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Review

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Comparative studies of endocannabinoid modulation of pain

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Cannabinoid-based therapies have long been used to treat pain, but there remain questions about their actual mechanisms and efficacy. From an evolutionary perspective, the cannabinoid system would appear to be highly conserved given that the most prevalent endogenous cannabinoid (endocannabinoid) transmitters, 2-arachidonyl glycerol and anandamide, have been found throughout the animal kingdom, at least in the species that have been analysed to date. This review will first examine recent findings regarding the potential conservation across invertebrates and chordates of the enzymes responsible for endocannabinoid synthesis and degradation and the receptors that these transmitters act on. Next, comparisons of how endocannabinoids modulate nociception will be examined for commonalities between vertebrates and invertebrates, with a focus on the medicinal leech *Hirudo verbana*. Evidence is presented that there are distinct, evolutionarily conserved anti-nociceptive and pro-nociceptive effects. The combined studies across various animal phyla demonstrate the utility of using comparative approaches to understand conserved mechanisms for modulating nociception.

This article is part of the Theo Murphy meeting issue 'Evolution of mechanisms and behaviour important for pain'.

1. Introduction

Nociception is likely one of the nervous system's most ancient and adaptively significant functions [1,2]. Consequently, there is considerable interest in the elements of nociception that have been conserved over the course of animal evolution. Comparative approaches to study and understand conserved signalling and modulatory processes might be leveraged to understand basic biological principles of nociception and develop potential therapeutics to treat pain. Conserved signalling processes in the cannabinoid neuromodulatory system may provide one such comparative opportunity. Interest has rapidly grown over the last 20 years for applications of cannabinoid-based therapies to treat pain, using both cannabis and cannabis-based derivatives (Δ-9-tetrahydrocannabinol, cannabidiol, cannabinol, etc.) [3] and drugs that selectively modulate the endocannabinoid system [4]. However, the efficacy of cannabinoid-based approaches to treat pain has been questionable and this is likely due to cannabinoids having more complicated effects on pain than were previously appreciated [5–7]. Consequently, the field of nociception/pain research would benefit from comparative studies of cannabinoid-mediated modulation, especially those using invertebrates in which it is possible to more readily monitor individual neurons and synapses and link changes in their properties to behavioural changes using physiological and/or genetic approaches. Evolution of cannabinoid signalling has been reviewed in the past [8,9]; however, this article will provide new information regarding the evolution of the cannabinoid receptor(s) and its effects relevant to nociception.

2. Evolution of endocannabinoid signalling

Endocannabinoids are a class of lipid neurotransmitter, which, owing to their hydrophobic nature, are synthesized on demand and released in an activity-dependent manner, most often by postsynaptic neurons, to serve as retrograde

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signals [10], although there is also evidence of an autocrine function [11]. The endocannabinoid system comprises the transmitters themselves, their receptors and the enzymes involved in their synthesis and degradation. The most well-studied and abundant endocannabinoids in the central nervous system (CNS) are 2-arachidonoyl glycerol (2-AG) and arachidonoyl ethanolamide (anandamide). In addition to their presence in vertebrates [12], these transmitters have been detected in a variety of invertebrates, including *Hydra vulgaris* (phylum Cnidaria) [13], *Caenorhabditis elegans*, *Caenorhabditis briggsae* and *Pelodera strongyloides* (Nematoda) [14], *Hirudo verbana* and *Theromyzon tessulatum* [15] (Annelida) [16], *Drosophila melanogaster* [17] and *Amblyomma americanum* [18] (Arthropoda) and *Ciona intestinalis* [19] (Chordata).

Anandamide and 2-AG have distinct synthesis and degradation pathways. The synthesis of both these endocannabinoids originates at a shared precursor, arachidonic acid. In total, four routes for anandamide biosynthesis, and two for 2-AG, have been proposed [20]. In general, the biochemical pathways for anandamide synthesis and metabolism are less well characterized than those for 2-AG, largely because it appears that the mechanism of anandamide synthesis is highly variable depending on the brain region and various up- and downstream factors [21].

The best understood synthesis routes for anandamide work through the processing of *N*-arachidonoyl phosphatidylethanolamine (NAPE) via a one-step cleavage by a phospholipase D (NAPE-PLD) or cleavage by a phospholipase C (NAPE-PLC) to an intermediate phospho-anandamide that is quickly dephosphorylated to liberate anandamide [22]. NAPE-PLD has been characterized throughout the animal kingdom, including protostomian invertebrates, e.g. *C. elegans* [23], and deuterostomian invertebrates, e.g. *Strongylocentrotus purpuratus* [8]. The synthesis of 2-AG appears to be more straightforward. The dominant pathway for 2-AG synthesis is via cleavage of phosphatidylinositol 4,5-bisphosphate by PLCβ to generate both inositol triphosphate and diacylglycerol (DAG), and DAG is hydrolysed by DAG lipase (DAGL) to produce 2-AG. Evidence suggests that 2-AG synthesis is well conserved. For 2016 alone, data mining revealed 263 full and partial gene sequences reported to encode DAGL in organisms all across the phylogenic tree [24]. For example, in *Hirudo*, complete transcripts have been found for both DAGL (accession no. KU500007) and monoacylglycerol lipase (MAGL, accession no. KY971276).

MAGL is considered the primary enzyme that degrades 2-AG [25]. MAGL-meditated degradation can influence 2-AG functions inside and outside of the nervous system (e.g. metabolism), regulating whether 2-AG can produce other effects by diffusing to receptor targets other than those in the immediate vicinity [26]. Some degradation enzymes are shared between 2-AG and anandamide, with varying affinities. Of these enzymes, fatty acid amide hydrolase (FAAH) is the primary enzyme that breaks down anandamide, but under some circumstances can also degrade 2-AG. MAGL and FAAH orthologues are widely present throughout the animal kingdom [8]. Altogether, taking a reductionist, comparative approach with invertebrate models could help elucidate the primary and/or conserved mechanisms of endocannabinoid biosynthesis and catabolism, particularly for anandamide. Invertebrate models may allow us to investigate different enzymatic pathways in isolation if certain routes for synthesis or degradation are absent in that model. Using these invertebrate models may also help parse out the distinct effects of anandamide and 2-AG in modulating nociception based on differences in the function and/or expression of their synthesizing and metabolizing enzymes.

The canonical endocannabinoid receptors are cannabinoid receptor type 1 (CB1) and type 2 (CB2), which are Class A G-protein coupled receptors (GPCRs) [27]. While 2-AG and anandamide both activate CB1 and CB2, anandamide is only a partial agonist at both of these receptors [28]. 2-AG and anandamide have also been reported to activate transient receptor potential vanilloid type 1 (TRPV1) channels [29–31] and peroxisome proliferator-activated receptors (PPARs) [32,33]. Finally, recent pharmacological studies indicate that the list of cannabinoid receptors may include orphan GPCRs, namely GPR18 and GPR55 [34], and GPCRs for other neurotransmitters such as serotonin [35] and opioid receptors [36].

A putative ancestral cannabinoid receptor was identified in the deuterostomian invertebrate *C. intestinalis* [37]. The predicted amino acid sequence for the *C. intestinalis* cannabinoid receptor (CICBR) consisted of 423 amino acids and displayed a similar sequence identity to human CB1 and CB2, suggesting an intermediate CB receptor structure that eventually underwent a gene duplication event (table 1). 2-AG [40,41] and anandamide [42] have also been reported to activate NeuroPeptide Receptors (NPR) 19 and 32 in *C. elegans*. Finally, mRNAs encoding two CB-like GPCRs have been reported in the mollusc *Lymnaea stagnalis* [43], joining the growing list of potential CB-like metabotropic receptors in protostomian invertebrates. However, if one compares the protein sequences for a subset of these invertebrate cannabinoid receptors with each other and with human cannabinoid receptors, there is a substantial amount of variation, which can be observed in table 1. Examination of the percentages of shared identity between the receptors varies from 33.45 to as little as 8.06%. Thus, it remains an open question whether and how the metabotropic cannabinoid receptors in primitive animals eventually evolved into the CB1 and CB2 receptors observed in mammals.

