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Caenorhabditis elegans as a model to explore the genetic underpinnings of human pain-related processes: cannabinoid and opioid neuropharmacology as an example

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Abstract

Caenorhabditis elegans has many traits that make it a valuable model for human neurobiology, including the study of pain-related processes. In particular, its genetic tractability can help uncover novel genetic factors involved in pain-related signal transduction. This can be beneficial for studying pain medications, such as cannabinoids and opioids. Here, we review how the pain-related impacts of cannabinoids/opioids have been assessed using behavioural assays (e.g., measuring feeding, locomotion, and nociception). Reviewed studies identified genetic factors responsible for both cannabinoid (e.g., endocannabinoid receptor *npr-19*) and opioid (e.g., opioid receptor *npr-17*) signalling, which were in turn used to characterize neurotransmission (e.g., monoaminergic, neuropeptidergic, and Hedgehog signalling) and complex modulators (e.g., TRP channels involved in cannabinoid signalling) contributing to cannabinoid/opioid signalling. Additionally, studies using these models were able to discover novel genetic components, including *frpr-13* (orthologous to human *GRP139*), involved in opioid sensitivity, and *ptr-25* (orthologous to human *PTCHD1*), involved in opioid tolerance. The pathways highlighted in this review represent clear paths for further investigation of the genetic mechanisms underlying individual differences in pain sensitivity, pain relief, and drug tolerance. Overall, this review demonstrates the value of *C. elegans* as a model for uncovering the genetic underpinnings of pain and its management.

Key words: *Caenorhabditis elegans*, cannabinoid, opioid, pain management, genetics

1. Introduction

1.1. *Caenorhabditis elegans* as a valuable model for nociception and antinociception

Throughout history, humans have used a variety of compounds to reduce and relieve pain. Amongst them are cannabinoids and opioids, both of which have variants that are still used for pain relief to this day. Despite their history, many aspects of their function and their complete signalling pathways remain unclear. While pain management and related processes are much more commonly studied in rodent models (Soliman et al. 2021), modelling nociception and antinociception in *C. elegans* nematode worms can provide insights into the underlying fundamental processes.

Caenorhabditis elegans has unique utility in high-throughput genetic behavioural screens. The genetic tractability for assays enables high-throughput drug and/or mutant screening with automated recording hardware (Swierczek et al. 2011; García-Garví and Sánchez-Salmerón 2025). Despite their small size and simple physiology, *C. elegans* also possesses a high degree of homology (60%–80%) to humans (Kaletta and Hengartner 2006). Behavioural analysis is supported by their well-characterized and simple nervous system: adult hermaphrodite animals have 302 neurons, divided into 118 morphologically distinct classes, with over 7600 highly reproducible synapses (White et al. 1986). This simple nervous system allows for interactions between specific neurons to be studied (Wu et al. 2022). Across these 302 neurons, *C. elegans* also possesses a high degree of neurotransmitter homology to humans (Bargmann 1998). In fact, *C. elegans* was proposed as a model for thermal nociception by Wittenburg and Baumeister (1999). Since then, it has been used to model conserved elements of nervous systems and the underlying biology of various pain-related processes, such as by elucidating the role of unsaturated fatty acids in TRPV-dependent sensory signalling (Kahn-Kirby et al. 2004), identifying poten-

tial TRPM analgesic targets (Husson et al. 2012), and investigating the interactions between monoamines and neuropeptides in behavioural modulation (Harris et al. 2010; Hapiak et al. 2013). Additionally, orthologues of human pain-related genes have been implicated in worm responses and adaptation to noxious stimuli (Jordan and Glauser 2023), demonstrating that behavioural assays can be used to assess the functions of these and other human pain-related genes.

1.2. Cannabinoids

Cannabinoids are a group of chemicals with psychoactive properties, including both phytocannabinoids and endocannabinoids. Phytocannabinoids such as tetrahydrocannabinol (THC) and cannabidiol (CBD) are derived from the *Cannabis sativa* plant, while endocannabinoids like N-arachidonoylethanolamine (AEA) and anandamide (2-arachidonoylglycerol; 2-AG) are produced within animals, including humans. Both of these classes of cannabinoids bind to the cannabinoid receptors in the nervous system (Devane et al. 1992; Mechoulam et al. 1995). Cannabis has been used for various medicinal effects, including pain relief, since at least the first century Before Common Era (BCE) (Pisanti and Bifulco 2019). Following the discovery of the endocannabinoid system (ECS) in the 1990s (Devane et al. 1992; Mechoulam et al. 1995), researchers began investigating its mechanisms and pharmacology. However, despite extensive study into the analgesic effects of cannabinoids, there remains a lack of high-quality evidence and scientific consensus regarding the analgesic potential of cannabinoids in clinical settings (Fisher et al. 2021; Nielsen et al. 2022). As such, there is a need for more high-quality evidence on the analgesic effects and side effects of cannabinoids, which both clinical and preclinical research can contribute towards. A systematic review of pre-clinical studies on cannabinoid use in animal models (Soliman et al. 2021) lends support for cannabinoids providing an analgesic effect in rodent model systems. However, there are still significant limitations in translating findings from rodent models of cannabinoid analgesia (Soliman et al. 2021), suggesting a need for additional complementary models, such as the biologically simpler *C. elegans*.

1.3. Opioids

There are three primary families of endogenous opioid peptides: enkephalins, endorphins, and dynorphins (Benarroch 2012). These are produced naturally within animals, including humans, and bind to the endogenous opioid receptors in the nervous system, which are divided into opioid receptors (μ-, δ-, and κ-), the nociceptin receptor (Corbett et al. 2006), and the opioid growth factor receptor (Zagon et al. 2000). Exogenous opioid agonists (derived from the opium poppy) have been used for medicinal purposes for thousands of years (Brownstein 1993); however, only since the 19th century have healthcare practitioners had access to isolated and purified opiate compounds like morphine and codeine. Since then, synthetic compounds including methadone, fentanyl, and fentanyl derivatives have been synthesized and used medicinally (Brownstein 1993). Additionally, opioid antagonists, such as naloxone and D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2 (CTOP), reversibly and competitively antagonize opioid receptors with varying affinities (Choi and Billings 2002). Now, opioids are commonly used for analgesia in clinical settings (Dowell 2022), yet many questions have arisen regarding how to balance the analgesic effects of opioids against the risk of abuse and dangerous side effects (Dowell 2022; Government of Canada 2025). Research into opioid effectiveness and safety has also involved more polypharmacy, with opioids and other compounds such as cannabinoids, yet more high-quality evidence is needed to support this strategy (Nielsen et al. 2022). *Caenorhabditis elegans* provides a model with which to explore the genetic underpinnings of opioid pharmacology.

1.4. Considerations for *Caenorhabditis elegans* as a model for nociception and antinociception

As a model organism for studying human pain and pain relief, *C. elegans* does possess limitations. Notably, nematodes do not experience pain as defined by the International Association for the Study of Pain (Raja et al. 2020). As such, *C. elegans* has more utility in identifying the underlying biological basis of aversive responses to pain-related stimuli (nociception and aversive behaviours), and behavioural/physiological sensitivity to pain medications, than it does in the study of the sensation of pain. In terms of neuronal connectivity and signalling, there are important differences as well: for example, *C. elegans* possess gap junction innexins (Bargmann 1998) as opposed to mammalian connexins, and gap junctions in *C. elegans* are heavily implicated in nociception (Sojka et al. 2025). These limitations mean that modelling human pain and its management in *C. elegans* involves more than simply replicating nociception and antinociception, and requires demonstrating functional as well as sequence homology.

1.5. Goal

Here, we review studies examining the effects of cannabinoids and opioids on nociception and the effects of mutations in specific genes on cannabinoid- or opioid-mediated antinociception. This review focuses on studies that accomplish this by analyzing worm behaviours. Both cannabinoids and opioids are covered due to their implications for human pain and its management. We aim to address translation to human contexts, providing information on gene orthology and function, as well as discuss the potential future directions for research using *C. elegans* as a model for pain-related processes.

2. Methods

We searched for published, peer-reviewed original research that (1) employed exogenous opioid agonists, cannabinoids, ECS modulators, and/or cannflavins; and (2) examined a behavioural output in *C. elegans*, with an emphasis on nociception and/or the antinociceptive effects of these compounds.

We identified 607 studies to be imported for screening: 351 from Web of Science and 256 from PubMed. We used

the search terms: (ALL = *C. elegans*) OR ALL=(*Caenorhabditis elegans*) OR ALL=(*C. elegans*) AND (ALL=(pain) OR ALL=(pain management) OR ALL=(analgesia) OR ALL=(nociception) OR ALL=(nocifensive)). Records were collected until 13 June 2025. From these, 175 duplicates were removed. The remaining 432 studies were abstract screened, of which 357 were determined to be irrelevant. Of the 75 that remained, 14 studies focused on the role of TRP channels in nociception, and 45 covered general nociception/nocifensive behaviours. The remainder were excluded due to being conference abstracts (2), being an evolutionary biology study (1), not conducting original research on *C. elegans* (1), only exploring pathogen avoidance (1), and not evaluating nociception in any form (1). These 75 were re-screened, focusing on studies incorporating behavioural assays in *C. elegans*, and filtering for the use of the compounds of interest. Re-screening narrowed the studies to 10 that met our criteria: four examining cannabinoids, one examining cannflavins, and five examining opioids.

3. Results

3.1. Drugs

The concentrations of pharmacological treatments employed in the reviewed studies are almost always higher than the concentrations used in humans. Due to the relative impermeability of the nematode cuticle, higher external concentrations are necessary to elicit a response. Dose–response curves were used by Oakes et al. (2017, 2019), Nieto-Fernandez et al. (2009), Wang et al. (2019), and Maza et al. (2022), to determine optimal concentrations for pharmacological treatments. Abdollahi et al. (2024), Boujenoui et al. (2024), and Lahaise et al. (2024) all employed a range of concentrations for endocannabinoid treatments, reporting effects for each dosage. The articles reviewed did not otherwise discuss controls for off-target effects.

