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Worms get the munchies: the endocannabinoid AEA induces hedonic amplification in *C. elegans* by modulating the activity of the AWC chemosensory neuron.

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Abstract

The mammalian endocannabinoid system, comprised of the endocannabinoids AEA (N-arachidonoyl-ethanolamine) and 2-AG (2-Arachidonoylglycerol), their receptors, CB1 and CB2, and their metabolic enzymes, is believed to integrate internal energy state and external food cues to modulate feeding. For example, cannabinoids can increase preference for more palatable, calorically dense food: a response called *hedonic amplification*, colloquially known as "the munchies." In mammals, cannabinoids can increase sensitivity to odors and sweet tastes, which may underlie amplification. We use *C. elegans*, an omnivorous bacterivore, as a model in which to investigate the neurophysiology of hedonic amplification. We found that exposure to AEA increases the worms' preference for strongly preferred (more palatable) bacteria over weakly preferred (less palatable) bacteria, mimicking hedonic amplification in mammals. Furthermore, AEA acts bidirectionally, increasing consumption of strongly preferred bacteria while decreasing consumption of weakly preferred bacteria. We also found that deletion of the putative CB1 homolog, *npr-19*, eliminates hedonic amplification, which can be rescued by expression of wild type *npr-19* or human CB1, establishing a humanized worm for cannabinoid signaling studies. Deletion of the olfactory neuron AWC, which directs chemotaxis to food, abolishes hedonic amplification. Consistent with this finding, calcium imaging revealed that AEA bidirectionally modulates AWC activity, increasing its responses to strongly preferred food and decreasing its response for weakly preferred food. In a GFP expression analysis, we found that *npr-19* is expressed in approximately 21 neuron classes but, surprisingly, not in AWC. Although AEA's effect could be mediated by NPR-19-expressing neurons presynaptic to AWC, nearly complete elimination of fast synaptic transmission, via the mutation *unc-13(e51)*, had no effect on modulation. Instead, it appears that AEA modulates AWC by activating one or more *npr-19*-expressing neurons that release a diffusible neuromodulator to which AWC is sensitive.

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Introduction

Maintenance of energy balance is critical to an animal's survival. An animal must take into account its hunger state and the available food in the environment to decide whether, where, and how much to feed. Integration of energy-state cues, chemosensory information about food, and food intake is mediated, in part, by the endocannabinoid system (ECS) (Matias & Di Marzo, 2007; Soria-Gómez et al., 2014). The mammalian ECS is composed of AEA (N-arachidonoylethanolamine) and 2-AG (2-Arachidonoylglycerol), their receptors, including CB1 and CB2, synthetic enzymes, and degradative enzymes. In humans, activation or blockade of cannabinoid receptors increases or decreases food intake, respectively (Colombo et al., 1998; Simiand et al., 1998). In addition to hyperphagia, cannabinoids have been shown to induce desire for highly palatable foods over calorically equivalent alternatives (Brown et al., 1977; Koch & Matthews, 2001). This effect, termed *hedonic amplification*, has been demonstrated in mice, rats, and primates, including humans (Arnone et al., 1997; Brown et al., 1977). Changes in feeding decisions may be due, in part, to cannabinoid modulation of sensory feeding cues, such as selectively increasing sensitivity to sweetness (Yoshida et al., 2010) or odor detection (Breunig et al., 2010; Soria-Gómez et al., 2014). However, much still remains to be learned about how cannabinoids mediate food choices.

A key challenge in studying cannabinoid signaling in the context of behavior, including food-choice, derives from the wide distribution of cannabinoid receptors across many interconnected brain areas. For example, cannabinoid receptors have been found at axon terminals of cortical glutamatergic neurons that project to the olfactory bulb, intrinsic cells in the olfactory bulb (Soria-Gómez et al., 2014), and type II taste cells (Yoshida et al., 2010). However, they are also found in the neocortex, pyriform cortex, hippocampus, amygdala, basal ganglia, thalamic nuclei, cerebellar cortex, and brainstem nuclei (Piomelli, 2003), and crosstalk between these areas and primary sensory neurons limits our ability to study how the initial sensory signal is altered by cannabinoids and propagated to downstream areas to modulate behavior.

To overcome this difficulty, we utilize the nematode *C. elegans* to study the effect of endocannabinoids on appetite. *C. elegans* has extensive homologies to human genes, including almost all the major components of the ECS, such as the main mammalian endocannabinoids AEA and 2-AG (Higgs et al., 2003; Lehtonen et al., 2008, 2011; Sugiura et al., 1995), their degradative enzymes, FAAH and MAGL (Y97E10AL.2 in worms) (Oakes et al., 2017) and, synthetic enzymes, NAPE-1 and DAGL (Harrison et al., 2014), respectively. In this study, we found that endocannabinoids induce hedonic amplification in *C. elegans*. Whereas *C. elegans* is

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an omnivorous bacterivore, chemotaxis assays have shown that, given a choice, it chooses certain bacteria species ("favored") over others ("non-favored") (Shtonda, 2006). We have found that exposure to the endocannabinoid AEA further increases the worm's attraction to favored food at the expense of non-favored food, indicating that *C. elegans* exhibits cannabinoid-mediated hedonic amplification, exactly as in mammals. Furthermore, the *C. elegans* receptor NPR-19, a putative homolog of CB1, was recently shown to function as a receptor for AEA and 2-AG in a heterologous expression system (Oakes et al., 2017). We now show that the *npr-19* gene is necessary for AEA's modulation of feeding decisions in the worm, and can be functionally replaced by the human CB1 gene. Finally, we show that cannabinoids modulate behavior by bidirectionally mediating activity of an olfactory neuron, AWC, in response to food, causing a stronger response to favored food and weaker response to non-favored food. This effect occurs via dense-core vesicle release from one or more *npr-19*-expressing neurons upstream of AWC. Together, our findings validate *C. elegans* as a model in which to study endocannabinoid modulation of food-seeking and appetitive behaviors, and provides insight into the role of chemosensation in cannabinoid-mediated changes in appetite.

Results

AEA alters feeding preferences in a manner resembling hedonic amplification. In humans and other mammals, cannabinoids have been shown not only to increase food intake, but also to selectively increase appetite for high-energy palatable food, a phenomenon known as hedonic amplification (Abel, 1975; Foltin et al., 1988; Mahler et al., 2007). Although *C. elegans* can use a wide variety of bacteria as a food source, it develops strong preferences early in life for species of bacteria that promote high population growth rates (Shtonda, 2006), henceforth "favored food."

C. elegans synthesizes at least seven different endocannabinoids, including the widely studied mammalian endocannabinoids *N*-arachidonoylethanolamine (AEA) and 2-Arachidonoylglycerol (2-AG). We pre-exposed adult N2 worms to AEA and measured food preferences utilizing a T-maze accumulation assay in which maze arms were baited with patches of favored and non-favored bacteria, *Comamonas* and *B. simplex*, respectively. Worms exposed to AEA exhibited a sustained increase in preference for favored bacteria (Fig. 1B, 1B') relative to unexposed controls. This increased preference could have resulted from increased accumulation of animals in favored food, decreased accumulation of animals in non-favored food, or both. Consistent with the latter, we found that AEA had a bidirectional effect on food accumulation,

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simultaneously increasing and decreasing the fraction of worms on the favored and non-favored food patches, respectively (Fig. 1C). Our ability to resolve preference changes was improved in a second set of experiments in which we adjusted the relative concentrations of favored and non-favored food such that worms unexposed to AEA were indifferent between the two offerings (Fig. 1D). Nevertheless, after exposure to AEA, worms still increased their preference for favored food (Fig. 1D). We replicated this experiment with a second pair of bacteria species, HB101 (favored) and DA837 (non-favored), and again found increased preference for favored food in AEA-exposed worms (Fig. 1E). Together, these data show that AEA causes a form of hedonic amplification in *C. elegans* that does not depend on particular bacteria concentrations or species; AEA reliably causes worms to more strongly prefer their favored food.