Including orphan GPCRs in this analysis provides potential evidence that may contribute to understanding the evolution of metabotropic cannabinoid receptors. Although the concept of whether to include these orphan GPCRs in the endocannabinoid system is relatively new, recent studies suggest that despite limited sequence similarity, some of these orphan GPCRs exhibit substantial pharmacological overlap with CB1 and CB2 [44]. Phylogenetic analysis of human GPCRs has reported some orphan GPCRs as having a common ancestor with CB1 and CB2 [45]. Insight into whether other receptors outside CB1/CB2 contribute to cannabinoid signalling can potentially be explored by leveraging invertebrate models. A more thorough analysis of the presence or absence of receptors that respond to cannabinoids in invertebrates and their resemblance to potential mammalian orthologues could clarify how these receptors evolved.

Given the variability in amino acid sequence similarity of the proposed invertebrate cannabinoid receptors to each other, to the CB1/CB2 receptors, and to the GPCRs shown in table 1, there are a number of possible evolutionary paths for the invertebrate cannabinoid receptors. First, one or more of these receptors may eventually have evolved into the canonical CB receptor first observed in *C. intestinalis*. Second, one or more of these receptors is an ancestor to the orphan GPCRs that are cannabinoid sensitive. Third, it is possible that one or more of the invertebrate receptors represents a 'hybrid' ancestor that

Table 1. Comparison of reported and predicted amino acid sequence identities and similarities for CB1 (accession no. P21554), CB2 (P34972), CICBR (F6X383), NPR-19 (Q17594), NPR-32 (O62403), GPR55 (Q9Y2T6), GPR18 (Q9Y2T6), CB1-like (LC093511.1) and CB2-like (LC093512.1) endocannabinoid receptors. Cells shaded in yellow indicate human sequences, green represents invertebrate sequences, and blue designates a comparison between human and invertebrate sequences. The per cent identities and similarities were computed using the Ident and Sim algorithms in the Sequence Manipulation Suite [2,38] following Multiple Sequence Comparison by Log-Expectation (MUSCLE) [39]. (Online version in colour.)

sequence 1	sequence 2	length (aa)	% identity	% similarity
CB1-like	CB2-like	556	33.45	42.81
CB1	CB2	474	31.01	45.36
GPR55	GPR18	345	23.19	38.55
CICBR	CB2	432	19.44	38.19
CICBR	CB1	521	18.43	32.82
NPR-19	CB2	437	15.56	32.27
NPR-19	CB2-like	508	15.35	29.33
CB1-like	CB2	498	15.06	28.92
CB2-like	CB1	557	14.72	29.26
CB2-like	CICBR	505	14.46	32.48
CB1-like	CB1	553	14.29	30.2
NPR-19	CICBR	461	14.1	31.89
CB1-like	CICBR	545	13.39	26.61
NPR-32	GPR18	422	13.03	25.59
CB1-like	GPR55	503	12.92	26.64
NPR-32	CB2-like	509	12.77	27.5
CB2-like	CB2	499	12.63	28.86
CB1-like	GPR18	496	12.3	26.61
NPR-32	CB2	440	12.05	25.45
CB2-like	GPR55	503	11.93	25.05
NPR-19	CB1-like	546	11.9	24.36
CICBR	NPR-32	442	11.76	27.38
CB2-like	GPR18	496	11.69	26.01
NPR-32	GPR55	428	11.68	22.43
GPR55	CB2	386	11.66	27.46
NPR-32	CB1-like	550	11.64	22.18
NPR-19	CB1	521	11.52	28.41
NPR-19	NPR-32	460	11.3	25.22
GPR18	CB2	375	11.2	34.13
CICBR	GPR55	438	10.27	27.85
CICBR	GPR18	428	9.81	26.4
NPR-19	GPR55	453	9.71	22.52
GPR18	CB1	485	9.69	23.3
NPR-19	GPR18	440	9.32	22.27
NPR-32	CB1	535	8.81	21.12
GPR55	CB1	496	8.06	22.38

eventually became both the CB1/CB2 and orphan GPCRs. Finally, it is possible that all of the metabotropic endocannabinoid receptors found in vertebrates and invertebrates are the result of convergent evolution. Using invertebrate model systems to resolve the evolutionary and functional relationships between the canonical CB receptors and the orphan receptors could contribute to understanding the cannabinoid modulation of nociception.

TRP channels, referred to as 'ionotropic cannabinoid receptors' or 'endovanilloid receptors,' are being increasingly reported to interact with endogenous and synthetic cannabinoid agonists [46]. TRP channels have well-documented conservation across a wide range of organisms and cell types [47]. In *C. elegans*, for example, proteins in each of the seven TRP subfamilies have been reported, including a series of TRPV channels—osmotic avoidance abnormal (*osm-9*) and (*osm-9*/capsaicin receptor-related) *ocr-1-4* genes [48]. Like CB1 receptors, activation of TRPV channels has been shown to initiate long-term depression (LTD) of excitatory or inhibitory synapses in a pre- or postsynaptic fashion [49–51]. There is also evidence of TRP channels functioning as endocannabinoid receptors in invertebrates [41,49,52,53]. TRP channels could themselves be a target for cannabinoid-based analgesics, but their role in endocannabinoid signalling in conjunction with the canonical cannabinoid receptors and in isolation needs to be more thoroughly investigated [54].

3. Cannabinoid neuromodulation in invertebrates

A number of studies report effects of synthetic cannabinoid receptor agonists and antagonists in protostomian invertebrates [43,55–60], although it is unclear how these drugs exert their effects given questions about what cannabinoid receptors invertebrates possess. There are also several studies describing the effects of endocannabinoids in protostomian invertebrates [13,17,52,61,62], but relatively few focus on cannabinoid modulation of nociception. In *C. elegans*, 2-AG and anandamide inhibited aversive responses to noxious chemical stimuli via the GPCR NPR-19, which is proposed to be an orthologue of CB1/CB2 receptors [40]. Interestingly, 2-AG exerts different effects on locomotion, increasing turning and reducing forward locomotion, which are mediated by TRPV- and TRPN-like cannabinoid receptors (*osm-9* and *trp-4* genes, respectively) [41]. The effects of 2-AG via TRPN receptors appear to be mediated by an increase in dopamine release, while the 2-AG–TRPV effects are mediated by increased serotonin release [41].

In *Hirudo*, 2-AG and anandamide reduce nocifensive responses to nociceptive stimuli but enhance responses to non-nociceptive mechanical stimuli [63]. These opposing effects of endocannabinoids are also observed at the synaptic level, where 2-AG and anandamide elicit LTD of nociceptive (N cell) synapses, but potentiate synapses made by non-nociceptive pressure-sensitive neurons (P cells) via a disinhibitory mechanism (figure 1) [49,64–66]. The synaptic and behavioural effects of endocannabinoids in *Hirudo* are mediated by a TRPV-like channel based on pharmacological studies in which TRPV1 antagonists block the effects of endocannabinoids and TRPV1 activators mimic or occlude endocannabinoid effects [49,67,68]. This combined with the recent *C. elegans* studies reinforces the idea that endocannabinoid signalling via TRPV channels may be evolutionarily conserved [31,29,41,69,70], although the involvement of a GPCR cannot be excluded in *Hirudo* at this time. Despite potential differences in receptors, endocannabinoid-mediated synaptic plasticity in *Hirudo* shares a number of features

