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## **Neural architecture of hunger-dependent multisensory decision making in *C. elegans***

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## SUMMARY

Little is known about how animals integrate multiple sensory inputs in natural environments to balance avoidance of danger with approach to things of value. Furthermore, the mechanistic link between internal physiological state and threat-reward decision making remains poorly understood. Here we confronted *C. elegans* worms with the decision whether to cross a hyperosmotic barrier presenting the threat of desiccation to reach a source of food odor. We identified a specific interneuron that controls this decision via top-down extrasynaptic aminergic potentiation of the primary osmosensory neurons to increase their sensitivity to the barrier. We also establish that food deprivation increases the worm's willingness to cross the dangerous barrier by suppressing this pathway. These studies reveal a potentially general neural circuit architecture for internal state control of threat-reward decision making.

## INTRODUCTION

Animals navigate complex natural environments containing both dangerous and valuable items, such as predators and food. The sensory cues that signal danger and reward are frequently transduced by different senses. In such circumstances, multisensory decision processes survey internal physiological state and balance attention to relevant sensory modalities to drive behavioral outputs (Rangel et al., 2008; Talsma et al., 2010). In human beings and other mammals, a key motif of multisensory integration is “top-down” control of perception (Manita et al., 2015; Talsma et al., 2010), wherein primary sensory cortex activity is modulated by feedback from higher-order regions (Fairhall and Macaluso, 2009; Fu et al., 2014; Zhang et al., 2014). Top-down control directs attention to the sensory modality signaling the most important features of a noisy environment given current internal physiological state (Berthoud, 2011; Rangel et al., 2008; Talsma, 2015). However, cellular and molecular mechanisms underlying internal state-dependent top-down attentional control in the context of threat-reward decision making remain unknown.

Here we employ the nematode worm *Caenorhabditis elegans* as a model system to investigate the molecular and cellular basis of multisensory decision making. In its natural environment in decomposing organic matter (Frezal and Felix, 2015), *C. elegans* must approach and obtain food while avoiding various threats, which include toxic chemicals and hyperosmotic concentrations of otherwise innocuous solutes. Worms detect threat and reward *via* primary sensory neurons that then propagate such information through an interneuron network to ultimately reach premotor command interneurons that direct locomotion (**Figure 1A**) (Varshney et al., 2011; White et al., 1986). The attractive food odor diacetyl is detected by the bilateral chemosensory neuron pair AWA (Sengupta et al., 1996). Aversive hyperosmolarity—which can

induce damage and death by desiccation (Culotti and Russell, 1978; Solomon et al., 2004)—is detected by the bilateral polymodal sensory neuron pair ASH (Hilliard et al., 2005; Kaplan and Horvitz, 1993). AWA and ASH propagate their sensory responses to an interconnected network of sensory interneurons (Ishihara et al., 2002; Luo et al., 2014; Shinkai et al., 2011). These sensory interneurons then provide inputs to the bilateral pair of RIM sensorimotor interneurons (Gordus et al., 2015; Guo et al., 2009; Piggott et al., 2011; White et al., 1986). RIM receives inhibitory synaptic inputs from sensory interneurons activated by attractive odors, such as diacetyl transduced by AWA (Gordus et al., 2015; Li et al., 2012). RIM receives excitatory synaptic inputs from sensory interneurons activated by aversive stimuli, such as hyperosmolarity transduced by ASH (Guo et al., 2009; Piggott et al., 2011). RIM, in turn, provides inhibitory synaptic inputs to forward command interneurons (Kawano et al., 2011; Pirri et al., 2009) and excitatory synaptic and gap-junction-coupled inputs to backward command interneurons (Gordus et al., 2015; Guo et al., 2009). This sensorimotor network topology and the signs of its synaptic connections ensure that aversive stimuli bias the worm towards backing up and changing direction, while attractive stimuli bias the worm towards continued forward motion. ASH also directly excites backward command interneurons, thus providing an alternative parallel feedforward pathway by which it can induce avoidance of danger (Guo et al., 2009).

Previous studies have implicated the AIY sensory interneurons in setting the multisensory threat-reward decision balance of approach to the attractive odor diacetyl when confronting a toxic  $\text{Cu}^{2+}$  barrier (Ishihara et al., 2002; Shinkai et al., 2011). Specifically, HEN-1 protein secreted by AIY acts on its receptor SCD-2 in another sensory interneuron pair AIA to regulate the decision to cross the  $\text{Cu}^{2+}$  barrier (Ishihara et al., 2002; Shinkai et al., 2011). Dopamine also regulates this threat-reward decision (Wang et al., 2014). While food deprivation

increases threat tolerance in this multisensory decision, the HEN-1 and dopamine receptor pathways are dispensable for this hunger effect (Ishihara et al., 2002; Wang et al., 2014). Thus, cellular and molecular mechanisms by which internal physiological state modulates threat-reward decision making in *C. elegans*, or indeed, any animal, remain unknown.

