressed[21].

Figure 2 – intracellular signalling pathways associated with CB1/CB2 receptor activation (reproduced with permission from Di Marzo *et al*[19]).



Stimulation of CB1/CB2 receptors on presynaptic membranes and their associated G-proteins causes inhibition of MAPK and subsequent inactivation of PKA, leading to changes in gene expression. CB1 receptors also inhibit influx of calcium ions, and promote efflux of potassium ions, which together result in a hyperpolarisation of the neuron and prevent neurotransmitter release.

Abbreviations: AC – adenylate cyclase, ATP – adenosine triphosphate, cAMP – cyclic AMP, MAPK – mitogen activated protein kinase, PKA – protein kinase A

Although CB1/2 work in a variety of ways, these mechanisms are probably best exemplified with regards to the peri-aqueductal gray (PAG) area of the brain, which as described above, has a role in descending modulation of pain. In a classic experiment, Mayer *et al* showed that electrical stimulation of the PAG causes analgesia in rats[22], and later studies showed that when the ventral area of the PAG was stimulated specifically, this could be blocked by the opioid antagonist naloxone[23], suggesting an opioid-dependent mechanism of descending pain modulation. However, when the dorsal or lateral aspects of the PAG were stimulated, naloxone was inefficacious at reducing analgesia[24], suggesting an opioid-independent mechanism here. In 1999, Walker *et al* administered a cannabinoid antagonist following dorsal and lateral PAG induced analgesia, and found that the effects were reversed[25]. They concluded that the CB1 receptor was responsible for descending pain modulation, based on its interaction with endogenous ligands like anandamide and their known intracellular effects[26].

Whilst CB1 tends to act centrally, as shown by its role in PAG-related descending pathways, CB2 tends to exert is effects peripherally[3]. For example, in a commonly used animal model for nociception[27], Ibrahim *et al* injected the chemical irritant formalin into the hindpaws of rats, and investigated the effects of administering a CB2 receptor agonist on nociception[28]. The study showed that CB2 activation mediates local keratinocytes to produce beta-endorphin, which acts on local μ-opioid receptors to increase the threshold for nociceptive signals to be propagated, decreasing the rats' experience of pain.

Although CB2 receptors act predominantly on the peripheral nervous system, Beltramo *et al* demonstrated expression of CB2 mRNA in the dorsal root ganglion and spinal cord of rats, and found that rats which had the CB1 gene knocked out benefitted from pain relief via central mechanisms, indicating a role of CB2 in neuropathic pain[29]. Indeed, one study has supported the hypothesis that dysfunctional CB2 expression in the ventral posteriolateral (VPL) nucleus of the thalamus – an area of neuron synapse in the spinothalamic tract – may partially explain elevated pain markers in neuropathic rats[30].

Other receptors

Elucidating the structure of CB1/2 receptors opened the gate for research on the ECS, but research findings are increasingly pointing towards interaction between endocannabinoids and other receptors involved in pain transduction.

One of the key receptors responsive to endocannabinoids such as anandamide is VR1. This is also known as the 'capsaicin receptor', as it is responds to the compound found in chilli peppers. Although most people's experience of capsaicin is buccal pain following spicy food, the compound paradoxically provides pain *relief* through the mechanisms of ion channel desensitisation[31] and substance P (a pain neurotransmitter) depletion at the dorsal root ganglion[32]. Anandamide is a full agonist to VR1 receptors[33], which could mean that it has the same potential as capsaicin in analgesia.

Cannabinoid agonists have been shown to interact with numerous other channels, including activating glycine and NMDA receptors, and inhibiting 5-HT3 and nicotinic acetylcholine receptors[34], suggesting a further potential role in homeostatic pain regulation.

As well as stimulating other receptors directly, cannabinoids may be indirectly linked to other pathways via the CB1 receptor through downstream signalling. Romero *et al* illustrated a link between CB1 activation and noradrenaline release, which stimulated anti-nociceptive pathways via peripheral adrenoreceptors[35].