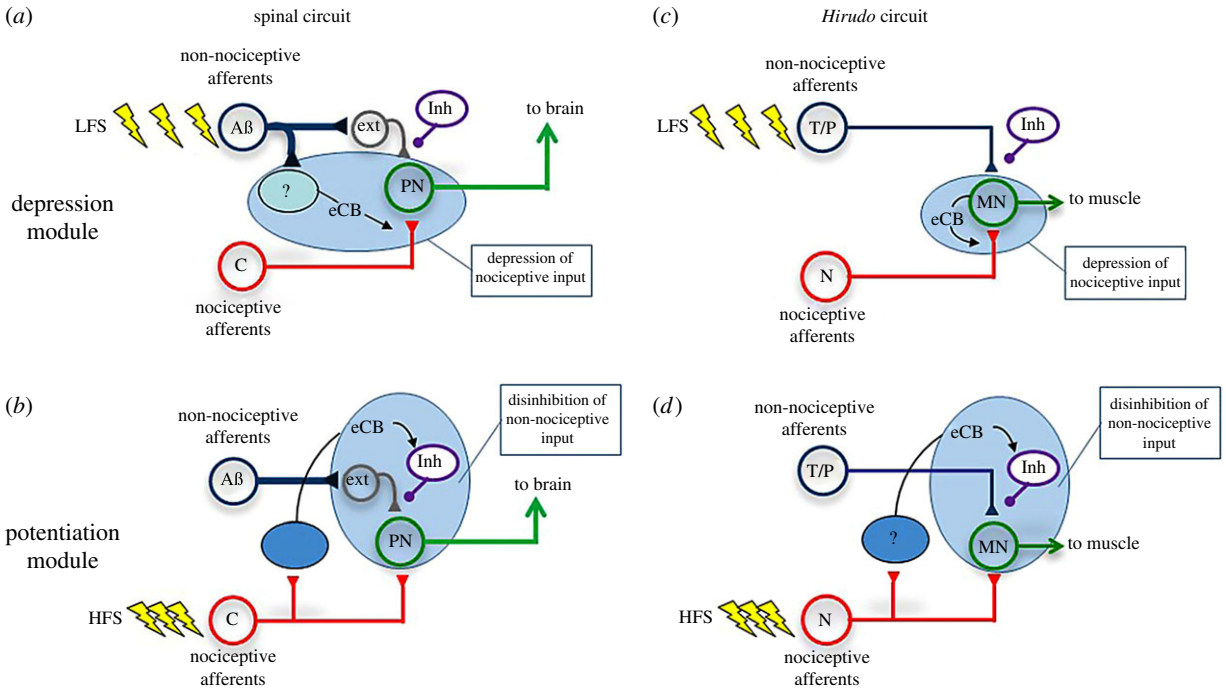

Figure 1. Evidence of distinct endocannabinoid modules. LFS (low-frequency stimulation) of non-nociceptive afferents activates a module for depression of nociceptive synapses via endocannabinoid release from (*a*) an unknown neuron (?) in the vertebrate spinal cord or from (*c*) a longitudinal motor neuron (MN) in *Hirudo*. HFS (high-frequency stimulation) of nociceptive afferents activates a module for potentiation/disinhibition of non-nociceptive input onto nociceptive circuits, although the source of endocannabinoids in both (*b*) the vertebrate spinal cord and (*d*) *Hirudo* is unknown (?). eCB, endocannabinoid; ext, excitatory interneuron; Inh, inhibitory neuron; PN, nociceptive projection neuron; T/P, touch/pressure mechanosensory cell. (Online version in colour.)

with mammalian synapses, including retrograde signalling, involvement of presynaptic Ca^{2+} and calcineurin signalling, and a requirement for transcription and translation-dependent processes [49,64,71–75].

4. Pro- and anti-nociceptive effects of cannabinoids

Cannabinoid therapies are generally thought to be analgesic, regardless of whether endocannabinoid- or phytocannabinoid-based treatments are used [76–80]. However, evidence from both preclinical and clinical studies indicates that cannabinoids can have pro-nociceptive effects as well [81–86]. This complicates the use of potential cannabinoid-based therapies to treat pain and is likely a major reason why there has been conflicting evidence about the utility of phytocannabinoid-based medicines to treat pain [5,6,87] as well as the failure of a clinical trial for an endocannabinoid-based analgesic [88]. Addressing this complexity requires understanding the cellular mechanisms that delineate the pro- versus anti-nociceptive effects and what conditions elicit one effect over the other.

How does one reconcile these opposing effects of cannabinoids? While nociceptive and non-nociceptive sensory pathways are generally segregated in their paths to the brain, non-nociceptive afferents do have access to nociceptive circuitry in the spinal cord [89–92]. These non-nociceptive inputs are polysynaptic and controlled by inhibitory neurons (figure 1*a*,*b*) that effectively gate non-nociceptive sensory input to nociceptive circuits (e.g. the nociceptive projection neurons) [90]. Nociceptive afferents, on the other hand, have direct monosynaptic input to many of these same projection neurons. Therefore, a potential mechanism for pro- versus anti-nociceptive effects is that cannabinoids have different effects on nociceptive and non-nociceptive afferent inputs to the spinal nociceptive circuits. Supporting this idea are rodent studies in which endocannabinoids are found to depress excitatory synapses made by nociceptive afferents (an anti-nociceptive effect; figure 1*a*) but disinhibit non-nociceptive afferent input (a pro-nociceptive effect; figure 1*b*) [82,93]. This hypothesis is illustrated in figure 1 where an endocannabinoid 'depression module' is shown to act on nociceptive signalling pathways, whereas a 'potentiation module' acts in non-nociceptive inputs.

Studies in *Hirudo* also provide evidence for these distinct effects by endocannabinoids on nociceptive and non-nociceptive pathways. In *Hirudo*, N cells elicit a defensive withdrawal reflex, whole-body shortening, in part via monosynaptic connections to local motor neurons (figure 1*c*,*d*). These motor neurons also receive monosynaptic input from non-nociceptive P cells; however, these synapses are weaker and greater P cell activation is required to elicit the withdrawal reflex [67,68,94,95]. Nevertheless, as in mammals, nociceptive and non-nociceptive afferents in *Hirudo* converge on common postsynaptic targets. Similar to observations in rodents, endocannabinoids depress N cell synapses (figure 1*c*) but enhance P cell synaptic transmission as a result of presynaptic disinhibition (figure 1*d*) [49,65,66]. These effects are functionally relevant, as endocannabinoids reduce behavioural responses elicited by either nociceptive stimuli or directly stimulating N cells [63,67]. On the other hand, endocannabinoids increase the magnitude of the shortening response elicited by P cell stimulation and reduce the threshold in response to non-nociceptive stimuli [63,68]. Thus, these dual mechanisms of endocannabinoids having anti-nociceptive effects via depression of nociceptive synapses and pro-nociceptive effects via disinhibition of non-nociceptive synapses appear to be conserved from invertebrates, to rodents and even to humans [82].

These findings suggest that while the non-nociceptive afferent inputs are sensitive to disinhibition, the nociceptive

pathways are not. One way this may arise is due to differences in the chloride gradients between nociceptive and non-nociceptive inputs, which determine whether neurons are inhibited or excited by GABA [7]. In *Hirudo*, the P cells have a relatively hyperpolarized Cl^- equilibrium potential (E_{Cl}) and are inhibited by GABA, while the N cells have a relatively depolarized E_{Cl} and are excited by GABA [65,66,96]. Consistent with this, P cell synapses can be disinhibited either by GABA receptor antagonists or by blocking the Cl^- exporter [65,66]. N cell synapses, however, cannot be disinhibited in this manner, and applying GABA receptor antagonists or blocking Cl^- importers actually decreases excitatory post-synaptic potential amplitude (disexcitation) [66]. These differences in Cl^- gradients directly impact how endocannabinoids affect P versus N cell synapses. P cell synapses are potentiated by endocannabinoids via a disinhibition mechanism, while N cell synapses are 'protected' from this disinhibition [66].