As discussed above, RIM is suitably positioned within the sensorimotor control network to regulate multisensory threat-reward decision making. RIM expresses the neuropeptide Pigment Dispersing Factor-2 (PDF-2) and its cognate G protein-coupled receptor (GPCR), PDFR-1 (Flavell et al., 2013; Janssen et al., 2008; Janssen et al., 2009; Meelkop et al., 2012). While PDF-1, another ligand of PDFR-1, has recently been shown to play a role in arousal-related behaviors (Barrios et al., 2012; Choi et al., 2013; Flavell et al., 2013), PDF-2 function is not well characterized. We found that PDFR-1 activation by PDF-2 in RIM decreases threat tolerance in the multisensory decision task. Unexpectedly, RIM does not control the decision via its known synaptic connections within the sensorimotor control network. Rather, RIM controls the decision by previously unknown extrasynaptic top-down tyraminerpic positive feedback onto the primary osmosensory ASH neurons. Our results also indicate that internal hunger state suppresses this positive feedback loop, thereby increasing threat tolerance of food-deprived worms. These studies reveal underlying molecular and cellular mechanisms of internal state control of multisensory threat-reward decision making in the worm, and suggest that top-down topological motifs of multisensory integration are similar in mammals and the vastly simpler worm.

## RESULTS

### Hunger modulates multisensory threat-reward decision making

To provide a controlled behavioral context for exploring mechanisms underlying multisensory decision making, we employed a task in which worms balance danger of osmotic desiccation with reward of food. A 1 cm diameter hyperosmotic fructose barrier ring is applied to a 5 cm diameter agar plate without food, and two 1  $\mu$ L spots of diluted diacetyl food odor are applied outside the ring (**Figure 1B**). After allowing five minutes for the hyperosmotic solution to absorb into the agar and diffuse to establish an osmotic gradient, ten worms are removed from food-containing plates and placed in the center of the ring (**Figure 1B**). The decision balance between retreating from versus exiting the barrier ring is quantified as the fraction of worms that exit the ring within 15 minutes of being placed.

Approximately 30% of worms exit a 2 M fructose ring in the absence of food odor (**Figure 1C**), driven by their intrinsic propensity to disperse (Culotti and Russell, 1978; Gray et al., 2005). The presence of two 1  $\mu$ L drops of 1:350 dilution of diacetyl in water outside the ring increases exiting to 80% (**Figure 1C**). This increase in exiting is abolished in *odr-10* null-mutant worms that lack the ODR-10 diacetyl receptor (Sengupta et al., 1996) (**Figure 1C**). Spots of more diluted diacetyl result in smaller increases in exiting (data not shown). Increasing the fructose ring concentration to 3 M abolishes exiting even in the presence of food odor (**Figure 1D**). To determine if internal physiological state modulates this multisensory threat-reward decision, we deprived worms of food before testing. The decisions of worms deprived of food for fifteen minutes are indistinguishable from those of non-deprived worms (**Figure 1D**). In

contrast, worms deprived of food for one hour increase exiting relative to non-deprived worms, and worms deprived of food for five hours increase exiting even further (**Figure 1D**).

### **PDF-2 neuropeptide regulates multisensory decision making**

As discussed above, the position of RIM in the sensorimotor control network is suitable for controlling multisensory threat-reward decision making (**Figure 1A**). While multiple neurons express either PDF-2 or its receptor PDFR-1, RIM expresses both (**Figure 2A**). PDF-1 and PDFR-1 null-mutant worms exhibit locomotor defects (Janssen et al., 2008; Meelkop et al., 2012) attributable to altered arousal state (Barrios et al., 2012; Choi et al., 2013; Flavell et al., 2013) that preclude testing in the multisensory decision assay. However, *pdf-2* null-mutant worms exhibit grossly normal locomotion (see below), allowing us to test whether the balance of threat-reward decision making is altered in *pdf-2* null-mutant worms. While wild-type worms readily exit a 2 M fructose ring to approach the source of food odor, they are almost completely contained by a 3 M ring (**Figure 2B**). In contrast, *pdf-2* null-mutant worms readily exit the 3 M ring to approach the source of food odor, although they are still contained by a 4 M ring (**Figure 2B**). Complete rescue of the wild-type decision balance by transgenic re-expression of *pdf-2* under the control of its own promoter sequence establishes the absence of PDF-2 peptide as the cause of the *pdf-2* null-mutant decision phenotype (**Figure 2B**). Interestingly, the *pdf-2* null mutation has no effect on avoidance of the osmotic ring in the absence of diacetyl, nor on attraction to diacetyl in the absence of the osmotic ring (**Figure 2B, C, D**). This absence of unisensory defects rules out primary sensory defects and suggests a specific role for PDF-2 in multisensory decision making.

To determine whether the *pdf-2* null mutation causes a general defect in locomotion in the multisensory decision paradigm, we used automated worm tracking to directly measure locomotor kinematics as worms navigate within the osmotic ring (**Figure 2E**). The radial distribution of *pdf-2* null-mutant worm trajectories was quantified during the first five minutes of the assay (before any *pdf-2* null-mutant worms exit the ring). Trajectories of *pdf-2* null-mutant worms are indistinguishable from wild-type worms (**Figure 2F**). There is also no detectable effect of the *pdf-2* null mutation on standard locomotor kinematic parameters, including reversal frequency, high angle “omega turn” frequency, reversal magnitude, and forward bout duration (**Figure S1B**). Exiting in the absence of food odor is unaffected in *pdf-2* null-mutant worms (**Figure 2B, C**), and there is no statistically significant effect of the *pdf-2* null mutation on locomotor kinematics within the ring in this unisensory context (**Figure S1C, D**). Taken together, these results indicate that a general defect in locomotion—such as might prevent reversal and retreat upon approaching the hyperosmotic ring—cannot account for the altered multisensory decision balance of *pdf-2* null-mutant worms.

