Reviewed Preprint
v1 • September 24, 2025
Not revised

Reviewed Preprint
v2 • August 24, 2026
Revised by authors

For correspondence:
dominique.glauser@unifr.ch

Competing interests: No competing interests declared

Funding: See page 23

Reviewing editor: Sonia Sen, Tata Institute for Genetics and Society, India

Starvation transforms signal encoding in *C. elegans* thermoresponsive neurons and suppresses heat avoidance via bidirectional glutamatergic and peptidergic signaling

Saurabh Thapliyal, Parvathi Sushama Gopinath, Dominique A Glauser
Department of Biology, University of Fribourg, Fribourg, Switzerland

eLife Assessment

This **important** study shows how hunger alters avoidance of harmful heat in C. elegans by reconfiguring the activity of key sensory neurons. The evidence is **convincing**, with well-designed behavioural, genetic, and imaging experiments that support the main conclusions. The work will be of interest to neuroscientists studying how internal states shape sensory processing and behaviour across species.

https://doi.org/10.7554/eLife.108246.2.sa3

Abstract

Animals must continuously adapt their behavioral outputs in response to changes in internal state, including nutritional state. Here, we show that starvation induces a profound and progressive suppression of thermonociceptive behavior in *Caenorhabditis elegans*. During early food deprivation (1-hr off food), the thermoresponsive sensory neurons AWCs mediate robust heat-evoked reversals over a broad range of stimulus intensities via glutamate and FLP-6 neuropeptide signaling, each covering distinct heat intensity ranges. After six hours of food deprivation (prolonged starvation), heat-evoked reversal responses are nearly abolished, independently of any external food odor cues. Starvation triggers a shift in the distribution of AWC heat-evoked calcium response polarity, transitioning from mostly excitatory responses to a more heterogeneous pattern combining both excitatory and inhibitory activities. This switch relies on ASI neurons, proposed to work as internal state-sensing neurons. INS-32 and NLP-18 neuropeptide signals from ASI switch from a reversal-promoting to a reversal-inhibiting effect. In addition, reversal-promoting glutamatergic transmission by AWC is antagonized by glutamatergic transmission from non-AWC neurons that suppresses FLP-6-dependent reversals. Our findings define a circuit logic by which nociceptive responsiveness gating by internal nutritional state is linked to dynamic modulation of sensory neuron activity patterns and orchestrated by bidirectional glutamatergic and neuropeptidergic signals. More broadly, this study illustrates how sensory systems integrate metabolic information to prioritize behavioral outputs under changing physiological conditions, providing mechanistic insight into the plastic coupling between sensation, internal state, and action selection.

Introduction

To ensure survival, animals must detect and avoid noxious stimuli, such as harmful chemicals, toxic gases, or extreme temperatures. These avoidance behaviors, often categorized as nociceptive, are conserved across phyla and essential for minimizing damage and guiding appropriate

behavioral responses. Like most animals studied so far, the nematode *Caenorhabditis elegans*, can produce robust innate escape behaviors and modulate these responses according to external and internal regulatory cues [1-4]. Many genes involved in mammalian pain perception and plasticity (such as the Transient Receptor Potential channels, CaM kinase and Calcineurin) are also implicated in worms, underscoring the evolutionary conservation of nociceptive pathways and the broad interest of the *C. elegans* model [5-9].

C. elegans is a small ectotherm, whose temperature equilibrates almost instantly with its surroundings. It can grow in a 15 to 25°C range. *C. elegans* produces robust avoidance behaviors in response to noxious temperature (which can be defined as temperature above 26°C) or to fast-raising thermal stimuli even below 26°C [10]. Upon acute thermal stimulation, forward-moving worms respond to head-targeted or whole animal heating by initiating backward locomotion (reversal event) and to tail-targeted heating by accelerating their forward locomotion [11]. Several thermo-responsive neurons have been characterized (see [12] for a review). Among these neurons, AFD neurons are the best characterized primary thermosensory neurons responding to temperature changes and mediating both thermotaxis in response to innocuous thermal cues and noxious heat avoidance response [13]. FLP neurons are tonic thermosensors whose activity level continuously reflects the current temperature, and which regulate animal speed and reorientation maneuvers [14, 15]. AWC neurons are polymodal sensory neurons responding to olfactory cues and to temperature in a context-dependent manner [16-18]. The pair of AWC sensory neurons asymmetrically differentiate into AWC^{ON} and AWC^{OFF}, which express specific markers and respond either similarly or differentially depending on the stimulus type [19]. Regarding AWC response to temperature change, both deterministic (stimulus-locked calcium elevations upon warming [18]), and stochastic responses, whose frequency can be modulated by warming [20], have been reported depending on experimental conditions. In addition, extremely powerful IR-laser based pulses (causing a 10°C elevation from 23 to 33°C within tens of milliseconds) were shown to trigger subtype-specific response: calcium elevation in AWC^{ON} and calcium decrease in AWC^{OFF} [21]. ASI sensory neurons have also been reported to respond to change in temperature in the innocuous range [22], but their implication in noxious-heat response is not known.

Neuromodulators, including neuropeptides and monoamines, play a central role in reconfiguring behavioral responses to sensory inputs based on context [15, 23-25]. In particular, feeding state has been shown to drive behavioral plasticity across a range of paradigms in *C. elegans*, including feeding-dependent changes in locomotion and sensory processing as a response to changes in the animal's internal physiological, sensory and/or metabolic states [26-29]. A reduction of nociceptive sensitivity or responsiveness during prolonged starvation could in principle help worms travel across harsher environments in order to find new food sources. Consistent with this potential ecological advantage, food deprivation-dependent reduction has been shown for the response to high osmolarity and chemical repellents [12, 30]. Examples of plasticity in the thermonociceptive response of *C. elegans* are so far essentially limited to the downregulation of noxious heat avoidance in response to persistent exposure to moderately noxious heat level (28°C) or repeated heat stimulations [7, 9, 31, 32]. The impact of prolonged starvation and the contribution of neuromodulation in the context-dependent regulation of thermonociceptive behaviors is largely unknown.

Here, we investigate how feeding state modulates heat avoidance behavior in *C. elegans*. We show that prolonged starvation significantly suppresses thermonociceptive responses, a plasticity not driven by external chemosensory cues but caused by nutrient availability. We identify a critical role for the AWC sensory neurons in mediating heat-evoked escape responses, and for the ASI neuroendocrine neurons in modulating these responses under starvation. Our data show that AWC neurons employ both glutamate and the neuropeptide FLP-6 to mediate avoidance, with distinct contributions from AWC^{ON} and AWC^{OFF} subtypes. Under early food deprivation, AWC responses to heat are stereotyped and excitatory, but under prolonged starvation, these responses become variable, reflecting a shift from mostly excitatory to a mix of excitatory and inhibitory responses. This shift depends on the ASI neurons, which modulate heat-evoked response via NLP-18 and INS-32 neuropeptides. Additionally, our findings suggest that glutamatergic signaling from

non-AWC sources also contribute to the suppression thermonociception following starvation. Together, our results suggest a neuromodulatory mechanism by which internal nutritional state reconfigures nociceptive behavior. Interestingly, the signaling molecules and neuromodulatory mechanisms into play are separable from the ones engaged in the regulation of thermotaxis after starvation [26, 33]. Our study demonstrates that even hard-wired escape responses are not immune to physiological context, and highlights a broader principle: survival strategies in simple animals balance external threats against internal needs through circuit-level plasticity.

Results

Starvation downregulates thermonociceptive responses in *C. elegans*

To assess how the feeding state modulates thermonociceptive behavior in *C. elegans*, we compared responses across different durations of food deprivation (Figure 1A). Synchronized first-day adult animals were stimulated with a series of 4-s infrared pulses of increasing heating power (100, 200, 300, 400 W), causing temperature increase of +2°C, +4°C +6°C and 8°C at the surface of the plate (Figure 1A). Fed animals on food produced robust heat-evoked reversal response to heat, but they also displayed a very elevated baseline of spontaneous reversals (~38%). A 1-hour off-food condition reduced spontaneous reversals (from ~38% to ~10%) and led to an attenuation of heat-evoked responses at low stimulus intensities, while responses to stronger stimuli remained comparable to those of fed animals. More prolonged food deprivation led to a striking progressive reduction in thermonociceptive responses at every heating level, with responses after 6 hours of starvation approaching baseline spontaneous reversal rates (Figure 1B and C). This suggests a robust inhibition of nociceptive behavior caused by prolonged starvation. To determine whether this attenuation was due to the absence of nutrients or chemosensory cues, we conducted similar starvation experiments in the presence of food odor, with OP50 bacteria present on the petri dish lid (Figure 1D). The reduction in thermonociceptive response persisted, indicating that the effect is driven by the internal starvation state rather than external olfactory input.

Although fed animals showed high sensitivity to noxious heat, they also displayed an elevated baseline of spontaneous reversals, which limited their utility as a control group by strongly reducing the dynamic range of heat-evoked reversal quantification and by complicating the quantitative comparison with food-deprivation conditions with much-reduced reversal baseline (Figure 1B). In addition, technical limitations in our calcium imaging setup would have prevented the intended follow-up analyses in fed animals. Based on these observations and technical considerations, we focused subsequent analyses, aiming at dissecting the circuit and molecular underpinnings of starvation-dependent plasticity, to the comparison of two off-food conditions with similar spontaneous reversal baseline: the *early food deprivation* condition (1-hour off-food, with high responsiveness to noxious heat) and the *prolonged starvation* (6-hour off-food with almost abolished noxious heat responsiveness).

Distinct roles of AWC and ASI neurons in heat-evoked response and starvation-dependent plasticity

To identify the neural substrates underlying thermonociceptive behavior and its modulation by starvation, we genetically ablated candidate thermosensory neurons (Figure 2). Surprisingly, ablation of AFD, the canonical thermosensory neuron, had no significant effect under either 1h or 6h food deprivation (Figure 2D and Figure 2-Supplement 1). Simultaneous ablation of AFD and FLP neurons induced an increase in spontaneous reversals, but only a mild reduction in heat-evoked responses and left starvation-dependent plasticity largely intact, suggesting a minor contribution of these neurons (Figure 2-Supplement 1). By contrast, ablation of AWC or ASI neurons had dramatic effects. Removal of AWC nearly abolished heat-evoked reversal behavior across all stimulus intensities and timepoints (Figure 2B and E). While $a this observation suggests that AWC plays an essential role in mediating the thermonociceptive response under both early food deprivation and prolonged starvation. Notably, in AWC-ablated animals, the residual

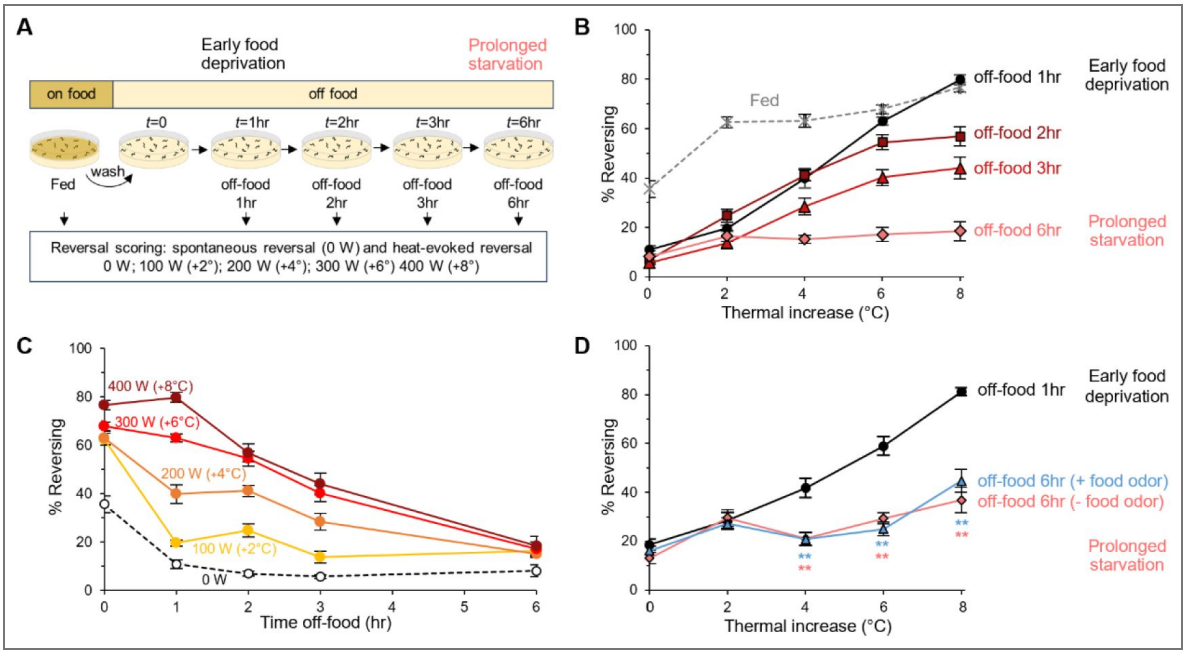


Figure 1. Starvation-dependent thermonociceptive plasticity in *C. elegans*

A. Schematic of the experimental procedure to quantify the impact of food deprivation on thermonociception in *C. elegans* adult hermaphrodites. Spontaneous and heat-evoked reversals were quantified in fed animals on food (Fed) or off food after 1, 2, 3, and 6 hr of food deprivation. Spontaneous reversals were measured as baseline reversal rate prior to any stimuli (0 W) and heat-evoked reversals were measured during a series of 4-s heat pulses at 100 W (+2°C), 200 W (+4°C), 300 W (+6°C) and 400 W (+8°C), respectively, delivered with an interstimulus interval of 20 s. **B-C**. Impact of food deprivation on thermonociceptive response, showing progressive attenuation of heat-evoked reversal response in the course of a 6-hr experiment. Results as average fraction of reversing animals (%) quantified in $N \geq 6$ assays, each scoring at least 50 worms. Error bars: S.E.M. The same dataset is presented as heat dose-response curves (B) and as time-course of the heat-evoked response decrease at each heating level (C). Spontaneous reversal rate corresponds to the baseline reversal response in the absence of heating stimuli (0 W, Thermal increase = 0 °C). **D.** Effect of prolonged starvation (off-food for 6 hr) in the presence or absence of food odor during the food-deprivation period, indicating that external food odor cues cannot prevent the response reduction. **, p>.01 versus the early food deprivation condition (off-food 1hr), by Bonferroni post-hoc tests.

response level was unaffected by starvation, suggesting that AWC might also be required for the expression of starvation-dependent plasticity. In contrast, ASI ablation had almost no effect under early food deprivation but almost entirely suppressed the starvation-induced reduction in heat-evoked reversals observed after prolonged starvation (Figure 2C and E). The persistence of robust heat-evoked responses in ASI-ablated animals indicates that ASI is specifically required for starvation-dependent attenuation of thermonociceptive behavior. Together, these data suggest that, under our experimental conditions, AWC is the primary driver of heat-evoked reversals upon early food deprivation, while ASI neurons selectively mediate their suppression under prolonged starvation.

AWC neurons mediate heat-evoked reversal via both glutamate and FLP-6 neuropeptides

We next sought to define the molecular mechanisms by which AWC neurons mediate robust heat-evoked reversals in the early food deprivation condition. AWC neurons are glutamatergic and express several neuropeptides, including FLP-6, INS-22 and NLP-5 [34, 35]. *eat-4* mutants lacking the vesicular glutamate transporter EAT-4 exhibited strong impairments in heat-evoked responses at high stimulus intensities (6 and 8°C thermal increases, Figure 3A). *flp-6* mutants displayed a marked deficit across the entire range of thermal increases (Figure 3B), while the impairment in *ins-22* and *nlp-5* mutants was less pronounced and more selectively affected the response to stimuli of intermediate intensities (Figure 3-Supplement 1). This indicates that different communication molecules are used over different thermal ranges to mediate heat-evoked reversals, which is in line with previous genetic analyses having recurrently shown that multiple genetic pathways are recruited for different levels of heat [6, 36].

Next, we focused on *eat-4* and *flp-6* mutants, showing the strongest phenotype. We addressed whether glutamate and FLP-6 signaling act dependently of each other in controlling heat-evoked reversal, by testing *eat-4;flp-6* double mutants. The residual response seen in each single mutant (Figure 3 A and B) was almost entirely abolished in the double mutant (Figure 3C). A two-way ANOVA for the highest heat stimuli with *eat-4* and *flp-6* genotypes as factors (two levels each: mutant or wild type) showed significant main effects of *eat-4* ($F_{(1,67)}$ =48.70, p<.001, η^2p=0.421) and *flp-6* ($F_{(1,67)}$ =58.50, p<.001, η^2p=0.466), respectively, but no interaction effects ($F_{(1,67)}$ =0.093, p=.761, η^2p=0.001). The significant cumulative effect of the two mutations indicates that the two signaling pathways act mostly independently of each other to mediate heat-evoked reversals.

Next, we carried out cell-specific rescue to demonstrate that AWC neurons represent a relevant source of glutamate and FLP-6 neuropeptides promoting heat-evoked reversals upon early food deprivation (off-food 1hr). Transgenic rescue experiments using either *[AWC^{OFF}::eat-4]* or *[AWC^{ON}::eat-4]* extrachromosomal array containing-lines showed that restoring *eat-4* expression in either AWC^{ON} or AWC^{OFF} alone was sufficient to recover heat-evoked responses to near wild-type levels (Figure 3E). Interestingly, *[AWC^{OFF}::eat-4]* transgene not only restored responsiveness but also enhanced reversals for low heating (+2°C) stimuli, potentially reflecting overexpression effects or altered signaling balance with other glutamatergic neurons.

Additionally, spontaneous reversal rates were restored only by *[AWC^{OFF}::eat-4]*. A similar rescue approach for *flp-6* expression in AWC revealed that only *[AWC^{OFF}::flp-6]*, not *[AWC^{ON}::flp-6]*, could rescue heat-evoked behavior, suggesting asymmetry in neuropeptidergic signaling (Figure 3F).

These results indicate that AWC neurons mediate thermonociceptive responses through both glutamatergic and FLP-6-dependent pathways, each covering distinct heat intensity ranges (Figure 3G). While either AWC subtype is sufficient for glutamatergic signaling, FLP-6 neuropeptide function appears more dependent on AWC^{OFF}.