Is increased accumulation under the influence of AEA associated with increased consumption of favored food? *C. elegans* swallows bacteria by means of rhythmic contractions of its pharynx, a muscular pump comprising its throat. Feeding rate can be quantified by measuring the frequency of pharyngeal pumps recorded electrically in electropharyngeograms (Lockery et al., 2012; Raizen & Avery, 1994). *C. elegans* prefers familiar bacteria species to unfamiliar ones, and the preference is associated with higher feeding rate in familiar food (Song et al., 2013). Mirroring the effect of AEA on accumulation, AEA exposure had a bidirectional effect on feeding rate, increasing and decreasing the rate of consumption of favored and non-favored food, respectively (Fig. 2). Thus, AEA appears to drive worms to both seek and consume favored foods more avidly.

AEA affects feeding by modulating AWC activity. Worms locate and accumulate in food patches through chemotaxis driven by 11 pairs of chemosensory neurons (Zaslaver et al., 2015). Of particular interest is the chemosensory neuron pair AWC. These neurons are excited by the sudden absence of food-related odors (Chalasani et al., 2007) and, when activated, generate behaviors that promote return into the food patch, such as backward locomotion, turning, and head straightening (Chalasani et al., 2007; Gordus et al., 2015; Gray et al., 2005; Kocabas et al., 2012). We found that worms lacking AWC neurons due to a mutation in a homeobox gene (*ceh-36* allele) which blocks their terminal differentiation, failed to display increased accumulation in favored food in response to AEA (Fig. 3A). Although the baseline preference for favored food is higher in ceh-36 mutants than in wildtype N2 worms (compare Fig. 3A and Fig. 1D), the absence of an AEA effect is unlikely to be due to a ceiling effect, as preference for favored food can be increased beyond these levels (see Fig. 1B'). Similarly, these worms also failed to display decreased pumping frequency in non-favored food (Supp.

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Fig. 1). These results implicate AWC neurons in both aspects of hedonic amplification in *C. elegans*.

To assess AEA's effect on AWC, we used calcium imaging to record the activity of AWC in response to favored and non-favored food. As expected, we found that AWC neurons respond to the removal of both favored and non-favored food (Fig. 3B). Preincubation with AEA has a bidirectional effect on AWC, increasing its response to favored food removal while decreasing its response to non-favored food removal (Fig. 3B, 3B'). As AWC activation triggers reversals and backward locomotion (Chalasani et al., 2007), this bidirectional action on AWC responses would lead to the increased accumulation of worms on favored food and decreased accumulation on non-favored food observed in feeding preference assays (Fig. 1B - 1D), causing animals to be more strongly retained on favored food than non-favored food.

The NPR-19 receptor is required for hedonic amplification. Although *C. elegans* has some of the same key components as the mammalian endocannabinoid system, including the synthetic and degradative enzymes, it lacks clear mammalian cannabinoid receptor orthologs (McPartland & Glass, 2003; Pastuhov et al., 2012). However, NPR-19 has been shown to function as a receptor for the endocannabinoids AEA and 2-AG in *C. elegans*, generating endocannabinoid-dependent current in a heterologous expression system (Oakes et al., 2017). NPR-19 was also shown to be necessary for cannabinoid-dependent behaviors (Oakes et al., 2017; Pastuhov et al., 2016). When we tested *npr-19*(ok2068) null worms, AEA failed to increase worms' preference for favored food (Fig. 4A). The phenotype could be rescued by re-expression of *npr-19* under its own endogenous promoter (Fig. 4A). Additionally, the increased preference for favored food could also be rescued by replacing *npr-19* with the human CB1 receptor (Fig. 4A).

To explore whether the pathways involved in hedonic amplification observed in the preference and feeding rate assays overlapped, we tested the *npr-19* mutants in the feeding assay. The increased feeding rate following AEA exposure observed in the N2 reference worm strain in the presence of favored food was abolished in *npr-19* mutants and rescued by re-expression of *npr-19* but surprisingly not CB1 (Supp. Fig. 1). This suggests that as with hedonic amplification observed in preference assays, *npr-19* is necessary for this phenotype. The decreased feeding rate observed in presence of non-favored food was unaffected in *npr-19* mutants, suggesting that it relies on a different pathway.

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We then asked whether *npr-19* is also required for AEA's modulation of AWC. Using calcium imaging, we found that in the absence of *npr-19,* AEA fails to bidirectionally modulate AWC activity (Fig. 4B). This implies that AEA modulates feeding preferences by acting on a pathway that requires both the NPR-19 receptor and AWC neurons.

AEA acts on AWC indirectly. Since AEA-mediated hedonic amplification and AEA's effect on AWC both require the NPR-19 receptor, we explored whether AEA acts on AWC directly, by binding NPR-19 on AWC. To determine which cells express the *npr-19* receptor, we used a p*npr-19*::GFP transgene and co-expressed *cho-1*::mCherry and *eat-4*::mCherry, two neuronal markers whose expression has been previously characterized (Pereira et al., 2015; Serrano-Saiz et al., 2013). We found widespread expression of the receptor in body wall muscles, as well as in 29 neurons in the head (27 - 31, 95% confidence interval, n = 20 worms imaged) and 8 neurons in the tail (7.8 - 8.5, 95% confidence interval, n = 22 worms imaged) (Fig. 5A, Supp. Table 1). We were able to confidently identify 21 of these neurons, divided into 11 cell types, and infer another 9 neurons in 6 classes with slightly less confidence (Fig. 5A, Supp. Table 2). Overall, 28 of the *npr-19*-expressing neurons colocalized with either *cho-1* or *eat-4*, and ~9 did not colocalize with either marker. Neurons expressing *npr-19* can roughly be divided into four groups: the pharyngeal nervous system, sensory neurons, motor neurons, and interneurons. Of the 14 types of neurons in the pharyngeal nervous system, 6 to 8 express *npr-19*. This is consistent with our data showing that pharyngeal pumping is modulated by AEA (Fig. 2). In addition, 4 to 8 types of sensory neurons were also labeled by *npr-19*::GFP, with function spanning olfaction, gustation, thermosensation, O_2 sensation, and mechanosensation, suggesting that cannabinoids can modulate the processing of a vast variety of stimuli. Finally, a number of motor and interneurons involved in navigation express *npr-19*. For example, the RIA interneuron modulates locomotion when animals are searching for food (Fig. 5A, Supp. Table 2) (Gray et al., 2005).