### **PDF-2 acts on PDFR-1 in RIM to set the threat-reward decision balance**

We next sought to determine both the cellular origin and target site of action of PDF-2 in regulating the threat-reward decision. Targeted re-expression of *pdf-2* in *pdf-2* null-mutant worms using a promoter specific to RIM and RIC, another interneuron pair near RIM in the anterior nerve ring (White et al., 1986), fully rescues the multisensory decision (**Figure 3A**). While only RIM normally expresses PDF-2 (Janssen et al., 2009), this indicates that PDF-2 secretion by RIM and RIC is sufficient to mediate wild-type decision balance. To determine the relevant PDFR-1-expressing targets of PDF-2, we employed membrane-tethered PDF-2 peptide (t-PDF-2) to activate PDFR-1 in specific neurons (**Figure 3B**). This approach has been

extensively validated *in vitro* and *in vivo* for cell-autonomous pharmacologically specific sustained activation of *Drosophila* PDFR and other class B1 neuropeptide receptors (Choi et al., 2012; Choi et al., 2009; Fortin et al., 2009; Krupp et al., 2013; Kunst et al., 2014). As noted earlier, RIM also expresses the PDF-2-receptor PDFR-1. Expression of t-PDF-2 in *pdf-2* null-mutant worms using either of two promoters whose expression overlaps solely in RIM fully rescues the decision balance (**Figure 3C**). Expression of t-PDF-2 in RIC, which does not express PDFR-1 (Barrios et al., 2012; Flavell et al., 2013; Janssen et al., 2008), and expression of a sequence-scrambled version of the tethered peptide (t-SCR) in RIM each fail to rescue the decision phenotype (**Figure 3C**). This confirms the cell-autonomy and pharmacological specificity of t-PDF-2 activation of PDFR-1 in RIM. Finally, expression of t-PDF-2 in RIM and RIC in a wild-type background does not suppress exiting of a 2 M fructose ring in the presence of diacetyl, suggesting that the PDF-2-PDFR-1 autocrine loop is maximally active in this state, thereby precluding any gain-of-function t-PDF-2 effect (**Figure S2**). Taken together, these data indicate that PDF-2 secretion by RIM and RIC and autocrine action of PDF-2 on PDFR-1 in RIM are each sufficient to mediate a wild-type decision balance, and establish RIM as a key locus of control of multisensory decision making in the worm.

### **Tyraminergetic positive feedback from RIM to ASH sets the threat-reward decision balance**

How does PDFR-1 activation in RIM decrease threat tolerance in the approach to food? One way in which RIM communicates with its downstream targets is *via* secretion of the biogenic amine tyramine, which is synthesized from tyrosine in a reaction catalyzed by the enzyme tyrosine decarboxylase (TDC), encoded by the *tdc-1* gene (**Figure 3D**) (Alkema et al., 2005). *tdc-1* null-mutant worms lack tyramine and phenocopy the *pdf-2* null-mutant phenotype, with worms now readily exiting the 3 M ring to approach the source of food odor, and with

unaltered avoidance of the osmotic ring on its own (**Figure 3E**). As for *pdf-2* null-mutant worms, locomotor kinematic defects do not account for the altered threat-reward decision balance of *tdc-1* null-mutant worms (**Figure S1**). *tdc-1* null-mutant worms also lack the biogenic amine octopamine, which is synthesized from tyramine in a reaction catalyzed by tyramine  $\beta$ -hydroxylase (TBH), encoded by the *tbh-1* gene (**Figure 3D**) (Alkema et al., 2005). However, *tbh-1* null-mutant worms, which synthesize tyramine but lack octopamine (Alkema et al., 2005), are unaffected (**Figure 3E**). This establishes that tyramine, and not octopamine, regulates the threat-reward decision balance. Additionally, double null-mutant *pdf-2; tdc-1* worms behave identically to both *pdf-2* and *tdc-1* single-mutant worms, suggesting that these genes function in the same genetic pathway to control the decision (**Figure 3E**). Finally, targeted re-expression of *tdc-1* in *tdc-1* null-mutant worms in RIM and RIC, but not RIC alone, fully rescues the multisensory decision (**Figure 3F**). RIM is the only tyraminerpic neuron in the sensorimotor control network (Alkema et al., 2005; Donnelly et al., 2013; Pirri et al., 2009), and the only other tyraminerpic neuroendocrine cell in the worm, *uv1*, controls egg-laying (Alkema et al., 2005; Jose et al., 2007). Taken together with the key role of PDFR-1 activation in RIM (**Figure 3C**), these results strongly support the conclusion that RIM controls threat-reward decision making, and that it exerts this control *via* tyraminerpic signaling to tyramine receptor-expressing targets.