3.1.1. Cannabinoids

Two endocannabinoids, AEA (Oakes et al. 2017; Abdollahi et al. 2024) and 2-AG (Oakes et al. 2017, 2019), were assayed in the reviewed cannabinoid studies. These compounds had been previously identified in *C. elegans* (Lehtonen et al. 2008). THC (Abdollahi et al. 2024; Boujenoui et al. 2024) and CBD (Boujenoui et al. 2024) were also employed to test the effects of exogenous cannabis-derived compounds. Additionally, we have included a study assessing the behavioural effects of cannflavins A and B (Lahaise et al. 2024). Cannflavins are chemically distinct from cannabinoids, but they are present in cannabis products and act as prostaglandin E2 inhibitors (Barrett et al. 1986) and are therefore relevant to pain management. In addition, the enzymatic inhibitors JZL184, 2-aminoethoxydiphenyl borate (2-APB), and URB597 were used to explore the effects of inhibited endocannabinoid biosynthetic processes on behaviours. JZL184 inhibits monoacylglycerol lipase (MAGL), the enzyme that breaks down 2-AG (Oakes et al. 2019), 2-APB has been shown to inhibit TRP channels at low concentrations (Xu et al. 2005), and URB597 is a selective inhibitor of fatty-acid amide hydrolase 1 (FAAH), an enzyme involved in the catabolism of AEA (Piomelli et al. 2006).

3.1.2. Opioids

In the five opioid studies, the opioid agonists morphine (Nieto-Fernandez et al. 2009; Cheong et al. 2015; Mills et al. 2016; Wang et al. 2019; Maza et al. 2022) and fentanyl (Wang et al. 2019; Maza et al. 2022) were used to evaluate opioid-mediated behaviours, and the selective κ-opioid receptor (KOR) agonist salvinorin A (Mills et al. 2016) was used to determine functional orthology of *C. elegans npr-17* to human *OPRK1*. The opioid peptides endomorphin 1 and 2 were used in Nieto-Fernandez et al. (2009), while dynorphin A and the nematode neuropeptides NLP 3.1, 3.2, and 3.3 were used in Mills et al. (2016) to characterize the endogenous agonists to NPR-17. Opioid antagonists were employed to observe whether they would abolish opioid-mediated behaviours. Specifically, naloxone (Nieto-Fernandez et al. 2009; Cheong et al. 2015; Mills et al. 2016; Wang et al. 2019), CTOP (Nieto-Fernandez et al. 2009), and the selective KOR antagonist norbinaltorphimine (nor-BNI) (Mills et al. 2016) were all employed.

3.2. Outcome assessments

3.2.1. Behavioural assays

Three cannabinoid/cannflavin studies used thermal avoidance (Abdollahi et al. 2024; Boujenoui et al. 2024; Lahaise et al. 2024), using a modified protocol from Margie et al. (2013). Avoidance assays with the noxious odorant 1-Octanol and crawling locomotory inhibition were also used in two studies (Oakes et al. 2017, 2019). Pharyngeal pumping and feeding rate using fluorescent beads (Kiyama et al. 2012) were both assayed in Oakes et al. (2017). The specific assays used are highlighted in Table 1.

Two opioid studies assayed swimming locomotory inhibition during prolonged exposure to opioids (Wang et al. 2019; Maza et al. 2022). Thermal avoidance was assayed in Nieto-Fernandez et al. (2009), pharyngeal pumping rate was assayed in Cheong et al. (2015), and an avoidance assay using the noxious odorant 1-Octanol was used in Mills et al. (2016). The specific assays used are highlighted in Table 1.

3.2.1.1. Thermal avoidance

Boujenoui et al. (2024) used thermal avoidance assays to evaluate the antinociceptive properties of CBD and THC. Worms were exposed to one of the two cannabinoids at varying concentrations for an hour preceding the assay. 100–300 young adult worms were then placed in the centre of marked nematode growth medium (NGM) assay plates. Each of the four quadrants was treated with the paralytic sodium azide, and the two test quadrants were heated with a metal tip to create a temperature gradient of 32–35 °C 2 mm around the tip. After 30 min, the plates were placed at 4 °C for at least an hour, and then the number of worms in each quadrant were counted; the thermal avoidance index was calculated from

Table 1. Pharmacology and behavioural assays employed to evaluate *Caenorhabditis elegans* responses to cannabinoids and opioids in papers of interest.

Citation	Ligands/drugs tested	*C. elegans* behavioural assays	Additional experiments
Cannabinoid			
Oakes et al. (2017)	3.2, 32, 320 μmol/L 2-AG, 3.2, 32, 320 μmol/L AEA, 320 μmol/L JZL184 (MAGL inhibitor), URB597 (FAAH inhibitor; concentration not reported)	1-Octanol avoidance, crawling locomotory inhibition, pharyngeal pumping, feeding	*Xenopus* oocyte patch-clamp
Oakes et al. (2019)	0.4, 8, 320 μmol/L 2-AG, 320 μmol/L JZL184, 10 μmol/L 2-APB (TRPV inhibitor)	1-Octanol avoidance, crawling locomotory inhibition	–
Lahaise et al. (2024)	0.25, 1, 5, 10, 25 μmol/L Cannflavins A and B	Thermal avoidance	Protein enrichment analysis
Boujenoui et al. (2024)	0.25, 1, 5, 10, 25 μmol/L THC, CBD	Thermal avoidance	Protein enrichment analysis
Abdollahi et al. (2024)	1, 5, 10, 25 μmol/L AEA, THC	Thermal avoidance	Protein enrichment analysis
Opioid			
Nieto-Fernandez et al. (2009)	10 nmol/L, 100 nmol/L, 1 μmol/L, 10 μmol/L, 100 μmol/L Morphine, endomorphine 1 and 2, naloxone, CTOP (concentrations not reported)	Thermal avoidance	–
Cheong et al. (2015)	0.5 mmol/L Morphine, 10 mmol/L naloxone	Pharyngeal pumping	Cultured human cell assays
Mills et al. (2016)	320 μmol/L Morphine, 320 μmol/L salvinorin A, 320 μmol/L naloxone, 320 μmol/L norbinaltorphimine, 1 μmol/L dynorphin A, 1 μmol/L NLP peptides NLP-3.1, NLP-3.2, and NLP-3.3	1-Octanol avoidance	–
Wang et al. (2019)	10 μmol/L Fentanyl, 300 μmol/L morphine, 20 μmol/L naloxone	Swimming locomotory inhibition	Cultured human cell assays, mouse behavioural assays and patch-clamp recordings
Maza et al. (2022)	10 μmol/L Fentanyl, 300 μmol/L morphine	Swimming locomotory inhibition	Cultured human cell assays, mouse behavioural assays and patch-clamp recordings

Note: THC, tetrahydrocannabinol; CBD, cannabidiol; AEA, N-arachidonoylethanolamine; MAGL, monoacylglycerol lipase; FAAH, fatty-acid amide hydrolase 1.

this. CBD was found to have antinociceptive effects (decreasing thermal avoidance) in wild-type (WT) worms, but not in *ocr-2* or *osm-9* null mutants, which lacked a functional copy of those TRPV family ion channels (Colbert et al. 1997; Tobin et al. 2002). Likewise, THC was found to have antinociceptive effects in WT, but not in *npr-19* or *npr-32* null mutants, which had been previously identified as endocannabinoid receptors mediating axon regeneration (Pastuhov et al. 2016). These results suggest that CBD targets vanilloid receptors, while THC targets cannabinoid receptors. Boujenoui et al. (2024) further investigated these results with proteomics studies, as covered in Section 3.2.2.

Abdollahi et al. (2024) assayed thermal avoidance using a similar protocol to evaluate the antinociceptive properties of AEA. Worms were exposed to AEA, capsaicin, or THC before the assays. While AEA was found to have antinociceptive effects in WT worms, these effects were significantly reduced though not eliminated in *ocr-2*, *osm-9*, *npr-19*, and *npr-32* null mutants. These results suggest that AEA targets both vanilloid and cannabinoid receptors. Abdollahi et al. (2024) further investigated these results with proteomics studies, as covered in Section 3.2.2.

Lahaise et al. (2024) assayed thermal avoidance using a similar protocol to evaluate the antinociceptive effects of cannflavins A and B. Both cannflavins had antinociceptive effects in WT worms, and in *osm-9*, *npr-19*, and *npr-32* null mutants. While cannflavin B also had antinociceptive effects in *ocr-2* null mutants, the antinociceptive effects of cannflavin A were notably reduced following exposure to noxious heat. Lahaise et al. (2024) further investigated these results with proteomics studies, as covered in Section 3.2.2.

Nieto-Fernandez et al. (2009) were the first to evaluate the antinociceptive effects of opioids in *C. elegans*, using a thermal avoidance protocol alongside morphine, the opioid peptides endomorphins 1 and 2, and naloxone and CTOP. A metal pen electronically heated to 33.0 ± 1.0 °C was presented in front of forward-moving worms, and reactions were classified according to how the worms responded. The proportion of worms exhibiting a rapid reflexive withdrawal in response to noxious heat was found to decrease following exposure to morphine, as well as endomorphins 1 and 2. Treatment with naloxone and CTOP following opioid exposure increased the proportion exhibiting nocifensive behaviour compared to worms treated with opioid agonists alone. This provided

evidence for endogenous *C. elegans* opioid signalling, which would be later established to be mediated by NPR-17 (Cheong et al. 2015).

3.2.1.2. 1-Octanol avoidance

In Oakes et al. (2017), worms were exposed to 2-AG or AEA on assay plates; after 10 min, 1-Octanol was presented in front of forward-moving worms, and the time until reversal was recorded. 2-AG and AEA were both found to inhibit reversals in response to 1-Octanol, as were JZL184 and URB597. This inhibition was found to be absent in *npr-19* null animals, as well as following *npr-19* RNAi knockdown in the URX sensory neurons, which are involved in aggregation (Cheung et al. 2004) and oxygen-seeking (Gray et al. 2004).

In Oakes et al. (2019), adult worms were exposed to 2-APB on assay plates and were assayed with the protocol from Oakes et al. (2017). It was found that 2-APB exposure in WT animals decreases sensitivity to 1-Octanol and mimics the phenotype of *osm-9* null animals. Oakes et al. (2019) also explored the effects of 2-AG on spontaneous reversals (occurring even in the absence of aversive stimuli), and found that 5 min of exposure to the compound stimulated reversals in WT, *npr-19* null, and *cat-2* (tyrosine hydroxylase, involved in dopamine biosynthesis) null, while *tph-1* (tryptophan hydroxylase, involved in serotonin biosynthesis) null, and *ser-4* ($G_{i/o}$-coupled serotonin receptor) null animals did not display this increase in spontaneous reversals, indicating that serotonin signalling is required for this process.