Surprisingly, however, AWC does not appear to express *npr-19* (Fig. 5A), suggesting that AEA does not affect AWC's responses directly, but modifies the signal sent to AWC from an upstream neuron. To test this hypothesis, we imaged AWC responses to food with and without AEA exposure in two synaptic mutant strains: *unc-13(*e51*)* and *unc-31(*e928*)*. In both mutants, AWC neurons still displayed normal responses to the removal of both favored and non-favored bacteria (Fig. 5B, 5C). In *unc-13* mutants, in which classical synaptic transmission is impaired (Richmond et al., 1999), AEA still bidirectionally modulates AWC activity, increasing its response to the removal of favored food and decreasing its response to the removal of non-

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favored food (Fig. 5B). We conclude that AEA's effect on AWC responses to food is not mediated by classical synaptic transmission. In *unc-31* mutants, in which dense-core vesicles transmission is decreased (i.e. monoaminergic and peptidergic transmission) (Speese et al., 2007), AEA fails to increase AWC's response to the removal of favored food, and increases, rather than decreases AWC's response to the removal of non-favored food (Fig. 5C). Together, these data suggest that AEA acts on *npr-19*-expressing neurons that, via a peptidergic or aminergic pathway, alter AWC's response to food. This causes a stronger retention of worms on favored food and weaker retention on non-favored food, thereby producing hedonic amplification (Fig. 6).

Discussion

Phytocannabinoids, such as THC, are well-known for their recreational use and psychoactive effects. Phytocannabinoids act by taking advantage of our endogenous cannabinoid system, which is comprised of the endocannabinoids 2-arachidonoylglycerol (2-AG) and *N*-arachidonoylethanolamine (AEA), their synthetic and degradative enzymes, and their receptors. One of the better known effects of THC is its modulation of feeding. Cannabinoids are known to cause hyperphagia (Williams et al., 1998; Williams & Kirkham, 1999) and hedonic amplification, which is defined as selective preference for more palatable foods over calorically equivalent alternatives. Cannabinoid-mediated hedonic amplification has been observed in humans (Foltin et al., 1988; Roberts et al., 2019), rats (De Luca et al., 2012; DiPatrizio & Simansky, 2008; Higgs et al., 2003; Jarrett et al., 2005; Mahler et al., 2007), and mice (Barbano et al., 2009).

It was long assumed that the nematode *Caenorhabditis Elegans* did not have a working endocannabinoid system because, even though it synthesizes both AEA and 2-AG (Lehtonen et al., 2008), no receptor could be identified (McPartland & Glass, 2003). However, with the recent discovery that the receptor NPR-19 binds AEA and 2-AG and is required for cannabinoid-mediated effects on behavior (Oakes et al., 2017; Pastuhov et al., 2016), we now know that *C. elegans* has a functional endocannabinoid system.

In this work, we show that *C. elegans* exhibits cannabinoid-mediated hedonic amplification. Whereas *C. elegans* is an omnivorous bacterivore, it exhibits preferences for certain bacteria over others; specifically, it prefers food that best supports growth (Shtonda, 2006). It was previously shown that *Comamonas* and HB101 are highly favored foods, whereas *B. Simplex* and DA837 are liked but not as much as highly-favored bacteria (Shtonda, 2006). We also found that *C. elegans* preferred favored bacteria over the non-favored bacteria, but more

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interestingly, excitation of the endogenous cannabinoid system by exposure to AEA made those preferences more pronounced (Fig. 1B). Since the preference index is calculated as the difference between the number of worms in the two foods divided by the total number of worms in the two foods, and the assay allows worms to move freely between patches of food, this shift in preference could have occurred due to an increased number of worms in favored food, a decreased number of worms in non-favored food, or both. We found that AEA caused both a decrease in the number of worms in non-favored food and an increase in favored food (Fig. 1C). This remarkably resembles cannabinoid-mediated hedonic amplification, wherein an animal chooses the more palatable option over alternatives. Even when we altered bacterial concentration to drive the worms' preference between the favored and non-favored food to approximately equal (by increasing the density of non-favored bacteria and decreasing the density of favored bacteria), preference for the favored food increased after exposure to AEA (Fig. 1D). Further, this effect persisted for a different pair of favored and non-favored bacteria (Fig. 1E), showing that hedonic amplification is a robust effect of cannabinoid action in *C. elegans*.

In mammals, cannabinoids act through interactions with the cannabinoid GPCRs, CB1 and CB2 (Matsuda et al., 1990; Munro et al., 1993). Of all the GPCRs in *C. elegans*, NPR-19 has the most amount of amino acid conservation to the human CB1/2 receptors (McPartland et al., 2006; Oakes et al., 2017; Pastuhov et al., 2016). It has now been shown that in *C. elegans*, cannabinoids inhibit the axon regeneration and nociception by acting on NPR-19 (Oakes et al., 2017; Pastuhov et al., 2016). We sought to see if cannabinoids altered feeding decisions by acting on NPR-19. Indeed, worms lacking the receptor failed to increase their preference for favored food after AEA exposure (Fig. 4A). Importantly, we could rescue AEA's effect on food preferences by expressing the human CB1 receptor in place of the NPR-19 receptor, thereby confirming the functional homology of these receptors.

Wildtype *C. elegans* lives in rotting vegetation and feeds on the bacteria in that vegetation. It is likely to be exposed to many different types of bacteria at once and must make decisions regarding whether to feed in a patch of bacteria or to search for a different type of bacteria. Many of the worm's 302 neurons are known to contribute to these decisions, as they sense odors, tastes, osmolarity, and oxygen, and then promote either dwelling behaviors in food, searching behaviors outside of food, or chemotaxis toward food. One such neuron is the odor-sensing AWC. AWC is an OFF neuron, meaning that it is hyperpolarized by the presence of food and depolarized by the disappearance of food scent (Chalasani et al., 2007). When AWC

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is activated, it generates behaviors that promote return into the food patch, such as backward locomotion, turning, and head straightening (Chalasani et al., 2007; Gordus et al., 2015; Gray et al., 2005; Kocabas et al., 2012). Our data shows that worms require functional AWC neurons for AEA's effect on food preference (Fig. 3A). Further, AEA exposure bidirectionally modulated AWC's response to the removal of food, making it respond more strongly to the removal of favored food, and less strongly to the removal of non-favored food (Fig. 3B). This is not the first time that sensory neurons have been implicated in the action of cannabinoids and their effect on feeding; previous work in mice has shown that endocannabinoids and exogenous cannabinoids increase food intake by acting on the olfactory system (Soria-Gómez et al., 2014), and that endocannabinoids selectively enhance sweet taste by increasing gustatory nerve responses to sweeteners (Yoshida et al., 2010). Thus, it appears that cannabinoids alter feeding through similar mechanisms in *C. elegans* as in mammals.

An interesting finding was that in spite of the fact that AWC is modulated by AEA in an NPR-19-dependent manner (Fig. 4B) and required for its effect on behavior, AWC does not express NPR-19 (Fig. 5A). Instead, it appears to inherit the cannabinoid signal from an upstream neuron that modulates AWC by releasing either peptides or aminergic neurotransmitters via dense core vesicles (Fig. 5C).

Together, these data present a mechanism for how cannabinoids produce a desire for highly palatable foods over less palatable alternatives in *C. elegans*. Worms roam freely between patches of bacteria and decide whether to stay in the current patch or search for another patch. After exposure to AEA, the cannabinoid binds to the NPR-19 receptor on one or more neurons, which then release a signaling molecule via dense core vesicles. These molecules modulate AWC such that it activates more strongly when a worm leaves a patch of favored food, causing a higher likelihood of a reversal response of the worm back into that patch. Conversely, AEA causes AWC to activate less strongly when a worm leaves a patch of non-favored food, causing a lower likelihood of a reversal response of the worm back into that patch. Therefore, AEA's effect on AWC increases the retention of worms in favored food and decreases retention in the non-favored food (Fig. 6).