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Affective components of ECS

When researching pain, it is important to differentiate between its nociceptive and affective qualities (see *box 1*). The latter comprises emotive attributes that we associate with actual or potential tissue damage, and can make the experience of pain significantly worse. So far, I have described how the ECS may interact with nociceptive component of pain through CB1, CB2 and a variety of other receptors. Given the well-known psychoactive components of phytocannabinoids like THC, what do we understand about the ability of the ECS to modulate the affective component of pain?

Studies using both positron emission tomography[36] and immunocytochemistry[37] have shown a high density of CB1 receptors in the frontal-limbic system of the brain, which is involved in behaviour and emotion. Using functional magnetic resonance imaging (fMRI), Lee *et al* explored the role of administering oral THC on the activity of the limbic system following a painful stimulus[38]. In comparison to placebo-treated subjects, those given oral THC reported a lower level of unpleasantness following pain, whilst still experiencing the same pain intensity. These subjective features were corroborated by a reduction in sensory-limbic functional connectivity on the fMRI[38], and the authors concluded that the analgesic properties of phytocannabinoids were mainly due to a dissociation between nociception and the affective component of pain, which could be replicated endogenously through endocannabinoids.

Finally, affective components of pain may be targeted indirectly. For example, Salomons *et al* have recently delineated a mechanism that explains the correlation between chronic pain and anxiety, suggesting that the latter decreased the sufferer's perceived control over their pain and exacerbated its affective component[39]. Could the well-documented anxiolytic effects of cannabinoids[40] underpin a viable mechanism for pain reduction? Furthermore, given the association between sleep deprivation and chronic pain[41], as well as the ability of cannabinoids to regulate sleep[42], could this represent a factor in the pathophysiology of chronic pain?

The ECS in clinical practice

Although our understanding of the ECS is far from comprehensive, research in pharmacology and drug delivery is optimising the potential of cannabinoids in clinical practice[43]. Animal models have elucidated some of the mechanisms underpinning pain reduction, but licensing drugs that are effective on humans is still in its early stages[44]. This is not only due to the physiological differences between animals used in models and human patients, but also because drug testing carries a high risk of exposing subjects to potential harm.

Despite these limitations, clinical trials have so far had promising results. A meta-analysis of 8 trials on the efficacy of cannabinoids on pain relief has shown 'moderate evidence' to support their use for the treatment of chronic pain[45]. The problem with licensing these drugs for official use stems from their adverse side

effect profile. Although binding of cannabinoids is sparse or absent in the brainstem, medulla and thalamus[3] (areas involved in vital physiological functions like breathing), which explains the absence of life-threatening sequelae following very high doses of cannabinoids, they have very common psychoactive side effects when delivered exogenously[46].

The psychedelic intoxication that cannabinoids such as THC induce restricts their widespread use in licensed pain relief. However, other phytocannabinoids are found in cannabis which exert significantly fewer psychoactive effects, including cannabidiol (CBD)[47]. CBD has a much lower affinity for CB1/2 receptors, and is postulated to work through enhancing effects of endogenous cannabinoids such as anandamide[47].

Drug development could adopt a novel approach to exploit the ECS in order develop treatment for pain relief. Just as CBD appears promising through manipulating *endogenous* cannabinoids, so medications which inhibit the breakdown of these endocannabinoids help target this narrow therapeutic window. FAAH inhibitors have been shown to produce analgesia in rats[48], and these medications have minimal side effects compared to exogenous cannabinoids[49].

A significant threat posed to the licensing of cannabis based medications is the smoked route of administration, not only because of the adverse side effects, but also because of reluctance of healthcare providers to prescribe such treatments[3]. Pharmakokinetics (how the body affects a particular drug) shows that whilst immediate bioavailability is lower when a cannabinoid is taken orally (compared to smoked), their elimination is longer and effects may last for up to nine hours more[50]. Oral administration of cannabinoid medication therefore presents a much more realistic method of delivery in the future.

In the UK, there are few cannabis-based medications which are currently licensed for pain relief, and those which can be prescribed carry strict indications on when to use them. Sativex was the first medication containing phytocannabinoids (THC and CBD) to become licensed for use in the UK in 2010[51]. It is an oromucosal spray effective for the treatment of chronic neuropathic pain[52], but is only licensed as a second-line medication for pain relief or spasticity in multiple sclerosis if gabapentin or baclofen offer 'inadequate relief or have intolerable side effects'[51]. Although clinical trials of the medication showed its efficacy and safety, it still carries uncommon psychoactive risks such as hallucinations.