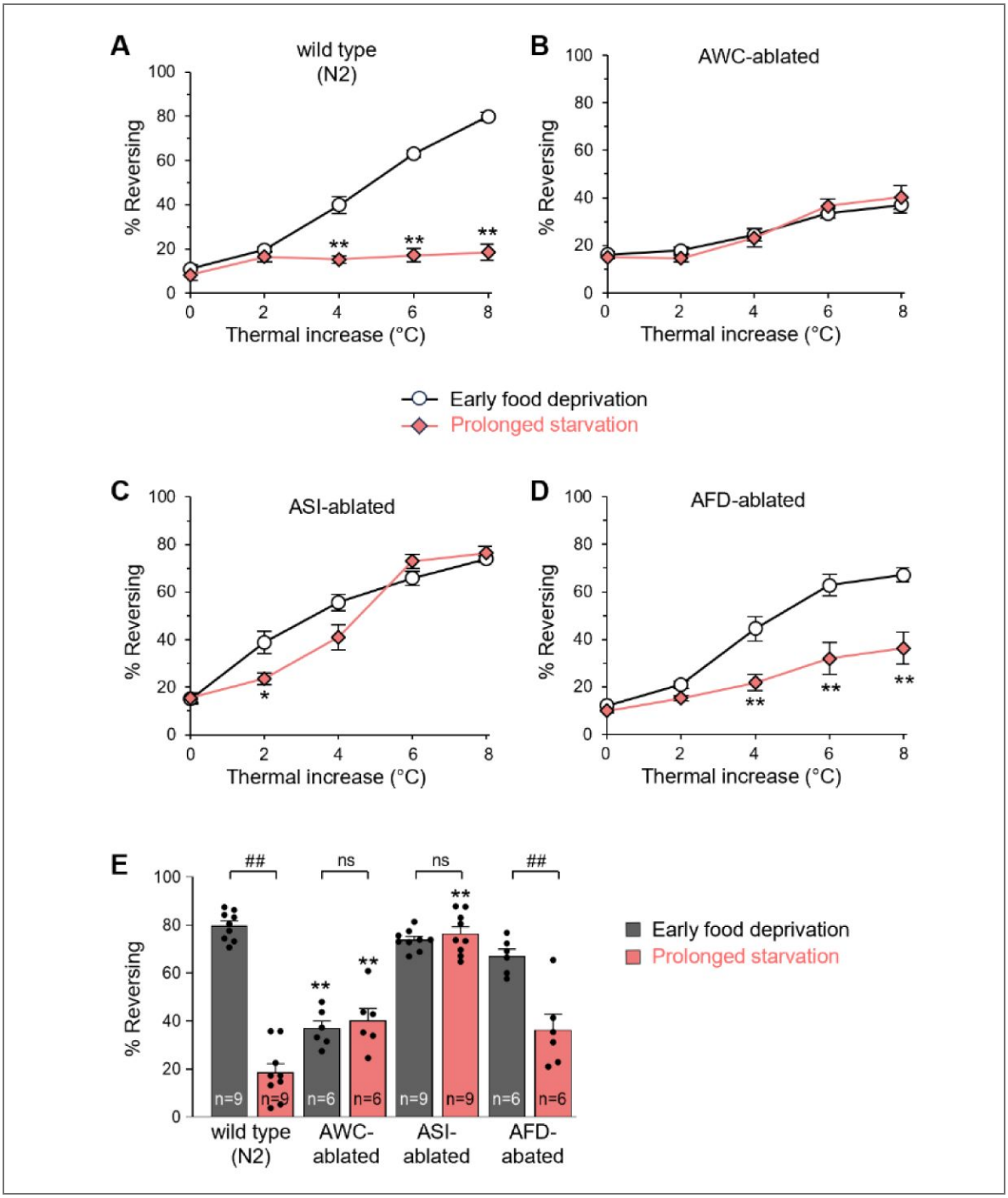


Figure 2. AWC mediates heat-evoked reversals upon early food deprivation and ASI mediates thermonociceptive plasticity upon starvation

Impact of the genetic ablation of AWC, ASI and AFD sensory neurons on thermonociceptive response and starvation-dependent plasticity. **A-D** Comparison of heat-evoked reversals after early food deprivation (off-food 1hr) and prolonged starvation (off-food 6 hr) in wild type and in transgenic animals with caspase-mediated ablation of indicated neurons. Results are presented as average +/- S.E.M. *, *p<.05,* and **, *p<.01* versus corresponding heat level in the early food deprivation condition, by Bonferroni post-hoc tests. **E** Comparison for the highest heating level (thermal increase = 8 °C) across the three genotypes presented in panel A to D. Bars as average, dots as individual assay scores, and error bars as S.E.M. *##, p<.01* between early food deprivation and prolonged starvation for each genotype; **, *p<.01* versus wild type (N2) at the corresponding timepoint, by Bonferroni post-hoc tests. The total number of assays (n) analyzed per condition, each scoring at least 50 worms, are indicated in panel E.

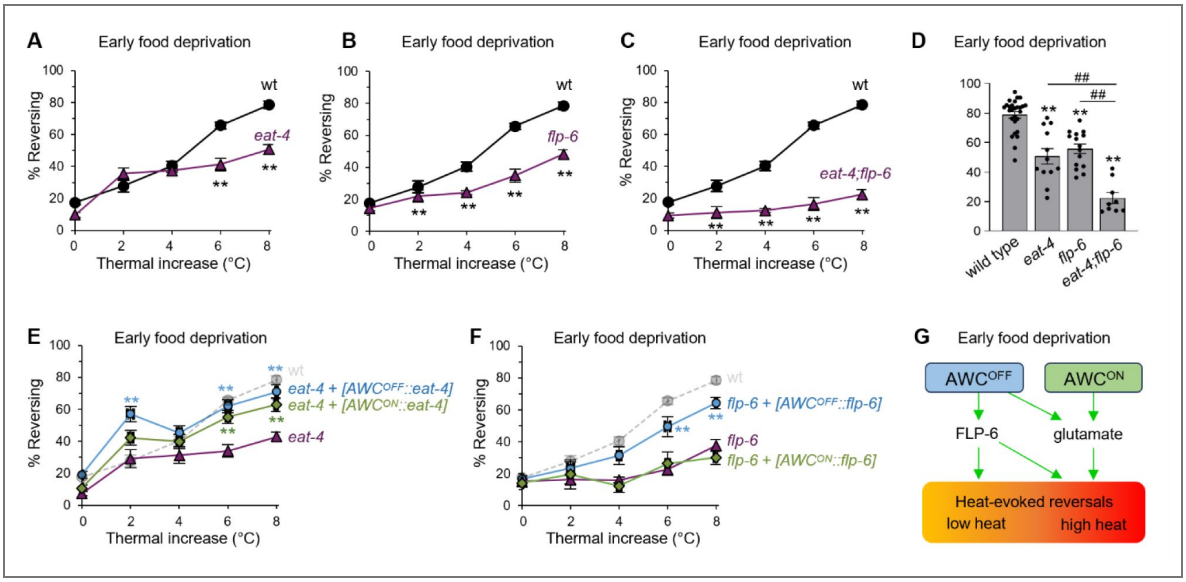


Figure 3. Differential engagement of glutamate and FLP-6 neuropeptides by AWC^{ON} and AWC^{OFF} in the control of spontaneous and heat-evoked reversal at various heat levels

A, B, C, E, F Comparison heat-evoked reversals after early food deprivation (off-food 1hr) in wild type, *eat-4(ky5)*, *flp-6(ok3056)*, *eat-4;flp-6* double mutants and in transgenic animals with AWC subtype-specific rescue of *eat-4* and *flp-6*, respectively. Results are presented as average +/- S.E.M. **, $p<.01$ versus wild type (A-C) and versus non-transgenic mutants (E and F) at respective thermal increase levels, by Bonferroni post-hoc tests. The number of assays (n), each scoring at least 50 worms, were : wild type, n=27; *eat-4*, n=12; *flp-6*, n=15; *eat-4;flp-6*, n=9; *eat-4+[AWC^{OFF}::eat-4]*, n=6; *eat-4+[AWC^{ON}::eat-4]*, n=9; *flp-6+[AWC^{OFF}::flp-6]*, n=7; *flp-6+[AWC^{OFF}::flp-6]*, n=8. **D** Epistasis analysis of *eat-4* and *flp-6* mutation effects on the heat-evoked reversal (thermal increase = 8 °C). Bars as average, dots as individual assay scores, and error bars as S.E.M. A two-way ANOVA showed no significant interaction of the two mutations and Bonferroni post-hoc tests confirmed significant cumulative effects of the two mutations (##, $p<.001$). **G** Visual model illustrating the specific contribution of AWC^{OFF} and AWC^{ON} to the regulation of heat-evoked reversals via the distributed action of glutamate and FLP-6 neuropeptide. A single wt control dataset is reported across panels.

Starvation alters the distribution of the AWC heat response polarity

To investigate if and how starvation modulates AWC activity at the cellular level, we recorded heat-evoked calcium dynamics in AWC^{ON} and AWC^{OFF} neurons using YC2.3 cameleon-expressing transgenic lines. We used a microfluidic setup to deliver defined thermal stimuli. From a baseline at 20°C, four stimuli reaching 22, 24, 26 and 28°C, respectively, were sequentially delivered, corresponding to the temperatures reached in the heat-evoked behavioral analysis (Figure 3, +2, +4, +6, +8°C, respectively). We examined responses under both early food deprivation and prolonged starvation conditions. In Figure 4A and B, we report average traces and individual traces as heat maps. Individual traces are essential to visualize the polarity of the stimulus-locked responses on a trial-by-trial and animal-by-animal basis. Globally, we noted a graded response of both AWC types, with the lowest stimulus (2 °C) causing either no response or comparatively weak response, but the higher stimuli (4, 6 and 8°C) causing obvious responses. We clustered traces as “calcium up” (traces showing mostly up regulation), “variable” (traced showing a mix of up and down regulation) and ‘calcium down’ (traces showing mostly down-regulation). The “calcium down” trials were often, but not always, followed by a “rebound” upregulation.

Upon early food-deprivation, both AWC neuron subtypes responded with a stimulus-locked intensity-dependent calcium increase ("calcium up") in most animals (45/56, 80% for AWC^{OFF} and 37/49, 77% for AWC^{ON}). The remaining traces showed either mixed ("variable") or "calcium down" profiles. The response peak histograms for both neuron subtypes show asymmetrical distributions with mostly excitatory responses (Figure 4C and D, upper histogram). After prolonged starvation, this response pattern shifted dramatically: the majority of traces became variable (i.e., including both calcium up and calcium down response along the stimulus series) or showed consistent calcium down-regulation (17/23, 74% for AWC^{OFF} and 14/17, 82% for AWC^{ON}, both changes being statistically significant by Fisher’s exact test: p=1.3E-5 and p=7.6E-5, respectively). The response peak histograms for both neuron subtypes (Figure 4C and D) now show much more symmetrical distributions, with equally frequent excitatory and inhibitory responses. Kruskal-Wallis tests showed significant distribution differences between early food deprivation and prolonged starvation (p<.001, for each neuron subtype).

The starvation-dependent response shift was reflected in the average traces (Figure 4A and B) as a decreased peak amplitude and rightward shift. But the modification of the average trace was largely caused by the redistribution in response types: the dynamic of individual *calcium up* and *calcium down* traces remaining similar in each condition (Figure 4, Supplement 1 A and B). Therefore, AWC neurons, which under early food deprivation produce very consistently excitatory responses to heat (calcium increases), enter a heterogeneous and less predictable response mode under prolonged starvation, where stimuli can trigger either excitatory or inhibitory responses.

In summary, starvation does not simply attenuate stimulus-locked calcium responses in AWC; it fundamentally alters their polarity distribution, shifting from a largely predictable excitation profile to a heterogeneous regime combining excitation and inhibition. We speculate that this transformation in thermal encoding might underlie, at least in part, the behavioral plasticity observed during starvation.

ASI is required to shift the distribution of the AWC heat response polarities

Because ASI neurons are required for the starvation-dependent thermonociceptive plasticity (Figure 2C), dispensable for noxious heat-evoked reversal response (Figure 2C), and have been previously shown to signal the feeding state of the animal [39], we hypothesized that ASI neurons might play a modulatory role to mediate the starvation impact on AWC activity patterns. To test this hypothesis, we recorded AWC^{ON} and AWC^{OFF} activity in ASI-ablated animals. In the early food-deprivation condition, 1hr off-food, ASI ablation produced no noticeable difference in AWC calcium activities (compare Figure 4 with 5). Strikingly different from the situation in

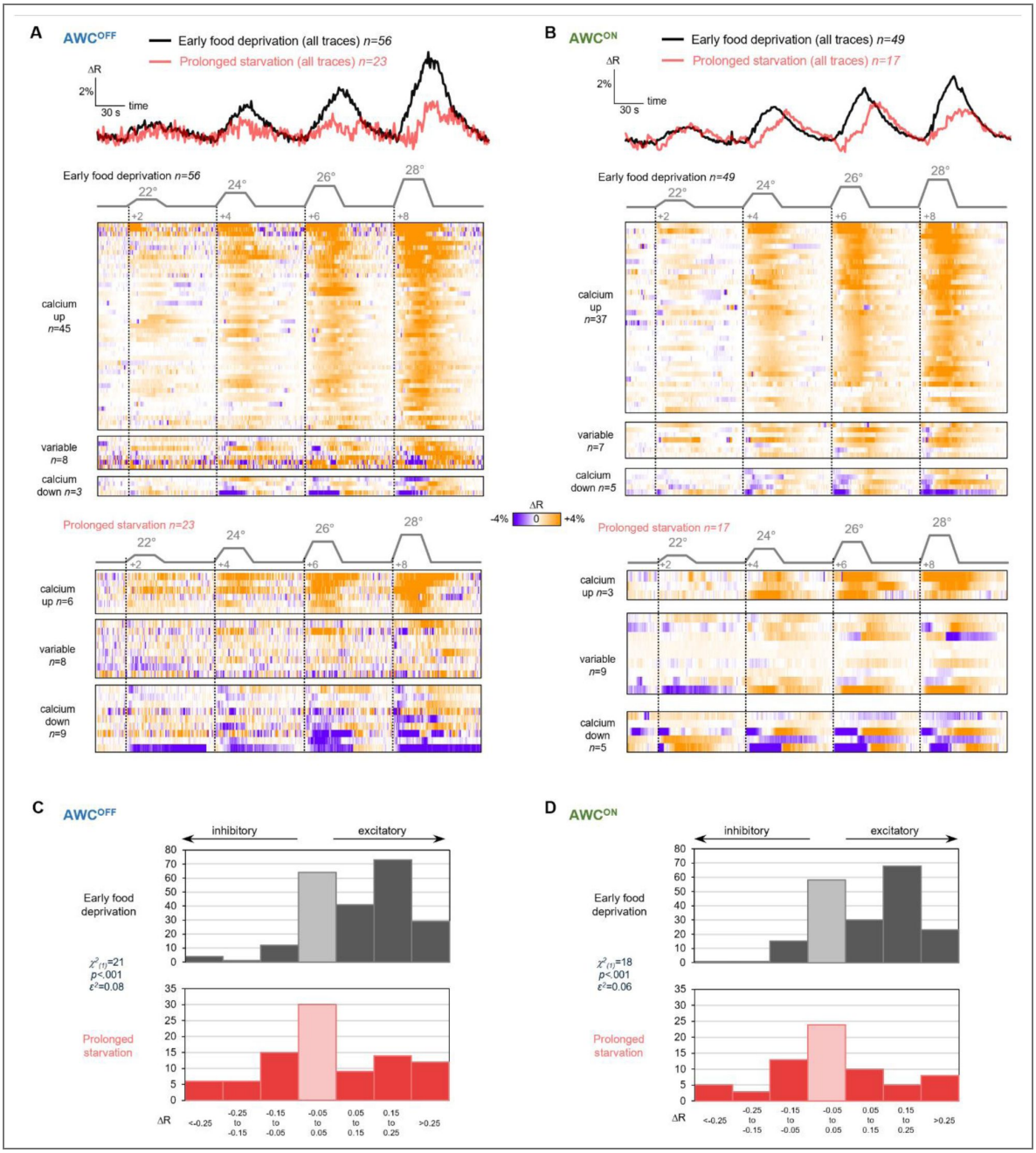


Figure 4. Starvation reconfigures AWC heat-evoked response polarity distribution from mostly excitatory to an heterogenous mix combining excitatory and inhibitory responses

A-B Calcium activity in AWC^{OFF} (left) and AWC^{ON} (right) in response to a series of four thermal up-steps. Upper plots show the average traces, lower heat maps show individual neuron traces, in the early food deprivation (off-food 1hr) and prolonged starved (off-food 6hr) conditions. **C-D** Histograms showing the distribution of calcium peak magnitudes (all thermal increase levels pooled) and highlighting the shift from mostly excitatory responses (early food deprivation, upper plot) to an equal mix of excitatory and inhibitory responses (prolonged starvation, lower plot) taking place for both AWC^{ON} and AWC^{OFF}. Results of Kruskal-Wallis non-parametric tests comparing the two food-deprivation timepoints are indicated to the left of each graph.

animals with intact ASI (Figure 4), starvation after 6hr off-food produced no noticeable effect in ASI-ablated animals, neither for AWC^{ON}, nor for AWC^{OFF} (Figure 5). In particular, the response polarity distributions remained asymmetrical with a very large majority of excitatory responses (Figure 5C and D). We conclude that the starvation-dependent shift in the distribution of AWC heat response polarity is mediated by the ASI neuromodulatory neurons.

Starvation-evoked thermonociceptivce plasticity is promoted by ASI-expressed neuropeptides, including NLP-18 and INS-32, and modulated by glutamatergic signaling

To elucidate the molecular signaling pathway underlying starvation-evoked thermonociceptive plasticity involving the AWC and ASI sensory neurons, we investigated the role of candidate neurotransmitters, including INS-1 which was previously shown to modulate thermotaxis in response to starvation [26], as well as other neurotransmitter/neuropeptides expressed in AWC and ASI.

First, we examined the phenotype of *ins-1* mutants, defective for starvation-evoked thermotaxis plasticity [26]. Interestingly, the thermonociceptive plasticity in these mutants was intact (Figure 6-Supplement 1), indicating that the molecular signaling controlling thermo-nociceptive plasticity is at least in part different from that engaged for negative thermotaxis plasticity as previously studied [26].

Second, we tested whether starvation-dependent plasticity was preserved in *eat-4* and *flp-6* mutant backgrounds, which we had suggested to represent the main AWC transmitters controlling heat-evoked reversals under the early food deprivation condition (Figure 3). Even if the heat-evoked response upon early food-deprivation was reduced relative to wild type in *flp-6* mutants, a significant further decline was seen after prolonged starvation (Figure 6B). In contrast, *eat-4* mutants displayed markedly elevated heat-evoked responses after prolonged starvation, even exceeding the response level seen in the early food deprivation condition for low heat stimuli (Figure 6C). This potentiated response in *eat-4* mutants was entirely dependent on an intact FLP-6 signaling, since reversal responses in *eat-4; flp-6* mutants were entirely abolished, like in *flp-6* single mutant (Figure 6C-E, a two-way ANOVA indicating a significant interaction effect between the two mutations: $F_{(1,70)}$ =0.093, p<.001, η^2p=0.247). Moreover, the potentiated response in *eat-4* single mutant could not be rescued by expressing *eat-4* rescue transgene selectively in either AWC^{OFF} or AWC^{ON} neurons (Figure 6F). Interestingly, AWC^{OFF}-specific rescue produced a further potentiation of heat-evoked reversal response to high heat stimuli (Figure 6F, 6 and 8°C thermal increases), aggravating the phenotype of *eat-4* mutants. These results are consistent with a model in which glutamatergic signaling regulates heat-evoked reversals in starved animals via two bidirectional drives (Figure 6G). On the one hand, glutamatergic signaling—originating from AWC^{OFF}—up-regulates reversals in response to high heat stimuli, thus contributing to prevent starvation-induced thermonociceptive plasticity. On the other hand, glutamatergic signaling—originating from neurons other than AWC— down-regulates reversals over a broad range of heat intensities, thus promoting starvation-induced thermonociceptive plasticity. The latter glutamatergic signaling inhibitory effect seems to be more dominant and to depend on intact FLP-6 signaling.