In Mills et al. (2016), NGM plates were first prepared with serotonin, tyramine, or octopamine (an invertebrate counterpart to norepinephrine (Roeder 1999)). Morphine, salvinorin A, naloxone, or nor-BNI was spread on top of the prepared plates immediately preceding the assay. Worms were incubated on these plates for 10 min (off food), 20 min (morphine, salvinorin A), or 30 min (serotonin, naloxone, octopamine, tyramine), before forward-moving worms were presented with 1-Octanol; time to reversal was recorded. Serotonin decreased the time to reversal and increased the proportion of animals that resumed forward movement following exposure to 1-Octanol. Naloxone and nor-BNI were found to abolish the effect of serotonin on aversive responses. The effects of morphine and salvinorin A were similar to those of serotonin in WT animals, aligning with what was observed in Nieto-Fernandez et al. (2009). Morphine's effects were greatly decreased in *npr-17* null mutants, supporting the conclusion from Cheong et al. (2015) that *npr-17* encodes a nematode opioid receptor.

3.2.1.3. Locomotory inhibition

Crawling locomotory inhibition was assayed in Oakes et al. (2017, 2019) using the method described in Sawin et al. (2000). Motility was quantified as the number of body bends recorded over a 20 s timeframe at 5 min intervals. Reversals were also assayed as described in WormBook (Hart 2006). Dopamine and serotonin signalling mutants, as well as TRP channel mutants, were assayed in Oakes et al. (2019). Both serotonergic and dopaminergic signalling, plus the TRP channels TRP-4 and OSM-9, are required for 2-AG-dependent inhibition of locomotion, while the cannabinoid receptor NPR-19 was not. It was also determined that the 2-AG-dependent increase in reversals was independent of *npr-19*, but required *tph-1* in the ADF neurons, *mod-1* (a serotonin-gated chloride channel), and *ser-1* (a G_q-coupled serotonin receptor).

Wang et al. (2019) and Maza et al. (2022) both employed swimming locomotory inhibition. Worms were immersed in a buffer containing morphine or fentanyl and body bends were automatically tracked. This was employed to first validate the transgenic murine μ-opioid receptor (tgMOR) strain as an opioid-responsive platform, which was in turn used in a forward genetic screen to identify mutants with abnormal responses to those two opioids. TgMOR will be discussed further in Section 3.3.2. Swimming locomotory inhibition was used again to characterize the responses of mutants identified from the screen. Wang et al. (2019) screened 600 000 F2 mutagenized worms and identified 900 that were hypo- or hyper-responsive to opioids. These were narrowed down to two strains that were superficially WT with abnormal sensitivity to opioids, one of which was investigated further. Maza et al. (2022) screened 27 mutants and identified one strain with abnormal opioid tolerance. These strains with abnormal opioid-dependent behaviours will be discussed in Section 3.3.2.

3.2.1.4. Pharyngeal pumping and feeding

In Oakes et al. (2017) cannabinoid sensitivity was assayed using pharyngeal pumping. Young adult worms were incubated on 2-AG-treated NGM plates for 10 minutes. Pharyngeal muscle contractions were counted over two minutes, which was used to determine pumping rate. WT worms were compared to mutant strains with and without cannabinoid exposure. Pharyngeal pumping was inhibited by high concentrations of 2-AG or AEA, a phenotype which was abolished in *npr-19* null mutants. Additionally, RNAi knockdown of *npr-19* in the M3 pharyngeal motor neurons mimicked the *npr-19* null phenotype, indicating that M3 expression of *npr-19* is responsible for the 2-AG- and AEA-mediated decrease in pharyngeal pumping.

Oakes et al. (2017) also employed a feeding assay to confirm that high concentrations of 2-AG and AEA inhibited feeding, and that *npr-19* null animals did not respond to either compound. This confirmed the findings from the pharyngeal pumping assay. This is in contrast to mammals, in which those compounds produce an appetite-stimulating effect (Di Marzo et al. 2001).

Cheong et al. (2015) employed a similar protocol to explore *C. elegans* endogenous opioid signalling: young adult worms were transferred to NGMSR plates (NGM plates with streptomycin sulfate, nystatin, and a greater agar percentage (Avery 1993)) with morphine spread over them. Pharyngeal pumping was observed for 1 min to determine pumping rate. Additionally, they conducted an RNAi screen of 115 neuropeptide genes, leading to the identification of NLP-24 as a key mediator of feeding, and subsequently to the identification of its receptor, NPR-17, as a nematode opioid receptor. WT worms were shown to increase their pharyngeal pumping rate following exposure to morphine, which was abolished in *npr-17* null animals. NPR-17 had previously been predicted to enable opioid receptor activity due to sequence similarity to a pre-

dicted *Brugia malayi* ORL1-like opioid receptor, with 68% identity (Harris et al. 2010).

3.2.2. Non-behavioural assays in *C. elegans*

Protein enrichment analyses (Abdollahi et al. 2024; Boujenoui et al. 2024; Lahaise et al. 2024), using mass spectrometry and KEGG pathway analysis, were used to identify enriched pathways following exposure to cannabinoid and cannflavin test compounds. Boujenoui et al. (2024) found that sub-pathways related to eukaryotic translation initiation were significantly enriched following exposure to CBD, elements of which had previously been implicated in the development of chronic pain in humans (Uttam et al. 2018). Additionally, worms exposed to THC were found to be significantly enriched in factors involved in nucleotide signalling and adaptive immunity. Factors involved in eukaryotic translation initiation were also found to be enriched following exposure to cannflavins A and B (Lahaise et al. 2024). Translation-related factors were found to be enriched yet again in Lahaise et al. (2024) following exposure to AEA. The presence of translation initiation as an enriched pathway throughout these proteomic analyses could suggest extensive remodelling in response to exposure to these compounds, in turn leading to durable effects.

Abdollahi et al. (2024) also employed a different proteomic approach, Thermal Proteome Profiling (TPP), to identify nematode protein targets of AEA. Proteins become more resistant to heat-induced unfolding when bound to a ligand; TPP uses this property to isolate and identify proteins bound to a ligand of interest (Savitski et al. 2014). This was used to confirm that NPR-32 and NPR-19 are the primary targets of AEA. Additional targets were the nematode TRPV channel OCR-2, a galectin orthologue LEC-2, the cathepsin-B orthologue CPR-4, the progranulin orthologue PGRN-1, and the transthyretin orthologue TTR-15.

3.3. Genetics

3.3.1. Orthology

The orthology of *C. elegans* and human genes is examined in Table 2.

3.3.2. Transgenes used to validate functional homology to human genes

Transgenic human *CNR1* driven by the *npr-19* promoter was employed in Oakes et al. (2017). *CNR1* expression was found to rescue 2-AG sensitivity in an *npr-19* null background. As such, the nematode *npr-19* is suspected to be orthologous to the human *CNR1* (Oakes et al. 2017).

In two opioid studies, a tgMOR strain expressing a transgenic (tg) murine μ-opioid receptor (MOR) was used as the basis for which to study other genes (Wang et al. 2019; Maza et al. 2022). TgMOR is structurally 94.00% homologous to the human μ-opioid receptor (*OPRM1*) (Altschul et al. 1997). This enabled the interrogation of MOR-mediated effects and signalling: Wang et al. (2019) used this in a genetic behavioural screen to identify the orphan G Protein-Coupled Receptor (GPCR) FRPR-13 as a mediator of opioid signalling; transgenic expression of the phylogenetically similar mammalian orphan GPCR GPR139 rescued tgMOR *frpr-13* null mutants, revealing the two genes to be functional orthologues. Maza et al. (2022) was able to recapitulate tolerance to opioids with a tgMOR strain, in turn using another genetic behavioural screen to identify the *PTCHD1/PTCHD4* homolog *ptr-25* as coding for a mediator of opioid tolerance.

Transgenic expression of mammalian genes of interest was also used to evaluate functional orthology between *C. elegans* and humans while studying novel GPCR targets. Wang et al. (2019) expressed human GPR139 with the *frpr-13* promoter in an *frpr-13* null background, rescuing tgMOR responses to fentanyl. Maza et al. (2022) also expressed human *PTCHD1*, a mediator of Hedgehog signalling (Noor et al. 2010), in a *ptr-25* null background. This partially rescued the tgMOR tolerance phenotype to fentanyl.

3.3.3. Pathway homology

Orthology by sequence similarity alone does not necessarily mean that cannabinoid- or opioid-mediated antinociception can be effectively modelled in *C. elegans*. To adequately model the complex signalling involved in these processes in worms, there must be multiple levels of similarity with human processes. Here, we highlight similarities between effects observed in nematodes and in human/mammalian contexts.

3.3.3.1. Dopamine

Dopamine was required for the cannabinoid-induced decrease in locomotory inhibition (Oakes et al. 2019). Dopaminergic neurons expressing CB_2 receptors are involved in many behaviours in mice (Liu et al. 2017). Dopaminergic signalling in the reward pathway also has implications for cannabinoid signalling: variation in *DRD2*, the gene coding for the D2 dopamine receptor, was found to be significant in one ancestral subgroup (European) in a genome-wide association study (GWAS) of cannabis use disorder (Levey et al. 2023).

While it was not interrogated in the studies covered by this review, dopaminergic signalling was identified as being a key mediator in opioid-conditioned cue preference through the NPR-17 pathway (Ide et al. 2022). This dopamine-mediated conditioned cue preference may be the nematode counterpart of the dopamine-mediated "wanting" that is characteristic of the mesocorticolimbic circuit (Berridge and Kringelbach 2015). This dopaminergic circuit has strong implications for opioid neuropharmacology: for example, dopaminergic neurons in the ventral tegmental area were found to be implicated in opioid-reward and associated behaviours (Fields and Margolis 2015) in mammals, and a meta-analysis (Kember et al. 2022) identified variation in *DRD2* in one ancestral subgroup (European Americans) as being significantly associated with opioid use disorder.

Table 2. *Caenorhabditis elegans* genes examined in studies, their human orthologues, and the mutant phenotypes, relative to wild type, observed.