What is the relevant tyramine receptor, and where and how does it act to mediate RIM control of the threat-reward decision balance? The worm genome encodes a hyperpolarizing tyramine-gated Cl<sup>-</sup> channel (LGC-55) expressed in one of the forward command interneurons, and previously shown to regulate locomotion (Pirri et al., 2009), as well as several tyramine GPCRs related to vertebrate adrenergic receptors, including TYRA-2, SER-2, and TYRA-3 (Donnelly et al., 2013; Rex and Komuniecki, 2002; Rex et al., 2004; Wragg et al., 2007). We

considered LGC-55 a particularly likely target of RIM tyramine signals in the control of threat-reward decision making, as it mediates direct synaptic inhibition of forward locomotor command interneurons by RIM (Alkema et al., 2005; Pirri et al., 2009). However, the threat-reward decision balance of both *lgc-55* and *tyra-3* null-mutant worms is indistinguishable from that of the wild-type (**Figure 4A**). *ser-2* null-mutant worms possibly exhibit altered multisensory decision balance, although not statistically distinguishable from the wild-type in our assays. *ser-2* null-mutant worms also exit the 3 M fructose ring in the absence of food odor more readily than wild-type, which could potentially be explained by previously described defects in avoidance and turning behaviors (Donnelly et al., 2013; Rex et al., 2004) (**Figure 4A**). Unlike the other tyramine receptor null-mutant worms, however, *tyra-2* null-mutant worms readily exit a 3 M ring to approach the source of food odor, phenocopying both *pdf-2* and *tdc-1* null-mutant worms (**Figure 4A**). Like *pdf-2* and *tdc-1* null-mutant worms, *tyra-2* null-mutant worms are unaffected in the unisensory avoidance-only context (**Figure 4A**). Furthermore, double null-mutant *pdf-2*; *tyra-2* worms behave identically to both *pdf-2* and *tyra-2* single-mutant worms, suggesting that these signaling modules operate in the same functional pathway to determine threat-reward decision balance (**Figure 4A**). Little is known about *tyra-2* function (Donnelly et al., 2013; Jin et al., 2016; Pirri et al., 2009; Rex et al., 2005), and our results now demonstrate that TYRA-2 is the receptor through which tyramine secreted by RIM controls threat-reward decision making.

*tyra-2* is expressed in a number of sensory neurons, including the osmosensory neuron ASH (Rex et al., 2005). Re-expression of *tyra-2* using either of two cell-specific promoters whose expression overlaps only in ASH rescues the wild-type decision balance in *tyra-2* null-mutant worms, while re-expression using a promoter active in other sensory neurons but not ASH has no effect (**Figure 4B**). Functional imaging of ASH  $Ca^{2+}$  responses to osmotic stimuli

revealed that exogenous tyramine pre-treatment increases ASH osmosensitivity, indicating neuronal activation (**Figure 4C, D, E**) (Zahratka et al., 2015). Tyramine potentiation of ASH osmosensitivity is abolished in *tyra-2* null-mutant worms (**Figure 4F, G, H**). Finally, we compared the time it takes *tyra-2* null-mutant worms to exit the ring in the standard multisensory assay and a time-shifted configuration in which the fructose ring is allowed five extra minutes to diffuse before the worms are placed (**Figure S3A**). Worms begin to exit the time-shifted configuration immediately after being placed, while worms begin to exit in the standard assay about five minutes after being placed (**Figure S3B, S3C**). Therefore exiting in the threat-reward decision assay is determined by the state of the osmotic barrier as each worm confronts it and the osmosensitivity of the ASH neuron as regulated by tyraminerpic feedback (**Figure S3B, S3C, S3D**). As RIM does not form synapses onto ASH (although ASH does synapse onto RIM) (White et al., 1986), taken together these results demonstrate that RIM controls multisensory threat-reward decision making by secreting tyramine to act extrasynaptically at a distance directly on ASH through TYRA-2 to potentiate ASH sensitivity to osmotic stimuli.

### **Computational modeling predicts slow, non-linear tyramine regulation of threat-reward decision making**

To characterize the interplay between neural activity, neuromodulatory activity, behavior, and the environment, we modeled each of these dynamic components *in silico* (Cohen and Sanders, 2014). Model worms contain a highly simplified minimal nervous system sufficient for decision making (**Figure 5A**; see **Experimental Procedures** and **Supplemental Methods** for details). Model parameters were fit to match the experimental exiting rates of **Figure 2B**. To illustrate the temporal evolution of the decision process, we plotted the locomotor trajectories and neural and neuromodulatory activity underlying the behavior of example simulated wild-type and *tyra-2* null-mutant worms