Third, since ASI is required for starvation-evoked plasticity but does not release classical neurotransmitters, we hypothesized that its function is mediated by neuropeptidergic signaling. We therefore screened for defects in starvation-dependent plasticity in mutants for neuropeptide genes known to be expressed in ASI: *ins-4, ins-6, ins-32,* and *nlp-18* [26, 34, 35, 40]. *ins-4* mutants exhibited normal plasticity, with robust responsiveness under early food deprivation and significantly reduced responses after prolonged starvation (Figure 7A). Mutants for *ins-6, ins-32,* and *nlp-18* all showed reduced responsiveness in the early food deprivation condition, but abnormally elevated responses after prolonged starvation (Figure 7A). This phenotype was qualitatively similar to the potentiation phenotype of *eat-4* mutants (Figure 6C). We performed further analyses with *ins-32* and *nlp-18* mutants, since they produced the most significant

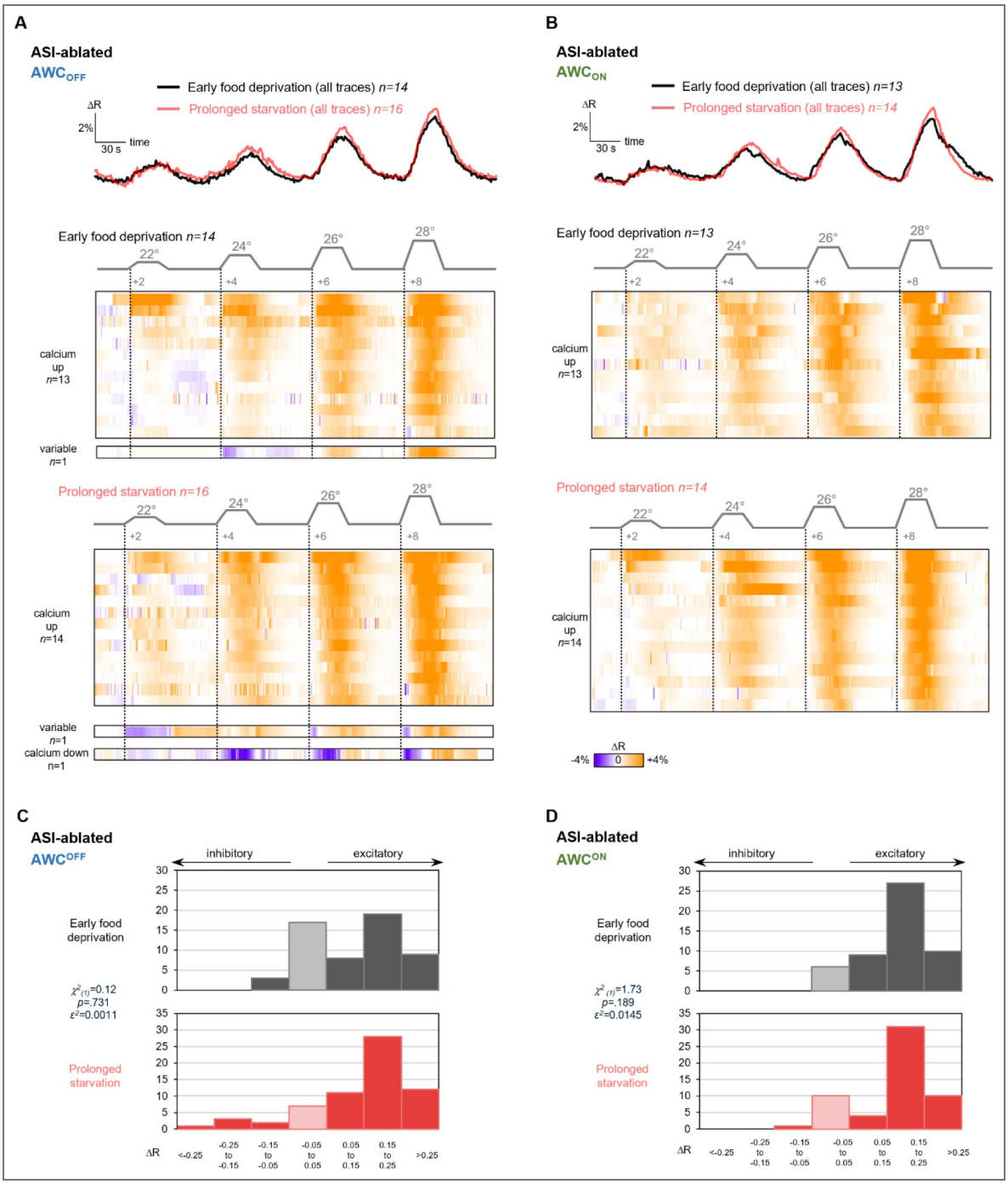


Figure 5. ASI ablation prevents AWC activity pattern reconfiguration upon starvation

(**A-B**) Calcium activity in AWC^{OFF} (A) and AWC^{ON} (B) in response to a series of four thermal up-steps in ASI-ablated animals. Upper plots show the average traces, lower heat maps show individual neuron traces, in the early food deprivation (off-food 1hr) and prolonged starved (off-food 6hr) conditions. The starvation impact seen in animals with intact ASI (Figure 4) is absent when ASI is ablated. **C-D** Histograms showing the distribution of calcium peak magnitudes (all thermal increase levels pooled) for both AWC^{ON} and AWC^{OFF}. Results of Kruskal-Wallis non-parametric tests comparing the two food-deprivation timepoints are indicated to the left of each graph.

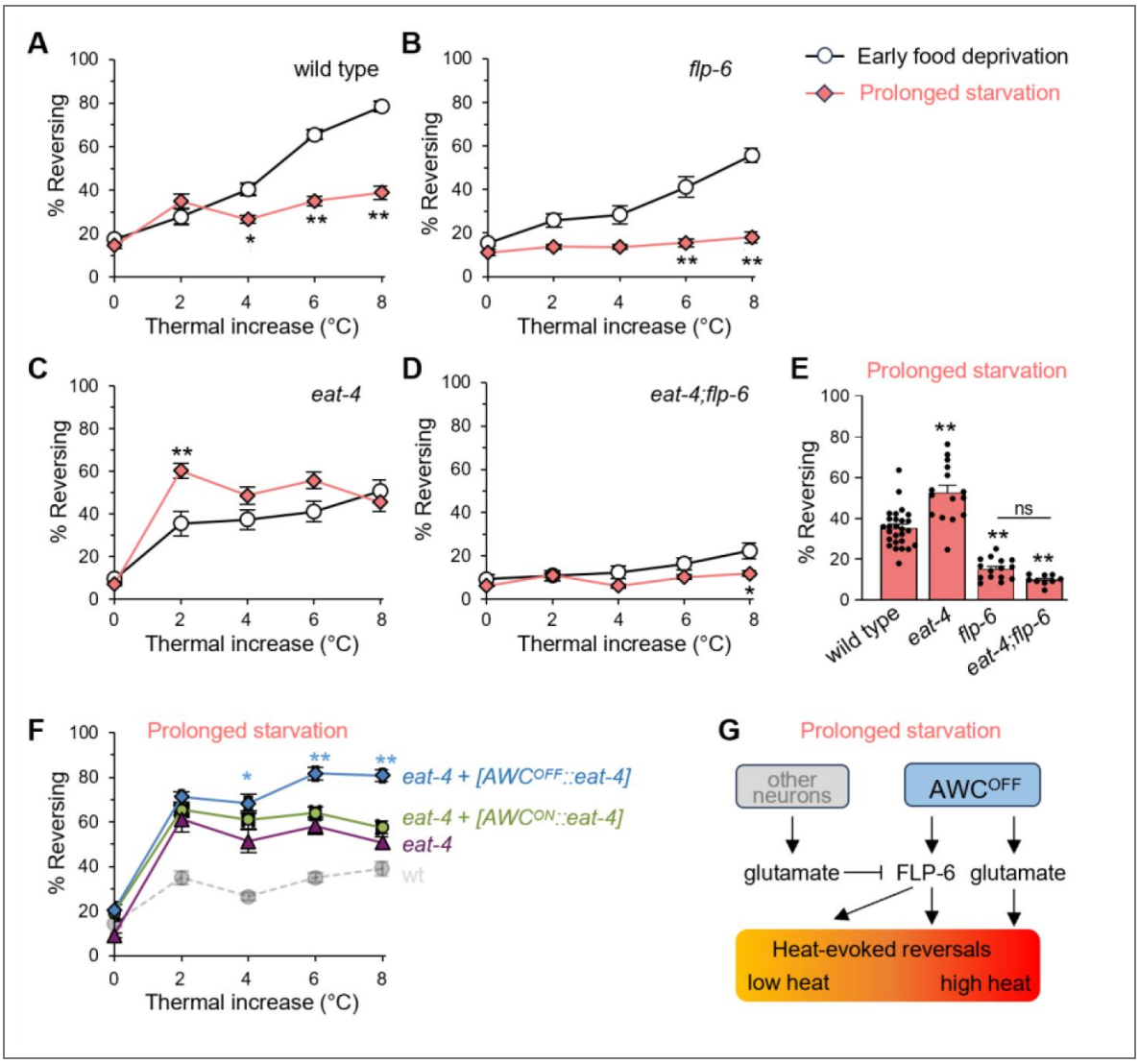


Figure 6. Bidirectional glutamate signaling actions modulate heat-evoked reversals following starvation

A-D Comparison of heat-evoked reversals after early food deprivation (off-food 1hr) and prolonged starvation (off-food 6hr) in wild type, *eat-4(ky5)*, *flp-6(ok3056)* and double mutant. **E** Heat-evoked reversal rate averaged over the four heating levels for the prolonged starvation condition. Same data as in panel A-D. **F** Analysis of transgenic animals with AWC subtype-specific rescue of *eat-4* after prolonged starvation (off-food 6hr). Results are presented as average +/- S.E.M. ***, p<.01* between the two food-deprivation timepoints (A-E) and versus non-transgenic mutants (F) at respective heat levels, by Bonferroni post-hoc tests. The number of assays (*n*), each scoring at least 50 worms, were: wild type, *n*=27; *eat-4*, *n*=15; *flp-6*, *n*=15; *eat-4+[AWC^{OFF}::eat-4]*, *n*=6; *eat-4+[AWC^{ON}::eat-4]*, *n*=9. **G** Visual model illustrating the bidirectional effect of glutamatergic signaling from AWC^{OFF} and from unidentified non-AWC neurons (other).

potentiation over a large range of heat intensities. To determine whether ASI is a relevant source of INS-32 and NLP-18 neuropeptides, we conducted ASI-specific rescue experiments in *ins-32* and *nlp-18* mutants. In *ins-32* mutant background, ASI-specific expression of *ins-32* produced a strong rescue effect, significantly restoring reversal response in early food-deprived animals (Figure 7B) and significantly reducing heat-evoked reversal response under the starvation condition (Figure 7C). In *nlp-18* mutant background, ASI-specific expression of *nlp-18* partially restored normal responsiveness under both the early food-deprivation and the prolonged starvation conditions (Figure 7 D and E). Although they do not exclude the possibility of contributions from other sources, these results suggest that ASI is a functionally relevant source of both INS-32 and NLP-18 to promote heat-evoked reversal upon early food-deprivation and to inhibit such reversals after prolonged starvation, (Figure 7F).

Collectively, our results (Figure 6 and 7) show that starvation-dependent thermonociceptive plasticity is orchestrated by multiple opposing signaling pathways in which neuropeptides and glutamate signals from different neurons produce context-dependent, bidirectional effects.

Discussion

Our study reveals that starvation profoundly modulates thermonociceptive behavior in *C. elegans* by engaging distinct neurons, altering neural response polarities, and mobilizing opposing neuromodulatory pathways. This behavioral plasticity, manifesting as a progressive attenuation of heat-evoked reversals under prolonged food deprivation, depends on a shift in the internal state that is not rescued by food-associated olfactory cues. By dissecting the underlying circuits and signaling mechanisms, we uncover two key regulatory nodes involving AWC and ASI neurons, which work in order to integrate thermal and nutritional cues into an adaptive behavioral output, as depicted in the visual model in Figure 8. AWC and ASI, as well as other (unidentified) neurons, use glutamatergic signaling and multiple neuropeptides to orchestrate this plastic response.

Starvation regulates thermonociceptive and negative thermotaxis plasticity via at least partly different mechanisms

Previous studies showed that AWC plays an important role in starvation-dependent plasticity in the negative thermotaxis behavior in an innocuous thermal range between 15 and 25°C [26, 33]. Negative thermotaxis involves the detection of thermal changes created by animal movement in spatial thermogradient (0.5°C/cm), the magnitude of the expected thermal changes approximating 0.01°C/s [26]. The starvation impact on negative thermotaxis was shown to (i) involve an up-regulation of AWC cell activity, (ii) rely on INS-1 neuropeptide produced in the intestine and (iii) to occur independently of ASI neurons. In contrast, our study used thermo-nociceptive stimuli, with faster raising thermal slopes (~0.5-2°C/s, hence 50-200 times faster than those occurring for thermotaxis) and covering noxious temperatures (up to 28°C). Our results indicate that the regulation of thermo-nociceptive response by starvation (i) is linked to a shift in the distribution of AWC activity response polarities from mostly excitatory to a mix of excitatory and inhibitory response, (ii) relies on ASI and specific neuropeptide produced in ASI, and (iii) works independently of INS-1 neuropeptide. Therefore, our study complements our understanding of the modulation of temperature-dependent behavior in *C. elegans* with previously undocumented mechanisms at the circuit, cellular and molecular levels.

AWC neurons are central drivers of heat-evoked reversal behavior via glutamate and neuropeptides

Our findings confirm that AWC sensory neurons are critical mediators of thermonociceptive behavior. Previous experiments carried out with extreme heating powers (10 °C raise within tens of milliseconds, [21]) had shown differential responsiveness in the two AWCs subtypes and a large behavioral impact was found in mutants affecting AWC asymmetries. The heating intensity range used in the present study (~0.5 to 2°C per second) triggered a similar withdrawal response, which

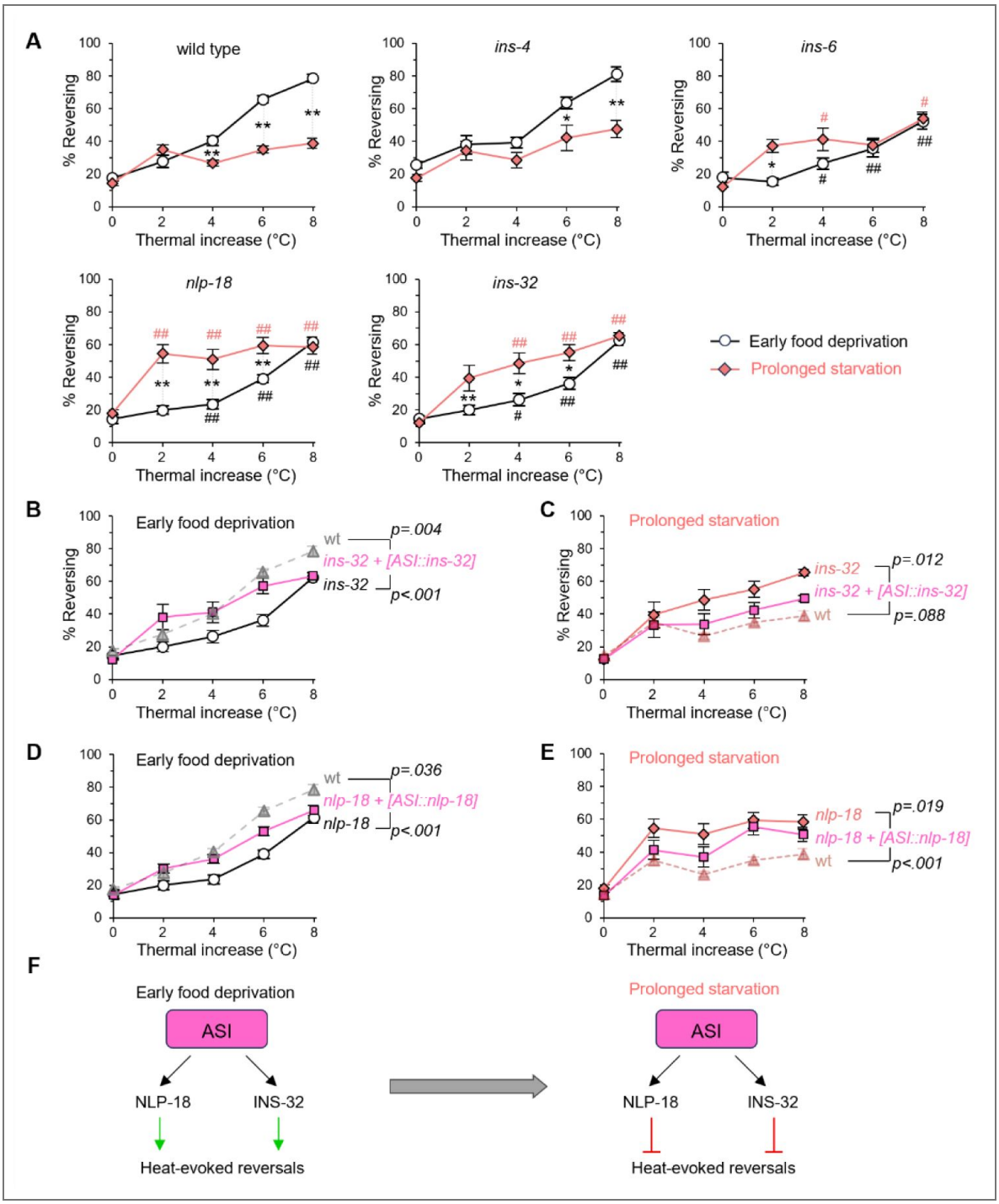


Figure 7. Starvation reconfigures neuropeptidergic signaling by ASI

A Comparison of heat-evoked reversals after early food deprivation (off-food 1hr) and prolonged starvation (off-food 6hr) in wild type, *ins-4(ok3534), ins-6(tm2008), nlp-18(ok1557)* and *ins-32(tm6109)*. *, *p<.05,* and **, *p<.01* between the two food-deprivation timepoints, by Bonferroni post-hoc tests. #, *p<.05,* and ##, *p<.01* versus wild type (N2) at the corresponding timepoint, by Bonferroni post-hoc tests. Data for wild type are the same as the one depicted in Figure 6. **B-E** Impact of transgenic rescue in *ins-32* (B-C) and *nlp-18* (D-E) background. Two-way ANOVA showing no significant heating power x genotype interaction, but a significant genotype main effect, post-hocs tests were conducted on the genotype factor only. Indicated *p* values were corrected with Bonferroni correction. **F** Model of the bidirectional actions and the sources for NLP-18 and INS-32 neuropeptides in the modulation of heat-evoked reversal. Red: reversal-suppressing pathway; Green: reversal-promoting pathway. Additional neuropeptides (such as INS-6) may also be involved, but in the absence of direct evidence for their origin from ASI, they were not included in this scheme.