Worm gene (allele)*	Human sequence orthologues	Mutant phenotype relative to WT	Worm gene function
Cannabinoids			
cat-1 (ok411)	*SLC18A2 SLC18A1*	Reduced 2-AG-dependent locomotory inhibition (Oakes et al. 2019)	Vesicular monoamine transporter; mediates monoamine transport into vesicles (Duerr et al. 1999)
cat-2 (n4547)	*TH*	Reduced 2-AG-dependent locomotory inhibition (Oakes et al. 2019)	Tyrosine hydroxylase; catalyzes step in dopamine biosynthesis (Lints and Emmons 1999)
cat-4 (ok342)	*GCH1*	Reduced 2-AG-dependent locomotory inhibition (Oakes et al. 2019)	GTP cyclohydrolase; involved in biosynthesis of catecholamines (Sawin et al. 2000)
ckr-2 (tm3082)	*CCKAR* *CCKBR*	No effect on aversive responses (Oakes et al. 2017)	G_q-coupled cholecystokinin receptor (Janssen et al. 2008)
dat-1 (ok157)	*SLC6A2 SLC6A3* *SLC6A4*†	No effect on 2-AG-dependent locomotory inhibition (Oakes et al. 2019)	Dopamine transporter; mediates dopamine reuptake (Jayanthi et al. 1998)
dop-1 (ok298, vs100)	*DRD5* *ADRA2B*† *DRD1*	No effect on cannabinoid-mediated inhibition of aversive responses (Oakes et al. 2017)	G_s-coupled D1-like dopamine receptor (Suo et al. 2002)
dop-3 (ok295)	*DRD2* *DRD4*† *DRD3*	Hypersensitive to 2-AG inhibition of aversive responses (Oakes et al. 2019)	$G_{i/o}$-coupled D2-like dopamine receptor (Chase et al. 2004)
dop-4 (tm1392)	*ADRA1B*†*ADRA1D* *ADRA1A* *HRH2*†	Reduced 2-AG-dependent locomotory inhibition (Oakes et al. 2019)	G_s-coupled D1-like dopamine receptor (Sugiura et al. 2005)
mod-1 (ok103)	*GABRE*†*GABRG2*† *GABRG1*†*GABRG3*	No effect on aversive responses with 2-AG (Oakes et al. 2019)	Serotonin-gated chloride channel (Ranganathan et al. 2000)
mod-5 (n3314)	*SLC6A4* *SLC6A3*†*SLC6A2*†	Enhanced 2-AG dependent locomotory inhibition (Oakes et al. 2017, 2019)	Serotonin transporter; mediates serotonin reuptake (Ranganathan et al. 2001)
npr-3 (tm1583)	*PRLHR*†	No effect on ZL184 or 2-AG inhibition of aversive responses (Oakes et al. 2017)	NPY-like G protein-coupled neuropeptide receptor (Keating et al. 2003)
npr-19 (ok2068)	—	Unchanged 2-AG-dependent locomotory inhibition (Oakes et al. 2019). Reduced JZL184 or 2-AG inhibition of aversive responses (URX neuron specific) (Oakes et al. 2017). Lower baseline heat sensitivity than WT (Lahaise et al. 2024). In null mutants, AEA (Abdollahi et al. 2024) and CBD decrease heat sensitivity, THC does not (Boujenoui et al. 2024)	Predicted to be G protein-coupled endocannabinoid receptor (Pastuhov et al. 2016; Oakes et al. 2017, 2019; Boujenoui et al. 2024; Abdollahi et al. 2024; Lahaise et al. 2024). Suspected to be orthologous to human *CNR1* by Oakes et al. (2017) due to sequence similarity and transgenic *CNR1* expression rescuing specific *npr-19* null phenotypes
npr-32 (ok2541)	*OPRL1*† *OPRK1*‡	Lower baseline heat sensitivity than WT (Lahaise et al. 2024). In null mutants, AEA (Abdollahi et al. 2024) and CBD decrease heat sensitivity, THC does not (Boujenoui et al. 2024)	Predicted to be G protein-coupled endocannabinoid receptor (Janssen et al. 2010; Boujenoui et al. 2024; Abdollahi et al. 2024; Lahaise et al. 2024)
ocr-2 (yz5, ky10)	*TRPV5* *TRPV6* *TRPV4*† *TRPV3* *TRPV1*† *TRPV2*†	Lower baseline heat sensitivity than WT (Lahaise et al. 2024). In null mutants, AEA (Abdollahi et al. 2024) and THC decrease heat sensitivity, CBD does not (Boujenoui et al. 2024)	TRPV-family ion channel (Tobin et al. 2002)
octr-1 (ok371)	*ADRA2A ADRA2C* *ADRA2B*	High doses of 2-AG required for inhibition of aversive responses (Oakes et al. 2017)	Octopamine receptor (Wragg et al. 2007)
osm-9 (yz6, ky10)	*TRPV5* *TRPV6* *TRPV4* *TRPV43* *TRPV1* *TRVP2*†	Lower baseline heat sensitivity than WT (Lahaise et al. 2024). In null mutants, AEA (Abdollahi et al. 2024) and THC decrease heat sensitivity, CBD does not (Boujenoui et al. 2024)	TRPV-family ion channel (Colbert et al. 1997)

Table 2. (*continued*).

Worm gene (allele)*	Human sequence orthologues	Mutant phenotype relative to WT	Worm gene function
ser-1 (ok345)	*HTR2C*† *HTR2A* *HTR2B*	No reversal increase when exposed to 2-AG (Oakes et al. 2019)	G_q-coupled serotonin receptor (Hamdan et al. 1999)
ser-2 (pk1357)	*HTR1A* *HTR1B*† *HTR1D*†	No effect on ZL184 or 2-AG inhibition of octanol avoidance (Oakes et al. 2017)	Tyramine receptor (Rex and Komuniecki 2002)
ser-4 (ok512)	*HTR1A* *HTR1D* *HTR1B* *HTR1E* *HTR1F* *HTR5A*†	2-AG-dependent locomotory inhibition reduced (Oakes et al. 2019). No effect on ZL184 or 2-AG inhibition of octanol avoidance (Oakes et al. 2017)	$G_{i/o}$-coupled serotonin receptor (Olde and McCombie 1997)
tph-1 (mg280, n4622)	*TPH2* *TPH1*	Slowed response to 2-AG, no reversal increase. Reduced 2-AG-dependent locomotory inhibition (Oakes et al. 2017, 2019)	Tryptophan hydroxylase; catalyzes the rate-limiting step in serotonin biosynthesis (Sze et al. 2000)
trp-4 (sy695)	*ANKRD44*†*ANKRD52*† *ANKRD28*† *ANK1*† *ANK2*† *ANK3*†	Reduced 2-AG-dependent locomotory inhibition (Oakes et al. 2019)	Mechanosensitive TRPN channel (Li et al. 2006)
Opioids			
egl-3	*PCSK2*	Decreased pharyngeal pumping in *eat-2* background (Cheong et al. 2015)	Proprotein convertase; necessary for neuropeptide processing (Husson et al. 2006)
egl-19 (bgg8)	*CACNA1D CACNA1F* *CACNA1C* *CACNA1S* *CACNA1A*†*CACNA1B*† *CANCA1E*†	Hypersensitive to fentanyl in tgMOR background (Wang et al. 2019)	Voltage gated calcium channel (Jospin et al. 2002)
frpr-13 (bgg9, bgg78)	—	Hypersensitive to fentanyl in tgMOR background (Wang et al. 2019); normal opioid tolerance (Maza et al. 2022)	G protein-coupled neuropeptide receptor (Wang et al. 2019). Suspected to be orthologous to human *GPR139* and *GPR142* by Wang et al. (2019) due to sequence similarity and phenotypic rescue experiments using transgenic expression
goa-1	*GNAO1* *GNAI3*† *GNAI2*† *GNAI1*† *GNAT2*†	No effect on basal aversive responses to octanol, abolished morphine or serotonin-dependent reversal stimulation (Mills et al. 2016)	G protein alpha subunit (Lochrie et al. 1991)
nlp-3 (tm2302)	—	Decreased pharyngeal pumping in *eat-2* and WT background (Cheong et al. 2015); Morphine still had effect on reversals in response to 1-Octanol (Mills et al. 2016)	Neuropeptide-like peptide (Li et al. 1999)
nlp-24 (tm2105)	—	Pharyngeal pumping mimicked *npr-17*-null (Cheong et al. 2015); Morphine still had effect on reversals in response to 1-Octanol (Mills et al. 2016)	Neuropeptide-like peptide (Nathoo et al. 2001)
npr-17 (tm3210, tm3225)	*MCHR1*† *MCHR2*† *SSTR3*‡ *SSTR2*‡ *SSTR5*‡ *SSTR1*‡ *SSTR4*‡	Effects of serotonin, morphine, salvinorin A, and NLP-3.3 on 1-Octanol avoidance abolished (Mills et al. 2016); effects of morphine/naloxone on pharyngeal pumping abolished (Cheong et al. 2015)	Predicted to be opioid-like receptor (Harris et al. 2010)
ptr-25 (bgg10, bgg25)	—	Resistant to opioid tolerance in tgMOR background (Maza et al. 2022)	Predicted to be a Hedgehog receptor (Maza et al. 2022). Suspected to be orthologous to human *PTCHD1* and *PTCHD4* by Maza et al. (2022) due to sequence similarity and phenotypic rescue experiments using transgenic expression

Table 2. (*concluded*).

Worm gene (allele)*	Human sequence orthologues	Mutant phenotype relative to WT	Worm gene function
rsbp-1 (vs163)	*R7BP*†	Hypersensitive to morphine and fentanyl in tgMOR background (Wang et al. 2019); no opioid tolerance developed (Maza et al. 2022)	R7 Regulator of G protein signalling (RGS) protein-binding protein (Porter and Koelle 2010)
ser-1 RNAi knockdown	*HTR2C*† *HTR2A* *HTR2B*	Increased time to initiate aversive responses (Mills et al. 2016)	G_q-coupled Serotonin receptor (Hamdan et al. 1999)

Note: OrthoList 2 uses six orthology programs: Ensembl Compara v87-89 (2016–2017) (Vilella et al. 2009), HomoloGene v68 (2014) (Wheeler et al. 2007), InParanoid v8 (2013) (Sonnhammer and Östlund 2015), OrthoMCL v5 (2011) (Li et al. 2003), OMA v1 (2016) (Altenhoff et al. 2015), and OrthoInspector v2 (2015) (Linard et al. 2015). Alliance of Genome Resources uses nine orthology programs; Ensembl Compara (Dyer et al. 2025), Hieranoid (Kaduk and Sonnhammer 2017), InParanoid (Persson and Sonnhammer 2023), OMA (Altenhoff et al. 2024), OrthoFinder (Emms and Kelly 2019), OrthoInspector (Nevers et al. 2019), PANTHER (Thomas et al. 2022), PhylomeDB (Fuentes et al. 2022), and SonicParanoid (Cosentino, Sriswasdi and Iwasaki 2024). WT, wild-type; THC, tetrahydrocannabinol; CBD, cannabidiol; AEA, N-arachidonoylethanolamine.
*Null alleles unless otherwise stated.
†Orthologues are only found in OrthoList 2 (Kim et al. 2018).
‡Orthologues are only found in Alliance of Genome Resources v8.2.0 (The Alliance of Genome Resources Consortium 2024). Those without either letter superscript were found in both databases. Orthologues are listed from the greatest number of programs showing orthology to the least.