With a growing understanding of the endocannabinoid system, we are beginning to develop new medications which no longer cross the narrow gap between the therapeutic and psychoactive effects of cannabinoids[43]. Future directions will aim to provide orally administered, highly bioavailable and non-psychoactive forms of phytocannabinoids to offer a much-needed alternative to current forms of pain medication.

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Conclusion

Chronic pain is a ubiquitous and debilitating problem in today's society, and medications currently used to treat this condition have limited efficacy. In the UK, our aging and comorbid population imposes a heavy burden on the NHS, and without a novel approach to chronic analgesia, pain specialists will soon struggle to cope with increasing referrals to their clinics. What we need is safe, effective drugs that GPs can prescribe so fewer patients are referred for complex pain treatments.

I have discussed one potential solution in the form of cannabis-based treatments. Humans have known about the pain relieving properties of cannabis for millennia[6], but our use of phytocannabinoids in medical practice has been stymied by their adverse psychoactive effects. Stigma surrounding cannabis use is rife: we often forget that medical professionals will readily prescribe heroin (diamorphine) to a woman in labour, but cause uproar if they support the use of cannabis as pain relief.

Fortunately, our growing understanding of the ECS offers a potential to harvest the expedient properties of phytocannabinoids for medical use, without succumbing to the effects of psychedelic intoxication. Given the complex interplay between the ECS and other pathways, we are still a long way off fully benefitting from this intricate and esoteric system, but current research is shaping treatments to provide millions of people worldwide with a new, effective and safe form of pain relief.

References

1. Nagasako, E.M., Oaklander, A.L. & Dworkin, R.H. Congenital insensitivity to pain: an update. *Pain* **101**, 213-219 (2003).
2. Elliott, A.M., Smith, B.H., Penny, K.I., Smith, W.C. & Chambers, W.A. The epidemiology of chronic pain in the community. *Lancet* **354**, 1248-1252 (1999).
3. Fine, P.G. & Rosenfeld, M.J. The endocannabinoid system, cannabinoids, and pain. *Rambam Maimonides medical journal* **4**, e0022 (2013).
4. Kalso, E., Edwards, J.E., Moore, R.A. & McQuay, H.J. Opioids in chronic non-cancer pain: systematic review of efficacy and safety. *Pain* **112**, 372-380 (2004).
5. Turk, D.C. Clinical effectiveness and cost-effectiveness of treatments for patients with chronic pain. *Clinical Journal of Pain* **18**, 355-365 (2002).
6. Aggarwal, S.K. et al. Medicinal use of cannabis in the United States: historical perspectives, current trends, and future directions. *Journal of opioid management* **5**, 153-68 (2009).
7. Gaoni, Y. & Mechoulam, R. Isolation, structure and partial synthesis of an active constituent of hashish. *Journal of the American Chemical Society* **86**, 1646-+ (1964).
8. 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).
9. Fride, E. & Mechoulam, R. Pharmacological activity of the cannabinoid receptor agonist, anandamide, a brain constituent. *European Journal of Pharmacology* **231**, 313-314 (1993).
10. Malfait, A.M. & Schnitzer, T.J. Towards a mechanism-based approach to pain management in osteoarthritis. *Nature Reviews Rheumatology* **9**, 654-664 (2013).
11. De Felice, M. et al. Engagement of descending inhibition from the rostral ventromedial medulla protects against chronic neuropathic pain. *Pain* **152**, 2701-2709 (2011).
12. Sherman, A.D. & Gebhart, G.F. Pain-induced alteration of glutamate in periaqueductal central gray and its reversal by morphine. *Life Sciences* **15**, 1781-1789 (1974).
13. Millan, M.J. Descending control of pain. *Progress in Neurobiology* **66**, 355-474 (2002).
14. Mao, J., Price, D.D., Lu, J., Keniston, L. & Mayer, D.J. Two distinctive antinociceptive systems in rats with pathological pain. *Neuroscience Letters* **280**, 13-16 (2000).
15. Kohnz, R.A. & Nomura, D.K. Chemical approaches to therapeutically target the metabolism and signaling of the endocannabinoid 2-AG and eicosanoids. *Chemical Society Reviews* **43**, 6859-6869 (2014).
16. Richardson, J.D., Aanonsen, L. & Hargreaves, K.M. SR 141716A, a cannabinoid receptor antagonist, produces hyperalgesia in untreated mice. *European Journal of Pharmacology* **319**, R3-R4 (1997).