Materials and Methods

Strains. Animals were cultivated under standard conditions (Brenner, 1974) using *E. coli* OP50 as a food source. The following strains were used for preference and feeding assays: N2 Bristol reference strain, FK311 (*ceh-36*(ks86)), RB1668 (*npr-19*(ok2068)), XL324 (ntIS1701[*npr-*

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19::CNR1::gfp-*npr-19*(1.1);*unc-122*::rfp]), XL325 (ntIS1702[*npr*-19::*npr-19*::gfp-*npr-19*(1.1)). The following strains were used for calcium imaging experiments: XL322 (ntIS1703[*str-2*::GCaMP6::wCherry;*unc-122*::dsRed]), XL327 (ntIs1703[*str-2*::GCaMP6::wCherry;*unc-122*::dsRed];*unc-13*(e51)), XL326 (ntIs1703[*str-2*::GCaMP6::wCherry;*unc-122*::dsRed];*unc-31*(e928)), XL346 (*npr-19*(ok2068); ntIs1912[*str-2*::GCaMP6::wCherry;*unc-122*::dsRed]). The following strains were used for identifying the npr-19 expression pattern: XL334 (otIs544[cho-1::SL2::mCherry::H2B+pha-1(+)];ntIS19114[npr-19::GFP1.1;unc-122::dsred]), XL335 (ntIS19114[npr-19::GFP1.1;unc-122::dsred];otIs518[eat-4::SL2::mCherry::H2B+pha-1(+)]). Young adults were used in all experiments.

Bacteria. The following bacterial strains were used in this study: DA1885 (*Bacillus simplex*), DA1877 (*Comamonas* sp), *E. Coli* HB101, *E. Coli* DA837. Bacteria were grown overnight at 37^{o}C, then concentrated by centrifugation, rinsed three times with either M9 medium (for EPG experiments) or A0 buffer (for behavioral/imaging experiments; $MgSO_4$ 1mM, $CaCl_2$ 1mM, HEPES 10mM, glycerol to 350 mOsm, pH 7), and resuspended to their final concentration. Concentration was defined as optical density at 600 nm (OD_{600}), as measured with a DSM cell density meter (Laxco, Bothell, WA, USA).

Animal preparation. Worms were washed five times in M9 for EPG experiments or A0 buffer (see above) for behavioral/imaging experiments. Worms were then incubated for 20 minutes with either background solution alone or background solution + 300 µM Arachidonoylethanolamide (AEA, Cayman chemical, Ann Arbor, MI, USA). The incubation time and relatively high concentration reflects the low permeability of the *C. elegans* cuticle to exogenous molecules.

Behavioral assays. Freshly poured NGM agar plates were dried in a dehydrator for 45 minutes at 45°C. A maze cut from foam sheets (Darice, Strongsville, OH, USA) using a laser cutter was then placed on each plate. Each end of the maze was seeded with 4.5 µl of bacteria. After transferring the animals to the starting points of the maze, 12 plates were placed on a flatbed scanner and simultaneously imaged every 15 minutes (Mathew et al., 2012; Stroustrup et al., 2013). The number of worms in the two patches of food and the hallway between them was counted manually and a preference index *i* calculated as follow:

$$i = (F-NF)/(F+NF)$$

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where F is the number of worms in the favored food patch, and NF the number of worms in the non-favored food patch. For experiments involving mutants, N2 reference strain animals were run in parallel with the mutants.

Electropharyngeograms. Pharyngeal pumping was measured electrophysiologically using a ScreenChip microfluidic system (Nemametrix, Eugene, OR, USA). Briefly, following pre-incubation as described above, worms were loaded into the microfluidic device with bacterial food (OD_{600} = 0.8) +/- AEA 300 µM. The low food concentration ensured that pumping frequency remained below maximum. Each animal was positioned between two electrodes to record electrical potential associated with pharyngeal pumping (Raizen & Avery, 1994). Worms were given two minutes to acclimate to the channel before acquiring one minute of EPG data. Data were processed using custom code written in Igor Pro (Wavemetrics, Lake Oswego, OR, USA).

Calcium imaging. After pre-incubation as described above, worms were immobilized in a custom microfluidic chip and presented with alternating 30-second epochs of buffer and bacteria (either *B. Simplex* or *Comamonas* at OD_{600} = 1, flow rate = 100 µl/min) for 3-minutes. Optical recordings of GCaMP6-expressing AWC neurons were performed on a Zeiss Axiovert 135, using a Zeiss Plan-Apochromat 40× oil, 1.4 numerical aperture objective, a X-Cite 120Q illuminator, a 470/40 excitation filter, and a 560/40 emission filter. Neurons were imaged at 3-10 Hz on an ORCA-ERA camera (Hamamatsu). Images were analyzed using custom code written in MATLAB. Results were expressed as the change in fluorescence ΔF/F based on the average fluorescence signal 10 seconds before the switch from food to buffer in a hand-drawn region of interest around the neuron. A subset of neurons did not respond to the stimulus. Non-responders were defined as worms in which ∆F/F was within the range of the response recorded during buffer-buffer control conditions. The percentage of non-responders did not vary between experimental conditions.

Expression profile. Worms were immobilized with 10mM sodium azide (NaN_3) and mounted on 5% agarose on glass slides. All images were acquired using a Zeiss confocal microscope (LSM800) with the ZEN software. Representative images are shown following maximum intensity projection of between 30 and 80 z-stacks. Image reconstruction was performed using ImageJ software (Collins, 2007). Identification of neurons was done based on published expression profiles of the *cho-1* (Pereira et al., 2015) and *eat-4* (Serrano-Saiz et al., 2013) genes in *C. elegans*.

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Statistics. Statistical significance was assessed by an ANOVA followed by a two-tailed Student's t-tests for normally distributed data, and Mann-Whitney tests for non-normally-distributed data.

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Competing interests

Shawn R. Lockery is co-founder and Chief Technology Officer of InVivo Biosystems, Inc., which manufactures instrumentation for recording electropharyngeograms. The other authors have no competing interests.

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Figure legends

Fig 1. *C. elegans* experiences AEA-mediated hedonic amplification.

A. Food preference was assessed with a T-maze whose arms were baited with equal concentrations (optical density: OD) of favored (blue, *comamonas*) and non-favored (orange, *B. simplex*) bacteria.

B. Preference was quantified as $i = (F - NF)/(F + NF)$, where F and NF are the number of worms in *Comamonas* and *B. simplex*, respectively. Animals display a marked and stable preference for favored food (n = 41). Pre-incubation in AEA further increases this preference (n = 40).

B'. Quantification of the average effect of AEA on food preference (p = 0.001).

C. AEA acts bidirectionally, increasing the fraction of worms in favored food and decreasing the fraction in non-favored food.

D. The effect of AEA is independent of bacteria concentration (p = 0.02), (Control: n = 48; AEA: n = 23).

E. AEA's effect is also independent of the pair of favored/non-favored bacteria (p = 0.023), (Control: n = 96; AEA: n = 35).

*p<0.05, **p<0.01, ***p<0.001 on two-tailed t-test, †p<0.05 after Bonferroni correction for multiple comparisons, n.s., not significant. Error bars represent SEM.

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Fig 2. AEA bidirectionally alters consumption of favored and non-favored food.

A. Representative traces from worms displaying the median pumping frequency of favored and non-favored food, with and without exposure to AEA.