(**Figure 5C, D**). This reveals that oscillatory changes in fructose and diacetyl concentration experienced by the worm as it undulates within the arena (**Figure 5C**) induce corresponding oscillatory changes in the activity of AWA, ASH, and RIM (**Figure 5D**) that are phase locked to the undulatory locomotor DMN-VMN pattern generator. In both wild-type and *tyra-2* null-mutant worms, the magnitude of RIM and ASH oscillatory activity is larger than the AWA signal, and decreases over time. However, the magnitude of this oscillatory activity in RIM and ASH is higher in wild-type worms than in *tyra-2* null-mutant worms (**Figure 5D, S4B, S4C**). Furthermore, in virtual *tyra-2* null-mutant worms, the magnitude of RIM and ASH activity decreases over the course of the simulation until it matches AWA activity, which permits exiting of the ring. After exiting, the AWA signal dominates, ASH becomes silent, and RIM becomes inhibited as the worm continuously ascends the diacetyl gradient (**Figure 5D**). The model thus predicts that RIM activity engages the RIM-ASH feedback loop, thereby decreasing threat tolerance and preventing exiting of the ring (**Figure 5D**). Importantly, the model also requires a non-linear threshold for RIM activation to trigger tyramine signaling to ASH and slow kinetics of accumulation and decay of the tyramine signal on a timescale of minutes to preclude exiting of wild-type worms even as the fructose barrier continues to diffuse and weaken (**Figure 5D, S4D**). The reduced activation of ASH and RIM in simulated *tyra-2* null-mutant worms, in which tyraminerpic RIM-ASH feedback is absent, encodes increased threat tolerance underlying increased propensity to exit the ring (**Figure 5D**). Our computational model thus suggests that the slow, continuous, and self-reinforcing enhancement of RIM and ASH activity by top-down tyraminerpic positive feedback determines threat tolerance that ultimately controls the decision balance.

## **RIM-ASH positive feedback underlies modulation of the threat-reward decision by internal hunger state**

Food deprivation increases threat tolerance in the multisensory decision paradigm (**Figure 1D**). We hypothesized it does so by suppressing RIM activity and thus the RIM-ASH tyraminerpic feedback pathway. As a first test of this hypothesis, we examined the effect of food deprivation on *tyra-2* null-mutant worms. In contrast to wild-type worms, *tyra-2* null-mutant worms fail to increase exiting after one hour of food deprivation, and rather maintain their already elevated threat tolerance (**Figure 6A**). This is consistent with the hypothesis that one hour of food deprivation increases threat tolerance by suppressing tyraminerpic RIM-ASH feedback, with *tyra-2* null-mutant worms failing to increase exiting because the RIM-ASH pathway is already fully suppressed. We also measured the effects of food deprivation on unisensory osmotic avoidance. We found that *tyra-2* null-mutant worms deprived of food for one hour exit more readily in the unisensory context, while wild-type worms are still retained (**Figure 6B**). This result also supports our model that food deprivation suppresses RIM activity and thus tyraminerpic RIM-ASH feedback. In our model, RIM inhibition induced by food deprivation disinhibits forward command interneurons and reduces activation of backward command interneurons. This renders retreat from the osmotic ring reliant upon RIM-ASH positive feedback in this context. Five hours of food deprivation further increases exiting of *tyra-2* null-mutant worms in the multisensory context, as seen in wild-type worms (**Figure 6A**). In the unisensory context, five hours of food deprivation further also increases exiting of both wild-type and *tyra-2* null-mutant worms (**Figure 6B**). These effects of more extended food deprivation on *tyra-2* null-mutant worms indicate the involvement of RIM-ASH-independent pathways for hunger-dependent increases in threat tolerance. Next we measured the effects of food deprivation

on unisensory attraction to food odor. We found that both wild-type and *tyra-2* null-mutant worms exhibit enhanced chemotaxis to diacetyl after one or five hours of food deprivation (**Figure 6C, S5**). While increased chemotaxis to diacetyl in hungry wild-type and *tyra-2* null-mutant worms could be driven by increased diacetyl sensitivity (Ryan et al., 2014), it is more likely that food deprivation increases attraction to diacetyl by effects on locomotion that result from suppressed RIM activity (Gordus et al., 2015; Gray et al., 2005).

To further test the hypothesis that food deprivation inhibits RIM, we turned to our computational model. We simulated food deprivation as duration-dependent tonic inhibition of RIM, keeping all other model parameters unchanged. We modeled one hour of food deprivation as RIM inhibition of  $\sim 0.03$  (in arbitrary units), because this level of RIM inhibition increases exiting of simulated wild-type worms to 50%, the same as real worms food-deprived for one hour (compare **Figure 6A** with **Figure 6D**). As RIM inhibition increases from 0 to  $\sim 0.03$ , simulated wild-type worms increase exiting more steeply than *tyra-2* null-mutant worms (**Figure 6D, S4E, S4F**, see **Supplemental Movie 1**). This qualitatively recapitulates the multisensory experimental results (**Figure 6A**). In the unisensory fructose-only context, as RIM inhibition increases from 0 to  $\sim 0.03$ , simulated wild-type worms increase exiting less steeply than *tyra-2* null-mutant worms (**Figure 6E**). This qualitatively recapitulates the unisensory experimental results (**Figure 6B**). We note that model parameters were selected solely to match simulated exiting rates (**Figure 5B**) to experimental exiting rates of non-food-deprived worms (**Figure 2B**), without any consideration of the effects of food deprivation. Nonetheless, the qualitative recapitulation of experimental effects of increasing food deprivation in both unisensory and multisensory contexts by simulating increasing inhibition of RIM strongly reinforce the

conclusion that food deprivation increases threat tolerance by inhibiting RIM and thereby suppressing RIM-ASH tyraminerpic feedback.