17. Zimmer, A., Zimmer, A.M., Hohmann, A.G., Herkenham, M. & Bonner, T.I. Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 5780-5785 (1999).
18. Lichtman, A.H., Shelton, C.C., Advani, T. & Cravatt, B.F. Mice lacking fatty acid amide hydrolase exhibit a cannabinoid receptor-mediated phenotypic hypoalgesia. *Pain* **109**, 319-327 (2004).
19. Di Marzo, V., Bifulco, M. & De Petrocellis, L. The endocannabinoid system and its therapeutic exploitation. *Nature Reviews Drug Discovery* **3**, 771-784 (2004).
20. Glass, M., Dragunow, M. & Faull, R.L.M. Cannabinoid receptors in the human brain: A detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. *Neuroscience* **77**, 299-318 (1997).
21. Svizenska, I., Dubovy, P. & Sulcova, A. Cannabinoid receptors 1 and 2 (CB1 and CB2), their distribution, ligands and functional involvement in nervous system structures - A short review. *Pharmacology Biochemistry and Behavior* **90**, 501-511 (2008).
22. Mayer, D.J., Wolfle, T.L., Akil, H., Carder, B. & Liebeski.Jc. Analgesia from electrical stimulation in brainstem of rat. *Science* **174**, 1351-& (1971).
23. Akil, H., Mayer, D.J. & Liebeskind, J.C. Antagonism of stimulation-produced analgesia by naloxone, a narcotic antagonist. *Science* **191**, 961-962 (1976).
24. Cannon, J.T., Prieto, G.J., Lee, A. & Liebeskind, J.C. Evidence for opioid and non-opioid forms of stimulation-produced analgesia in the rat.n*Brain Research* **243**, 315-321 (1982).
25. Walker, J.M., Huang, S.M., Strangman, N.M., Tsou, K. & Sanudo-Pena, M.C. Pain modulation by release of the endogenous cannabinoid anandamide. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 12198-12203 (1999).
26. Vogel, Z. et al. Anandamide, a brain endogenous compound, interacts specifically with cannabinoid receptors and inhibits adenylate cyclase. *Journal of Neurochemistry* **61**, 352-355 (1993).
27. Hunskaar, S. & Hole, K. The formalin test in mice - dissociation between inflammatory and non-inflammatory pain. *Pain* **30**, 103-114 (1987).
28. Ibrahim, M.M. et al. CB2 cannabinoid receptor activation produces antinociception by stimulating peripheral release of endogenous opioids. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 3093-3098 (2005).
29. Beltramo, M. et al. CB2 receptor-mediated antihyperalgesia: possible direct involvement of neural mechanisms. *European Journal of Neuroscience* **23**, 1530-1538 (2006).
30. Jhaveri, M.D. et al. Evidence for a novel functional role of cannabinoid CB2 receptors in the thalamus of neuropathic rats. *European Journal of Neuroscience* **27**, 1722-1730 (2008).
31. Bhave, G. et al. cAMP-dependent protein kinase regulates desensitization of the capsaicin receptor (VR1) by direct phosphorylation. *Neuron* **35**, 721-731 (2002).