Does a similar mechanism translate to vertebrates? Most studies suggest that both nociceptive and non-nociceptive sensory neurons in mammals have elevated intracellular Cl^- levels and are depolarized by GABA/glycine [97–101]. However, some experiments using intact dorsal root ganglion (DRG) neurons suggest that non-nociceptive afferents have low E_{Cl} and are inhibited by GABA/glycine and that only nociceptors are depolarized by these transmitters [102,103]. Further evidence that nociceptive and non-nociceptive afferents have different Cl^- gradients is found in a study in which blocking GABA receptors increased synaptic transmission by Aβ afferents via disinhibition (indicative of a low E_{Cl}), but reduced transmission by nociceptive afferents (disexcitation; indicative of a low E_{Cl}) [104], similar to what is observed in *Hirudo*.

5. Accessing different endocannabinoid modules

Given the evidence for pro- and anti-nociceptive effects by cannabinoids, the next question is how are these different effects normally elicited within the nervous system? Evidence from both rodent and human studies indicates that strong activation of nociceptors produces endocannabinoid-mediated allodynia (illustrated in figure 1*b*) [82]. This is supported by studies in *Hirudo* where high-frequency stimulation (HFS) of the N cells or noxious stimuli applied to the skin elicits endocannabinoid-mediated potentiation of P cell synapses (figure 1*d*) and sensitization of P cell-elicited shortening [66]. Furthermore, *in vivo* studies have shown that injury-induced sensitization to non-nociceptive stimuli in *Hirudo* is also endocannabinoid-dependent (M. Jorgenson and B. D. Burrell 2018, unpublished observation). Therefore, nociceptive stimuli appear to be an evolutionarily conserved mechanism for producing cannabinoid-mediated sensitization to non-nociceptive stimuli.

Low-frequency stimulation (LFS) of non-nociceptive afferents in *Hirudo* elicits both endocannabinoid-mediated depression of N cell synapses and decreases in the magnitude of the N cell-elicited shortening response (figure 1*c*) [49,64,67]. A comparable process is also observed in rodents, where repetitive stimulation of Aβ-fibres produces endocannabinoid-dependent depression of C-fibre synapses and reduces mechanical hypersensitivity following nerve injury (figure 1*a*) [105,106]. The contribution of cannabinoid signalling to the anti-nociceptive effects of repetitive non-nociceptive stimulation may be relevant to analgesic therapies, e.g. transcutaneous electrical nerve stimulation (TENS) or spinal cord stimulation (SCS).

Functionally, these different patterns of activity should only elicit one endocannabinoid 'module', either potentiation or depression. This appears to be the case in *Hirudo* (figure 1*c*,*d*). LFS does not produce endocannabinoid-mediated synaptic potentiation, possibly because the threshold for activation of the potentiation module has not been reached [66]. HFS might be expected to activate both the potentiation and depression modules; however, NMDA receptor-mediated long-term potentiation of nociceptive synapses appears to inhibit endocannabinoid-LTD in these same nociceptive synapses [107]. Whether other species have similar activity-dependent mechanisms to control what form of endocannabinoid-mediated modulation is produced is not known.

While cannabinoid-mediated potentiation appears to contribute to nociceptive sensitization, little consideration has been given to understanding why repetitive non-nociceptive stimuli should reduce nociceptive signalling. One potential explanation is that repetitive non-nociceptive stimulation is producing a form of generalized habituation in which both the activated stimulus–response pathway and an inactive but related pathway habituate [108–113]. In *Hirudo*, habituation to non-nociceptive stimuli leads to a decrease in response to nociceptive stimuli that meets the requirements for generalization of habituation [114]. Direct habituation to the non-nociceptive stimuli was not cannabinoid-mediated, but the generalized habituation to nociceptive stimuli did require endocannabinoid signalling, similar to the synaptic and behavioural effects of non-nociceptive LFS described above.

This habituation mechanism may represent an important evolutionarily conserved process for regulating pain. Increased aversive/defensive responses following injury are protective and have adaptive value [2,115]. At some point, however, animals must resume normal behavioural patterns or risk reductions in fitness due to loss of feeding or reproductive opportunities. Perhaps as animals begin to recover and become more active following an injury, the normal somatosensory stimuli that result from this activity initiate habituation processes that reduce sensitized responses to innocuous and noxious stimuli simultaneously. This may be relevant from a clinical perspective in part because understanding habituation of nocifensive behaviours could lead to novel approaches to treat pain, but also because nociceptive habituation is disrupted in some human chronic pain conditions [116–120]. Studies in which the capacity for endocannabinoid-mediated habituation to reverse nociceptive sensitization and the adaptive value of this process are an obvious direction for future research.

Activation of different receptors provides an additional element for accessing different endocannabinoid modules. As shown in table 1, there is evidence of multiple cannabinoid receptors, which may have distinct properties (e.g. activate different intracellular signalling cascades) and produce different modulatory effects at the physiological and behaviour level. Evidence for such receptor-based endocannabinoid modules is found in the recent studies by Komunieki and co-workers in which distinct forms of modulation of monoamine release and locomotion are produced by 2-AG and anandamide activation of GPCR- versus TRP-type cannabinoid receptors in *C. elegans* [40,41].

6. Concluding remarks

Endocannabinoid modulation of nociception exhibits remarkable conservation from the synaptic to behavioural level across the animal kingdom. The major cannabinoid transmitters,

2-AG and anandamide, are found widely in vertebrates and invertebrates as are the enzymes involved in synthesis and degradation of these transmitters. There also appear to be commonalities in terms of synaptic plasticity and the intracellular signalling mechanisms involved. Remarkably, it is at the level of cannabinoid receptors where the most variability is observed. Earlier studies of invertebrate genome databases indicated that protostomian invertebrates lacked orthologues of the canonical cannabinoid receptors [8,9,121]. However, candidates for an invertebrate metabotropic cannabinoid receptor have been recently uncovered. Whether these have direct relations to CB1/CB2, are part of a family of ancestral proteins that eventually produced the canonical and/or orphan GPCR cannabinoid receptors, or are the result of convergent evolution is not clear at this time. At the circuit and behavioural level, there is clear evidence of conservation of the pro- and anti-nociceptive effects of endocannabinoids. Collectively, these studies illustrate the utility of harnessing comparative approaches to understand the basic biology of nociception from the cellular to behavioural level.

Data accessibility. This article has no additional data.