### **Tyramine supplementation and direct inhibition of RIM each recapitulate hunger state modulation of the threat-reward decision *via* RIM-ASH positive feedback**

The results presented thus far support a model in which food deprivation increases threat tolerance by inhibiting RIM and thereby suppressing RIM-ASH tyraminerpic feedback. To provide additional experimental tests of this model, we directly manipulated neural activity within the circuit. To exogenously induce tyraminerpic potentiation of ASH, we added tyramine to the assay plate. Our model predicts that exogenous tyramine will increase threat sensitivity and thereby promote retreat from the ring in multisensory contexts *via tyra-2* (**Figure 7A**). Exogenous tyramine has no effect on wild-type worms, which already exhibit maximum threat sensitivity (**Figure 7B**). In contrast, exogenous tyramine suppresses exiting of *tdc-1* null-mutant worms exiting to wild-type levels (**Figure 7B**). Importantly, the effect of exogenous tyramine is completely eliminated in *tyra-2* null-mutant worms (**Figure 7B**). We next tested the effect of exogenous tyramine on wild-type worms food-deprived for one hour. Our model predicts that one-hour food deprivation increases exiting *via* suppression of RIM-ASH tyraminerpic feedback; therefore, the presence of tyramine, regardless of the worm's internal state, should suppress exiting. Indeed, wild-type worms food-deprived for one hour behave like sated worms in the presence of exogenous tyramine (**Figure 7C**). Again, *tyra-2* null-mutant worms are unaffected (**Figure 7C**). Tyramine supplementation completely reverses increased exiting of one-hour food-deprived wild-type worms, thus further indicating that suppression of RIM-ASH tyraminerpic feedback underlies increased threat tolerance of hungry worms.

Complementarily, our model predicts that direct inhibition of RIM activity should increase exiting (**Figure 7D**). Transgenic expression of a histamine-gated chloride channel (His-Cl) specifically in RIM allows for controlled silencing of RIM contingent upon the presence of exogenously applied histamine, as worms do not endogenously use histamine as a neurotransmitter (Pokala et al., 2014). In the absence of exogenous histamine, worms expressing His-Cl in RIM behave like wild-type worms and fail to exit the ring (**Figure 7E**). Direct inhibition of RIM by addition of 10 mM histamine to the assay plate increases exiting to ~50%, identical to the behavior of *tdc-1* and *tyra-2* null-mutant worms (**Figure 7E**). Inhibition of RIM by addition of 30 mM histamine to the plate did not increase exiting any further (**Figure 7E**). Therefore while the absolute level to which RIM is inhibited by histamine is unknown, activation of His-Cl and histamine-mediated inhibition of RIM are apparently saturated by 10 mM histamine. Our model predicts that one hour of food deprivation increases exiting by inhibiting RIM activity; therefore, as with *tyra-2* null-mutant worms, no further increase in exiting of hungry worms is predicted when RIM is directly inhibited. In the absence of histamine, like wild-type worms, worms expressing His-Cl in RIM increase exiting after one hour of food-deprivation (**Figure 7F**). As predicted by our model, direct inhibition of RIM with histamine has no further effect on exiting of these one-hour food-deprived worms (**Figure 7F**). Taken together, these effects of exogenous tyramine and direct inhibition of RIM demonstrate that suppression of RIM-ASH tyraminerpic feedback through RIM inhibition quantitatively accounts for the entirety of increased threat tolerance induced by one hour of food deprivation.

## DISCUSSION

Here we demonstrate that tyraminerpic RIM-ASH positive feedback controls multisensory threat-reward decision making in *C. elegans*. PDFR-1 activation by PDF-2 in RIM (**Figure 2, 3**) and extrasynaptic tyraminerpic potentiation of ASH (**Figure 4**) set the decision balance between retreat from an osmotic threat and approach to food odor. We devised a minimal computational neural network model to recapitulate the decision *in silico*. Our model predicts that integration of multisensory inputs in RIM nonlinearly determines the magnitude of the tyramine feedback signal. Using this model, we simulated the dynamic changes in neural and neuromodulatory activity that occur in freely moving worms during decision making, and determined the slow timescale at which RIM-ASH tyraminerpic feedback must act to implement the decision (**Figure 5, S4D**). Our experimental and computational results establish that food deprivation increases threat tolerance through suppression of RIM-ASH positive feedback by inhibition of RIM (**Figures 6, 7, S4E, S4F**). These studies provide an integrated neuroendocrine circuit architecture for internal state control of multisensory threat-reward decision making.