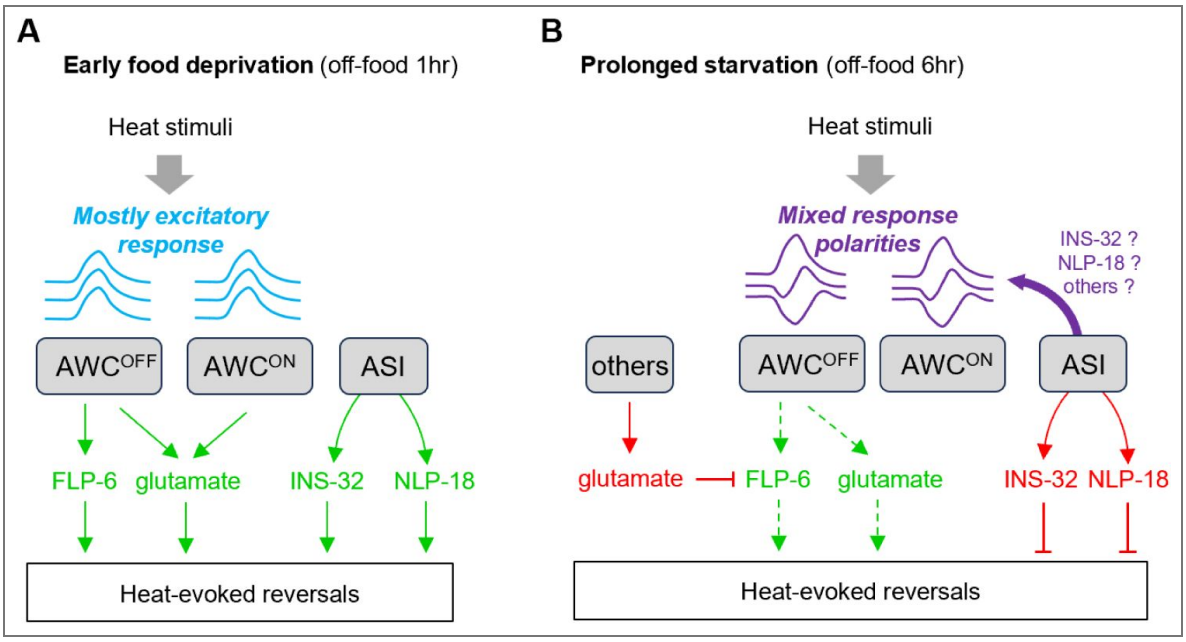


Figure 8. Visual model of the cellular and molecular signaling pathways mediating and modulating heat-evoked reversals according to food deprivation duration

A Situation upon early food deprivation (off-food 1hr). The two AWC neurons produce mostly stimulus-locked calcium elevations in response to heat stimuli and make a major contribution to heat-evoked reversals. Glutamate signaling from AWC^{ON} and AWC^{OFF}, as well as FLP-6 neuropeptide signaling from AWC^{OFF} mediates heat-evoked reversals. INS-32 and NLP-18 neuropeptide from ASI neurons, as well as potentially from additional unidentified sources (not depicted), also promote heat-evoked reversals. **B** Situation following prolonged starvation (off-food 6hr). Starvation causes several functional changes. First, AWC calcium activity pattern shifts from a mostly excitatory response (calcium elevations) to less predictable response patterns combining calcium elevations (excitation), calcium decreases (inhibition) and variable responses; this effect is mediated by ASI (purple arrow). Second, INS-32 and NLP-18 neuropeptides switch from a reversal-promoting effect to a reversal-suppressing effect. One simple model for INS-32 and NLP-18 action would be that they mediate the ASI effect on AWC activity patterns (purple arrow), but this possibility remains hypothetical. Third, glutamatergic signaling switches from a mostly reversal-promoting effect to a reversal-suppressing effect; this switch is associated with different glutamate sources (AWC and non-AWC neurons, respectively). Of note, residual reversal response upon starvation might be mediated by FLP-6 and glutamate from AWC^{OFF}. In summary, our findings highlight a complex reconfiguration of the thermoresponsive circuit following starvation, which includes thermosensory encoding change and complex neuromodulation using bidirectional signaling molecules that produce reversal-promoting (green pathways) or reversal-suppressing effects (red pathways) in a context-dependent manner.

almost entirely relied on the presence of intact AWC neurons. However, under our experimental conditions, we did not find major differences between AWC^{ON} or AWC^{OFF} subtypes in terms of calcium dynamics, nor did mutations affecting their asymmetry produce radical effects. Therefore, noxious heat-evoked activity in AWC varies widely according to context, which is in line with previous literature [18, 20, 41]. Interestingly, the role of AWC is distinct from that of AFD and FLP neurons, which are canonically linked to thermosensation and nociception [5, 14, 42, 43], but contribute only modestly to heat-evoked behavior in our assay conditions with between 1 and 6 hrs of food deprivation.

We further demonstrate that AWC neurons use both glutamatergic and neuropeptidergic signals to encode heat intensity, with glutamate (via EAT-4) primarily driving responses to stronger stimuli and the FLP-6 neuropeptide acting over a broader thermal range. Functional asymmetry between AWC^{ON} and AWC^{OFF} emerges in our rescue experiments, with AWC^{OFF} more effective in restoring heat-evoked behavior, particularly through FLP-6. This suggests that AWC^{OFF} may have privileged access to neuropeptidergic regulatory machinery or downstream targets, although glutamatergic signaling appears largely redundant across AWC subtypes. Together with previous findings, our results refine our appreciation of how AWC responds to heat and how developmental asymmetries might modulate aversive response to thermal cues over a very broad intensity range.

Starvation reshapes AWC heat stimulus-locked response from predominantly excitatory to a pattern combining excitatory and inhibitory responses

A key observation of this work is that prolonged starvation transforms heat stimulus-locked AWC calcium responses from intensity-dependent excitatory response patterns to a more heterogeneous response mode. This shift is not merely a quantitative attenuation but a qualitative reorganization of response states, including frequent calcium downregulation or variable traces, which in the same animal can comprise both calcium up- and down-regulation of calcium levels during a single stimulus train. We speculate that this transition from mostly excitatory responses to a mix of excitatory/inhibitory response profiles reflects a fundamental reconfiguration of sensory processing under prolonged nutrient deprivation. In an early food-deprived state, AWC neurons may signal heat intensity in a consistent manner to promote aversion. Under prolonged starvation, however, dampened and variable AWC responses may bias the animal away from escape behaviors, thus prioritizing food-seeking over threat avoidance. This state-dependent reconfiguration may serve as a flexible strategy to balance risk avoidance and metabolic needs, as elaborated below.

ASI neurons modulate AWC dynamics and promote plasticity via neuropeptides

Our data demonstrate that ASI neurons are dispensable for baseline thermonociceptive responses but essential for starvation-induced behavioral plasticity. Ablation of ASI fully abolished the starvation-dependent attenuation of heat-evoked reversals and prevented the pattern shift in AWC calcium response polarities. These results suggest that ASI gates the modulation of AWC activity based on internal nutritional state. ASI might also modulate the circuit downstream of AWC. Interestingly, in two previous studies, starvation-evoked down-regulation of AWC-dependent thermotactic behavior was intact when ASI was either transiently or chronically inhibited [26, 33]. Therefore, starvation most likely uses a different circuitry to modulate noxious-heat evoked reversal than for thermotaxis modulation.

We further identify two ASI-expressed neuropeptides, NLP-18 and INS-32, as relevant mediators of this plasticity. Mutants for the corresponding pro-neuropeptide genes displayed abnormal behavioral adaptation to starvation, and transgenic expression of either peptide in ASI partially rescues the plasticity defect. ASI was previously shown to mediate starvation-evoked phenotypic plasticity regarding developmental trajectories (via TGFbeta and the insulin-like peptide DAF-28 [44, 45]), food-seeking exploration in starved larvae (via NLP-24 opioid-like neuropeptide [46]), the

curvature taken during head-touch evoked escape response (via NLP-18 [47]), and ASH-mediated octanol avoidance response (via multiple NLP neuropeptides [48]). Our findings now add context-dependent aversive thermosensory behavior regulation to the growing list of satiety signaling and stress integration-related functions regulated by ASI neurons. It remains to be determined how ASI integrates systemic cues of metabolic status and how its neuropeptidergic outputs interact with AWC-neural pathway at the synaptic or circuit level to regulate noxious heat avoidance. Another open question is whether ASI action takes place during development (prior to starvation), or more acutely with active signaling after starvation.

Opposing neuromodulatory influences shape behavioral flexibility in order to balance protection and exploration strategies

The balance between starvation-induced suppression and preservation of thermonociceptive behavior appears to be controlled by a set of signaling molecules, whose effect can be bidirectional, operating in a context-dependent manner. Upon early food deprivation, neuropeptides, including INS-32 released from ASI and NLP-18 from an unidentified source, as well as glutamatergic signals from AWC neurons promote thermonociceptive behaviors (Figure 8A). Upon prolonged starvation, the same signaling molecules can produce the opposite effect and suppress this behavior (Figure 8B). For NLP-18 and glutamate the differential effect is associated with a potentially different source, e.g., non-AWC neurons for glutamate. For INS-32, our cell-specific rescue experiment suggests that ASI is a functionally relevant source in the two contexts. However, we cannot rule out the implication of additional neurons, nor an overexpression effect. While additional studies will be needed to decipher the context-dependent mode of action of each of these signaling molecules, our findings suggest a regulatory architecture in which bidirectional neuromodulatory signals integrate metabolic context with sensory drive to determine behavioral priorities. Rather than globally silencing the thermonociceptive pathway, starvation might well reconfigure it through a layered combination of neuronal state shifts, circuit-level inhibition, and peptide-based tuning. This complexity may afford the animal greater flexibility in selecting context-appropriate responses under competing environmental and internal pressures. From an ecological and evolutionary perspective, the downregulation of nociceptive responses during prolonged starvation likely reflects a strategic shift in behavioral priorities. In the face of acute noxious stimuli, animals typically favor rapid escape to minimize injury, a protective response. However, under prolonged food deprivation, such protection may come at the cost of missed opportunities for locating food. In this context, a heightened aversive response could lead to excessive avoidance of mildly threatening environments that may nevertheless contain nutritional resources. In addition, because worm exploration strategies rely on modulating locomotion speed, the duration of forward locomotion bouts and the frequency of re-orientation events, often associated with reversals, the down-regulation of reversal response is directly relevant to the rate of animal dispersion in the environment [49, 50]. By reducing the sensitivity or reliability of nociceptive pathways, particularly through modulating AWC output, *C. elegans* may adopt a more exploratory behavioral mode, tolerating greater risk to increase the chances of finding food. This trade-off between protection and exploration mirrors similar state-dependent shifts observed in other species [51, 52] suggesting a conserved strategy wherein internal metabolic needs dynamically reshape threat evaluation and behavioral responses.

Methods

C. elegans maintenance and strains list

C. elegans strains were maintained according to standard techniques on nematode growth medium (NGM) agar plates seeded with OP50. Animal synchronization was made by treating gravid adults with standard hypochlorite-based procedure. File S1 includes a list of strains used in the present study. The lines with genetical neuron ablation were previously described [8, 22, 53].

Cloning and transgenesis

Promoter-containing Entry plasmids (Multi-site Gateway slot 1) were constructed by PCR using N2 genomic DNA as template and primers flanked by attB4 and attB1r recombination sites; the PCR product being cloned into pDONR-P4-P1R vector (Invitrogen) by BP recombination. Coding sequence-containing Entry plasmids (Multi-site Gateway slot 2) were constructed by PCR using N2 cDNA as template and primers flanked by attB1 and attB2 recombination sites; the PCR product being cloned into pDONR_221 vector (Invitrogen) by BP recombination. Expression plasmids for transgenesis were created through LR recombination reactions (Gateway LR Clonase, Invitrogen) as per the manufacturer's instructions. DNA prepared with a GenElute HP Plasmid miniprep kit (Sigma) was microinjected in the gonad to generate transgenic lines according to a standard protocol [54]. Rescue constructs included SL2::mCherry transgenes, the expression pattern of which was verified using epifluorescence microscopy, as previously described [55]. File S1 includes a list of plasmids and primers used in this study.

Behavioral assays

Preparation of worms

All the behavioral experiments were performed in young adult animals cultivated at 20°C. For the Fed condition, 80 animals were transferred to a NGM plate fully covered with OP50 bacterial lawn 18 h before the experiment. For starved condition, fed animals were washed 3 times in 1.5 mL collection tubes with M9 (pre-equilibrated at the growth temperature of worms) to remove OP50. During washes, worms were left to settle to the bottom of the tubes by gravity. About 80 animals were then plated on unseeded NGM plates with a drop of M9 and left to air dry for 5 min. Duration of starvation was considered from the time of the start of the wash. During starvation, animals were kept in the incubator at 20°C. Food-odor experiments were performed by adding OP50 bacteria on the inward side of the petri dish lid during the starvation period.

Heat-stimulation protocol

Heat stimulation was delivered using previously described INFERNO system [31]. The stimulation program consisted of a baseline period of 40 s without any heat stimulation (to determine spontaneous reversal rate), 4 s with 100 W heating (1 IR lamp turned on), 20 s of interstimulus interval (ISI), 4 s with 200 W heating (2 lamps turned on), 20 s of ISI, 4 s with 300 W heating (3 lamps turned on), 20 s of ISI and 4 s with 400 W heating (4 lamps turned on) as previously described. Thermal increases were measured with Type K thermal probes linked to Data Logger (Pico Technology). For these four heating power levels, the peak temperatures reached at the surface of the plate were ~22, ~24, ~26 and ~28°C, respectively, corresponding to thermal slope approximating 0.5, 1, 1.5 and 2 °C/s, respectively.

Movie recording and analysis

During the stimulation program, worm plates were filmed using a DMK 33U×250 camera and movies acquired with the IC capture software (The Imaging Source), at a 1600×1800 pixel resolution, at 8 frames per second, and the resulting .AVI file was encoded as Y800 8-bit monochrome. Behavioral recordings were analyzed using the Multi-Worm Tracker 1.3.0 (MWT) [56]. A custom Python script was used to flag reversal events and report the frame during which they occurred. The movie time course was separated into 4-s bins and the fraction of animals reversing in each bin was extracted as the primary output for subsequent analyses.

Calcium imaging

Worm preparation and thermal stimulation

The imaging was performed 1 h and 6 h after food deprivation. Animals cultivated at 20°C were glued, maintained at 20°C for 90 s of baseline imaging acquisition and subjected to 30-s heat stimulation at 22°C, 24°C, 26°C and 28°C using CherryTemp microfluidic system by Cherry Biotech,

with 60-s ISI. The system works by shifting between two fluidic loops that each pass through a thermalization block located in the vicinity of the specimen. It takes between 4 and 6 s to achieve 80% of the thermal shift between baseline (20°) and the higher temperature target.

Data acquisition and trace processing

Calcium imaging was performed using a Leica DMI6000B inverted epifluorescence microscope equipped with a HCX PL Fluotar L40x/0.60 CORR dry objective, a Leica DFC360FX CCD camera, an EL6000 light source, and equipped with fast filter wheels for FRET imaging (excitation filter: 427 nm (BP 427/10); emission filters 472 (BP 472/30) and 542 nm (BP 542/27) [14]. Calcium levels in AWC^{ON} and AWC^{OFF} neurons were monitored using cameleon YC2.3 sensor. Imaging YFP/CFP emission ratio (R) of the YC2.3 cameleon indicator was used to analyze relative changes in response to short-lasting stimuli. Baseline drift was estimated by linear interpolation between consecutive baseline periods (average over 10 s prior to each stimulus) and subtracted from the raw ratiometric trace (to obtain ΔR values), effectively removing slow, non-physiological signal components (due to differential photobleaching of the two fluorophores) while preserving rapid stimulus-evoked calcium responses. Baseline-corrected ΔR traces were used for visualization and comparative analyses, including heat maps of response amplitude and directions.

Manual trace categorization

Trace categorization as *calcium up*, *calcium down* or *variable* was done manually (not blind to the condition). First, the polarity of calcium signal change was assessed on a trial-by trial-basis. For each trial, we considered the direction of the first visible peak following thermal stimulation and labeled it as either *calcium up trial, calcium down trial,* or *undefined polarity trial* (when no obvious direction could be delineated due to limited signal change or to low signal-to-noise ratio). A visible peak was defined as a peak whose magnitude was at least twice as large as the signal variation (noise) seen in the immediately preceding 10-s baseline period and whose duration was at least 2 frames. Second, traces were categorized as *calcium up* (with three or more *calcium up* trails), as *calcium down traces* (with three or more *calcium down* trails) and as *variable* (other cases). Globally, this manual categorization used arbitrary signal-to-noise cutoff and criteria to qualitatively assess traces and sort them for visualization.

Peak intensities histograms

To complement the manual trace categorization with a more quantitative approach, we computed the response magnitude in each by averaging the ΔR signal in the 15-s post stimulus (this ΔR signal already subtracts the local baseline signal). The distributions of response magnitudes (which could be either positive for excitatory responses or negative for inhibitory responses) were presented in histograms.

Statistical analyses

We used Jamovi (Version 2.6.44) to conduct Kruskal-Wallis non-parametric tests (to compare calcium peak distribution differences) and ANOVAs, followed by Bonferroni post hoc tests (to compare behavioral scores). Multiple-comparison corrections took account of repeated comparisons to the same controls. Some analyses are reported across multiple figure panels, in which case the same control data was plotted several times, with indication in the respective figure legends. Replicate numbers (n) are defined in the Figures. These n were determined in agreement with previous studies using similar measures. No *a priori* power analyses were performed. Results of statistical tests, including p-values and effect sizes, are included in File S2.

Figure supplements

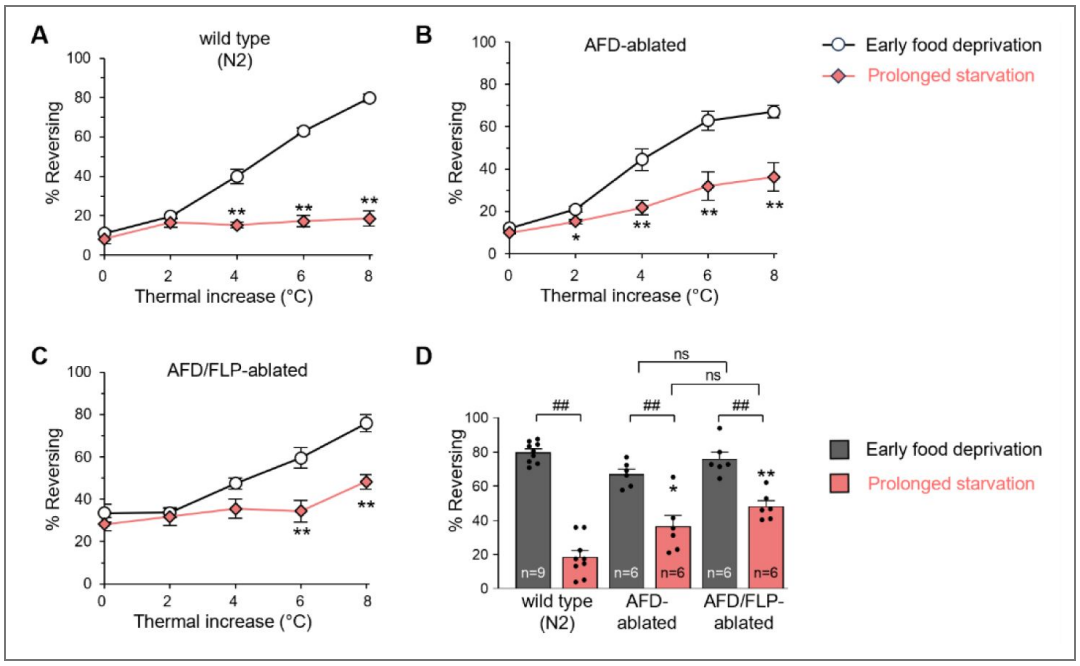


Figure 2-supplement 1. Heat-evoked reversal upon early food deprivation and starvation-dependent plasticity are largely intact in AFD and FLP-ablated animals Impact of the genetic ablation of candidate thermo-responsive neurons on thermonociceptive response and starvation-dependent plasticity. **A-C** Comparison of heat-evoked reversals after early food deprivation (off-food 1hr) and starvation (off-food 6 hr) in wild type and in transgenic animals with caspase-mediated ablation of indicated neurons. Results are presented as average +/- S.E.M. *, *p<.05,* and **, *p<.01* versus corresponding heat level in the early food deprivation condition, by Bonferroni post-hoc tests. **D** Comparison for the highest heating level (thermal increase = 8 °C) across the three genotypes presented in panel A to C. Bars as average, dots as individual assay scores, and error bars as S.E.M. ##, *p<.01* between early food deprivation and prolonged starvation for each genotype; **, *p<.01* versus wild type (N2) at the corresponding timepoint, by Bonferroni post-hoc tests. The total number of assays (n) analyzed per condition, each scoring at least 50 worms, are indicated in panel D. The wild type (N2) dataset is the same as in Figure 2D. Data reported in the two figures were acquired in parallel and multiple comparison corrections were made in a single analysis.