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References

1. Walters ET. 2016 Pain-capable neural substrates may be widely available in the animal kingdom. *Anim. Sentience* **1**, 37.
2. Williams AC. 2016 What can evolutionary theory tell us about chronic pain? *Pain* **157**, 788–790. (doi:10.1097/j.pain.0000000000000464)
3. Elikkottil J, Gupta P, Gupta K. 2009 The analgesic potential of cannabinoids. *J. Opioid Manag.* **5**, 341–357. (doi:10.5055/jom.2009.0034)
4. Di Marzo V. 2009 The endocannabinoid system: its general strategy of action, tools for its pharmacological manipulation and potential therapeutic exploitation. *Pharmacol. Res.* **60**, 77–84. (doi:10.1016/j.phrs.2009.02.010)
5. Hill KP, Palastro MD, Johnson B, Ditre JW. 2017 Cannabis and pain: a clinical review. *Cannabis Cannabinoid Res.* **2**, 96–104. (doi:10.1089/can.2017.0017)
6. De Vita MJ, Moskal D, Maisto SA, Ansell EB. 2018 Association of cannabinoid administration with experimental pain in healthy adults: a systematic review and meta-analysis. *JAMA Psychiatry* **75**, 1118–1127. (doi:10.1001/jamapsychiatry.2018.2503)
7. Christie MJ, Mallet C. 2009 Endocannabinoids can open the pain gate. *Sci. Signal.* **2**, e57. (doi:10.1126/scisignal.288pe57)
8. Elphick MR. 2012 The evolution and comparative neurobiology of endocannabinoid signalling. *Phil. Trans. R Soc. B* **367**, 3201–3215. (doi:10.1098/rstb.2011.0394)
9. McPartland JM, Norris RW, Kilpatrick CW. 2007 Coevolution between cannabinoid receptors and endocannabinoid ligands. *Gene* **397**, 126–135. (doi:10.1016/j.gene.2007.04.017)
10. Katona I, Freund TF. 2008 Endocannabinoid signaling as a synaptic circuit breaker in neurological disease. *Nat. Med.* **14**, 923–930. (doi:10.1038/nm.f.1869)
11. Wu N, Tasker JG. 2017 Nongenomic glucocorticoid suppression of a postsynaptic potassium current via emergent autocrine endocannabinoid signaling in hypothalamic neuroendocrine cells following chronic dehydration. *eNeuro* **4**, ENEURO.0216–17.2017. (doi:10.1523/ENEURO.0216-17.2017)
12. Salzet M, Stefano GB. 2002 The endocannabinoid system in invertebrates. *Prostaglandins Leukotrienes Essent. Fatty Acids* **66**, 353–361. (doi:10.1054/plef.2001.0347)
13. De Petrocellis L, Melck D, Bisogno T, Milone A, Di Marzo V. 1999 Finding of the endocannabinoid signalling system in *Hydra*, a very primitive organism: possible role in the feeding response. *Neuroscience* **92**, 377–387. (doi:10.1016/S0306-4522(98)00749-0)
14. Lehtonen M, Reisner K, Auriola S, Wong G, Callaway JC. 2008 Mass-spectrometric identification of anandamide and 2-arachidonoylglycerol in nematodes. *Chem. Biodivers.* **5**, 2431–2441. (doi:10.1002/cbdv.200890208)
15. Stefano GB, Salzet B, Salzet M. 1997 Identification and characterization of the leech CNS cannabinoid receptor: coupling to nitric oxide release. *Brain Res.* **753**, 219–224. (doi:10.1016/S0006-8993(96)01484-9)
16. Matias I *et al.* 2001 Evidence for an endocannabinoid system in the central nervous system of the leech *Hirudo medicinalis*. *Mol. Brain Res.* **87**, 145–159. (doi:10.1016/S0169-328X(00)00290-4)
17. Khaliullina H, Bilgin M, Sampaio JL, Shevchenko A, Eaton S. 2015 Endocannabinoids are conserved inhibitors of the Hedgehog pathway. *Proc. Natl Acad. Sci. USA* **112**, 3415–3420. (doi:10.1073/pnas.1416463112)
18. Fezza F, Dillwith JW, Bisogno T, Tucker JS, Di Marzo V, Sauer JR. 2003 Endocannabinoids and related fatty acid amides, and their regulation, in the salivary glands of the lone star tick. *Biochim. Biophys. Acta* **1633**, 61–67. (doi:10.1016/S1388-1981(03)00087-8)
19. Matias I, McPartland JM, Di Marzo V. 2005 Occurrence and possible biological role of the endocannabinoid system in the sea squirt *Ciona intestinalis*. *J. Neurochem.* **93**, 1141–1156. (doi:10.1111/j.1471-4159.2005.03103.x)
20. Lu H-C, Mackie K. 2016 An introduction to the endogenous cannabinoid system. *Biol. Psychiatry* **79**, 516–525. (doi:10.1016/j.biopsych.2015.07.028)
21. Maccarrone M. 2017 Metabolism of the endocannabinoid anandamide: open questions after 25 years. *Front. Mol. Neurosci.* **10**, 166. (doi:10.3389/fnmol.2017.00166)
22. Liu J *et al.* 2006 A biosynthetic pathway for anandamide. *Proc. Natl Acad. Sci. USA* **103**, 13345. (doi:10.1073/pnas.0601832103)
23. Harrison N, Lone MA, Kaul TK, Reis Rodrigues P, Ogungbe IV, Gill MS. 2014 Characterization of *N*-acyl phosphatidylethanolamine-specific phospholipase-D isoforms in the nematode *Caenorhabditis elegans*. *PLoS ONE* **9**, e113007. (doi:10.1371/journal.pone.0113007)
24. Yuan D, Wu Z, Wang Y. 2016 Evolution of the diacylglycerol lipases. *Prog. Lipid Res.* **64**, 85–97. (doi:10.1016/j.plipres.2016.08.004)
25. Ueda N, Tsuboi K, Uyama T, Ohnishi T. 2011 Biosynthesis and degradation of the endocannabinoid 2-arachidonoylglycerol. *Biofactors* **37**, 1–7. (doi:10.1002/biof.131)
26. Murataeva N, Straiker A, Mackie K. 2014 Parsing the players: 2-arachidonoylglycerol synthesis and degradation in the CNS. *Br. J. Pharmacol.* **171**, 1379–1391. (doi:10.1111/bph.12411)
27. Ibsen MS, Connor M, Glass M. 2017 Cannabinoid CB_1 and CB_2 receptor signaling and bias. *Cannabis Cannabinoid Res.* **2**, 48–60. (doi:10.1089/can.2016.0037)
28. Gonsiorek W *et al.* 2000 Endocannabinoid 2-arachidonyl glycerol is a full agonist through human type 2 cannabinoid receptor: antagonism by anandamide. *Mol. Pharmacol.* **57**, 1045.
29. Zygmunt PM, Petersson J, Andersson DA, Chuang H, Sørgård M, Di Marzo V, Julius D, Högestätt ED. 1999 Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature* **400**, 452. (doi:10.1038/22761)
30. De Petrocellis L, Bisogno T, Maccarrone M, Davis JB, Finazzi-Agro A, Di Marzo V. 2001 The activity of

anandamide at vanilloid VR1 receptors requires facilitated transport across the cell membrane and is limited by intracellular metabolism. *J. Biol. Chem.* **276**, 12856–12863. (doi:10.1074/jbc.M008555200)