Tyramine secreted by RIM regulates multisensory decision making by modulating ASH sensitivity extrasynaptically at a distance. Interestingly, our modeling studies predict that the extrasynaptic tyramine signal must both accumulate and decay slowly, modulating ASH sensitivity over long time-scales of multiple minutes in order to appropriately implement the threat-reward decision balance. The computational model is agnostic as to how the slow kinetics of tyramine signaling are implemented. We propose that this slow tyramine signaling functions as a form of memory for the worm by suppressing sensory adaptation to dangerous stimuli sensed over time, a feature which could be advantageous for worms navigating changing environmental conditions. Slow-acting diffusion and accumulation of extrasynaptic signals that

act at a distance like tyramine are likely better suited for this purpose than more fast-acting synaptic signals. In addition, tyraminerpic RIM-ASH positive feedback could determine the worm's tolerance to a variety of threats, as the ASH neuron is polymodal and senses multiple noxious cues (Hilliard et al., 2005; Kaplan and Horvitz, 1993).

We conclusively demonstrate that suppression of RIM-ASH tyraminerpic feedback through RIM inhibition quantitatively accounts for the entirety of increased threat tolerance induced by one hour of food deprivation. Our studies indicate that RIM is a metabolic modulator of threat-reward decision making. Interestingly, we found that the direct synaptic connections that RIM forms with downstream command locomotor interneurons driving basic attraction and aversion behaviors are dispensable for modulation of threat-reward decision-making. In the decision studied here, the only function of RIM is to provide tyraminerpic feedback to ASH based on metabolic state. Extended food deprivation beyond one hour then recruits other currently unknown RIM-ASH-independent pathways for hunger-dependent increases in threat tolerance. It was recently shown that in RIM and RIC, expression of NKAT-1, an enzyme in the biosynthetic pathway of kynurenic acid, a metabolite whose levels vary with hunger state, underlies hunger modulation of feeding rate (Lemieux et al., 2015). This provides a potential cellular mechanism linking organismic metabolic state to RIM excitability, thereby controlling tyramine secretion, and thus determines the likelihood of exiting the ring during the decision task.

Cell-specific rescue and t-PDF-2 expression experiments support the existence of a PDF-2-PDFR-1 autocrine loop in RIM. Interestingly, autocrine PDF signaling also occurs in the *Drosophila* circadian control network (Choi et al., 2012). Activation of autocrine positive feedback loops, however, are probably not easily reversible – therefore, it is likely that these

cascades are only initiated beyond a threshold level of activation modulated by externally derived signals or internal cellular state. Such a mechanism would reinforce the stability of distinct, long-lasting behavioral states, such as behaviors relevant to night versus day, or, in this case, biasing threat-reward decision making in the threat-tolerant or threat-sensitive directions. Therefore, we propose that by flipping a potentially stable switch, the PDF-2-PDFR-1 autocrine loop biases the worm towards threat-sensitive decisions and importantly, eliminates ambivalence when confronted with various situations.

How does RIM-ASH positive feedback control threat-reward decision making? The worm sensory interneuron network downstream of primary sensory neurons captures multisensory information (Ishihara et al., 2002; Luo et al., 2014; Shinkai et al., 2011), and RIM is downstream of this network (Gordus et al., 2015; Guo et al., 2009; Piggott et al., 2011; White et al., 1986). Thus we propose that RIM excitability is set by PDF-2-PDFR-1 autocrine loop activity, and RIM activity fluctuates dynamically as it responds to specific combinations of diacetyl and osmotic stimuli as the worm navigates the decision arena. Ultimately, the worm makes a probabilistic choice between retreat and exit based on the stochastic, instantaneous activity state of RIM, with stochasticity of RIM activity having been previously observed (Gordus et al., 2015). Longer time-scale tyraminerpic feedback increases the probability that instantaneous RIM, and therefore ASH, activity states are high. Thus by acutely modifying forward and backward locomotor-control neuron activity through feedforward channels or, on longer time scales, indirectly through tyraminerpic RIM-ASH feedback, RIM activity transforms multisensory inputs into a motor decision (**Figure S6**).

As discussed previously, in human beings and other mammals, a key feature of multisensory integration is internal-state-dependent “top-down” control of perception (Manita et

al., 2015; Talsma et al., 2010) by higher-order brain regions (Fairhall and Macaluso, 2009; Fu et al., 2014; Zhang et al., 2014). Interestingly, in the human brain, aminergic neuromodulatory systems like locus coeruleus are involved in directing attention to food-related cues in a hunger-dependent manner (Mohanty et al., 2008). However, analysis of neural mechanisms in the context of an approach-avoidance decision in human and mammalian systems are limited by technical constraints. Here we describe an integrated circuit that demonstrates a mechanism by which internal physiological hunger state drives a shift in the decision balance of approach and avoidance. The molecular, cellular, and network motifs described here in the worm may be relevant in more complex nervous systems.

## EXPERIMENTAL PROCEDURES

All *C. elegans* strains were maintained on Nematode Growth Medium (NGM) agar plates with *E. coli* OP50 as a food source. Transgenic strains were constructed by injection of plasmid DNA into the germline using standard methods. At least three independent lines were generated, tested, and results aggregated for each transgene.