Figure 3-Supplement 1. Alteration of heat-evoked reversals in *nlp-5* and *ins-22* mutants

A-B Comparison of heat-evoked reversals after early food deprivation (off-food 1hr) in wild type, *nlp-5(ok1981)* and *ins-22(ok3616)*. Results are presented as average +/- S.E.M. *, *p<.05* and **, *p<.01* versus wild type by Bonferroni post-hoc tests. The number of assays (*n*), each scoring at least 50 worms, were: wild type, *n*=27; *nlp-5*, *n*=6; *ins-22*, *n*=6. A single wt control dataset is reported across panels in this Figure and in Figure 3.

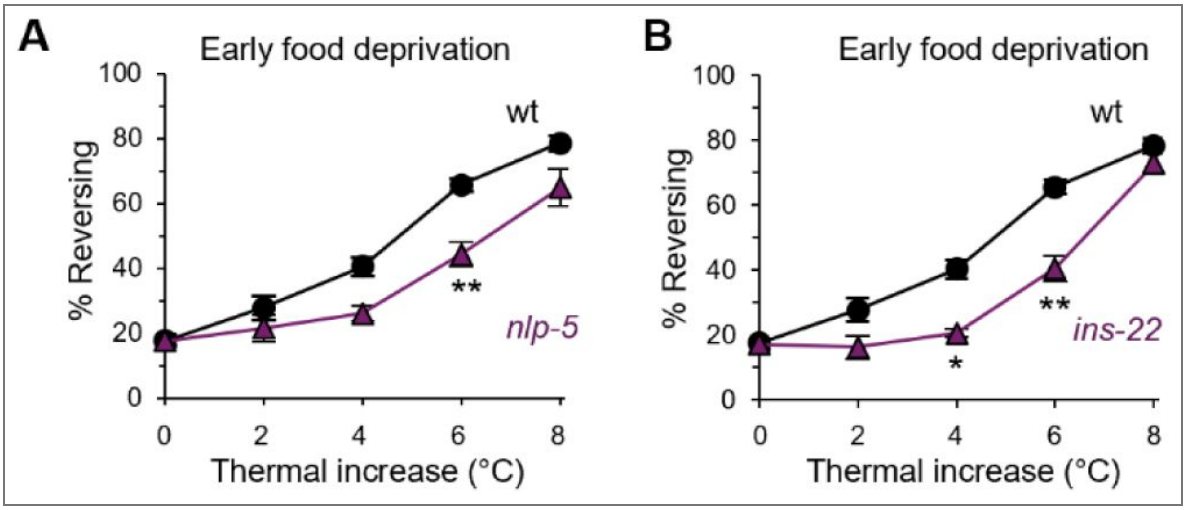
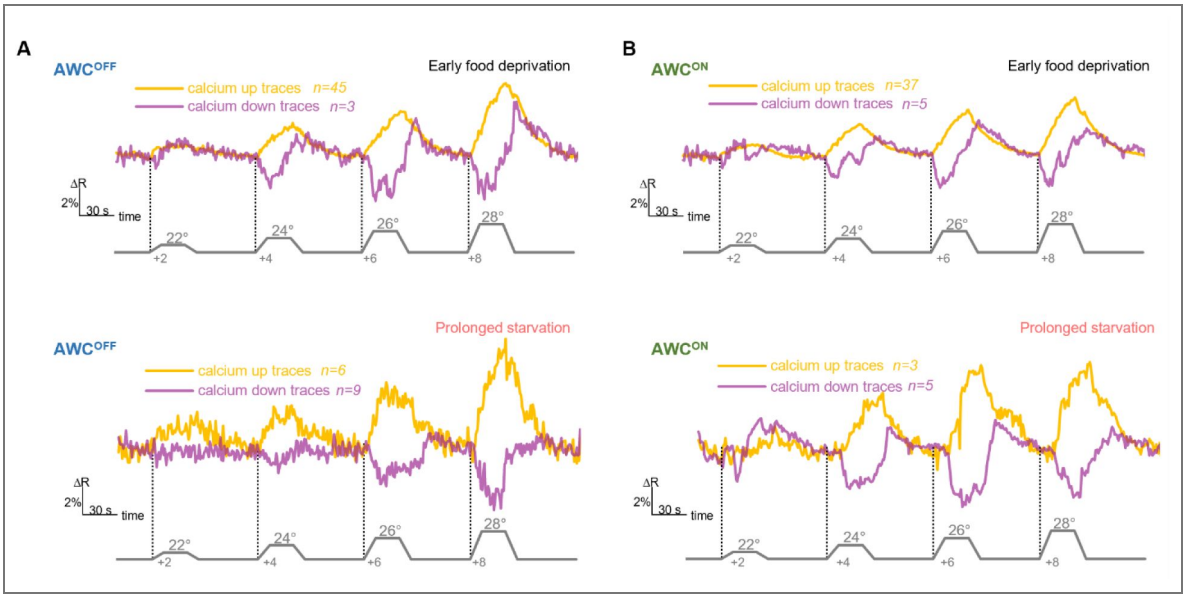


Figure 4-supplement 1. Similar kinetics of calcium up and calcium down responses under the early food deprivation and the prolonged starvation condition

A-B From the same dataset as in Figure 4 A-B, average traces showing the dynamics of *calcium up* (excitatory) or *calcium down* (inhibitory) response types with similar amplitude and globally comparable shapes.



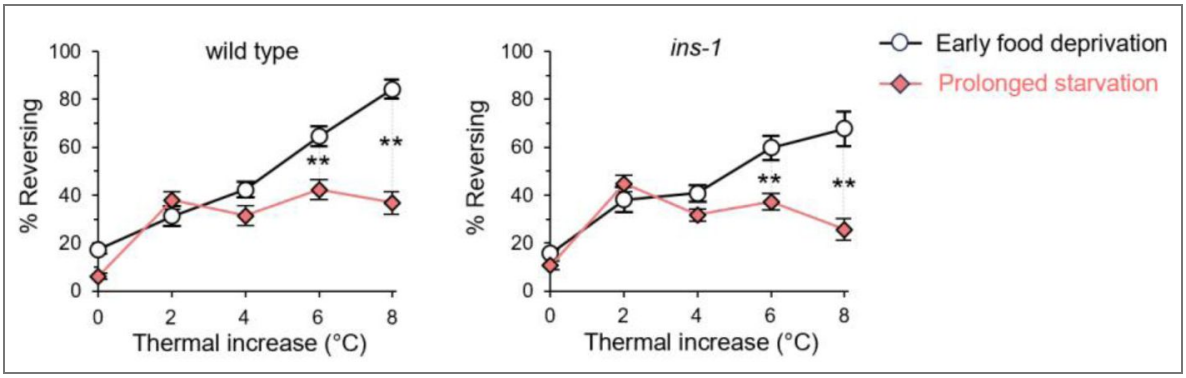


Figure 6-Supplement 1. Intact starvation-induced thermonociceptive plasticity in *ins-1* mutants

Comparison of heat-evoked reversals after early food deprivation (off-food 1hr) and prolonged starvation (off-food 6hr) in wild type (N2), and *ins-1(nj32)*. *, *p<.05,* and **, *p<.01* between the two food deprivation timepoints, by Bonferroni post-hoc tests. #, *p<.05,* and ##, *p<.01* versus wild type (N2) at the corresponding timepoint, by Bonferroni post-hoc tests.

Data availability

The data associated with this article are included in the manuscript, the figure and supplements (with raw data in File S2).

Acknowledgements

We are grateful to Lisa Schild and Laurence Bulliard for expert technical support, to Aurore Jordan for the cloning of AWC-specific promoter plasmids and for the participation in calcium trace acquisition. Some strains were provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440). Some strains were provided by NBRP, which is funded by the Japanese government. The study was supported by the Swiss National Science Foundation (BSSGI0_155764, PP00P3_150681, and 310030_197607 to DAG).

Additional information

Data availibility statement

The data associated with this article are included in the manuscript, the figure and supplements (with raw data in File S2).

Material availibility statement

The strains and plasmids generated during the present study should be requested to the corresponding author.

Funding

Funder	Grant reference number	Author
Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung (SNF)	BSSGI0_155764	Dominique A Glauser
Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung (SNF)	PP00P3_1506	Dominique A Glauser
Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung (SNF)	310030_197607	Dominique A Glauser

Author ORCID iDs

Dominique A Glauser: https://orcid.org/0000-0002-3228-7304

Additional files

File S1.

File S2.

References

1. **Ezcurra M,** et al. (2011) Food sensitizes C. elegans avoidance behaviours through acute dopamine signalling. *EMBO J* **30**:1110-1122 https://doi.org/10.1038/emboj.2011.22 | PubMed
2. **Ryu L,** et al. (2018) Feeding state regulates pheromone-mediated avoidance behavior via the insulin signaling pathway in Caenorhabditis elegans. *Embo j* **37** https://doi.org/10.15252/embj.201798402 | PubMed
3. **Burrell BD** (2017) Comparative biology of pain: What invertebrates can tell us about how nociception works. *J Neurophysiol* **117**:1461-1473 https://doi.org/10.1152/jn.00600.2016 | PubMed

4. **Byrne Rodgers J,** Ryu WS (2020) Targeted thermal stimulation and high-content phenotyping reveal that the C. elegans escape response integrates current behavioral state and past experience. *PLOS One* **15**:e0229399 https://doi.org/10.1371/journal.pone.0229399 | PubMed
5. **Liu S,** Schulze E, Baumeister R (2012) Temperature- and Touch-Sensitive Neurons Couple CNG and TRPV Channel Activities to Control Heat Avoidance in Caenorhabditis elegans. *PLoS One* **7**:e32360 https://doi.org/10.1371/journal.pone.0032360 | PubMed
6. **Jordan A,** Glauser DA (2023) Distinct clusters of human pain gene orthologs in Caenorhabditis elegans regulate thermo-nociceptive sensitivity and plasticity. *Genetics* **224** https://doi.org/10.1093/genetics/iyad047 | PubMed
7. **Rudgalvyte M,** et al. (2025) Antagonist actions of CMK-1/CaMKI and TAX-6/calcineurin along the C. elegans thermal avoidance circuit orchestrate adaptation of nociceptive response to repeated stimuli. *eLife* **14** https://doi.org/10.7554/elife.103497 | PubMed
8. **Glauser DA,** et al. (2011) Heat avoidance is regulated by transient receptor potential (TRP) channels and a neuropeptide signaling pathway in Caenorhabditis elegans. *Genetics* **188**:91-103 https://doi.org/10.1534/genetics.111.127100 | PubMed
9. **Schild LC,** et al. (2014) The balance between cytoplasmic and nuclear CaM kinase-1 signaling controls the operating range of noxious heat avoidance. *Neuron* **84**:983-96 https://doi.org/10.1016/j.neuron.2014.10.039 | PubMed
10. **Wittenburg N,** Baumeister R (1999) Thermal avoidance in Caenorhabditis elegans: an approach to the study of nociception. *Proc Natl Acad Sci U S A* **96**:10477-82 https://doi.org/10.1073/pnas.96.18.10477 | PubMed
11. **Mohammadi A,** et al. (2013) Behavioral response of Caenorhabditis elegans to localized thermal stimuli. *BMC Neurosci* **14**:66 https://doi.org/10.1186/1471-2202-14-66 | PubMed
12. **Glauser DA,** Goodman MB (2016) Molecules empowering animals to sense and respond to temperature in changing environments. *Curr Opin Neurobiol* **41**:92-98 https://doi.org/10.1016/j.conb.2016.09.006 | PubMed
13. **Goodman MB,** Sengupta P (2018) The extraordinary AFD thermosensor of C. elegans. *Pflugers Arch* **470**:839-849 https://doi.org/10.1007/s00424-017-2089-5 | PubMed
14. **Saro G,** et al. (2020) Specific Ion Channels Control Sensory Gain, Sensitivity, and Kinetics in a Tonic Thermonociceptor. *Cell Rep* **30**:397-408. https://doi.org/10.1016/j.celrep.2019.12.029 | PubMed
15. **Thapliyal S,** Beets I, Glauser DA (2023) Multisite regulation integrates multimodal context in sensory circuits to control persistent behavioral states in C. elegans. *Nat Commun* **14**:3052 https://doi.org/10.1038/s41467-023-38685-1 | PubMed
16. **Bargmann CI** (2006) Chemosensation in C. elegans. *WormBook* 1-29 https://doi.org/10.1895/wormbook.1.123.1 | PubMed
17. **Colbert HA,** Bargmann CI (1995) Odorant-specific adaptation pathways generate olfactory plasticity in C. elegans. *Neuron* **14**:803-12 https://doi.org/10.1016/0896-6273(95)90224-4 | PubMed
18. **Kuhara A,** et al. (2008) Temperature sensing by an olfactory neuron in a circuit controlling behavior of C. elegans. *Science* **320**:803-7 https://doi.org/10.1126/science.1148922 | PubMed
19. **Zaslaver A,** et al. (2015) Hierarchical sparse coding in the sensory system of Caenorhabditis elegans. *Proc Natl Acad Sci U S A* **112**:1185-9 https://doi.org/10.1073/pnas.1423656112 | PubMed
20. **Biron D,** et al. (2008) An olfactory neuron responds stochastically to temperature and modulates Caenorhabditis elegans thermotactic behavior. *Proc Natl Acad Sci U S A* **105**:11002-7 https://doi.org/10.1073/pnas.0805004105 | PubMed
21. **Kotera I,** et al. (2016) Pan-neuronal screening in Caenorhabditis elegans reveals asymmetric dynamics of AWC neurons is critical for thermal avoidance behavior. *eLife* **5** https://doi.org/10.7554/elife.19021 | PubMed

22. **Beverly M,** Anbil S, Sengupta P (2011) Degeneracy and neuromodulation among thermosensory neurons contribute to robust thermosensory behaviors in Caenorhabditis elegans. *J Neurosci* **31**:11718-27 https://doi.org/10.1523/jneurosci.1098-11.2011 | PubMed

23. **Harris G,** et al. (2011) Dissecting the serotonergic food signal stimulating sensory-mediated aversive behavior in C. elegans. *PLoS One* **6**:e21897 https://doi.org/10.1371/journal.pone.0021897 | PubMed

24. **Mills H,** et al. (2012) Monoamines and neuropeptides interact to inhibit aversive behaviour in Caenorhabditis elegans. *Embo j* **31**:667-78 https://doi.org/10.1038/emboj.2011.422 | PubMed

25. **Oranth A,** et al. (2018) Food Sensation Modulates Locomotion by Dopamine and Neuropeptide Signaling in a Distributed Neuronal Network. *Neuron* **100**:1414-1428. https://doi.org/10.1016/j.neuron.2018.10.024 | PubMed

26. **Takeishi A,** et al. (2020) Feeding state functionally reconfigures a sensory circuit to drive thermosensory behavioral plasticity. *eLife* **9** https://doi.org/10.7554/elife.61167 | PubMed

27. **Rengarajan S,** et al. (2019) Feeding state sculpts a circuit for sensory valence in Caenorhabditis elegans. *Proceedings of the National Academy of Sciences of the United States of America* **116**:1776-1781 https://doi.org/10.1073/pnas.1807454116 | PubMed

28. **Banerjee N,** et al. (2023) Differential processing of a chemosensory cue across life stages sharing the same valence state in Caenorhabditis elegans. *Proceedings of the National Academy of Sciences of the United States of America* **120**:e2218023120 https://doi.org/10.1073/pnas.2218023120 | PubMed

29. **Ghosh DD,** et al. (2016) Neural Architecture of Hunger-Dependent Multisensory Decision Making in C. elegans. *Neuron* **92**:1049-1062 https://doi.org/10.1016/j.neuron.2016.10.030 | PubMed

30. **Ezcurra M,** et al. (2016) Neuropeptidergic Signaling and Active Feeding State Inhibit Nociception in Caenorhabditis elegans. *J Neurosci* **36**:3157-69 https://doi.org/10.1523/jneurosci.1128-15.2016 | PubMed

31. **Lia AS,** Glauser DA (2020) A system for the high-throughput analysis of acute thermal avoidance and adaptation in C. elegans. *J Biol Methods* **7**:e129 https://doi.org/10.14440/jbm.2020.324 | PubMed

32. **Ippolito D,** Thapliyal S, Glauser DA (2021) Ca2+/CaM binding to CaMKI promotes IMA-3 importin binding and nuclear translocation in sensory neurons to control behavioral adaptation. *eLife* **10**:e71443 https://doi.org/10.7554/eLife.71443 | PubMed

33. **Yeon J,** Takeishi A, Sengupta P (2021) Chronic vs acute manipulations reveal degeneracy in a thermosensory neuron network. *MicroPubl Biol* **2021** https://doi.org/10.17912/micropub.biology.000355 | PubMed

34. **Ghaddar A,** et al. (2023) Whole-body gene expression atlas of an adult metazoan. *Sci Adv* **9**:eadg0506 https://doi.org/10.1126/sciadv.adg0506 | PubMed

35. **Taylor SR,** et al. (2021) Molecular topography of an entire nervous system. *Cell* **184**:4329-4347. https://doi.org/10.1016/j.cell.2021.06.023 | PubMed

36. **Ghosh R,** et al. (2012) Multiparameter behavioral profiling reveals distinct thermal response regimes in Caenorhabditis elegans. *BMC Biol* **10**:85 https://doi.org/10.1186/1741-7007-10-85 | PubMed

37. **Lesch BJ,** et al. (2009) Transcriptional regulation and stabilization of left-right neuronal identity in C. elegans. *Genes Dev* **23**:345-58 https://doi.org/10.1101/gad.1763509 | PubMed

38. **Troemel ER,** Sagasti A, Bargmann CI (1999) Lateral signaling mediated by axon contact and calcium entry regulates asymmetric odorant receptor expression in C. elegans. *Cell* **99**:387-98 https://doi.org/10.1016/s0092-8674(00)81525-1 | PubMed

39. **Gallagher T,** et al. (2013) ASI regulates satiety quiescence in C. elegans. *J Neurosci* **33**:9716-24 https://doi.org/10.1523/jneurosci.4493-12.2013 | PubMed

40. **Chen Y,** Baugh LR (2014) Ins-4 and daf-28 function redundantly to regulate C. elegans L1 arrest. *Developmental Biology* **394**:314-326 https://doi.org/10.1016/j.ydbio.2014.08.002 | PubMed

41. **Flavell SW,** Raizen DM, You YJ (2020) Behavioral States. *Genetics* **216**:315-332 https://doi.org/10.1534/genetics.120.303539 | PubMed

42. **Kimura KD,** et al. (2004) The C. elegans Thermosensory Neuron AFD Responds to Warming. *Current Biology* **14**:1291-1295 https://doi.org/10.1016/j.cub.2004.06.060 | PubMed

43. **Chatzigeorgiou M,** Schafer William R (2011) Lateral Facilitation between Primary Mechanosensory Neurons Controls Nose Touch Perception in C. elegans. *Neuron* **70**:299-309 https://doi.org/10.1016/j.neuron.2011.02.046 | PubMed