3.3.3.2. *Serotonin*

Similar to dopamine, serotonin was required for both the cannabinoid-induced decrease in locomotory inhibition (Oakes et al. 2019) and sensitivity to noxious stimuli (Oakes et al. 2017). Aguiar et al. (2024) found that serotonin-induced peripheral nociception was reversed by CB_1 and CB_2 receptor antagonists in mice, supporting the pathway described in Oakes et al. (2017) and in Oakes et al. (2019), in which serotonin acts in series with endocannabinoid signalling to modulate nociception. Additionally, it has been shown that endocannabinoids modulate monoaminergic neurotransmission (Peters et al. 2021). This modulation is complex and happens both presynaptically through CB_1 receptors, and indirectly through alternative receptors and pathways; this complex modulation could be responsible for the upregulated expression of pathways involved in translation initiation observed in Boujenoui et al. (2024), Lahaise et al. (2024), and Abdollahi et al. (2024).

The predicted opioid receptor (NPR-17) and the opioid-like neuropeptide (NLP-24) were found to be key mediators of the effect of serotonin on *C. elegans* responses to noxious chemical stimuli in Mills et al. (2016), acting in series with *ser-1*-mediated serotonergic signalling in a key sensory neuron pair (ASI neurons). It has also been shown that serotonin application can induce peripheral antinociception through its actions on opioid signalling (Sasaki et al. 2021). D'Addario et al. (2007) also identified hippocampal serotonin signalling as a regulator of dynorphin mRNA levels. Serotonergic signalling does not necessarily complement the effects of opioids: methadone has been reported to lower serotonin transporter availability in addiction-treatment patients on methadone compared to patients not on methadone (Yeh et al. 2012). Similarly, Isensee et al. (2017) demonstrated that $NA_v1.7$ knockout mice, with lifelong lowered pain sensitivity, had downregulated G_s-coupled serotonin receptors and upregulated μ-opioid signalling. Mills et al. (2016) interrogated the effects of the G_s-coupled *ser-5* and *ser-7*, as well as the $G_{i/o}$-coupled *ser-1*, and found that only *ser-1* had a role in modulating responses to noxious chemical stimuli. Possible mechanisms for this complex modulation in *C. elegans* could be the serotonin-mediated cross-inhibition between different pairs of sensory neurons when exposed to specific noxious stimuli (Guo et al. 2015), or simply the different excitatory and inhibitory GPCRs involved.

3.3.3.3. *TRP channels*

Boujenoui et al. (2024) found that the TRP channels OSM-9 and OCR-2 were responsible for CBD-mediated antinociception, while Abdollahi et al. (2024) found that TRP channels and cannabinoid receptors acted in parallel in mediating the antinociceptive effects of AEA. Li et al. (2021) found that in HEK293 cells, AEA (and N-arachidonoyl-dopamine, NADA) binds to the transgenic murine TRP channel TRPV1 in addition to cannabinoid receptors. Additionally, endogenous epoxides of NADA and its serotonin analog, NA5HT, both bind to TRPV1, and NA5HT epoxide is a full CB_1 agonist (Arnold et al. 2021). Endocannabinoid epoxides have not yet been conclusively identified in *C. elegans*; however, *C. elegans* has enzymes capable of synthesizing (Keller et al. 2014) and breaking down (Harris et al. 2008) these epoxides. TRP channels could complement cannabinoid receptors in this system, with AEA and endocannabinoid epoxides acting at both TRP channels and endocannabinoid receptors to mediate antinociception.

3.3.3.4. *Hedgehog*

Maza et al. (2022) identified the G protein-coupled Hedgehog receptor *ptr-25* and its human orthologue *PTCHD1* as being involved in mediating tolerance to opioids. Hedgehog signalling (through the more well-characterized orthologues of *PTCH1*) has been previously implicated in the development of opioid tolerance in mice (Liu et al. 2018) and in rats (Babcock et al. 2011), which Maza et al. (2022) were able to recapitulate in their opioid tolerance model. It is important to note that *C. elegans* lacks direct orthologues to Sonic Hedgehog (*SHH*) and to Smoothened (*SMO*), yet Hedgehog-related proteins (with a conserved HOG domain, and a domain functionally similar to Hedge) are still involved in many developmental processes (Zugasti et al. 2005). As shown by Maza et al. (2022), despite these differences in protein sequence, transgenic expression of a human Hedgehog receptor (*PTCHD1*) in *C. elegans* can fulfill the role of a nematode patched-related receptor in the

development of opioid tolerance; overexpression of *ptr-25* in HEK293T cells also resulted in opioid-induced MOR internalization. *Caenorhabditis elegans* has 60 Hedgehog-related proteins (Serra et al. 2024); identifying which ones are involved in nematode opioid signalling presents a possible avenue for further study.

3.3.3.5. *Opioid peptides*

Multiple studies have identified and employed NPR-17 as a nematode opioid receptor (Cheong et al. 2015; Mills et al. 2016), with neuropeptide-like peptides derived from NLP-24 and NLP-3 as its agonists. These neuropeptides have conserved YGG- sequences, also found in mammalian opioid peptides. Interestingly, treatment with the peptide products of NLP-24 containing YGGY- sequences, which were able to activate human MOR and KOR in HEK293T cells, failed to elicit a response from nematode NPR-17, while the GPYGYG- containing peptide did elicit a response (Cheong et al. 2015). This indicates that, while these peptides share a potentially conserved sequence, the NPR-17 receptor itself is sufficiently different to human MOR and KOR that it responds to different endogenous ligands. However, the NPR-17 receptor has been shown to be similar enough to human opioid receptors that a knocked-in human MOR has been shown to rescue specific *npr-17* null phenotypes in the context of food preference (Kim et al. 2024). Pop et al. (2024) also identified the predicted opioid peptide NLP-27, involved in the nematode response to fungal infection, which has a similar conserved YGGYG- sequence; however, this peptide has yet to be established as an NPR-17 agonist.

4. Conclusion

The genetic tractability, simple nervous system, and varied, quantifiable behaviours enable the use of *C. elegans* for modelling cannabinoid- or opioid-dependent antinociception as well as more complex phenomena arising from that, such as tolerance. As a model organism, *C. elegans* possesses endogenous cannabinoid- and opioid-like signalling; if endogenous signalling is insufficient, expression of mammalian receptors behind selective promoters has been shown to rescue endogenous signalling and/or add additional functionality in modelling complex phenomena. Monoaminergic neurotransmission is required for many cannabinoid- or opioid- induced changes in nociception, locomotion, and feeding, while less well-studied pathways such as the Hedgehog signalling pathway contribute to the development of tolerance.

Forward RNAi and mutagenesis screens have identified novel genes associated with opioid effectiveness and tolerance; the study of nematode cannabinoid signalling could benefit from forward genetic approaches to cannabinoid effects in worms. Additional efforts could be directed to translating insights gained in *C. elegans* to humans through further use of transgenes. *Caenorhabditis elegans* has proven itself as a model for nociception, antinociception, and drug tolerance with cannabinoids and opioids. Further research can expand upon this, using *C. elegans* to model other pain-related processes.

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References

Abdollahi, M., Castaño, J.D., Salem, J.B., and Beaudry, F. 2024. Anandamide modulates thermal avoidance in Caenorhabditis elegans through vanilloid and cannabinoid receptor interplay. Neurochem. Res. **49**: 2423–2439. doi:10.1007/s11064-024-04186-w.

Aguiar, D.D., Petrocchi, J.A., da Silva, G.C., Lemos, V.S., e Castor, M.G.M., de Castro Perez, A., et al. 2024. Participation of the cannabinoid system and the NO/cGMP/KATP pathway in serotonin-induced peripheral antinociception. Neurosci. Lett. **818**: 137536. doi:10.1016/j.neulet.2023.137536.

Altenhoff, A.M., Škunca, N., Glover, N., Train, C.-M., Sueki, A., Piližota, I., et al. 2015. The OMA orthology database in 2015: function predictions, better plant support, synteny view and other improvements. Nucleic Acids Res. **43**: D240–D249. doi:10.1093/nar/gku1158.

Altenhoff, A.M., Warwick Vesztrocy, A., Bernard, C., Train, C.-M., Nicheperovich, A., Prieto Baños, S., et al. 2024. OMA orthology in 2024: improved prokaryote coverage, ancestral and extant GO enrichment, a revamped synteny viewer and more in the OMA Ecosystem. Nucleic Acids Res. **52**: D513–D521. doi:10.1093/nar/gkad1020.

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D.J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. **25**: 3389–3402. doi:10.1093/nar/25.17.3389.

Arnold, W.R., Carnevale, L.N., Xie, Z., Baylon, J.L., Tajkhorshid, E., Hu, H., and Das, A. 2021. Anti-inflammatory dopamine-and serotonin-based endocannabinoid epoxides reciprocally regulate cannabinoid receptors and the TRPV1 channel. Nat. Commun. **12**: 926. doi:10.1038/s41467-021-20946-6.

Avery, L. 1993. The genetics of feeding in Caenorhabditis elegans. Genetics, **133**: 897–917. doi:10.1093/genetics/133.4.897.

Babcock, D.T., Shi, S., Jo, J., Shaw, M., Gutstein, H.B., and Galko, M.J. 2011. Hedgehog signaling regulates nociceptive sensitization. Curr. Biol. **21**: 1525–1533. doi:10.1016/j.cub.2011.08.020.