B. Average pumping frequency of worms on favored and non-favored food before and after exposure to AEA shows that AEA causes an increase of pumping on favored food ($p = 0.026$) and a decrease in pumping on non-favored food ($p = 0.001$).

*p<0.05, **p<0.01, ***p<0.001, n.s., not significant on Mann-Whitney Tests. Error bars represent SEM. Favored food: n = 66 control, n = 67 AEA; Non-favored food: n = 99 control, n = 99 AEA.

Fig 3. AEA affect activation of olfactory neuron AWC, which is required for AEA-mediated hedonic amplification.

A. *ceh-36* mutants, which do not have functional AWC neurons, do not exhibit hedonic amplification in the food preference assay ($p = 0.778$) (Control: n = 24; AEA: n = 21).

B. Average traces of the normalized calcium signal of AWC ($\Delta F/F$ +/- SEM) in response to withdrawal of food shows that pre-incubation with AEA causes an increase in AWC activation when removing favored food (blue, *Comamonas*) (Control: n = 28; AEA: n = 32) but a decrease in activation when removing non-favored food (orange, *B. simplex*) (Control: n = 30; AEA: n = 29).

B'. The maximum amplitude of the normalized calcium signal of AWC ($\Delta F/F$ +/- SEM) after pre-incubation with AEA is significantly increased to the removal of favored food ($p = 0.01$) and significantly decreased to the removal of non-favored food ($p = 0.03$).

*p<0.05, **p<0.01, ***p<0.001, n.s., not significant on a two-tailed t-test. Error bars or shading represent SEM.

Fig 4. Putative CB1 homolog, npr-19, is required for AEA's effect on behavior and AWC activation.

A. AEA-mediated hedonic amplification in preference assays is eliminated in npr-19-null worms ($p = 0.492$; n = 24 for both conditions). Amplification is rescued by expression of wildtype npr-19 ($p = 0.001$; n = 24 for both conditions) or human CB1 gene ($p = 0.00013$; n = 27 for both conditions) driven by the *npr-19* promoter.

B. Average traces of the normalized calcium signal of AWC ($\Delta F/F$ +/- SEM) in response to withdrawal of favored (blue, *comamonas*) or non-favored (orange, *B. simplex*) food show that

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AEA fails to bidirectionally modulate AWC activity in *npr-19* null worms (Favored: p = 0.532; n = 35 for both conditions; Non-favored: p = 0.312; Control: n = 37, AEA: n = 26).
*p<0.05, **p<0.01, ***p<0.001, n.s., not significant in two-tailed t-tests. Error bars or shading represent SEM.

Fig 5. AEA acts on AWC indirectly.
A. Worms expressing GFP under the *npr-19* promoter and mCherry under the *eat-4* (left, glutamate transporter) or *cho-1* (right, cholinergic transporter) promoters shows that *npr-19* is expressed in a number of neurons, including M3, MI, RIA, ASG, RMD, URA, PHC, ALN, and PLN. It is not, however, expressed in AWC.
B. Average traces of the normalized calcium signal of AWC (ΔF/F +/- SEM) in response to withdrawal of favored (blue, *comamonas*) (Control: n = 26; AEA: n = 27) or non-favored (*B. simplex*, orange) (Control: n = 32; AEA: n = 33) food show that AEA's bidirectional effect on AWC activation is cell autonomous, as it persists in the synaptic mutant *unc-13* (Favored: p = 0.0023; Non-favored: p = 0.0177).
C. AEA's modulation of AWC activity is altered in worms carrying the *unc-31* mutation, which have reduced dense-core vesicle release. (Favored: p = 0.0885, Control: n = 25, AEA: n = 24; Non-favored: p = 0.0204; Control: n = 19, AEA: n = 26).
*p<0.05, **p<0.01, ***p<0.001, n.s., not significant on two-tailed t-tests. Error bars or shading represent SEM.

Fig 6. A model for how AEA affects worms' feeding preferences, leading to hedonic amplification. When AEA binds to the NPR-19 receptor on a neuron upstream of AWC, that neuron then, via dense-core vesicle release, modulates AWC's activity such that it activates more strongly when favored food odor disappears and less strongly when non-favored food odor disappears. As AWC causes worm return in food patches, this bidirectional modulation leads to stronger retention of worms on favored food and weaker retention on non-favored food.

Supp. Fig. 1. Average pumping frequency of worms on favored and non-favored food before and after exposure to AEA. In the food consumption assay, *ceh-36* worms, which do not have a functional AWC, do not exhibit the AEA-induced decrease in pumping on non-favored bacteria (p = 0.6323; Control: n = 90, AEA: n = 88), but still show an increase in pumping on favored bacteria (p < 0.0001; Control: n = 76, AEA: n = 53). In *npr-19* null worms, the increased consumption of favored food observed in N2 wildtype worms after AEA exposure

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is absent (p = 0.5157; Control: n = 86, AEA: n = 77), whereas the decreased consumption of non-favored food after AEA exposure is independent of npr-19 (p = 0.0004; Control: n = 61, AEA: n = 86). *npr-19* rescue worms display a normal increase in pumping on favored food (p = 0.0156; Control: n = 76, AEA: n = 95) but no decrease in pumping on non-favored food (p = 0.1535; Control: n = 97, AEA: n = 97). CB1 fails to rescue AEA's effect on feeding (Favored: p = 0.6919; Control: n = 100, AEA: n = 86; Non-favored: p = 0.7934; Control: n = 111, AEA: n = 113).

*p<0.05, **p<0.01, ***p<0.001, n.s., not significant on Mann-Whitney Tests. Error bars represent SEM.

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Figure 1.