44. **Makino M,** et al. (2021) Regulation of Satiety Quiescence by Neuropeptide Signaling in Caenorhabditis elegans. *Front Neurosci* **15**:678590 https://doi.org/10.3389/fnins.2021.678590 | PubMed

45. **Li W,** Kennedy SG, Ruvkun G (2003) daf-28 encodes a C. elegans insulin superfamily member that is regulated by environmental cues and acts in the DAF-2 signaling pathway. *Genes Dev* **17**:844-58 https://doi.org/10.1101/gad.1066503 | PubMed

46. **Park JY,** et al. (2020) A novel functional cross-interaction between opioid and pheromone signaling may be involved in stress avoidance in Caenorhabditis elegans. *Sci Rep* **10**:7524 https://doi.org/10.1038/s41598-020-64567-3 | PubMed

47. **Chen L,** et al. (2024) CKR-1 orchestrates two motor states from a single motoneuron in C. elegans. *iScience* **27**:109390 https://doi.org/10.1016/j.isci.2024.109390 | PubMed

48. **Hapiak V,** et al. (2013) Neuropeptides amplify and focus the monoaminergic inhibition of nociception in Caenorhabditis elegans. *J Neurosci* **33**:14107-16 https://doi.org/10.1523/jneurosci.1324-13.2013 | PubMed

49. **Glauser DA** (2013) How and why Caenorhabditis elegans uses distinct escape and avoidance regimes to minimize exposure to noxious heat. *Worm* **2**:e27285 https://doi.org/10.4161/worm.27285 | PubMed

50. **Schild LC,** Glauser DA (2013) Dynamic switching between escape and avoidance regimes reduces Caenorhabditis elegans exposure to noxious heat. *Nat Commun* **4** https://doi.org/10.1038/ncomms3198 | PubMed

51. **Lee Y-F,** Kuo Y-M, Chu W-C (2016) Energy state affects exploratory behavior of tree sparrows in a group context under differential food-patch distributions. *Frontiers in Zoology* **13**:48 https://doi.org/10.1186/s12983-016-0180-y | PubMed

52. **Grunwald Kadow IC** (2019) State-dependent plasticity of innate behavior in fruit flies. *Current Opinion in Neurobiology* **54**:60-65 https://doi.org/10.1016/j.conb.2018.08.014 | PubMed

53. **Russell J,** et al. (2014) Humidity sensation requires both mechanosensory and thermosensory pathways in Caenorhabditis elegans. *Proc Natl Acad Sci U S A* **111**:8269-74 https://doi.org/10.1073/pnas.1322512111 | PubMed

54. **Evans TC** (2006) Transformation and microinjection. *WormBook* https://doi.org/10.1895/wormbook.1.108.1

55. **Marques F,** et al. (2020) Tissue-specific isoforms of the single C. elegans Ryanodine receptor gene unc-68 control specific functions. *PLoS Genet* **16**:e1009102 https://doi.org/10.1371/journal.pgen.1009102 | PubMed

56. **Swierczek NA,** et al. (2011) High-throughput behavioral analysis in C. elegans. *Nat Methods* **8**:592-8 https://doi.org/10.1038/nmeth.1625 | PubMed

Peer reviews

Reviewer #1 (Public review):

This study by Thapliyal and Glauser investigates the neural mechanisms that contribute to the progressive suppression of thermonociceptive behavior that is induced under conditions of starvation. Several previous studies have demonstrated that when starved, C. elegans alters its preferences for a variety of sensory cues, including CO2, temperature, and odors, in order to prioritize food seeking over other behavioral drives. The varied mechanisms that

underlie the ability of internal states to alter behavioral responses are not fully understood, however there is growing evidence for a role by neuropeptidergic signaling as well as capacity for functionally distinct microcircuits, formed by distinct internal states, to trigger similar behavior outcomes.

Within the physiological range of C. elegans (~15-25C), starvation triggers a profound reduction in temperature-driven thermotaxis behaviors. This reduction involves the recruitment of the amphid sensory neuron pair AWC. The AWC neurons primarily act to sense appetitive chemosensory cues, however under starvation conditions begin to display temperature responses that previous studies have linked to the reduction in thermotaxis navigation. Here, Thapliyal and Glauser investigate the impact of starvation on thermonociceptive responses, innate escape behaviors that are triggered by exposure to noxious temperatures above 26C or rapid thermal stimuli below 26C. They compare the strength of thermonociceptive behaviors, specifically heat-triggered reversals, in worms experiencing either early food deprivation (1 hour off food) or prolonged starvation (6 hours off food). Their experiments demonstrate a progressive loss of heat-triggered reversals that is mediated by AWC and ASI neurons, as well as both glutamateric and neuropeptidergic signaling.

At the level of neural activity, this study reports that the transition from early food deprivation to prolonged starvation reconfigures the temperature-driven activity of AWC neurons from mostly excitatory to a heterogenous mix combining excitatory and inhibitory responses. This finding is interesting in light of previous work that reported the opposite transition in temperature-driven AWC responses when comparing well-fed worms to those kept from food for 3 hours. Specifically, these differences highlight the differences between temperature responses within the C. elegans physiological temperature range (previous studies) and their noxious temperature response (this study). This study also identifies neural and genetic mechanisms that contribute to differences in thermonociceptive responses at +1 versus +6 hours starvation; interestingly, these mechanisms are also partially distinct from those that contribute to differences in negative thermotaxis behaviors in well-fed and +3 hours starvation worms. A limitation of this manuscript is that these differences are not particularly acknowledged or addressed, other than the hypothesis that independent mechanisms underlie negative thermotaxis versus thermonociceptive stimuli.

In this revised article, the authors commanding knowledge of the distinction between thermotaxis navigation (especially negative thermotaxis) and thermonociceptive behaviors is communicated with an admirable depth and clarity to the readers; this study's new findings are helpfully contextualized within the previous literature.

This study represents an important addition to the growing evidence that C. elegans sensory behaviors are strongly impacted by internal states, and that neuropeptigergic signaling plays a key role in mediating behavioral plasticity. To that end, the authors have provided compelling evidence of their claims.

https://doi.org/10.7554/eLife.108246.2.sa2

Reviewer #3 (Public review):

Thapliyal, Gopinath, and Glauser show that starvation alters how C. elegans respond to noxious thermal stimuli. Using targeted neural ablation, mutant analysis, and live-cell functional imaging the authors demonstrate that hunger changes the properties of AWC sensory neurons, which sense noxious heat. The authors further show that effects of hunger on nociception require ASI neurons, which are known to respond to hunger and mediate effects of food deprivation on behavior. Finally, the study uses mutant analysis to implicate glutamate and specific neuropeptides in thermal nociception and in modulation of nociceptors by hunger-responsive neurons.

The study clearly shows a strong effect of hunger on nociception and documents a striking effect of hunger on the intrinsic properties of AWC sensory neurons, which respond to noxious heat. The study also clearly and compellingly demonstrates that ablation of hunger-responsive ASI neurons blocks effects of hunger on nociceptive AWCs. These data, which constitute the kernel of the manuscript, are striking and exciting. This revised manuscript analyzes effects of starvation on AWC physiology and clearly shows that starvation alters the way AWCs respond to thermal stimuli by decreases the probability that AWCs will be activated and increases the probability that they will be inhibited. New data also identify ASI-derived neuropeptides that are required for modulation of AWCs by starvation. This study reveals a mechanistic link between an animal's metabolic state and sensory processing and establishes modulation of AWC function as a powerful model to study the molecular basis of this link.

https://doi.org/10.7554/eLife.108246.2.sa1

Author response:

The following is the authors' response to the original reviews.

We believe that the manuscript has been substantially strengthened through the revision process. The main changes are summarized below:

We substantially revised the Introduction and Discussion sections to better position our work relative to previous studies on starvation-dependent thermotaxis plasticity, neuropeptidergic modulation, and AWC function.

We clarified throughout the manuscript the distinction between negative thermotaxis in innocuous thermal ranges and thermonociceptive responses to noxious heat. We now discuss more explicitly that these behaviors involve at least partly distinct molecular, cellular, and circuit-level mechanisms.

We performed new experiments in *ins-1* mutants. Unlike what was previously reported for thermotaxis plasticity, *ins-1* does not appear required for starvation-dependent thermonociceptive plasticity in our paradigm (new Figure 6—figure supplement 1).

We revised the analysis and terminology used for AWC calcium imaging data. We no longer use the "deterministic/stochastic" terminology and instead describe a starvation-induced shift from predominantly excitatory responses to a mixed distribution of excitatory and inhibitory responses. We also added new quantitative analyses and histogram representations of response distributions, as directly suggested by reviewers, to better illustrate this point.

We performed new genetic interaction experiments using *eat-4*; *flp-6* double mutants. These analyses revealed that glutamatergic and FLP-6 signaling act largely in parallel to mediate heat-evoked reversals after early food deprivation, while prolonged starvation reveals a hierarchical interaction between these pathways.

We revised and clarified the mechanistic model figures accordingly, particularly regarding the proposed ASI → AWC signaling pathway and the role of ASI-derived neuropeptides.

We improved the presentation and statistical rigor throughout the manuscript, including:

- Replacement of heating power values by corresponding temperature increases,

- Clarification of the rationale for using the 1-hour off-food condition as reference,

- Expanded statistical reporting and multiple-comparison procedures,

- Additional methodological details for calcium imaging, rescue validation, and cell ablation approaches,

- Clarification of replotted datasets in figure legends.

We also simplified the manuscript by removing experiments whose interpretation remained ambiguous (notably the *nsy-1* and *nsy-7* analyses).

Below, we provide a detailed point-by-point response to all reviewer comments.

> ***Public Reviews:***
>
> ***Reviewer #1 (Public review):***
>
> *This study by Thapliyal and Glauser investigates the neural mechanisms that contribute to the progressive suppression of thermonociceptive behavior that is induced under conditions of starvation. Several previous studies have demonstrated that when starved, C. elegans alters its preferences for a variety of sensory cues, including CO2, temperature, and odors, in order to prioritize food seeking over other behavioral drives. The varied mechanisms that underlie the ability of internal states to alter behavioral responses are not fully understood; however, there is growing evidence for a role of neuropeptidergic signaling as well as the capacity for functionally distinct microcircuits, formed by distinct internal states, to trigger similar behavior outcomes.*
>
> *Within the physiological range of C. elegans (~15-25{degree sign}C), starvation triggers a profound reduction in temperature-driven thermotaxis behaviors. This reduction involves the recruitment of the amphid sensory neuron pair AWC. The AWC neurons primarily act to sense appetitive chemosensory cues; however, under starvation conditions begin to display temperature responses that previous studies have linked to the reduction in thermotaxis navigation. Here, Thapliyal and Glauser investigate the impact of starvation on thermonociceptive responses, innate escape behaviors that are triggered by exposure to noxious temperatures above 26{degree sign}C or rapid thermal stimuli below 26{degree sign}C. They compare the strength of thermonociceptive behaviors, specifically heat-triggered reversals, in worms experiencing either early food deprivation (1 hour off food) or prolonged starvation (6 hours off food). Their experiments demonstrate a progressive loss of heattriggered reversals that is mediated by AWC and ASI neurons, as well as both glutamatergic and neuropeptidergic signaling.*
>
> *At the level of neural activity, this study reports that the transition from early food deprivation to prolonged starvation reconfigures the temperature-driven activity of AWC neurons from largely deterministic to stochastic. This finding is interesting in light of previous work that reported the opposite transition (from stochastic to deterministic) in temperature-driven AWC responses when comparing well-fed worms to those kept from food for 3 hours. This study also identifies neural and genetic mechanisms that contribute to differences in thermonociceptive responses at +1 versus +6 hours of starvation; confusingly, these mechanisms are partially distinct from those that*

> *contribute to differences in negative thermotaxis behaviors in well-fed and +3 hours of starvation worms (Takeishi et al, 2020). A limitation of this manuscript is that these differences are not particularly acknowledged or addressed, other than the hypothesis that independent mechanisms underlie negative thermotaxis versus thermonociceptive stimuli. However, this suggestion is not experimentally verified.*

We thank this reviewer for pointing to the interest of our work. The difference between previous work focusing on negative thermotaxis in the range of innocuous temperatures and our work focusing on thermo-nociceptive response is important and indeed deserves further clarification and a deeper discussion in the manuscript.

Two major empirical evidence for a distinction between negative thermotaxis (as assessed in previous studies) and thermonociceptive plasticity (as assessed in our paradigm) were already included in the initial article version. First, we reported a decrease in average response in AWC neurons due to a shift in the distribution of response polarities from mostly up-response to a mix of 'up-response' and 'downresponse' after starvation, while previous results showed an increase in response probability of AWCs after starvation (Takeishi et al, 2020). Second, contrary to starvation-evoked thermotaxis adaptation, ASI neurons are required to orchestrate starvation-evoked plasticity in thermonociception. These observations already indicate differences at the circuit and cellular level. For the revision, we conducted further experiments to address the molecular level. We tested *ins-1* mutants (see also specific point 3 by reviewer 2, below) and deepened this aspect in the discussion section of the revised manuscript. Previous study found that INS-1 signaling from the intestine is a major mediator of negative thermotaxis plasticity. In contrast, our new data show that INS-1 peptide does not seem critical in regulating starvation-dependent thermonociceptive plasticity (see new Figure 6-supplement 1). Taken together these three lines of empirical evidence support the notion that negative thermotaxis and thermonociceptive starvation-evoked plasticity involves at least partially distinct mechanisms and it seems therefore inappropriate to qualify this notion as purely hypothetical.

Modification in the revised manuscript include extended introduction about the known thermotaxis regulation mechanisms (Introduction section), new Figure 6-supplement 1 about *ins-1* and accompanying text in the result section as well as extended discussion about these differences (Discussion section)

Multiple additional aspects of this study make the results difficult to synthesize with existing knowledge, including

> *(1) Differences in - and insufficient discussion of - the magnitude and kinetics of thermal stimuli;*

We have included a better description of the stimuli characteristics in the revised methods section. The discussion section was deepened to better emphasize that different types of thermal stimuli have been used in different studies.

> *(2) This study's use of "heating power" rather than temperature values when presenting behavioral results;*

Thanks for noting this point, which was indeed an unnecessary complication in the result display of the initial manuscript. We have changed 'power values' to corresponding 'temperature increase' in the revised figures.

> *(3) The use of +1 hours starvation as a baseline instead of well-fed worms. Indeed, this last point reflects a noticeable experimental result that differs from previous studies, namely that at room temperature, the basal movements of well-fed and starved worms are not different. Such a surprising result warrants further quantification of worm mobility in general and could have prompted a set of experiments directly testing*

> *previously published thermal conditions to demonstrate that the new effects reported arise specifically from the use of thermonociceptive stimuli, as hypothesized.*

The consideration of on-food and off-food behavioral state is an important point indeed. We found that, at room temp, worms shift from dwelling on food (a state with high spontaneous reversal rate) to global search off-food (a state with low spontaneous reversal, after 1hr starvation) (see Figure 1B). Therefore, unlike the reviewer's statement, the reported data highlighted key differences in the basal locomotion of worms in fed and 1hr starved conditions. Furthermore, these behavioral states have been characterized very deeply using high-content worm behavioural tracking in our recent publication: Thapliyal et al. 2023 (PMID: 37236963). Our choice of using 1hr as a baseline is primarily driven by the fact that an elevated baseline of spontaneous reversals on food decreased the dynamic range to monitor changes in heat-evoked reversals. 1-hour early food deprivation reduced spontaneous reversals and led to a mild attenuation of heat-evoked responses at low stimulus intensities, while responses to stronger stimuli remained comparable to those of fed animals. Additionally, we observed a clear progressive decrease in heat-evoked reversals with increased duration of food-deprivation, which we further used to dissect the mechanism underlying this plasticity. The choice of the 1hr food deprivation timepoint as a reference is further justified below.

> *Finally, a previous report (Yeon et al, 2021) demonstrated differences in the impact of chronic versus acute neural silencing on starvation-dependent plasticity in the context of negative thermotaxis. We therefore wonder whether similar developmental compensation impacts the neural circuits that contribute to starvation-dependent plasticity in the thermonociceptive responses.*

Indeed, this is an interesting question. Our conclusions are so far based on ablation (with chronic effects). In order to gain insight on this question, future studies could address the impact of chronic vs acute silencing approach in starvation-dependent thermonociceptive responses. We have added an opening on this question in the discussion, as follows:

"Another open question is whether ASI action takes place during development (prior to starvation), or more acutely with active signaling after starvation."

> *A weakness of this manuscript is that the introduction is insufficiently scholarly in terms of citations and the description of current knowledge surrounding the impact of internal state on sensory behavior, particularly given previous work on the impact of feeding state on thermosensory behavioral plasticity (Takeshi et al 2020, Yeon et al 2021) and chemosensory valence (Banerjee et al 2023, Rengarajan et al 2019, etc).*

To address this weakness, we have revised the introduction section of the manuscript and cited previous relevant research on the impact of internal states on animal behavior, including the papers suggested by the reviewer. We note that 2 out of 4 suggested citations were already present in the initial manuscript (though in the discussion section).

> *Similarly, the authors' commanding knowledge of the distinction between thermotaxis navigation (especially negative thermotaxis) and thermonociceptive behaviors could be communicated in more depth and clarity to the readers, in order to contextualize this study's new findings within the previous literature.*

As mentioned above, we have deepened this aspect in the discussion section of the manuscript (with a dedicated paragraph). It is quite clear that starvationinduced plasticity in negative thermotaxis and thermonociceptive behaviors engage distinct mechanisms (at least in part). These differences include distinct alterations in AWC calcium activity, role of ASI neurons and INS-1 neuropeptide.

> *Nevertheless, this study represents a solid addition to the growing evidence that C. elegans sensory behaviors are strongly impacted by internal states, and that neuropeptidergic signaling plays a key role in mediating behavioral plasticity. To that end, the authors have provided solid evidence of their claims.*

We thank this reviewer for the efforts in evaluating our manuscript, for the positive assessment of our work, and for highlighting some weaknesses which, we believe, have been addressed through the revision.

> ***Reviewer #2 (Public review):***
>
> *In this work, Thapliyal and Glauser tried to provide a mechanistic understanding by which animals modulate their neural circuit responses to control nociceptive behavior on the basis of the dynamic internal feeding state. It is an important study that adds to the growing body of evidence coming from multiple model systems. They have used elegant genetics, behavioral, and Ca-imaging experiments to demonstrate how the auxiliary thermosensory neuron pair, AWC, and one of the internal state-sensing interneuron pairs, ASI, respond to dynamic internal starvation state to modulate behavioral response to noxious heat. Interestingly, these neuron pairs use distinct molecular mechanisms along with some other unidentified neurons to suppress heat-induced reversal response under short-term and prolonged starvation. The experiments are well performed, supporting most of the claims and providing an important framework for future studies.*
>
> *I have some queries that, if answered, will certainly enhance the study.*
>
> *(1) The results suggest that ASI is one of the primary drivers for the starvation-evoked behavioral plasticity, which regulates AWC activity under prolonged starvation. It raises many important questions, including: (a) how starvation modulates ASI response to heat?, and (b) under prolonged starvation, whether ASI also promotes other, non-AWC, glutamatergic inhibitory neurons to suppress heat-induced reversal, and how?*

We agree with this reviewer that the mechanisms by which ASI detects and mediates starvation-evoked changes in our model is a very interesting (unsolved) question. However, addressing these questions empirically represents a substantial body of work that would go beyond the scope of the present report. E.g., is temperature-dependent activity in ASI even relevant? At present, we envision that ASI could either work acutely (during heat stimuli) or be modulated over much longer time frames (hours of starvation) as an internal state sensor. Therefore, there will be quite some exploration needed before we figure out the ASI-level regulation more fully (including the critical temporal aspect regarding cell activity, as well as quantitative and qualitative transmission aspects). It will be very interesting in future work to address these questions.