31. Zygmunt PM *et al.* 2013 Monoacylglycerols activate TRPV1 – a link between phospholipase C and TRPV1. *PLoS ONE* **8**, e81618. (doi:10.1371/journal.pone.0081618)
32. Sun Y, Alexander SPH, Garle MJ, Gibson CL, Hewitt K, Murphy SP, Kendall DA, Bennett AJ. 2007 Cannabinoid activation of PPAR alpha; a novel neuroprotective mechanism. *Br. J. Pharmacol.* **152**, 734–743. (doi:10.1038/sj.bjp.0707478)
33. Kozak KR, Gupta RA, Moody JS, Ji C, Boeglin WE, Dubois RN, Brash AR, Marnett LJ. 2002 15-Lipoxygenase metabolism of 2-arachidonylglycerol: generation of a peroxisome proliferator-activated receptor α agonist. *J. Biol. Chem.* **277**, 23 278–23 286. (doi:10.1074/jbc.M201084200)
34. Morales P, Reggio PH. 2017 An update on non-CB_1, non-CB_2 cannabinoid related G-protein-coupled receptors. *Cannabis Cannabinoid Res.* **2**, 265–273. (doi:10.1089/can.2017.0036)
35. Viñals X *et al.* 2015 Cognitive impairment induced by Delta9-tetrahydrocannabinol occurs through heteromers between cannabinoid CB_1 and serotonin $5\text{-}HT_{2A}$ receptors. *PLoS Biol.* **13**, e1002194. (doi:10.1371/journal.pbio.1002194)
36. Bushlin I, Gupta A, Stockton SD, Miller LK, Devi LA. 2012 Dimerization with cannabinoid receptors allosterically modulates delta opioid receptor activity during neuropathic pain. *PLoS ONE* **7**, e49789. (doi:10.1371/journal.pone.0049789)
37. Elphick MR, Satou Y, Satoh N. 2003 The invertebrate ancestry of endocannabinoid signalling: an orthologue of vertebrate cannabinoid receptors in the urochordate *Ciona intestinalis*. *Gene* **302**, 95–101. (doi:10.1016/S0378-1119(02)01094-6)
38. Stothard P. 2000 The sequence manipulation suite: JavaScript programs for analyzing and formatting protein and DNA sequences. *Biotechniques* **28**, 1102–1104. (doi:10.2144/00286ir01)
39. Chojnacki S, Cowley A, Lee J, Foix A, Lopez R. 2017 Programmatic access to bioinformatics tools from EMBL-EBI update: 2017. *Nucleic Acids Res.* **45**(W1), W550–W553. (doi:10.1093/nar/gkx273)
40. Oakes MD, Law WJ, Clark T, Bamber BA, Komuniecki R. 2017 Cannabinoids activate monoaminergic signaling to modulate key *C. elegans* behaviors. *J. Neurosci.* **37**, 2859–2869. (doi:10.1523/JNEUROSCI.3151-16.2017)
41. Oakes M, Law WJ, Komuniecki R. 2019 Cannabinoids stimulate the TRP channel-dependent release of both serotonin and dopamine to modulate behavior in *C. elegans*. *J. Neurosci.* **39**, 4142–4152. (doi:10.1523/JNEUROSCI.2371-18.2019)
42. Pastuhov SI, Matsumoto K, Hisamoto N. 2016 Endocannabinoid signaling regulates regenerative axon navigation in *Caenorhabditis elegans* via the GPCRs NPR-19 and NPR-32. *Genes Cells* **21**, 696–705. (doi:10.1111/gtc.12377)
43. Sunada H, Watanabe T, Hatakeyama D, Lee S, Forest J, Sakakibara M, Ito E, Lukowiak K. 2017 Pharmacological effects of cannabinoids on learning and memory in *Lymnaea*. *J. Exp. Biol.* **220**, 3026–3038. (doi:10.1242/jeb.159038)
44. Howlett AC, Abood ME. 2017 CB_1 and CB_2 receptor pharmacology. *Adv. Pharmacol.* **80**, 169–206. (doi:10.1016/bs.apha.2017.03.007). Elsevier.
45. Morales P, Isawi I, Reggio PH. 2018 Towards a better understanding of the cannabinoid-related orphan receptors GPR3, GPR6, and GPR12. *Drug Metab. Rev.* **50**, 74–93. (doi:10.1080/03602532.2018.1428616)
46. Muller C, Morales P, Reggio PH. 2019 Cannabinoid ligands targeting TRP channels. *Front. Mol. Neurosci.* **11**, 487. (doi:10.3389/fnmol.2018.00487)
47. Minke B. 2010 The history of the *Drosophila* TRP channel: the birth of a new channel superfamily. *J. Neurogenet.* **24**, 216–233. (doi:10.3109/01677063.2010.514369)
48. Xiao R, Xu XZS. 2009 Function and regulation of TRP family channels in *C. elegans*. *Pflugers Arch.* **458**, 851–860. (doi:10.1007/s00424-009-0678-7)
49. Yuan S, Burrell BD. 2010 Endocannabinoid-dependent LTD in a nociceptive synapse requires activation of a presynaptic TRPV-like receptor. *J. Neurophysiol.* **104**, 2766–2777. (doi:10.1152/jn.00491.2010)
50. Brown TE, Chirila AM, Schrank BR, Kauer JA. 2013 Loss of interneuron LTD and attenuated pyramidal cell LTP in Trpv1 and Trpv3 KO mice. *Hippocampus* **23**, 662–671. (doi:10.1002/hipo.22125)
51. Chávez AE, Chiu CQ, Castillo PE. 2010 TRPV1 activation by endogenous anandamide triggers postsynaptic long-term depression in dentate gyrus. *Nat. Neurosci.* **13**, 1511–1518. (doi:10.1038/nn.2684)
52. Leung HT, Tseng-Crank J, Kim E, Mahapatra C, Shino S, Zhou Y, An L, Doerge RW, Pak WL. 2008 DAG lipase activity is necessary for TRP channel regulation in *Drosophila* photoreceptors. *Neuron* **58**, 884–896. (doi:10.1016/j.neuron.2008.05.001)
53. Kahn-Kirby AH, Dantzker JLM, Apicella AJ, Schafer WR, Browse J, Bargmann CI, Watts JL. 2004 Specific polyunsaturated fatty acids drive TRPV-dependent sensory signaling in vivo. *Cell* **119**, 889–900. (doi:10.1016/j.cell.2004.11.005)
54. Storozhuk MV, Zholos AV. 2018 TRP channels as novel targets for endogenous ligands: focus on endocannabinoids and nociceptive signalling. *Curr. Neuropharmacol.* **16**, 137–150. (doi:10.2174/1570159X15666170424120802)
55. Lemak MS, Bravarenko NI, Bobrov MY, Bezuglov VV, Ierusalimsky VN, Storozhuk MV, Malyshev AY, Balaban PM. 2007 Cannabinoid regulation in identified synapse of terrestrial snail. *Eur. J. Neurosci.* **26**, 3207–3214. (doi:10.1111/j.1460-9568.2007.05945.x)
56. Rawls SM, Gomez T, Raffa RB. 2007 An NMDA antagonist (LY 235959) attenuates abstinence-induced withdrawal of planarians following acute exposure to a cannabinoid agonist (WIN 52212-2). *Pharmacol. Biochem. Behav.* **86**, 499–504. (doi:10.1016/j.pbb.2007.01.010)
57. Rawls SM, Gerber K, Ding Z, Roth C, Raffa RB. 2008 Agmatine: identification and inhibition of methamphetamine, kappa opioid, and cannabinoid withdrawal in planarians. *Synapse* **62**, 927–934. (doi:10.1002/syn.20571)
58. Li Q, Burrell BD. 2009 Two forms of long-term depression in a polysynaptic pathway in the leech CNS: one NMDA receptor-dependent and the other cannabinoid dependent. *J. Comp. Physiol. A* **195**, 831–841. (doi:10.1007/s00359-009-0462-3)
59. Reis Rodrigues P, Kaul TK, Ho J-H, Lucanic M, Burkewitz K, Mair WB, Held JM, Bohn LM, Gill MS. 2016 Synthetic ligands of cannabinoid receptors affect dauer formation in the nematode *Caenorhabditis elegans*. *G3 (Bethesda)* **6**, 1695–1705. (doi:10.1534/g3.116.026997)
60. Jimenez-Del-Rio M, Daza-Restrepo A, Velez-Pardo C. 2008 The cannabinoid CP55,940 prolongs survival and improves locomotor activity in *Drosophila melanogaster* against paraquat: implications in Parkinson's disease. *Neurosci. Res.* **61**, 404–411. (doi:10.1016/j.neures.2008.04.011)
61. Li Q, Burrell BD. 2011 Associative, bidirectional changes in neural signaling utilizing NMDA receptor- and endocannabinoid-dependent mechanisms. *Learn. Mem.* **18**, 545–553. (doi:10.1101/lm.2252511)
62. Lucanic M, Held JM, Vantipalli MC, Klang IM, Graham JB, Gibson BW, Lithgow GJ, Gill MS. 2011 *N*-Acylethanolamine signalling mediates the effect of diet on lifespan in *Caenorhabditis elegans*. *Nature* **473**, 226. (doi:10.1038/nature10007)
63. Summers T, Hanten B, Peterson W, Burrell B. 2017 Endocannabinoids have opposing effects on behavioral responses to nociceptive and non-nociceptive stimuli. *Sci. Rep.* **7**, 5793. (doi:10.1038/s41598-017-06114-1)
64. Yuan S, Burrell BD. 2013 Endocannabinoid-dependent long-term depression in a nociceptive synapse requires coordinated presynaptic and postsynaptic transcription and translation. *J. Neurosci.* **33**, 4349–4358. (doi:10.1523/JNEUROSCI.3922-12.2013)
65. Higgins A, Yuan S, Wang Y, Burrell BD. 2013 Differential modulation of nociceptive versus non-nociceptive synapses by endocannabinoids. *Mol. Pain* **9**, 26. (doi:10.1186/1744-8069-9-26)
66. Wang Y, Burrell BD. 2016 Differences in chloride gradients allow for three distinct types of synaptic modulation by endocannabinoids. *J. Neurophysiol.* **116**, 619–628. (doi:10.1152/jn.00235.2016)
67. Yuan S, Burrell BD. 2013 Nonnociceptive afferent activity depresses nocifensive behavior and nociceptive synapses via an endocannabinoid-dependent mechanism. *J. Neurophysiol.* **110**, 2607–2616. (doi:10.1152/jn.00170.2013)
68. Wang Y, Burrell BD. 2018 Endocannabinoid-mediated potentiation of nonnociceptive synapses contributes to behavioral sensitization. *J. Neurophysiol.* **119**, 641–651. (doi:10.1152/jn.00092.2017)
69. Gibson HE, Edwards JG, Page RS, Van Hook MJ, Kauer JA. 2008 TRPV1 channels mediate long-term