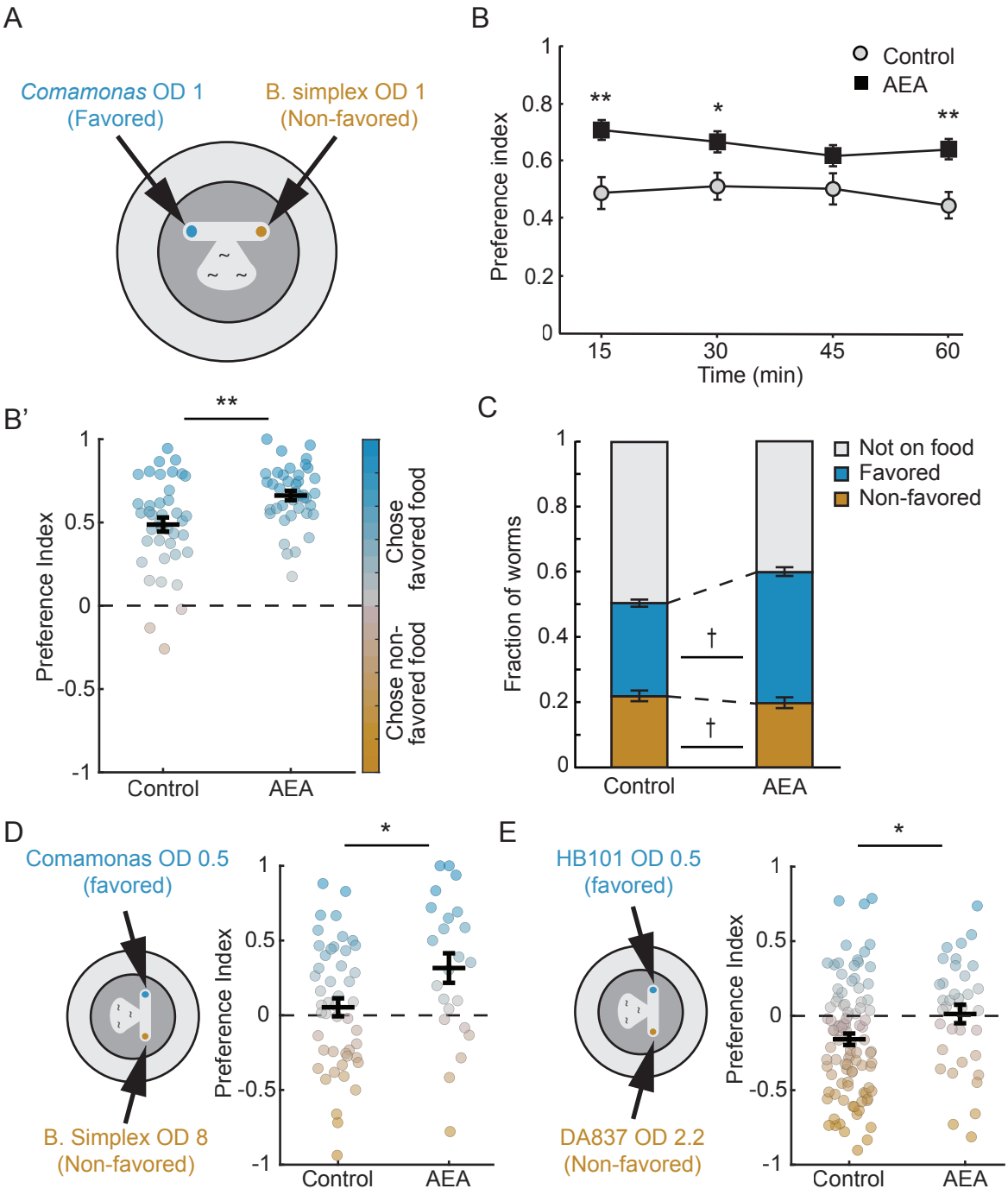

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Figure 2.

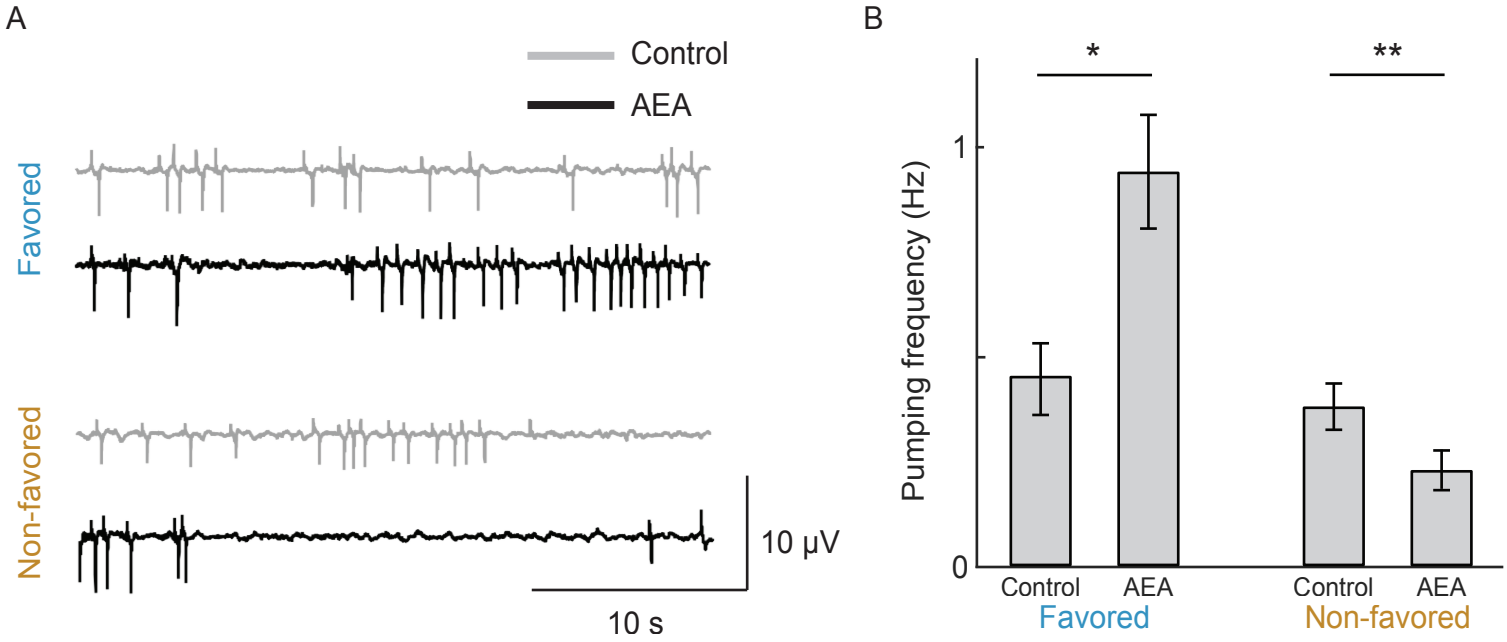

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Figure 3.