> *(2) How does ASI regulate AWC activity? In the proposed model (Figure 8) authors suggested an independent, unknown signal, other than INS-32 and NLP-18, from ASI to regulate AWC activity. However, from the results, the existence of another signal is not very clear.*

Thanks for raising this point, which reveals a weakness in our graphical representation (in Fig. 8) that was not properly conveying our point. Our current work shows INS-32 and NLP-18 to be important in modulating heat-evoked reversals upon starvation. However, at the moment, we don't know if INS-32, NLP-18, both, and/or other neuropeptides from ASI modulate AWC activity patterns. The calcium imaging experiments in single, double and potentially triple mutants would answer these questions but are not within our current reach, given the time needed to carry out these experiments. However, we acknowledge this point and have changed the figure and its legend to state that the arrow connecting ASI to AWC activity pattern could potentially reflect the action of these neuropeptides.

> *(3) Previously, Takeishi et. al. showed that ins-1 dynamically modulates AWC-AIAmediated thermotaxis behavior based on the feeding state of the animal. It raises questions whether ins-1 also contributes to noxious heat-induced reversal behavior.*

We thank the reviewer for this question. We have now quantified the phenotype of *ins-1* mutant in our paradigm. Our data shows that INS-1 neuropeptide is not critical in mediating starvation-evoked thermonociceptive plasticity, unlike plasticity in thermotaxis behavior (See Figure 6- Supplement 1). Together with the differential activity patterns in AWC and the differential need for ASI neurons, these new data further consolidate the notion that starvation-evoked thermotaxis adaptation and noxious-heat avoidance engage separable molecular, cellular and circuit-level modulatory mechanisms. A specific discussion paragraph was added too.

> *(4) Experiments with AWC fate conversion mutants (nsy-1 and nsy-7) were very good ideas; however, the results obtained were confusing. flp-6 mutant data suggest AWCoff would be essential for heat-induced reversal, especially at the low intensity stimulus level. However, the nsy-1 mutant-forming two AWCon neurons showed complete rescue at the low heat level, which is quite opposite. Similarly, although less prominent, eat-4 rescue experiments suggested both nsy-1 and nsy-7 should behave normally at high heat conditions, which was not the result observed.*

We appreciate this comment and the legit attempt to infer what we should expect from a worm with two AWCon or two AWCoff, respectively. From previous studies so far, it's not quite clear if cellular properties of newly formed AWCs in *nsy-1* and *nsy-7* mutants, including response to sensory cues, formed synapses and their partners, expression of neuromodulator and gap junctions, synaptic output are similar or different. We think further studies are required to first establish if FLP-6 and glutamate signaling (expression, release and action) from altered AWCs in *nsy-1* and *nsy-7* mutants are the same or different. Therefore, direct comparison between cell fate conversion mutants with *flp-6* and glutamate would rely on too many assumptions at this stage. Considering this comment, the limited additional value of the data with *nsy1* and *nsy-7* mutants (in the absence of additional analyses) and the confusion it could trigger, we have decided to remove these non-essential data of the manuscript.

> ***Reviewer #3 (Public review):***
>
> *Summary:*
>
> *Thapliyal and Glauser show that hunger alters how C. elegans responds to noxious thermal stimuli. Using targeted neural ablation, mutant analysis, and live-cell functional imaging, the authors demonstrate that hunger changes the properties of AWC sensory neurons, which sense noxious heat. The authors further show that the effects of hunger on nociception require ASI neurons, which are known to respond to hunger and mediate the effects of food deprivation on behavior. Finally, the study uses mutant analysis to implicate glutamate and specific neuropeptides in thermal nociception and in the modulation of nociceptors by hungerresponsive neurons.*
>
> *Strengths:*
>
> *The study clearly shows a strong effect of hunger on nociception and documents a striking effect of hunger on the intrinsic properties of AWC sensory neurons, which respond to noxious heat. The study also clearly and compellingly demonstrates that ablation of hunger-responsive ASI neurons blocks the effects of hunger on nociceptive AWCs. These data, which constitute the kernel of the manuscript, are striking and exciting.*
>
> *Weaknesses:*

> *The study has some weaknesses that the authors should address.*
>
> *(1) Ablation of AWC neurons alters the basal sensitivity to noxious heat stimuli. This should be clearly noted in the description of the result and warrants some discussion.*

We thank this reviewer for raising this legitimate point. We have clarified this aspect in the results section of the revised manuscript, reading as follows:

"Removal of AWC nearly abolished heat-evoked reversal behavior across all stimulus intensities and timepoints (Figure 2B and E). While one should keep in mind that potential indirect developmental effects might take place in neuro-ablation lines, this observation suggests that AWC plays an essential role in mediating the thermonociceptive response under both early food deprivation and prolonged starvation. Notably, in AWC-ablated animals, the residual response level was unaffected by starvation, suggesting that AWC might also be required for the expression of starvation-dependent plasticity."

The contrast with known function of the best-characterized sensory neurons mediating thermal nociception (AFD and FLP) is discussed as follows:

"...Therefore, noxious heat-evoked activity in AWC varies widely according to context, which is in line with previous literature [18, 20, 41]. Interestingly, the role of AWC is distinct from that of AFD and FLP neurons, which are canonically linked to thermosensation and nociception [5, 14, 42, 43], but contribute only modestly to heat-evoked behavior in our assay conditions with between 1 and 6 hrs of food deprivation."

> *(2) Throughout the study, it seems that data are replotted in multiple figure panels. The authors should clearly indicate in the figure legends when this occurs. Also, the authors should ensure that statistical tests requiring multiple comparisons are correctly implemented and reflect the number of times experimental data are compared to a single set of control data.*

Thanks for raising this important point. We have clarified this aspect in the revised figure legends of the manuscript, and in the method section. In some instances, we reconducted some analyses to be perfectly rigorous in multiple comparison accounting. This did not lead to significantly different conclusions. The one exception was that the small effect of *eat-4* mutation on spontaneous reversal went below significance threshold. We therefore removed this aspect of the result reporting and of the corresponding interpretation scheme, which became slightly simpler (Figure 3). Globally, this makes the story more focused.

> *(3) How ASIs modulate AWCs remains unclear. The authors find that loss of INS-6, an insulin-like peptide provided by ASIs, partially recapitulates the effect of ASI ablation. This observation is not further developed, and instead, the authors characterize other secreted factors that seem to mediate sensitization of animals to noxious heat stimuli. While it is interesting that there are multiple opposing inputs into the nociceptor circuit, the essential connection between ASIs and AWCs that underlies the foundational observations in Figures 1 and 2 is not sufficiently characterized.*

Whereas we agree that how ASI modulates AWCs is only partially solved by our study, we should emphasize that our work identified two ASI-expressed neuropeptides that function to decrease reversal response after starvation: INS-32 and NLP-18. We initially set a lower priority on INS-6 because the reversal response level in starved mutants appeared lower than that in *nlp-18* and *ins-32*. It is important to note that *ins-32* and *nlp-18* are not 'generally potentiated' mutants, but display reversal upregulation selectively following starvation, which placed them as strong candidates to selectively mediate ASI regulation. This said, it is also true that these two mutants (and *ins-6* too) display reduced responsiveness at the early food deprivation time point. Therefore, none of the neuropeptide mutants was strictly

identical to ASI ablated line, suggesting that the peptides might also work via non-ASI cells at the early food deprivation timepoint.

Following this reviewer's comment, we have attempted to complement our story with the idea of using a similar approach and rescue *ins-6* with its endogenous promoter or ASI-specific promoter. Unfortunately, we failed to obtain rescue effects, and therefore these data (with a negative result) remain inconclusive (as we cannot guarantee that the rescue constructs were functional). We decided to keep these data aside in the revised manuscript. Globally, our point made graphically in Figure 7F remains valid. We have complemented the figure legend to mention that INS-6 could also potentially work from ASI, but it is not depicted as no ASI-specific data are available. In summary, our data suggests that the connection between ASI and AWC(s) might be established by the integrated action of multiple peptides and their receptors. Further calcium imaging experiments in single, double and potentially triple mutant(s) of peptides and receptors would be required go deeper in this question, which could be performed in future work.

"...Additional neuropeptides (such as INS-6) may also be involved, but in the absence of direct evidence for their origin from ASI, they were not included in this scheme."

> *(4) The assertion that 'starvation reshapes AWC responses from deterministic to stochastic' is not clearly supported by the data. AWC neurons seem capable of showing different responses to thermal stimuli, and the probabilities associated with these responses change after fasting. The different kinds of responses are seen under basal and fasted conditions.*

We thank this reviewer for the comment. There is an activity response shift that is quite solidly described, including with new quantitative analyses of distributions (histograms in new Fig. 4CD and new Fig. 5C-D, accompanied by Kruskal-Wallis tests). Yet, we totally agree that the wording choice was inappropriate. We have furthermore changed our terminology to avoid using the terms "stochastic" or "deterministic" that were indeed a cause of confusion. We now use the terms "stimulus-locked responses" and describe the shift as "shift from mostly excitatory responses to a mix of both excitatory and inhibitory responses". We have also included detailed methodology for characterization of traces and statistical analysis in the revised method section of the manuscript, together with the new analyses on peak polarity distribution.

> ***Recommendations for the authors:***
>
> ***Reviewing Editor Comments:***
>
> *The reviewers agree that the study is clearly presented and makes good use of behavioral, genetic, and imaging approaches to link starvation state with changes in AWC and ASI function. To strengthen the manuscript and ensure clarity for readers, we ask you to address the following points in revision:*
>
> *(1) Positioning and citations.*
>
> *Clarify how your findings relate to Takeishi 2020, where the opposite trend in AWC activity was reported, and make a clear distinction between thermonociception and thermotaxis. The introduction should also include additional citations in two specific areas: prior work on AWC and noxious thermal stimuli, and studies demonstrating starvation-dependent behavioral changes via altered neuropeptide release (e.g., Banerjee 2023; Rengarajan 2019).*

We have clarified this aspect with extension of the work cited in the introduction and extensive rewriting of the discussion sections.

Our data shows that mechanisms underlying starvation dependent changes in thermonociception and thermotaxis show differences at the molecular, cellular and circuit levels. First, we see a decrease in average response in AWC neurons due to shift from mostly excitatory to a mix of excitatory and inhibitory responses in response to noxious heat after starvation, while previous study found an increase in response probability of AWCs after starvation (Takeishi et al, 2020). Second, contrary to thermotaxis behavior ASI neurons are required to orchestrate starvation evoked plasticity in thermonociception. And, finally, previous study found that INS-1 signaling from the intestine regulates thermotaxis behavioral plasticity while INS-1 peptide does not seem critical in regulating starvation-dependent thermonociceptive plasticity (new data in Figure 6 supplement 1).

We have revised the introduction section of the manuscript and cited previous relevant research on AWC and noxious thermal stimuli and studies demonstrating starvationdependent behavioral changes via altered neuropeptide release including the papers suggested by reviewers. The extended discussion section reads as follows:

“Starvation regulates thermonociceptive and negative thermotaxis plasticity via at least partly different mechanisms

Previous studies showed that AWC plays an important role in starvationdependent plasticity in the negative thermotaxis behavior in an innocuous thermal range between 15 and 25°C [26, 33]. Negative thermotaxis involves the detection of thermal changes created by animal movement in spatial thermogradient (0.5°C/cm), the magnitude of the expected thermal changes approximating 0.01°C/s [26]. The starvation impact on negative thermotaxis was shown to (i) involve an up-regulation of AWC cell activity, (ii) rely on INS1 neuropeptide produced in the intestine and (iii) to occur independently of ASI neurons. In contrast, our study used thermo-nociceptive stimuli, with faster raising thermal slopes (~0.5-2°C/s, hence 50-200 times faster than those occurring for thermotaxis) and covering noxious temperatures (up to 28°C). Our results indicate that the regulation of thermo-nociceptive response by starvation (i) is linked to a shift in the distribution of AWC activity response polarities from mostly excitatory to a mix of excitatory and inhibitory response, (ii) relies on ASI and specific neuropeptide produced in ASI, and (iii) works independently of INS-1 neuropeptide. Therefore, our study complements our understanding of the modulation of temperature-dependent behavior in *C. elegans* with previously undocumented mechanisms at the circuit, cellular and molecular levels.”

> *(2) ASI → AWC mechanism.*
>
> *Because ASI is central to your conclusions, please expand on how ASI is thought to act on AWC and/or other neurons. If an additional ASI signal is proposed beyond INS-32/NLP-18, mark this as speculative unless further rationale can be provided, and adjust the model figure accordingly.*

We have revised Figure 8 and its legend to clarify what is still hypothetical in the way ASI could affect AWC activity patterns and reversals. Note that the figure was also modified to integrate the conclusions made from epistasis analysis of *eat-4* and *flp-6*.

> *(3) AWC response description.*
>
> *The data support a shift in response distributions rather than a categorical switch from "deterministic to stochastic." Please adjust the language accordingly and provide a clear description of how traces were classified as "up, variable, or down," ideally with a quantification of the distributional shift.*

We agree that the term “stochastic” can convey different things, and because it was used for something different for AWC in the past, we should have avoided it. We have revised the

nomenclature. What we observe can indeed be better described as a shift in the response polarity distribution. The article was revised accordingly. We also included the quantitative analysis and histogram representation, suggested in one of the specific comments, and added detailed methodology on the categorization of traces.

When ASI is intact, we see a shift from mostly excitatory responses to an ~equal mix of excitatory and inhibitory responses (new Figure 4C-D). This effect is lost when ASI is ablated (new Figure 5C-D).

> *(4) Presentation and statistics.*
>
> *In figure legends, indicate where datasets are replotted across panels and confirm that multiple-comparison corrections take account of repeated comparisons to the same controls.*

We have included these details in the revised figure legends, and a statement in the method section.

> *(5) Methods clarity.*
>
> *Provide justification for using 1-h off-food as the baseline, with quantification of baseline mobility/reversal rates. Expand the calcium-imaging methods to describe the processing pipeline (ΔR calculation, baseline period, drift correction), and add a brief rationale if the approach deviates from common normalization procedures. Clarify how cell ablations were performed and verified for specificity, and how cell-specific rescues were confirmed. Please also acknowledge the potential for developmental compensation with chronic ablation.*

The justification of using 1hr off-food as baseline was made more prominent in the revised manuscript.

Revised result section:

“Starvation downregulates thermonociceptive responses in *C. elegans*

To assess how the feeding state modulates thermonociceptive behavior in *C. elegans*, we compared responses across different durations of food deprivation (Figure 1A). Synchronized first-day adult animals were stimulated with a series of 4-s infrared pulses of increasing heating power (100, 200, 300, 400 W), causing temperature increase of +2°C, +4°C +6°C and 8°C at the surface of the plate (Figure 1A). Fed animals on food produced robust heat-evoked reversal response to heat, but they also displayed a very elevated baseline of spontaneous reversals (~38%). A 1-hour off-food condition reduced spontaneous reversals (from ~38% to ~10%) and led to an attenuation of heat-evoked responses at low stimulus intensities, while responses to stronger stimuli remained comparable to those of fed animals. More prolonged food deprivation led to a striking progressive reduction in thermonociceptive responses at every heating level, with responses after 6 hours of starvation approaching baseline spontaneous reversal rates (Figure 1B and C). This suggests a robust inhibition of nociceptive behavior caused by prolonged starvation. To determine whether this attenuation was due to the absence of nutrients or chemosensory cues, we conducted similar starvation experiments in the presence of food odor, with OP50 bacteria present on the petri dish lid (Figure 1D). The reduction in thermonociceptive response persisted, indicating that the effect is driven by the internal starvation state rather than external olfactory input.

Although fed animals showed high sensitivity to noxious heat, they also displayed an elevated baseline of spontaneous reversals, which limited their utility as a control group by strongly reducing the dynamic range of heat-evoked reversal quantification and by complicating the quantitative comparison with food-deprivation conditions with much-reduced reversal

baseline (Figure 1B). In addition, technical limitations in our calcium imaging setup would have prevented the intended follow-up analyses in fed animals. Based on these observations and technical considerations, we focused subsequent analyses, aiming at dissecting the circuit and molecular underpinnings of starvation-dependent plasticity, to the comparison of two off-food conditions with similar spontaneous reversal baseline: the early food deprivation condition (1-hour off-food, with high responsiveness to noxious heat) and the prolonged starvation (6-hour off-food with almost abolished noxious heat responsiveness)."

In addition, the method section was modified as follows:

- Calcium imaging details were added regarding ΔR calculation, baseline period, drift correction.

- We now explicitly refer to the original respective articles describing the neuroablation lines.

- We clarify that cell-specific transgene expression for rescue was confirmed using SL2::mCherry co-marker

In the result section, we now explicitly address potential developmental compensation in genetic ablation backgrounds in the result section as follows: "…one should keep in mind that potential indirect developmental effects might take place in neuro-ablation lines".

> ***Reviewer #1 (Recommendations for the authors):***
>
> *(1) The data availability statement is missing from the reviewed manuscript and should be included.*

Thanks, we have included the data availability statement in the revised manuscript.

> *(2) We request additional information on how n's were determined for individual experiments, as well as the inclusion of post-hoc power measurements for all quantification.*

n were determined in agreement with previous studies using similar measures. No a priori power analyses were performed. A posteriori power analyses are not informative beyond the reported effect sizes and p-values (now reported in File S2). We clarified this in the statistical subsection of the method section.

> *(3) In many cases, the specific statistical tests used are not clear or justified; more details should be provided, including the non-post-hoc test used. Are all tests one-way ANOVAs? For comparisons across genotype and starvation duration, two-way ANOVAs would likely be more appropriate. Also, the authors switch between Bonferroni post-hoc tests and Holm-Bonferroni post-hoc tests. What determined the use of one versus another?*

We have now clarified the statistical analyses used and provided full details in File S2. We have now more systematically applied two-way ANOVAs across all relevant analyses (with detailed parameters reported in File S2). When particularly relevant (e.g epistasis analysis between *eat-4* and *flp-6* mutations) the results of the two-way ANOVAs, is also explicitly stated in the result section.

We also note that all multiple-comparison corrections were performed using the Bonferroni method. Previous mentions of Holm-Bonferroni correction were inaccuracies, and we apologize for this confusion; these mentions have now been corrected throughout the manuscript.

> *(4) The use of heating power instead of the temperature experienced by the worms is an unwelcome abstraction. We strongly recommend revisiting that choice.*

We do agree. We have revised the figures to label the axis with temperature increase.

> *(5) For calcium imaging, how are the traces categorized into "calcium up", "calcium down", or "no change"? Were those determined blindly - i.e., by individuals unaware of the experimental condition? Did the response direction need to be consistent across different temperatures? Did the change from baseline need to hit a specific threshold, consistent with previous studies in the field (i.e., +/- 3xSD for a minimum amount of time)? We encourage the authors to include these details in their methods section.*

We have complemented the method section to clarify the criteria for the qualitative classification of traces. More importantly, new quantitative peak polarities comparisons were added (see specific points below and above, about histograms).