Bargmann, C.I. 1998. Neurobiology of the *Caenorhabditis elegans* genome. Science, **282**: 2028–2033. doi:10.1126/science.282.5396.2028.

Barrett, M.L., Scutt, A.M., and Evans, F.J. 1986. Cannflavin A and B, prenylated flavones fromCannabis sativa L. Experientia, **42**: 452–453. doi:10.1007/BF02118655.

Benarroch, E.E. 2012. Endogenous opioid systems: current concepts and clinical correlations. Neurology, **79**: 807–814. doi:10.1212/WNL.0b013e3182662098.

Berridge, K.C., and Kringelbach, M.L. 2015. Pleasure systems in the brain. Neuron, **86**: 646–664. doi:10.1016/j.neuron.2015.02.018.

Boujenoui, F., Nkambeu, B., Salem, J.B., Castano Uruena, J.D., and Beaudry, F. 2024. Cannabidiol and tetrahydrocannabinol antinociceptive activity is mediated by distinct receptors in Caenorhabditis elegans. Neurochem. Res. **49**: 935–948. doi:10.1007/s11064-023-04069-6.

Brownstein, M.J. 1993. A brief history of opiates, opioid peptides, and opioid receptors. Proc. Natl. Acad. Sci. **90**: 5391–5393. doi:10.1073/pnas.90.12.5391.

Chase, D.L., Pepper, J.S., and Koelle, M.R. 2004. Mechanism of extrasynaptic dopamine signaling in Caenorhabditis elegans. Nat. Neurosci. **7**: 1096–1103. doi:10.1038/nn1316.

Cheong, M.C., Artyukhin, A.B., You, Y.-J., and Avery, L. 2015. An opioid-like system regulating feeding behavior in C. elegans. eLife, **4**: e06683. doi:10.7554/eLife.06683.

Cheung, B.H., Arellano-Carbajal, F., Rybicki, I., and de Bono, M. 2004. Soluble guanylate cyclases act in neurons exposed to the body fluid to promote *C. elegans* aggregation behavior. Curr. Biol. **14**: 1105–1111. doi:10.1016/j.cub.2004.06.027.

Choi, Y.S., and Billings, J.A. 2002. Opioid antagonists: a review of their role in palliative care, focusing on use in opioid-related constipation. J. Pain Symptom Manage. **24**: 71–90. doi:10.1016/S0885-3924(02)00424-4.

Colbert, H.A., Smith, T.L., and Bargmann, C.I. 1997. OSM-9, A novel protein with structural similarity to channels, is required for olfaction, mechanosensation, and olfactory adaptation incaenorhabditis elegans. J. Neurosci. **17**: 8259–8269. doi:10.1523/JNEUROSCI.17-21-08259.1997.

Corbett, A.D., Henderson, G., McKnight, A.T., and Paterson, S.J. 2006. 75 years of opioid research: the exciting but vain quest for the Holy Grail. Br. J. Pharmacol. **147**. doi:10.1038/sj.bjp.0706435.

Cosentino, S., Sriswasdi, S., and Iwasaki, W. 2024. SonicParanoid2: fast, accurate, and comprehensive orthology inference with machine learning and language models. Genome Biol. **25**: 195. doi:10.1186/s13059-024-03298-4.

D'Addario, C., Di Benedetto, M., Izenwasser, S., Candeletti, S., and Romualdi, P. 2007. Role of serotonin in the regulation of the dynorphinergic system by a κ-opioid agonist and cocaine treatment in rat CNS. Neuroscience, **144**: 157–164.

Devane, W.A., Hanuš, L., Breuer, A., Pertwee, R.G., Stevenson, L.A., Griffin, G., et al. 1992. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science, **258**: 1946–1949. doi:10.1126/science.1470919.

Di Marzo, V., Goparaju, S.K., Wang, L., Liu, J., Bátkai, S., Járai, Z., et al. 2001. Leptin-regulated endocannabinoids are involved in maintaining food intake. Nature, **410**: 822–825. doi:10.1038/35071088.

Dowell, D. 2022. CDC Clinical Practice Guideline for prescribing opioids for pain—United States, 2022. MMWR Recomm. Rep. **71**:1–95. doi:10.15585/mmwr.rr7103a1.

Duerr, J.S., Frisby, D.L., Gaskin, J., Duke, A., Asermely, K., Huddleston, D., et al. 1999. The cat-1 gene of Caenorhabditis elegansEncodes a vesicular monoamine transporter required for specific monoamine-dependent behaviors. J. Neurosci. **19**: 72–84. doi:10.1523/JNEUROSCI.19-01-00072.1999.

Dyer, S.C., Austine-Orimoloye, O., Azov, A.G., Barba, M., Barnes, I., Barrera-Enriquez, V.P., et al. 2025. Ensembl 2025. Nucleic Acids Res. **53**: D948–D957. doi:10.1093/nar/gkae1071.

Emms, D.M., and Kelly, S. 2019. OrthoFinder: phylogenetic orthology inference for comparative genomics. Genome Biol. **20**: 238. doi:10.1186/s13059-019-1832-y.

Fields, H.L., and Margolis, E.B. 2015. Understanding opioid reward. Trends Neurosci. **38**: 217–225. doi:10.1016/j.tins.2015.01.002.

Fisher, E., Moore, R.A., Fogarty, A.E., Finn, D.P., Finnerup, N.B., Gilron, I., et al. 2021. Cannabinoids, cannabis, and cannabis-based medicine for pain management: a systematic review of randomised controlled trials. Pain, **162**: S45–S66. doi:10.1097/j.pain.0000000000001929.

Fuentes, D., Molina, M., Chorostecki, U., Capella-Gutiérrez, S., Marcet-Houben, M., and Gabaldon, T. 2022. PhylomeDB V5: an expanding repository for genome-wide catalogues of annotated gene phylogenies. Nucleic Acids Res. **50**: D1062–D1068. doi:10.1093/nar/gkab966.

García-Garví, A., and Sánchez-Salmerón, A.-J. 2025. High-throughput behavioral screening in Caenorhabditis elegans using machine learning for drug repurposing. Sci. Rep. **15**: 26140.

Government of Canada. 2025. Key findings: opioid- and stimulant-related harms in Canada. Public Health Agency of Canada. Available from https://health-infobase.canada.ca/substance-related-harms/opioids-stimulants/ [accessed 30 September 2025].

Gray, J.M., Karow, D.S., Lu, H., Chang, A.J., Chang, J.S., Ellis, R.E., et al. 2004. Oxygen sensation and social feeding mediated by a C. elegans guanylate cyclase homologue. Nature, **430**: 317–322. doi:10.1038/nature02714.

Guo, M., Wu, T.-H., Song, Y.-X., Ge, M.-H., Su, C.-M., Niu, W.-P., et al. 2015. Reciprocal inhibition between sensory ASH and ASI neurons modulates nociception and avoidance in *Caenorhabditis elegans*. Nat. Commun. **6**: 5655. doi:10.1038/ncomms6655.

Hamdan, F.F., Ungrin, M.D., Abramovitz, M., and Ribeiro, P. 1999. Characterization of a novel serotonin receptor from *Caenorhabditis elegans*: cloning and expression of two splice variants. J. Neurochem. **72**: 1372–1383. doi:10.1046/j.1471-4159.1999.721372.x.

Hapiak, V., Summers, P., Ortega, A., Law, W.J., Stein, A., and Komuniecki, R. 2013. Neuropeptides amplify and focus the monoaminergic inhibition of nociception in *Caenorhabditis elegans*. J. Neurosci. **33**: 14107–14116. doi:10.1523/JNEUROSCI.1324-13.2013.

Harris, G., Mills, H., Wragg, R., Hapiak, V., Castelletto, M., Korchnak, A., and Komuniecki, R.W. 2010. The monoaminergic modulation of sensory-mediated aversive responses in *Caenorhabditis elegans* requires glutamatergic/peptidergic cotransmission. J. Neurosci. **30**: 7889–7899. doi:10.1523/JNEUROSCI.0497-10.2010.

Harris, T.R., Aronov, P.A., Jones, P.D., Tanaka, H., Arand, M., and Hammock, B.D. 2008. Identification of two epoxide hydrolases in *Caenorhabditis elegans* that metabolize mammalian lipid signaling molecules. Arch. Biochem. Biophys. **472**: 139–149. doi:10.1016/j.abb.2008.01.016.

Hart, A.C. 2006. Behavior. WormBook, In: WormBook: The Online Review of C. Elegans Biology [Internet]. WormBook. doi:10.1895/wormbook.1.87.1.

Husson, S.J., Clynen, E., Baggerman, G., Janssen, T., and Schoofs, L. 2006. Defective processing of neuropeptide precursors in *Caenorhabditis elegans* lacking proprotein convertase 2 (KPC-2/EGL-3): mutant analysis by mass spectrometry. J. Neurochem. **98**: 1999–2012. doi:10.1111/j.1471-4159.2006.04014.x.

Husson, S.J., Costa, W.S., Wabnig, S., Stirman, J.N., Watson, J.D., Spencer, W.C., et al. 2012. Optogenetic analysis of a nociceptor neuron and network reveals ion channels acting downstream of primary sensors. Curr. Biol. **22**: 743–752. doi:10.1016/j.cub.2012.02.066.

Ide, S., Kunitomo, H., Iino, Y., and Ikeda, K. 2022. Caenorhabditis elegans exhibits morphine addiction-like behavior via the opioid-like receptor NPR-17. Front. Pharmacol. **12**: 802701. doi:10.3389/fphar.2021.802701.

Isensee, J., Krahé, L., Moeller, K., Pereira, V., Sexton, J.E., Sun, X., et al. 2017. Synergistic regulation of serotonin and opioid signaling contributes to pain insensitivity in Na_v 1.7 knockout mice. Sci. Signal, **10**: eaah4874. doi:10.1126/scisignal.aah4874.

Janssen, T., Lindemans, M., Meelkop, E., Temmerman, L., and Schoofs, L. 2010. Coevolution of neuropeptidergic signaling systems: from worm to man. Ann. N.Y. Acad. Sci. **1200**: 1–14. doi:10.1111/j.1749-6632.2010.05506.x.

Janssen, T., Meelkop, E., Lindemans, M., Verstraelen, K., Husson, S.J., Temmerman, L., et al. 2008. Discovery of a cholecystokinin-gastrin-like signaling system in nematodes. Endocrinology, **149**: 2826–2839. doi:10.1210/en.2007-1772.