32. Burks, T.F., Buck, S.H. & Miller, M.S. Mechanisms of depletion of substance P by capsaicin. *Federation Proceedings* **44**, 2531-2534 (1985).
33. De Petrocellis, L. et al. The activity of anandamide at vanilloid VR1 receptors requires facilitated transport across the cell membrane and is limited by intracellular metabolism. *Journal of Biological Chemistry* **276**, 12856-12863 (2001).
34. Pertwee, R.G. et al. International union of basic and clinical pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB1 and CB2. *Pharmacological Reviews* **62**, 588-631 (2010).
35. Romero, T.R.L., Resende, L.C., Guzzo, L.S. & Duarte, I.D.G. CB1 and CB2 cannabinoid receptor agonists induce peripheral antinociception by activation of the endogenous noradrenergic system. *Anesthesia and Analgesia* **116**, 463-472 (2013).
36. Burns, H.D. et al. F-18 MK-9470, a positron emission tomography (PET) tracer for in vivo human PET brain imaging of the cannabinoid-1 receptor. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 9800-9805 (2007).
37. Eggan, S.M. & Lewis, D.A. Immunocytochemical distribution of the cannabinoid CB1 receptor in the primate neocortex: A regional and laminar analysis. *Cerebral Cortex* **17**, 175-191 (2007).
38. Lee, M.C. et al. Amygdala activity contributes to the dissociative effect of cannabis on pain perception. *Pain* **154**, 124-134 (2013).
39. Salomons, T.V., Nusslock, R., Detloff, A., Johnstone, T. & Davidson, R.J. Neural emotion regulation circuitry underlying anxiolytic effects of perceived control over pain. *Journal of Cognitive Neuroscience* **27**, 222-233 (2015).
40. Haller, J., Varga, B., Ledent, C. & Freund, T.F. CB1 cannabinoid receptors mediate anxiolytic effects: convergent genetic and pharmacological evidence with CB1-specific agents. *Behavioural Pharmacology* **15**, 299-304 (2004).
41. Smith, M.T. & Haythornthwaite, J.A. How do sleep disturbance and chronic pain inter-relate? Insights from the longitudinal and cognitive-behavioral clinical trials literature. *Sleep Medicine Reviews* **8**, 119-132 (2004).
42. Carley, D.W., Pavlovic, S., Janelidze, M. & Radulovacki, M. Functional role for cannabinoids in respiratory stability during sleep. *Sleep* **25**, 391-398 (2002).
43. Iversen, L. & Chapman, V. Cannabinoids: a real prospect for pain relief? *Current Opinion in Pharmacology* **2**, 50-55 (2002).
44. Holdcroft, A., Hargreaves, K.M., Rice, A.S.C. & Pertwee, R.G. Cannabinoids and pain modulation in animals and humans. *Proceedings of the 9th World Congress on Pain* **16**, 915-926 (2000).
45. Whiting, P.F. et al. Cannabinoids for medical use: a systematic review and meta-analysis. *Jama-Journal of the American Medical Association* **313**, 2456-2473 (2015).
46. Nahas, G., Harvey, D.J., Sutin, K., Turndorf, H. & Cancro, R. A molecular basis of the therapeutic and psychoactive properties of cannabis (Delta(9)-tetrahydrocannabinol). *Progress in Neuro-Psychopharmacology & Biological Psychiatry* **26**, 721-730 (2002).

47. Mechoulam, R., Parker, L.A. & Gallily, R. Cannabidiol: An overview of some pharmacological aspects. *Journal of Clinical Pharmacology* **42**, 11S-19S (2002).
48. de Novellis, V. et al. The analgesic effect of N-arachidonoyl-serotonin, a FAAH inhibitor and TRPV1 receptor antagonist, associated with changes in rostral ventromedial medulla and locus coeruleus cell activity in rats. *Neuropharmacology* **55**, 1105-1113 (2008).
49. Panlilio, L.V. et al. Effects of fatty acid amide hydrolase (FAAH) inhibitors on working memory in rats. *Psychopharmacology* **233**, 1879-1888 (2016).
50. Grotenhermen, F. Pharmacokinetics and pharmacodynamics of cannabinoids. *Clinical Pharmacokinetics* **42**, 327-360 (2003).
51. MS Trust. Sativex Fact Sheet. http://www.nhs.uk/ipgmedia/national/multiple%20sclerosis%20trust/assets/sativex.pdf (2011).
52. Nurmikko, T.J. et al. Sativex successfully treats neuropathic pain characterised by allodynia: A randomised, double-blind, placebo-controlled clinical trial. *Pain* **133**, 210-220 (2007).