> *(6) For the experiments showing that exposure to food odor does not prevent response reduction, we suggest that feeding worms heat-killed bacteria would be a helpful control for the importance of bacterial nutritional status. In addition, showing that the impact of starvation was reversible with re-feeding would have been a useful experiment in line with standard experimental design in the starvation field.*

Thanks, indeed with our current work we cannot pinpoint the role of additional sensory cues (except food odor) to be mediating starvation-evoked plasticity. Together with refeeding, these are all extremely interesting questions that we aim to answer and potentially link with ASI and AWC activity in our future work.

> *(7) For the various AWC rescue experiments, we found it curious that there wasn't an AWCon+off rescue, only each neuron individually.*

Previous studies have identified similar or opposite responses of both AWCs for distinct sensory cues. Though our calcium imaging experiments point to both AWC on and off having similar response patterns to heat, we cannot rule out the possibility that their output (ability of control reversals) is distinct possibly due to recruited neuromodulators. Therefore, in the present work, we examined where these cell types act via the same or distinct combinations of neuromodulators to control reversals.

> ***Reviewer #2 (Recommendations for the authors):***
>
> *Experiments suggested:*
>
> *(1) The authors should look into the Ca-dynamics in ASI.*

How does the spontaneous and heat-evoked activity of ASI differ in fed, early food-deprivation and prolong starvation and its link to releases of neuromodulators, modified AWC activity to alter output of thermal nociception are very interesting questions. However, these questions are extremely exploratory (see argumentation above in response to the public review) and addressing them goes beyond the scope of our current manuscript.

> *(2) The authors should check AWC activity in ins-32 and nlp-18 mutant animals.*

In this study, we focused on the roles of INS-32 and NLP-18 released from ASI in modulating heat-evoked reversals, as these mutants exhibit relatively strong behavioral phenotypes. However, these effects remain less pronounced than those observed following ASI ablation. In addition, we cannot exclude the contribution of additional signaling molecules, including INS-6 and other neuropeptides.

A comprehensive analysis of AWC activity in this context would require calcium imaging across multiple genetic backgrounds, including single, double, and potentially higher order peptide and receptor mutants, combined with cell-specific rescue experiments. While we

appreciate the suggestion, such an approach would represent a substantial extension of the present work and will be important to pursue in future studies to further elucidate the underlying mechanisms.

> *(3) Short-term food deprivation completely eliminated heat heat-induced reversal response to 100W stimulus, while the response to 400W stimulus remained unaffected. This suggests fed, short-term starvation, and prolonged starvation are three distinct states, and authors should also test the response of AWC and ASI ablated animals in the fed conditions.*

We agree that analyzing thermal nociception in fed states, in addition to short-term and prolonged food deprivation states is an important and interesting question, as these 3 conditions likely represent 3 distinct internal states that may recruit different neural pathways.

Several reasons led us to set the fed condition aside for this study, and we realize we insufficiently explain them in the initial manuscript. There are 2 main reasons.

(1) It is of paramount importance to consider the 'baseline' reversal rate (spontaneous reversals not triggered by heat, but visible in our dataset as the first point in the 'dose-response' curve). In Fed animals spontaneous reversal rate is very high (~38%) compared to the 1hr and 6hr food-deprivation conditions (>10%). This has two consequences: first a decreased dynamic range for quantify heat-evoked reversal, and, second, the difficulty in judging quantitative differences in heat-evoked reversals with such major differences in baseline reversals.

(2) Experimentally, assessing calcium responses in truly fed animals presents technical challenges. With our current setup, animals must be removed from food for at least ~5 minutes prior to recording (followed by ~5 minutes of imaging), which effectively corresponds to a "freshly starved" condition rather than a fully fed state. While previous studies have used serotonin to mimic aspects of the fed state, such manipulations can be difficult to interpret in this context.

A systematic comparison including fully fed animals, as well as AWC- and ASI-ablated conditions across these states, would be a valuable direction for future work, in particular once the methodological barriers associated with point 2, have been overcome.

We have clarified these choices in the result section as follows:

"Starvation downregulates thermonociceptive responses in *C. elegans*

To assess how the feeding state modulates thermonociceptive behavior in *C. elegans*, we compared responses across different durations of food deprivation (Figure 1A). Synchronized first-day adult animals were stimulated with a series of 4-s infrared pulses of increasing heating power (100, 200, 300, 400 W), causing temperature increase of +2°C, +4°C +6°C and 8°C at the surface of the plate (Figure 1A). Fed animals on food produced robust heat-evoked reversal response to heat, but they also displayed a very elevated baseline of spontaneous reversals (~38%). A 1-hour off-food condition reduced spontaneous reversals (from ~38% to ~10%) and led to an attenuation of heat-evoked responses at low stimulus intensities, while responses to stronger stimuli remained comparable to those of fed animals. More prolonged food deprivation led to a striking progressive reduction in thermonociceptive responses at every heating level, with responses after 6 hours of starvation approaching baseline spontaneous reversal rates (Figure 1B and C). This suggests a robust inhibition of nociceptive behavior caused by prolonged starvation. To determine whether this attenuation was due to the absence of nutrients or chemosensory cues, we conducted similar starvation experiments in the presence of food odor, with OP50 bacteria present on the petri dish lid (Figure 1D). The

reduction in thermonociceptive response persisted, indicating that the effect is driven by the internal starvation state rather than external olfactory input.

Although fed animals showed high sensitivity to noxious heat, they also displayed an elevated baseline of spontaneous reversals, which limited their utility as a control group by strongly reducing the dynamic range of heat-evoked reversal quantification and by complicating the quantitative comparison with food-deprivation conditions with muchreduced reversal baseline (Figure 1B). In addition, technical limitations in our calcium imaging setup would have prevented the intended follow-up analyses in fed animals. Based on these observations and technical considerations, we focused subsequent analyses, aiming at dissecting the circuit and molecular underpinnings of starvationdependent plasticity, to the comparison of two off-food conditions with similar spontaneous reversal baseline: the early food deprivation condition (1-hour off-food, with high responsiveness to noxious heat) and the prolonged starvation (6-hour off-food with almost abolished noxious heat responsiveness)."

> *(4) The authors should test the effect of ins-1 in noxious heat-mediated dynamic reversal behavior.*

We thank the reviewer for this valuable suggestion. We have now tested the phenotype of *ins-1* mutants in our paradigm. Our data shows that INS-1 neuropeptide is not critical in mediating starvation-evoked thermonociceptive plasticity unlike plasticity in thermotaxis behavior (New Figure 6 Sup1). This molecular aspect adds to our initially presented evidence at the cell activity and circuit levels, that noxiousevoked reversal and thermotaxis behaviors are regulated in a clearly separable manner.

> *(5) Whether Glutamate and flp-6 work in parallel or in the same pathway to regulate reversals?*

We thank the reviewer for this question. We tested *eat-4*; *flp-6* double mutants and found:

(1) After short-term food deprivation (1hr), *flp-6* and *eat-4* separately contribute to heat-evoked reversal at high & low heat and they act in parallel pathways to explain ~90% of animal responsiveness (new version of Fig 3)

Corresponding new text:

"Next, we focused on *eat-4* and *flp-6* mutants, showing the strongest phenotype. We addressed whether glutamate and FLP-6 signaling act dependently of each other in controlling heat-evoked reversal, by testing *eat-4*; *flp-6* double mutants. The residual response seen in each single mutant (Figure 3 A and B) was almost entirely abolished in the double mutant (Figure 3C). A two-way ANOVA for the highest heat stimuli with *eat-4* and *flp-6* genotypes as factors (two levels each: mutant or wild type) showed significant main effects of *eat-4* ($F_{(1,67)}$ =48.70, p<.001, η^2p=0.421) and *flp-6* ($F_{(1,67)}$ =58.50, p<.001, ηp=0.466), respectively, but no interaction effects ($F_{(1,67)}$ =0.093, p=.761, η^2p=0.001). The significant cumulative effect of the two mutations indicates that the two signaling pathways act mostly independently of each other to mediate heat-evoked reversals."

(2) After prolong starvation (6hr), *flp-6* mutation has a dominant impact on plasticity and *eat-4* mutation cannot cause loss of plasticity, pointing to a hierarchy in this context (new version of Fig. 6, including revised hierarchy in the model in panel G, and also revised model in Fig.

8).

Corresponding revised text:

"Second, we tested whether starvation-dependent plasticity was preserved in *eat-4* and *flp6* mutant backgrounds, which we had suggested to represent the main AWC transmitters controlling heat-evoked reversals under the early food deprivation condition (Figure 3). Even

if the heat-evoked response upon early food-deprivation was reduced relative to wild type in *flp-6* mutants, a significant further decline was seen after prolonged starvation (Figure 6B). These results indicate that starvation-dependent plasticity can operate independently of FLP-6. In contrast, *eat-4* mutants displayed markedly elevated heat-evoked responses after prolonged starvation, even exceeding the response level seen in the early food deprivation condition for low heat stimuli (Figure 6C). This potentiated response in *eat-4* mutants was entirely dependent of an intact FLP-6 signaling, since reversal responses in *eat-4*; *flp-6* mutants were entirely abolished, like in *flp-6* single mutant (Figure 6C-E, a two-way ANOVA indicating a significant interaction effect between the two mutations: $F_{(1,70)}$ =0.093, p<.001, η^2p=0.247). Moreover, the potentiated response in *eat-4* single mutant could not be rescued by expressing *eat-4* rescue transgene selectively in either AWC^{OFF} or AWC^{ON} neurons (Figure 6F). Interestingly, AWC^{OFF}-specific rescue produced a further potentiation of heat-evoked reversal response to high heat stimuli (Figure 6F, 6 and 8°C thermal increases), aggravating the phenotype of *eat-4* mutants. These results are consistent with a model in which glutamatergic signaling regulates heat-evoked reversals in starved animals via two bidirectional drives (Figure 6G). On the one hand, glutamatergic signaling—originating from AWC^{OFF}—up-regulates reversals in response to high heat stimuli, thus contributing to prevent starvation-induced thermonociceptive plasticity. On the other hand, glutamatergic signaling—originating from neurons other than AWC— down-regulates reversals over a broad range of heat intensities, thus promoting starvation-induced thermonociceptive plasticity. The latter glutamatergic signaling inhibitory effect seems to be more dominant and to depend on intact FLP-6 signaling."

> *(6) The authors should perform flp-6 and eat-4 mutant/rescue experiments in the nsy-1 and nsy-7 background to clarify the results.*

Our results indicate that glutamate release via EAT-4 from both AWC^{ON} and AWC^{OFF}, as well as FLP-6 from AWC^{OFF}, contributes to heat-evoked reversals regulation. The experiments suggested by the reviewer would, in principle, provide further insight into the interaction between AWC subtype identity and the respective roles of glutamatergic and peptidergic signaling.

However, as discussed in more details above in the public review, the extent and nature of $AWC^{ON/OFF}$ remodeling in *nsy-1* and *nsy-7* mutant backgrounds remain incompletely understood. This introduces significant uncertainty in interpreting results obtained from combining these mutations with *eat-4* and *flp-6* manipulations. As a result, such experiments would be difficult to interpret in a definitive manner at this stage.

We therefore consider this an important direction for future work, once the roles of *nsy1* and *nsy-7* in AWC subtype specification and function are more clearly established. As our preliminary results with *nsy-1* and *nsy-7* mutants added more confusion than clarity, we have chosen to set them aside (former Fig. 3-figure supplement 2 has been removed).

> *Minor comments:*
>
> *(1) What is food odor? The experiment should be clearly mentioned.*

Thanks for spotting this unintended omission. Food odor experiments were performed by adding OP50 bacteria on the inward side of the petri dish lid instead of the NGM surface. We have added this description in the method section of the revised manuscript.

> *(2) Panel 3C is coming before 3B. This should be rearranged.*

Thanks for pointing this out. Panel arrangement was entirely reorganized in revise Fig. 3, with the addition of *eat-4 x flp-6* genetic interaction analysis.

> *(3) In Figure 6, if the panels are arranged horizontally, it would be easier to follow.*

Thanks for pointing this out. Panel arrangement was entirely reorganized in revise Fig. 6, with the addition of *eat-4 flp-6* genetic interaction analysis.

> ***Reviewer #3 (Recommendations for the authors):***
>
> *(1) The authors should consider moving measurements of AFD-ablated animals into the main Figure 1. AFD is a well-known thermosensor, and it is worth showing that responses to noxious thermal stimuli persist in animals lacking AFD.*

Thank you for this suggestion. We have moved measurements of AFDablated animals to the main figure (revised Fig. 2).

> *(2) AFD ablation does affect responses to noxious heat. The authors could consider ablating/silencing AFDs and AWCs simultaneously to determine whether these two neurontypes account for the behavior.*

We agree that investigating the combinatorial contributions of thermosensory neurons, including AFD and AWC, to thermal nociception is an important and interesting question. In principle, simultaneous ablation or silencing of these neuron types could indeed reveal unexpected interactions.

In our experimental paradigm, however, we observe only a minimal contribution of AFD neurons to heat-evoked reversals, whereas ablation of AWC nearly abolishes the response. Based on these observations, we chose to focus the present study on AWC, which appears to play a more prominent role in this behavior.

A more detailed dissection of the potential interactions between AFD and AWC, including combinatorial manipulations, would be a valuable direction for future work. In particular, the possibility that AFD exerts a modulatory influence remains an interesting hypothesis to explore.

> *(3) Given that EAT-4/VGLUT and FLP-6 neuropeptides each contribute to nociception, the authors should consider testing an eat-4; flp-6 double mutant to determine whether this combination of neurochemical signals accounts for AWC signaling to downstream circuits.*

We thank the reviewer for this suggestion, which is similar to point 5 of Reviewer 2 (above).

We tested *eat-4*; *flp-6* double mutants and found:

(1) After short-term food deprivation (1hr), *flp-6* and *eat-4* separately contribute to heat evoked reversal at high & low heat and they act in parallel pathways to explain ~90% of animal responsiveness (new version of Fig 3)

Corresponding new text:

“Next, we focused on *eat-4* and *flp-6* mutants, showing the strongest phenotype. We addressed whether glutamate and FLP-6 signaling act dependently of each other in controlling heat-evoked reversal, by testing *eat-4*;*flp-6* double mutants. The residual response seen in each single mutant (Figure 3 A and B) was almost entirely abolished in the double mutant (Figure 3C). A two-way ANOVA for the highest heat stimuli with *eat-4* and *flp-6* genotypes as factors (two levels each: mutant or wild type) showed significant main effects of *eat-4* ($F_{(1,67)}$ =48.70, p<.001, η^2p=0.421) and *flp-6* ($F_{(1,67)}$ =58.50, p<.001, η^2p=0.466), respectively, but no interaction effects ($F_{(1,67)}$ =0.093, p=.761, η^2p=0.001). The significant cumulative effect of the two mutations indicates that the two signaling pathways act mostly independently of each other to mediate heat-evoked reversals.”

(2) After prolong starvation (6hr), *flp-6* mutation has a dominant impact on plasticity and *eat-4* mutation cannot cause loss of plasticity, pointing to a hierarchy in this context (new version of Fig. 6, including revised hierarchy in the model in panel G, and also revised model in Fig. 8).

Corresponding revised text:

"Second, we tested whether starvation-dependent plasticity was preserved in *eat-4* and *flp6* mutant backgrounds, which we had suggested to represent the main AWC transmitters controlling heat-evoked reversals under the early food deprivation condition (Figure 3). Even if the heat-evoked response upon early food deprivation was reduced relative to wild type in *flp-6* mutants, a significant further decline was seen after prolonged starvation (Figure 6B). These results indicate that starvation-dependent plasticity can operate independently of FLP-6. In contrast, *eat-4* mutants displayed markedly elevated heat-evoked responses after prolonged starvation, even exceeding the response level seen in the early food deprivation condition for low heat stimuli (Figure 6C). This potentiated response in *eat-4* mutants was entirely dependent of an intact FLP-6 signaling, since reversal responses in *eat-4*; *flp-6* mutants were entirely abolished, like in *flp-6* single mutant (Figure 6C-E, a two-way ANOVA indicating a significant interaction effect between the two mutations: $F_{(1,70)}$ =0.093, p<.001, $\eta^2 p$=0.247). Moreover, the potentiated response in *eat-4* single mutant could not be rescued by expressing *eat-4* rescue transgene selectively in either AWC^{OFF} or AWC^{ON} neurons (Figure 6F). Interestingly, AWC^{OFF}-specific rescue produced a further potentiation of heat-evoked reversal response to high heat stimuli (Figure 6F, 6 and 8°C thermal increases), aggravating the phenotype of *eat-4* mutants. These results are consistent with a model in which glutamatergic signaling regulates heat-evoked reversals in starved animals via two bidirectional drives (Figure 6G). On the one hand, glutamatergic signaling—originating from AWC^{OFF}—up-regulates reversals in response to high heat stimuli, thus contributing to prevent starvation-induced thermonociceptive plasticity. On the other hand, glutamatergic signaling—originating from neurons other than AWC— down-regulates reversals over a broad range of heat intensities, thus promoting starvation-induced thermonociceptive plasticity. The latter glutamatergic signaling inhibitory effect seems to be more dominant and to depend on intact FLP-6 signaling."

> *(4) The authors should consider representing AWC responses to thermal stimuli as histograms to illustrate how fasting increases the probability of some responses and decreases the probability of others.*

We thank this reviewer for the suggestion. The proposed histograms nicely convey the concept of "shift in response polarity distribution" that we observed (using the new terminology we now use, instead of using the term "stochastic"). To create such histograms, we computed the magnitude of the peaks on a trial-by-trial basis. When ASI is intact, we see a shift from mostly excitatory responses to an ~equal mix of excitatory and inhibitory responses (new Figure 4C-D). This effect is lost when ASI is ablated (new Figure 5C-D).

> *(5) It seems important to better understand the ins-6 mutant phenotype and determine whether ASI-to-AWC signaling involves this insulin-like peptide (ILP). The authors should consider using some of the tools available for disrupting ILP signaling to more clearly demonstrate that a specific neurochemical signal mediates modulation of AWCs by ASIs.*

Our work identified two ASI-expressed neuropeptides that function to decrease reversal response after starvation: INS-32 and NLP-18. We initially set a lower priority on INS-6 because the reversal response level in starved mutants appeared lower than that in *nlp-18* and *ins-32*. It is important to note that *ins-32* and *nlp-18* are not 'generally potentiated' mutants, but display reversal up-regulation selectively following starvation, which placed them as strong candidates to selectively mediate ASI regulation. This said, it is also true that

these two mutants (and *ins-6* too) display reduced responsiveness at the early food deprivation time point. Therefore, none of the neuropeptide mutants was strictly identical to ASI, suggesting that the peptides might also work via non-ASI cells at the early food deprivation timepoint (as follow up data indicated at least for *nlp-18*).

Following this reviewer's comment (and the similar one in the public review), we have attempted to complement our story with the idea of using a similar approach and rescue *ins-6* with its endogenous promoter or ASI-specific promoter. Unfortunately, we failed to obtain rescue effects, and therefore these data remain inconclusive (as we cannot guarantee that the rescue constructs were functional). We decided to keep these data aside in the revised manuscript. Globally, our point made graphically in Figure 7F remains valid. We have complemented the figure legend to mention that INS6 could also potentially work from ASI, but it is not depicted as no ASI-specific data are available. In summary, our data suggests that the connection between ASI and AWC(s) might be established by the integrated action of multiple peptides and their receptors. Further calcium imaging experiments in single, double and potentially triple mutant(s) of peptides and receptors would be required to go deeper in this question, which could be performed in future work.

https://doi.org/10.7554/eLife.108246.2.sa0