Jayanthi, L.D., Apparsundaram, S., Malone, M.D., Ward, E., Miller, D.M., Eppler, M., and Blakely, R.D. 1998. The *Caenorhabditis elegans* Gene*T23G5.5* encodes an antidepressant- and cocaine-sensitive dopamine transporter. Mol. Pharmacol. **54**: 601–609. doi:10.1016/S0026-895X(24)13059-3.

Jordan, A., and Glauser, D.A. 2023. Distinct clusters of human pain gene orthologs in Caenorhabditis elegans regulate thermo-nociceptive sensitivity and plasticity. Genetics **224**: iyad047. doi:10.1093/genetics/iyad047.

Jospin, M., Jacquemond, V., Mariol, M.-C., Ségalat, L., and Allard, B. 2002. The L-type voltage-dependent Ca2+ channel EGL-19 controls body wall muscle function in Caenorhabditis elegans. J. Cell Biol. **159**: 337–348. doi:10.1083/jcb.200203055.

Kaduk, M., and Sonnhammer, E. 2017. Improved orthology inference with hieranoid 2. Bioinformatics, **33**: 1154–1159. doi:10.1093/bioinformatics/btw774.

Kahn-Kirby, A.H., Dantzker, J.L.M., Apicella, A.J., Schafer, W.R., Browse, J., Bargmann, C.I., and Watts, J.L. 2004. Specific polyunsaturated fatty acids drive TRPV-dependent sensory signaling In vivo. Cell, **119**: 889–900. doi:10.1016/j.cell.2004.11.005.

Kaletta, T., and Hengartner, M.O. 2006. Finding function in novel targets: C. elegans as a model organism. Nat. Rev. Drug Discovery, **5**: 387–399. doi:10.1038/nrd2031.

Keating, C.D., Kriek, N., Daniels, M., Ashcroft, N.R., Hopper, N.A., Siney, E.J., et al. 2003. Whole-genome analysis of 60 G protein-coupled receptors in Caenorhabditis elegans by gene knockout with RNAi. Curr. Biol. **13**: 1715–1720. doi:10.1016/j.cub.2003.09.003.

Keller, J., Ellieva, A., Ma, D.K., Ju, J., Nehk, E., Konkel, A., et al. 2014. CYP-13A12 of the nematode Caenorhabditis elegans is a PUFA-epoxygenase involved in behavioural response to reoxygenation. Biochem. J. **464**: 61–71. doi:10.1042/BJ20140848.

Kember, R.L., Vickers-Smith, R., Xu, H., Toikumo, S., Niarchou, M., Zhou, H., et al. 2022. Cross-ancestry meta-analysis of opioid use disorder uncovers novel loci with predominant effects in brain regions associated with addiction. Nat. Neurosci. **25**: 1279–1287. doi:10.1038/s41593-022-01160-z.

Kim, A.T., Li, S., Kim, Y., You, Y.-J., and Park, Y. 2024. Food preference-based screening method for identification of effectors of substance use disorders using *Caenorhabditis elegans*. Life Sci. **345**: 122580. doi:10.1016/j.lfs.2024.122580.

Kim, W., Underwood, R.S., Greenwald, I., and Shaye, D.D. 2018. OrthoList 2: a new comparative genomic analysis of human and Caenorhabditis elegans genes. Genetics, **210**: 445–461. doi:10.1534/genetics.118.301307.

Kiyama, Y., Miyahara, K., and Ohshima, Y. 2012. Active uptake of artificial particles in the nematode Caenorhabditis elegans. J. Exp. Biol. **215**: 1178–1183. doi:10.1242/jeb.067199.

Lahaise, M., Boujenoui, F., and Beaudry, F. 2024. Cannflavins isolated from Cannabis sativa impede Caenorhabditis elegans response to noxious heat. Naunyn Schmiedeberg's Arch. Pharmacol. **397**: 535–548. doi:10.1007/s00210-023-02621-3.

Lehtonen, M., Reisner, K., Auriola, S., Wong, G., and Callaway, J.C. 2008. Mass-spectrometric identification of anandamide and 2-arachidonoylglycerol in nematodes. Chem. Biodiversity **5**: 2431–2441. doi:10.1002/cbdv.200890208.

Levey, D.F., Galimberti, M., Deak, J.D., Wendt, F.R., Bhattacharya, A., Koller, D., et al. 2023. Multi-ancestry genome-wide association study of cannabis use disorder yields insight into disease biology and public health implications. Nat. Genet. **55**: 2094–2103. doi:10.1038/s41588-023-01563-z.

Li, C., Nelson, L.S., Kim, K., Nathoo, A., and Hart, A.C. 1999. Neuropeptide gene families in the nematode Caenorhabditis elegans. Ann. N.Y. Acad. Sci. **897**: 239–252. doi:10.1111/j.1749-6632.1999.tb07895.x.

Li, L., Stoeckert, C.J., and Roos, D.S. 2003. OrthoMCL: identification of ortholog groups for eukaryotic genomes. Genome Res. **13**: 2178–2189. doi:10.1101/gr.1224503.

Li, W., Feng, Z., Sternberg, P.W., and Shawn Xu, X.Z. 2006. A C. elegans stretch receptor neuron revealed by a mechanosensitive TRP channel homologue. Nature **440**: 684–687. doi:10.1038/nature04538.

Li, Y., Chen, X., Nie, Y., Tian, Y., Xiao, X., and Yang, F. 2021. Endocannabinoid activation of the TRPV1 ion channel is distinct from activation by capsaicin. J. Biol. Chem. **297**: 101022. doi:10.1016/j.jbc.2021.101022.

Linard, B., Allot, A., Schneider, R., Morel, C., Ripp, R., Bigler, M., et al. 2015. OrthoInspector 2.0: software and database updates. Bioinformatics, **31**: 447–448. doi:10.1093/bioinformatics/btu642.

Lints, R., and Emmons, S.W. 1999. Patterning of dopaminergic neurotransmitter identity among Caenorhabditis elegans ray sensory neurons by a tgfβfamily signaling pathway and a Hox gene. Development, **126**: 5819–5831. doi:10.1242/dev.126.24.5819.

Liu, Q.-R., Canseco-Alba, A., Zhang, H.-Y., Tagliaferro, P., Chung, M., Dennis, E., et al. 2017. Cannabinoid type 2 receptors in dopamine neurons inhibits psychomotor behaviors, alters anxiety, depression and alcohol preference. Sci. Rep. **7**: 17410. doi:10.1038/s41598-017-17796-y.

Liu, S., Yao, J.-L., Wan, X.-X., Song, Z.-J., Miao, S., Zhao, Y., et al. 2018. Sonic hedgehog signaling in spinal cord contributes to morphine-induced hyperalgesia and tolerance through upregulating brain-derived neurotrophic factor expression. J. Pain Res. **11**: 649–659. doi:10.2147/JPR.S153544.

Lochrie, M.A., Mendel, J.E., Sternberg, P.W., and Simon, M.I. 1991. Homologous and unique G protein alpha subunits in the nematode Caenorhabditis elegans. Cell Regul. **2**: 135–154. doi:10.1091/mbc.2.2.135.

Margie, O., Palmer, C., and Chin-Sang, I. 2013. C. elegans chemotaxis assay. J. Vis. Exp. 50069.

Maza, N., Wang, D., Kowalski, C., Stoveken, H.M., Dao, M., Sial, O.K., et al. 2022. Ptchd1 mediates opioid tolerance via cholesterol-dependent effects on μ-opioid receptor trafficking. Nat. Neurosci. **25**: 1179–1190. doi:10.1038/s41593-022-01135-0.

Mechoulam, R., Ben-Shabat, S., Hanus, L., Ligumsky, M., Kaminski, N.E., Schatz, A.R., et al. 1995. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem. Pharmacol. **50**: 83–90. doi:10.1016/0006-2952(95)00109-D.

Mills, H., Ortega, A., Law, W., Hapiak, V., Summers, P., Clark, T., and Komuniecki, R. 2016. Opiates modulate noxious chemical nociception through a complex monoaminergic/peptidergic cascade. J. Neurosci. **36**: 5498–5508. doi:10.1523/JNEUROSCI.4520-15.2016.

Nathoo, A.N., Moeller, R.A., Westlund, B.A., and Hart, A.C. 2001. Identification of neuropeptide-like protein gene families in Caenorhabditis elegans and other species. Proc. Natl. Acad. Sci. **98**: 14000–14005. doi:10.1073/pnas.241231298.

Nevers, Y., Kress, A., Defosset, A., Ripp, R., Linard, B., Thompson, J.D., et al. 2019. OrthoInspector 3.0: open portal for comparative genomics. Nucleic Acids Res. **47**: D411–D418. doi:10.1093/nar/gky1068.

Nielsen, S., Picco, L., Murnion, B., Winters, B., Matheson, J., Graham, M., et al. 2022. Opioid-sparing effect of cannabinoids for analgesia: an

updated systematic review and meta-analysis of preclinical and clinical studies. Neuropsychopharmacology, **47**: 1315–1330. doi:10.1038/s41386-022-01322-4.

Nieto-Fernandez, F., Andrieux, S., Idrees, S., Bagnall, C., Pryor, S.C., and Sood, R. 2009. The effect of opioids and their antagonists on the nociffensive response of Caenorhabditis elegans to noxious thermal stimuli. Invert. Neurosci. **9**: 195–200. doi:10.1007/s10158-010-0099-5.

Noor, A., Whibley, A., Marshall, C.R., Gianakopoulos, P.J., Piton, A., Carson, A.R., et al. 2010. Disruption at the *PTCHD1* locus on Xp22.11 in Autism spectrum disorder and intellectual disability. Sci. Transl. Med. **2**. doi:10.1126/scitranslmed.3001267.

Oakes, M., Law, W.J., and 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.

Oakes, M.D., Law, W.J., Clark, T., Bamber, B.A., and 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.

Olde, B., and McCombie, W.R., 1997. Molecular cloning and functional expression of a serotonin receptor fromCaenorhabditis elegans. J. Mol. Neurosci. **8**: 53–62. doi:10.1007/BF02736863.

Pastuhov, S.Iv., Matsumoto, K., and 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.

Persson, E., and Sonnhammer, E.L., 2023. InParanoiDB 9: ortholog groups for protein domains and full-length proteins. J. Mol. Biol. **435**: 168001. doi:10.1016/j.jmb.2023.168001.