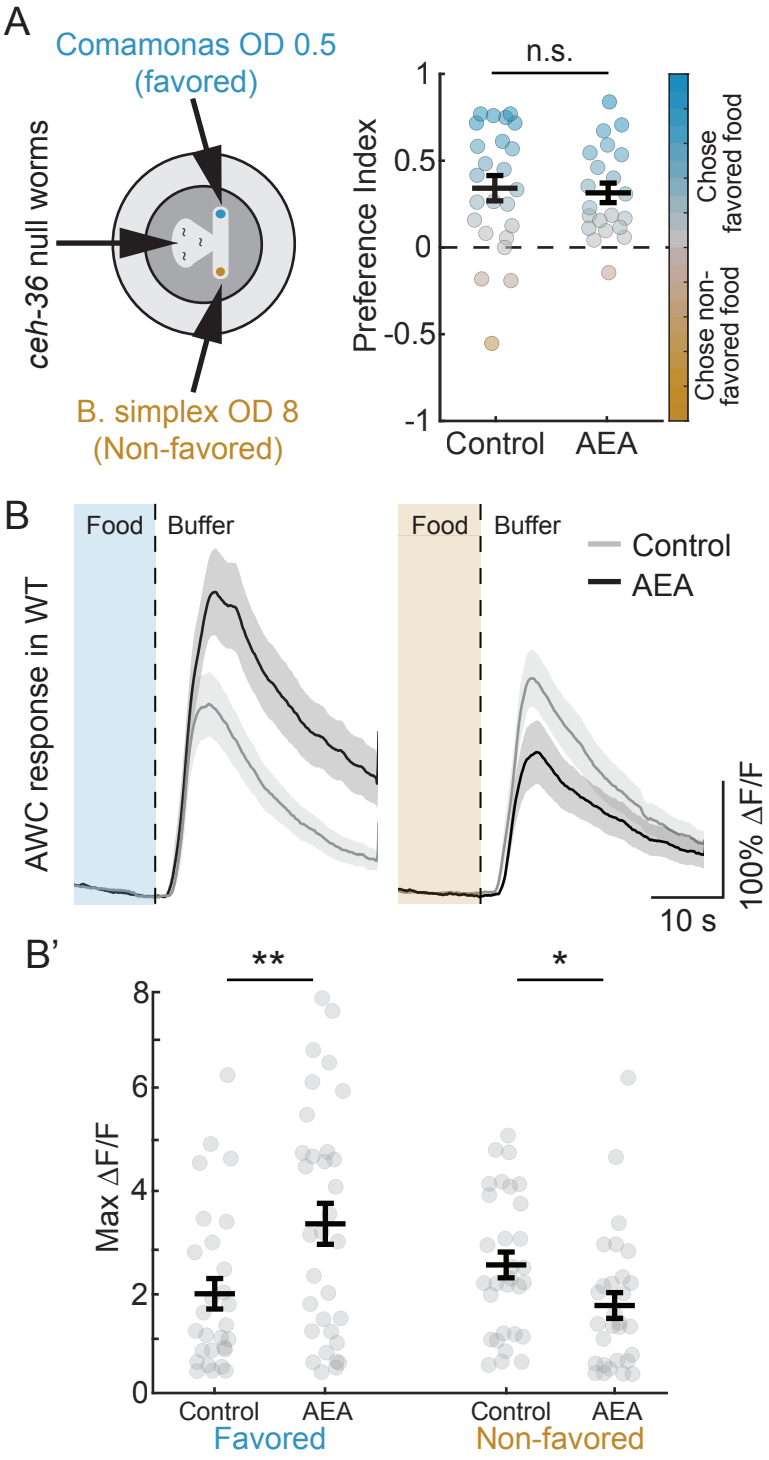

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Figure 4.

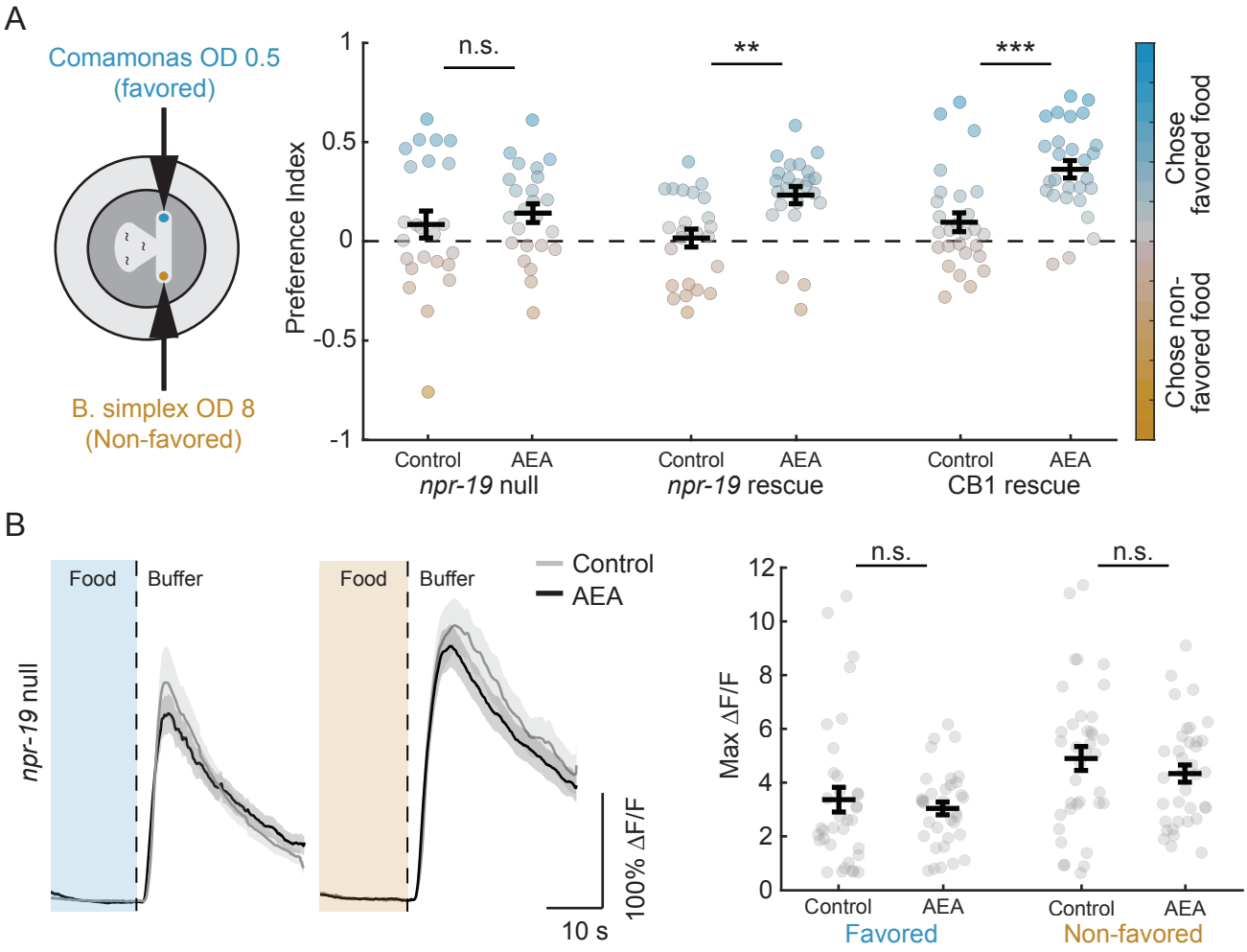

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Figure 5.