depression at synapses on hippocampal interneurons. *Neuron* **57**, 746–759. (doi:10.1016/j.neuron.2007.12.027)

70. Edwards JG. 2014 TRPV1 in the central nervous system: synaptic plasticity, function and pharmacological implications. In *Capsaicin as a therapeutic molecule* (ed. OME Abdel-Salam), pp. 77–104. Basel, Switzerland: Springer.
71. Jensen T, Edwards JG. 2012 Calcineurin is required for TRPV1-induced long-term depression of hippocampal interneurons. *Neurosci. Lett.* **510**, 82–87. (doi:10.1016/j.neulet.2012.01.006)
72. Heifets BD, Castillo PE. 2009 Endocannabinoid-signaling and long-term synaptic plasticity. *Annu. Rev. Physiol.* **71**, 283–306. (doi:10.1146/annurev.physiol.010908.163149)
73. Yin HH, Davis MI, Ronesi JA, Lovinger DM. 2006 The role of protein synthesis in striatal long-term depression. *J. Neurosci.* **26**, 11 811–11 820. (doi:10.1523/JNEUROSCI.3196-06.2006)
74. Adermark L, Talani G, Lovinger DM. 2009 Endocannabinoid-dependent plasticity at GABAergic and glutamatergic synapses in the striatum is regulated by synaptic activity. *Eur. J. Neurosci.* **29**, 32–41. (doi:10.1111/j.1460-9568.2008.06551.x)
75. Yuan S, Burrell BD. 2012 Long-term depression of nociceptive synapses by non-nociceptive afferent activity: role of endocannabinoids, Ca^{2+}, and calcineurin. *Brain Res.* **1460**, 1–11. (doi:10.1016/j.brainres.2012.04.030)
76. Gregg LC *et al.* 2012 Activation of type 5 metabotropic glutamate receptors and diacylglycerol lipase-α initiates 2-arachidonoylglycerol formation and endocannabinoid-mediated analgesia. *J. Neurosci.* **32**, 9457–9468. (doi:10.1523/JNEUROSCI.0013-12.2012)
77. Nyilas R, Gregg LC, Mackie K, Watanabe M, Zimmer A, Hohmann AG, Katona I. 2009 Molecular architecture of endocannabinoid signaling at nociceptive synapses mediating analgesia. *Eur. J. Neurosci.* **29**, 1964–1978. (doi:10.1111/j.1460-9568.2009.06751.x)
78. Kinsey SG, Long JZ, O'Neal ST, Abdullah RA, Poklis JL, Boger DL, Cravatt BF, Lichtman AH. 2009 Blockade of endocannabinoid-degrading enzymes attenuates neuropathic pain. *J. Pharmacol. Exp. Ther.* **330**, 902–910. (doi:10.1124/jpet.109.155465)
79. Rahn EJ, Hohmann AG. 2009 Cannabinoids as pharmacotherapies for neuropathic pain: from the bench to the bedside. *Neurotherapeutics* **6**, 713–737. (doi:10.1016/j.nurt.2009.08.002)
80. Noyes Jr R, Brunk S, Baram D, Canter A. 1975 Analgesic effect of delta-9-tetrahydrocannabinol. *J. Clin. Pharmacol.* **15**, 139–143. (doi:10.1002/j.1552-4604.1975.tb02348.x)
81. Carey LM, Slivicki RA, Leishman E, Cornett B, Mackie K, Bradshaw H, Hohmann AG. 2016 A pro-nociceptive phenotype unmasked in mice lacking fatty-acid amide hydrolase. *Mol. Pain* **12**. (doi:10.1177/1744806916649192)
82. Pernia-Andrade AJ *et al.* 2009 Spinal endocannabinoids and CB_1 receptors mediate C-fiber-induced heterosynaptic pain sensitization. *Science* **325**, 760–764. (doi:10.1126/science.1171870)
83. Strangman NM, Patrick SL, Hohmann AG, Tsou K, Walker JM. 1998 Evidence for a role of endogenous cannabinoids in the modulation of acute and tonic pain sensitivity. *Brain Res.* **813**, 323–328. (doi:10.1016/S0006-8993(98)01031-2)
84. Ueda M, Iwasaki H, Wang S, Murata E, Poon KYT, Mao J, Martyn JAJ. 2014 Cannabinoid receptor type 1 antagonist, AM251, attenuates mechanical allodynia and thermal hyperalgesia after burn injury. *Anesthesiology* **121**, 1311. (doi:10.1097/ALN.0000000000000422)
85. Beaulieu P. 2006 Effects of nabilone, a synthetic cannabinoid, on postoperative pain. *Can. J. Anesth.* **53**, 769–775. (doi:10.1007/BF03022793)
86. Walter C, Oertel BG, Lötsch J. 2015 THC may reproducibly induce electrical hyperalgesia in healthy volunteers. *Eur. J. Pain* **19**, 516–518. (doi:10.1002/ejp.575)
87. Kraft B. 2012 Is there any clinically relevant cannabinoid-induced analgesia? *Pharmacology* **89**, 237–246. (doi:10.1159/000337376)
88. Huggins JP, Smart TS, Langman S, Taylor L, Young T. 2012 An efficient randomised, placebo-controlled clinical trial with the irreversible fatty acid amide hydrolase-1 inhibitor PF-04457845, which modulates endocannabinoids but fails to induce effective analgesia in patients with pain due to osteoarthritis of the knee. *Pain* **153**, 1837–1846. (doi:10.1016/j.pain.2012.04.020)
89. Arcourt A, Lechner SG. 2015 Peripheral and spinal circuits involved in mechanical allodynia. *Pain* **156**, 220–221. (doi:10.1097/01.j.pain.0000460818.62406.38)
90. Torsney C, MacDermott AB. 2006 Disinhibition opens the gate to pathological pain signaling in superficial neurokinin 1 receptor-expressing neurons in rat spinal cord. *J. Neurosci.* **26**, 1833–1843. (doi:10.1523/JNEUROSCI.4584-05.2006)
91. Petitjean H *et al.* 2015 Dorsal horn parvalbumin neurons are gate-keepers of touch-evoked pain after nerve injury. *Cell Rep.* **13**, 1246–1257. (doi:10.1016/j.celrep.2015.09.080)
92. Lu Y *et al.* 2013 A feed-forward spinal cord glycinergic neural circuit gates mechanical allodynia. *J. Clin. Invest.* **123**, 4050–4062. (doi:10.1172/JCI70026)
93. Kato A, Punnakkal P, Pernía-Andrade AJ, Von Schoultz C, Sharopov S, Nyilas R, Katona I, Zeilhofer HU. 2012 Endocannabinoid-dependent plasticity at spinal nociceptor synapses. *J. Physiol.* **590**, 4717–4733. (doi:10.1113/jphysiol.2012.234229)
94. Shaw BK, Kristan Jr WB. 1997 The neuronal basis of the behavioral choice between swimming and shortening in the leech: control is not selectively exercised at higher circuit levels. *J. Neurosci.* **17**, 786–795. (doi:10.1523/JNEUROSCI.17-02-00786.1997)
95. Baylor DA, Nicholls JG. 1969 Chemical and electrical synaptic connexions between cutaneous mechanoreceptor neurones in the central nervous system of the leech. *J. Physiol.* **203**, 591–609. (doi:10.1113/jphysiol.1969.sp008881)
96. Sargent PB, Yau KW, Nicholls JG. 1977 Extrasynaptic receptors on cell bodies of neurons in central nervous system of the leech. *J. Neurophysiol.* **40**, 446–452. (doi:10.1152/jn.1977.40.2.446)
97. Duchen MR. 1986 Excitation of mouse motoneurones by GABA-mediated primary afferent depolarization. *Brain Res.* **379**, 182–187. (doi:10.1016/0006-8993(86)90274-X)
98. Willis Jr WD. 1999 Dorsal root potentials and dorsal root reflexes: a double-edged sword. *Exp. Brain Res.* **124**, 395–421. (doi:10.1007/s002210050637)
99. Sung KW, Kirby M, Mcdonald MP, Lovinger DM, Delpire E. 2000 Abnormal $GABA_A$ receptor-mediated currents in dorsal root ganglion neurons isolated from Na-K-2Cl cotransporter null mice. *J. Neurosci.* **20**, 7531–7538. (doi:10.1523/JNEUROSCI.20-20-07531.2000)
100. Mao S, Garzon-Muvdi T, Di Fulvio M, Chen Y, Delpire E, Alvarez FJ, Alvarez-Leefmans FJ. 2012 Molecular and functional expression of cation-chloride cotransporters in dorsal root ganglion neurons during postnatal maturation. *J. Neurophysiol.* **108**, 834–852. (doi:10.1152/jn.00970.2011)
101. Wei B, Kumada T, Furukawa T, Inoue K, Watanabe M, Sato K, Fukuda A. 2013 Pre- and post-synaptic switches of GABA actions associated with Cl^- homeostatic changes are induced in the spinal nucleus of the trigeminal nerve in a rat model of trigeminal neuropathic pain. *Neuroscience* **228**, 334–348. (doi:10.1016/j.neuroscience.2012.10.043)
102. Price TJ, Hargreaves KM, Cervero F. 2006 Protein expression and mRNA cellular distribution of the NKCC1 cotransporter in the dorsal root and trigeminal ganglia of the rat. *Brain Res.* **1112**, 146–158. (doi:10.1016/j.brainres.2006.07.012)
103. Gilbert D, Franjic-Würtz C, Funk K, Gensch T, Frings S, Möhrlen F. 2007 Differential maturation of chloride homeostasis in primary afferent neurons of the somatosensory system. *Int. J. Dev. Neurosci.* **25**, 479–489. (doi:10.1016/j.ijdevneu.2007.08.001)
104. Melin C, Jacquot F, Dallel R, Artola A. 2013 Segmental disinhibition suppresses C-fiber inputs to the rat superficial medullary dorsal horn via the activation of $GABA_B$ receptors. *Eur. J. Neurosci.* **37**, 417–428. (doi:10.1111/ejn.12048)
105. Sdrulla AD *et al.* 2015 Electrical stimulation of low-threshold afferent fibers induces a prolonged synaptic depression in lamina II dorsal horn neurons to high-threshold afferent inputs in mice. *Pain* **156**, 1008–1017. (doi:10.1097/01.j.pain.0000460353.15460.a3)
106. Yang F *et al.* 2016 Activation of cannabinoid CB1 receptor contributes to suppression of spinal nociceptive transmission and inhibition of mechanical hypersensitivity by Aβ-fiber stimulation. *Pain* **157**, 2582–2593. (doi:10.1097/j.pain.0000000000000680)
107. Yuan S, Burrell BD. 2019 Interaction between NMDA receptor- and endocannabinoid-mediated modulation of nociceptive synapses. *Sci. Rep.* **9**, 1373. (doi:10.1038/s41598-018-37890-z)
108. Glanzman DL, Groves PM, Thompson RF. 1972 Stimulus generalization of habituation in spinal interneurons. *Physiol. Behav.* **8**, 155–158. (doi:10.1016/0031-9384(72)90145-X)