Peters, K.Z., Cheer, J.F., and Tonini, R., 2021. Modulating the neuromodulators: dopamine, serotonin, and the endocannabinoid system. Trends Neurosci. **44**: 464–477. doi:10.1016/j.tins.2021.02.001.

Piomelli, D., Tarzia, G., Duranti, A., Tontini, A., Mor, M., Compton, T.R., et al. 2006. Pharmacological profile of the selective FAAH inhibitor KDS-4103 (URB597). CNS Drug Rev. **12**: 21–38. doi:10.1111/j.1527-3458.2006.00021.x.

Pisanti, S., and Bifulco, M., 2019. Medical *Cannabis* : a plurimillennial history of an evergreen. J. Cell. Physiol. **234**: 8342–8351. doi:10.1002/jcp.27725.

Pop, M., Klemke, A.-L., Seidler, L., Wernet, N., Steudel, P.L., Baust, V., et al. 2024. Caenorhabditis elegans neuropeptide NLP-27 enhances neurodegeneration and paralysis in an opioid-like manner during fungal infection. iScience **27**: 109484. doi:10.1016/j.isci.2024.109484.

Porter, M.Y., and Koelle, M.R. 2010. RSBP-1 is a membrane-targeting subunit required by the gα_q -specific but not the gα_o -specific R7 regulator of G protein signaling in *Caenorhabditis elegans*. Mol. Biol. Cell **21**: 232–243. doi:10.1091/mbc.e09-07-0642.

Raja, S.N., Carr, D.B., Cohen, M., Finnerup, N.B., Flor, H., Gibson, S., et al. 2020. The revised International Association for the Study of Pain definition of pain: concepts, challenges, and compromises. Pain, **161**: 1976–1982. doi:10.1097/j.pain.0000000000001939.

Ranganathan, R., Cannon, S.C., and Horvitz, H.R. 2000. MOD-1 is a serotonin-gated chloride channel that modulates locomotory behaviour in C. elegans. Nature, **408**: 470–475. doi:10.1038/35044083.

Ranganathan, R., Sawin, E.R., Trent, C., and Horvitz, H.R. 2001. Mutations in the Caenorhabditis elegans serotonin reuptake transporter MOD-5 reveal serotonin-dependent and -independent activities of fluoxetine. J. Neurosci. **21**: 5871–5884. doi:10.1523/JNEUROSCI.21-16-05871.2001.

Rex, E., and Komuniecki, R.W. 2002. Characterization of a tyramine receptor from Caenorhabditis elegans. J. Neurochem. **82**: 1352–1359. doi:10.1046/j.1471-4159.2002.01065.x.

Roeder, T. 1999. Octopamine in invertebrates. Prog. Neurobiol. **59**: 533–561. doi:10.1016/S0301-0082(99)00016-7.

Sasaki, M., Kamiya, Y., Bamba, K., Onishi, T., Matsuda, K., Kohno, T., et al. 2021. Serotonin plays a key role in the development of opioid-induced hyperalgesia in mice. J. Pain, **22**: 715–729. doi:10.1016/j.jpain.2020.12.008.

Savitski, M.M., Reinhard, F.B.M., Franken, H., Werner, T., Savitski, M.F., Eberhard, D., et al. 2014. Tracking cancer drugs in living cells by thermal profiling of the proteome. Science, **346**: 1255784. doi:10.1126/science.1255784.

Sawin, E.R., Ranganathan, R., and Horvitz, H.R. 2000. C. elegans locomotory rate is modulated by the environment through a dopaminergic pathway and by experience through a serotonergic pathway. Neuron, **26**: 619–631. doi:10.1016/S0896-6273(00)81199-X.

Serra, N.D., Darwin, C.B., and Sundaram, M.V. 2024. Caenorhabditis elegans Hedgehog-related proteins are tissue- and substructure-specific components of the cuticle and precuticle. Genetics, **227**: iyae081. doi:10.1093/genetics/iyae081.

Sojka, S.E., Ezak, M.J., Polk, E.A., Bischer, A.P., Neyland, K.E., Wojtovich, A.P., and Ferkey, D.M. 2025. An extensive gap junction neural network modulates Caenorhabditis elegans aversive behavior. Genes, **16**: 260. doi:10.3390/genes16030260.

Soliman, N., Haroutounian, S., Hohmann, A.G., Krane, E., Liao, J., Macleod, M., et al. 2021. Systematic review and meta-analysis of cannabinoids, cannabis-based medicines, and endocannabinoid system modulators tested for antinociceptive effects in animal models of injury-related or pathological persistent pain. Pain, **162**: S26–S44. doi:10.1097/j.pain.0000000000002269.

Sonnhammer, E.L., and Östlund, G. 2015. InParanoid 8: orthology analysis between 273 proteomes, mostly eukaryotic. Nucleic Acids Res. **43**: D234–D239. doi:10.1093/nar/gku1203.

Sugiura, M., Fuke, S., Suo, S., Sasagawa, N., Van Tol, H.H.M., and Ishiura, S. 2005. Characterization of a novel D2-like dopamine receptor with a truncated splice variant and a D1-like dopamine receptor unique to invertebrates from *Caenorhabditis elegans*. J. Neurochem. **94**: 1146–1157. doi:10.1111/j.1471-4159.2005.03268.x.

Suo, S., Sasagawa, N., and Ishiura, S. 2002. Identification of a dopamine receptor from *Caenorhabditis elegans*. Neurosci. Lett. **319**: 13–16. doi:10.1016/S0304-3940(01)02477-6.

Swierczek, N.A., Giles, A.C., Rankin, C.H., and Kerr, R.A. 2011. High-throughput behavioral analysis in C. elegans. Nat. Methods, **8**: 592–598. doi:10.1038/nmeth.1625.

Sze, J.Y., Victor, M., Loer, C., Shi, Y., and Ruvkun, G. 2000. Food and metabolic signalling defects in a Caenorhabditis elegans serotonin-synthesis mutant. Nature, **403**: 560–564. doi:10.1038/35000609.

The Alliance of Genome Resources Consortium. 2024. Updates to the Alliance of Genome Resources Central Infrastucture. Genetics, **227**: iyae049. doi:10.1093/genetics/iyae049.

Thomas, P.D., Ebert, D., Muruganujan, A., Mushayahama, T., Albou, L., and Mi, H. 2022. PANTHER : making genome-scale phylogenetics accessible to all. Protein Sci. **31**: 8–22. doi:10.1002/pro.4218.

Tobin, D.M., Madsen, D.M., Kahn-Kirby, A., Peckol, E.L., Moulder, G., Barstead, R., et al. 2002. Combinatorial expression of TRPV channel proteins defines their sensory functions and subcellular localization in C. elegans neurons. Neuron, **35**: 307–318. doi:10.1016/S0896-6273(02)00757-2.

Uttam, S., Wong, C., Price, T.J., and Khoutorsky, A. 2018. eIF4E-dependent translational control: a central mechanism for regulation of pain plasticity. Front. Genet. **9**: 470. doi:10.3389/fgene.2018.00470.

Vilella, A.J., Severin, J., Ureta-Vidal, A., Heng, L., Durbin, R., and Birney, E. 2009. EnsemblCompara GeneTrees: complete, duplication-aware phylogenetic trees in vertebrates. Genome Res. **19**: 327–335. doi:10.1101/gr.073585.107.

Wang, D., Stoveken, H.M., Zucca, S., Dao, M., Orlandi, C., Song, C., et al. 2019. Genetic behavioral screen identifies an orphan anti-opioid system. Science, **365**: 1267–1273. doi:10.1126/science.aau2078.

Wheeler, D.L., Barrett, T., Benson, D.A., Bryant, S.H., Canese, K., Chetvernin, V., et al. 2007. Database resources of the national center for biotechnology information. Nucleic Acids Res. **36**: D13–D21. doi:10.1093/nar/gkm1000.

White, J.G., Southgate, E., Thomson, J.N., and Brenner, S. 1986. The structure of the nervous system of the nematode Caenorhabditis elegans: the mind of a worm. Phil. Trans. R. Soc. Lond. **314**: 340.

Wittenburg, N., and Baumeister, R. 1999. Thermal avoidance in Caenorhabditis elegans: an approach to the study of nociception. Proc. Natl. Acad. Sci. **96**: 10477–10482. doi:10.1073/pnas.96.18.10477.

Wragg, R.T., Hapiak, V., Miller, S.B., Harris, G.P., Gray, J., Komuniecki, P.R., and Komuniecki, R.W. 2007. Tyramine and octopamine independently inhibit serotonin-stimulated aversive behaviors in Caenorhabditis elegans through two novel Amine receptors. J. Neurosci. **27**: 13402–13412. doi:10.1523/JNEUROSCI.3495-07.2007.

Wu, J.-J., Yin, S.-W., Liu, H., Li, R., Huang, J.-H., Wang, P.-Z., et al. 2022. Positive interaction between ASH and ASK sensory neurons accelerates nociception and inhibits behavioral adaptation. iScience, **25**: 105287. doi:10.1016/j.isci.2022.105287.

Xu, S.-Z., Zeng, F., Boulay, G., Grimm, C., Harteneck, C., and Beech, D.J. 2005. Block of TRPC5 channels by 2-aminoethoxydiphenyl borate: a differential, extracellular and voltage-dependent effect. Br. J. Pharmacol. **145**: 405–414. doi:10.1038/sj.bjp.0706197.

Yeh, T.L., Chen, K.C., Lin, S.-H., Lee, I.H., Chen, P.S., Yao, W.J., et al. 2012. Availability of dopamine and serotonin transporters in opioid-dependent users—a two-isotope SPECT study. Psychopharmacology (Berl.) **220**: 55–64. doi:10.1007/s00213-011-2454-6.

Zagon, I.S., Verderame, M.F., Allen, S.S., and McLaughlin, P.J. 2000. Cloning, sequencing, chromosomal location, and function of cDNAs encoding an opioid growth factor receptor (OGFr) in humans. Brain Res. **856**: 75–83. doi:10.1016/S0006-8993(99)02330-6.

Zugasti, O., Rajan, J., and Kuwabara, P.E. 2005. The function and expansion of the Patched- and Hedgehog-related homologs in *C. elegans*. Genome Res. **15**: 1402–1410. doi:10.1101/gr.3935405.