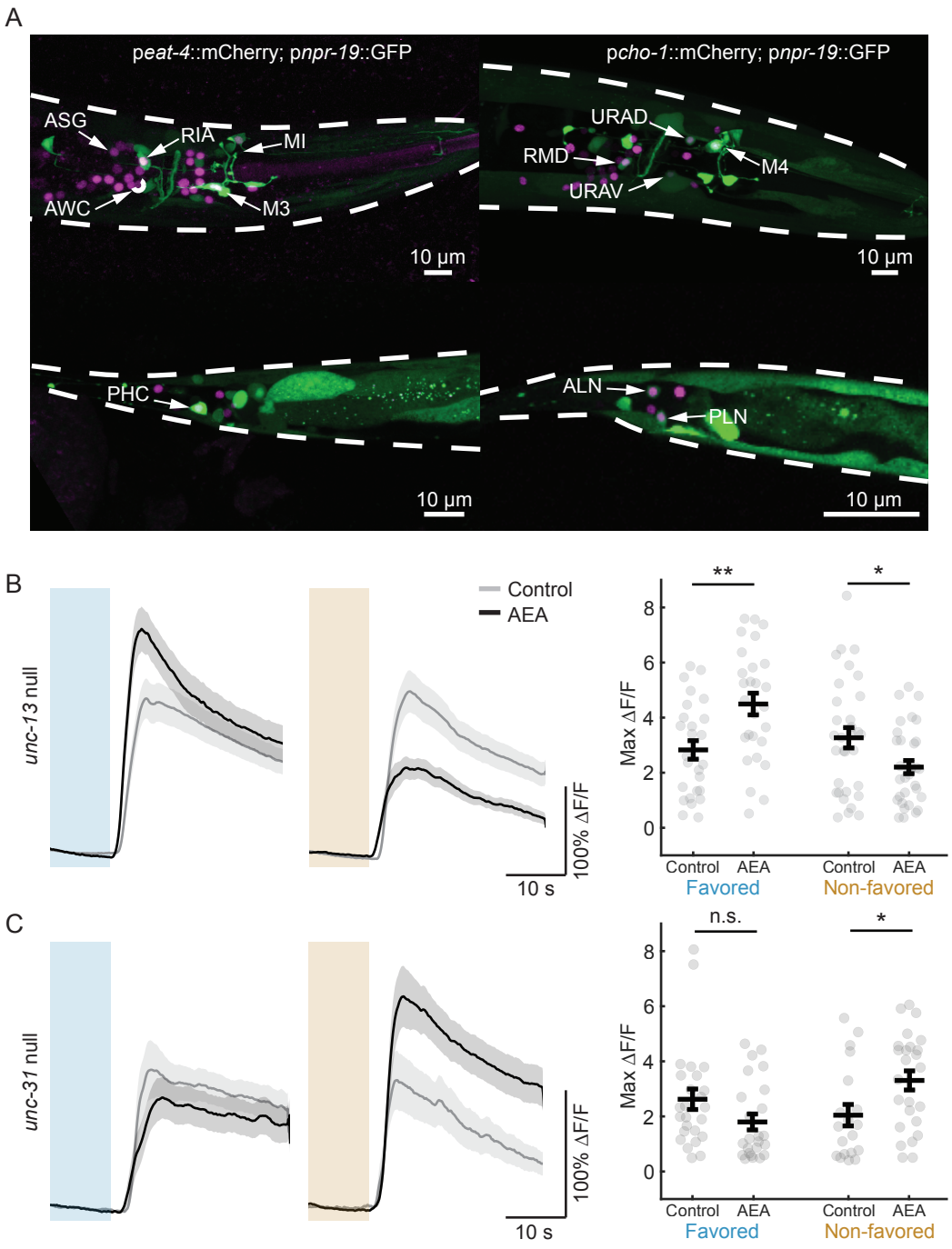

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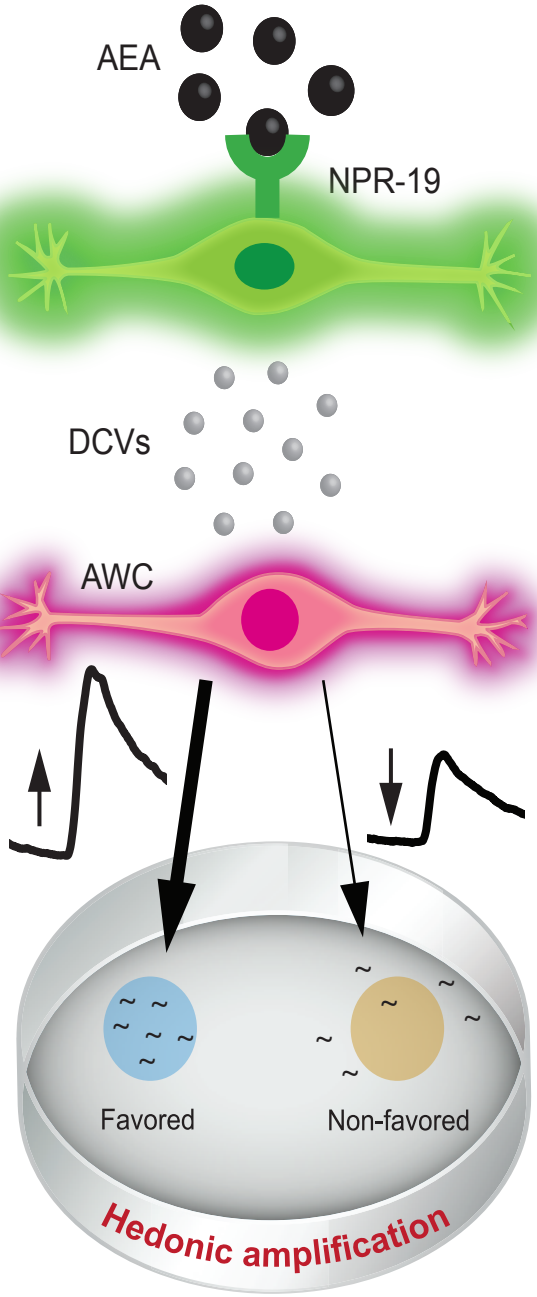
Figure 6.



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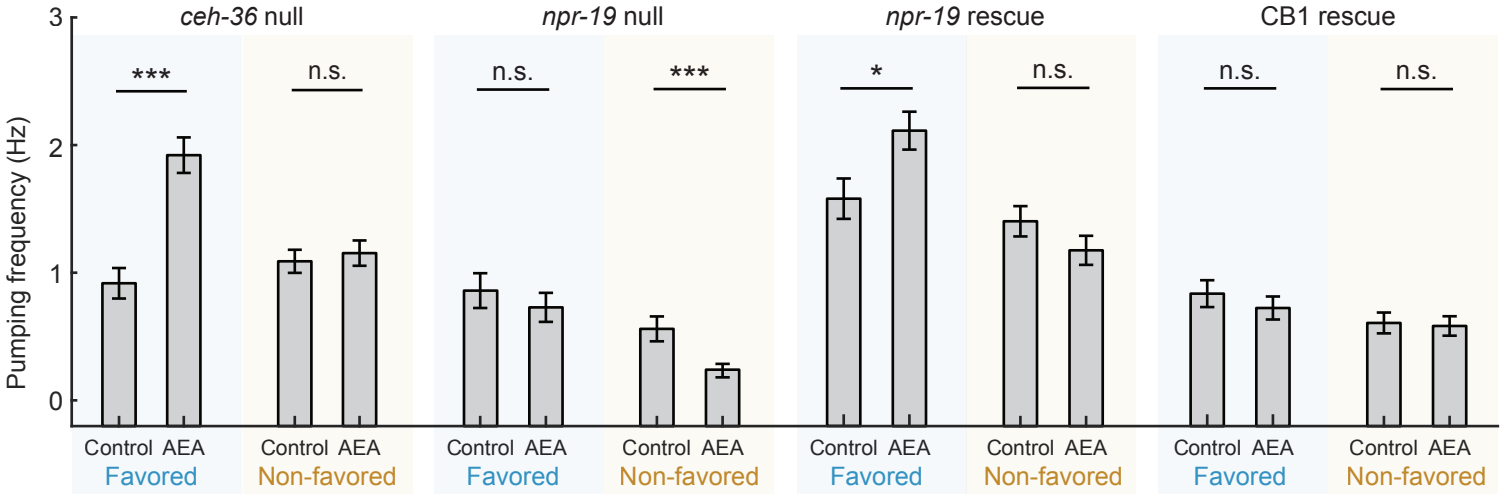
Supplementary Figure 1.



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Supp. Table 1. Quantification of npr-19::GFP positive cells.

worm	GFP puncta	mCherry puncta	mCherry puncta (AIY cutoff)
1	28	95	75
2	22	70	70
3	33	69	65
4	30	56	78
5	28	73	74
6	33	64	63
7	28	77	78
8	29	60	57
9	36	63	65
10	26	60	64
AVG	29.3	68.7	68.9
SD	3.973523485	11.29454145	7.156193898

Supp. Table 2. Identification of *npr-19*-expressing neurons in the head and tail.

Neuron	**Evidence**	**Function**
M3 L/R	colocalized eat-4	pharyngeal
M4	colocalized cho-1	pharyngeal
MI	colocalized eat-4	pharyngeal
I2	colocalized eat-4	pharyngeal
URA D/V L/R	colocalized cho-1	motor
RMD L/R	colocalized cho-1	motor
RIA L/R	colocalized eat-4	interneuron
ALN L/R	colocalized cho-1	sensory
PLN L/R	colocalized cho-1	sensory
URX L/R	colocalized cho-1	sensory
ASG L/R	colocalized eat-4	sensory