109. Frost WN, Brown GD, Getting PA. 1996 Parametric features of habituation of swim cycle number in the marine mollusc *Tritonia diomedea*. *Neurobiol. Learn. Mem.* **65**, 125–134. (doi:10.1006/nlme.1996.0015)
110. Goldberg JI, Lukowiak K. 1984 Transfer of habituation in *Aplysia*: contribution of heterosynaptic pathways in habituation of the gill-withdrawal reflex. *J. Neurobiol.* **15**, 395–411. (doi:10.1002/neu.480150602)
111. Burrell BD, Sahley CL. 1998 Generalization of habituation and intrinsic sensitization in the leech. *Learn. Mem.* **5**, 405–419.
112. Poon CS, Young DL. 2006 Nonassociative learning as gated neural integrator and differentiator in stimulus-response pathways. *Behav. Brain Funct.* **2**, 29. (doi:10.1186/1744-9081-2-29)
113. Rankin CH *et al.* 2009 Habituation revisited: an updated and revised description of the behavioral characteristics of habituation. *Neurobiol. Learn. Mem.* **92**, 135–138. (doi:10.1016/j.nlm.2008.09.012)
114. Hanson A, Burrell BD. 2018 Are the persistent effects of 'gate control' stimulation on nociception a form of generalization of habituation that is endocannabinoid-dependent? *Neurobiol. Learn. Mem.* **155**, 361–370. (doi:10.1016/j.nlm.2018.09.001)
115. Crook RJ, Dickson K, Hanlon R, Walters E. 2014 Nociceptive sensitization reduces predation risk. *Curr. Biol.* **24**, 1121–1125. (doi:10.1016/j.cub.2014.03.043)
116. Albu S, Gómez-Soriano J, Avila-Martin G, Taylor J. 2015 Deficient conditioned pain modulation after spinal cord injury correlates with clinical spontaneous pain measures. *Pain* **156**, 260–272. (doi:10.1097/01.j.pain.0000460306.48701.f9)
117. Avramidis T, Bougea A, Hadjigeorgiou G, Thomaides T, Papadimitriou A. 2017 Blink reflex habituation in migraine and chronic tension-type headache. *Neurol. Sci.* **38**, 993–998. (doi:10.1007/s10072-017-2885-x)
118. Coppola G, Di Lorenzo C, Schoenen J, Pierelli F. 2013 Habituation and sensitization in primary headaches. *J. Headache Pain* **14**, 65. (doi:10.1186/1129-2377-14-65)
119. Peters ML, Schmidt AJ, Van den Hout MA. 1989 Chronic low back pain and the reaction to repeated acute pain stimulation. *Pain* **39**, 69–76. (doi:10.1016/0304-3959(89)90176-0)
120. Smith BW, Tooley EM, Montague EQ, Robinson AE, Cosper CJ, Mullins PG. 2008 Habituation and sensitization to heat and cold pain in women with fibromyalgia and healthy controls. *Pain* **140**, 420–428. (doi:10.1016/j.pain.2008.09.018)
121. McPartland JM, Agraval J, Gleeson D, Heasman K, Glass M. 2006 Cannabinoid receptors in invertebrates. *J. Evol. Biol.* **19**, 366–373. (doi:10.1111/j.1420-9101.2005.01028.x)