### *Behavioral assays*

For unisensory and multisensory assays, 10 $\mu$ L of fructose solution is applied in a 1cm diameter ring centered on a standard NGM plate (Culotti and Russell, 1978). For multisensory assays, 1 $\mu$ L diluted diacetyl was applied to each side of the plate, at least 1cm from the ring border. Ten (unless otherwise indicated) adult worms were transferred inside the ring. Fifteen minutes later, the number of worms outside the ring were counted. For the food deprivation assays, worms were transferred inside the ring from another plate, where they were kept without food for the indicated time interval. For this and other assays, see **Supplemental Experimental Procedures** for more information. At least five independent assays were performed for each condition. One- or two-way ANOVA, as appropriate, was used to analyze statistical differences, with *post-hoc* Tukey-Kramer tests performed for pairwise comparisons between all pairs of genotypes. We did not include all previously tested genotypes/conditions each time a new genotype/condition was tested. Therefore identical data was presented in multiple figure panels.

### *Imaging*

Calcium imaging of ASH neurons was performed on young adult worms immobilized in an olfactory chamber as described previously (Chronis et al., 2007). Imaged animals expressed

the genetically-encoded calcium sensor GCaMP3 from the *sra-6* promoter (Tian et al., 2009). Prior to loading into the olfactory chamber worms were bathed in neuronal buffer for 10 min either in the presence or absence of 50mM tyramine. Animals were exposed to the test stimulus for 20 sec. Averaged fluorescence was calculated for a region of interest (ROI) surrounding the ASH soma. The percent change in fluorescence for each frame was calculated relative to the corrected fluorescence of the ROI just prior to stimulus onset. See **Supplemental Experimental Procedures** for more details.

### *Computational model*

Model animals possess a simplified nervous system comprising sensory neurons AWA and ASH, interneuron RIM, and two reciprocally inhibited motor neurons denoted DMN and VMN, which activate dorsal and ventral body bends, respectively. AWA and ASH sensory neurons respond with transient activation to changes in diacetyl or fructose stimuli, respectively. Activation of model sensory neurons AWA and ASH provides differentially weighted inhibitory and excitatory inputs onto RIM, respectively. RIM integrates these sensory inputs and inhibits motoneurons DMN and VMN to bias dorsal versus ventral bends, thereby inducing gradual steering of the worm. Additionally, RIM activity positively increases the likelihood of pirouettes, which are modeled as instantaneous step changes in angular heading. The simulation also includes RIM-ASH tyraminerpic positive feedback. Tyraminerpic potentiation of ASH in the model is only engaged above a threshold level of RIM activation. *tyra-2* null-mutant worms are modeled as lacking this tyraminerpic feedback to ASH. Model parameters were manually calibrated until exiting rates of simulated wild-type and *tyra-2* null-mutant worms matched experimental exiting rates in multisensory and unisensory contexts for 2 M, 3 M, and 4 M fructose. The virtual decision arena comprises a continuously diffusing fructose gradient ring and

time-invariant diacetyl gradients originating from two spots outside the ring. Each simulation begins with a single worm in the center of the virtual arena with a randomly selected initial heading, and the simulation is allowed to proceed for fifteen virtual minutes. See **Supplemental Experimental Procedures** for additional details.

## **Author Contributions**

DDG, TS, NC, MRK, MNN designed study and experiments. DDG, SH, DLC performed experiments. TS and NC performed computational modeling. DDG, TS, LYM, DLC, NC, MRK, and MNN analyzed the data. DDG and MNN wrote the paper with input from the other authors.

## **Author Information**

The authors declare no conflicts of interest.

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## **Figure 1. Hunger state modulates a multisensory threat-reward decision**

**(A)** Schematic of information flow in the *C. elegans* sensorimotor control network. The AWA primary sensory neuron that responds to attractive stimuli like diacetyl food odor indirectly inhibits the RIM interneuron. The ASH primary sensory neuron that responds to aversive stimuli like hyperosmolarity indirectly excites RIM. RIM directly inhibits forward command interneurons and directly excites backward command interneurons to control the balance between forward and backward locomotion. ASH also bypasses RIM to directly excite backward command interneurons.

**(B)** Schematic of the decision making assay. Worms (black squiggles) are placed in the center of a hyperosmotic fructose ring (red circle) with the food odor diacetyl (yellow spots) outside the ring. As each worm ascends the attractive diacetyl gradient, it encounters the aversive hyperosmotic ring, where it either proceeds to exit or retreats to remain in the ring. The decision balance is quantified as the percent of worms that exit the ring during a fifteen minute trial.

**(C)** Wild-type and *odr-10* null-mutant worm tolerance of 2 M fructose ring in presence or absence of food odor. *odr-10* null-mutant worms lack the olfactory receptor that senses diacetyl.

**(D)** Effect of increasing food deprivation on decision to exit a 3 M fructose ring in the presence of food odor.

(Identical data is presented in multiple data sets, as all previously tested genotypes/conditions were not tested every time we extended our analysis to additional genotypes/conditions. Unless otherwise indicated, data in all Figures represent the average of at least five independent assays with ten worms per assay. All statistical comparisons are by one- or two-way ANOVA, as

appropriate, with Tukey-Kramer paired-comparison test applied to all pairs of genotypes. Error bars denote s.e.m.; \*\*\*,  $p \leq 0.001$ .)

**Figure 2. PDF-2 neuropeptide regulates multisensory decision making**

(A) RIM is potentially a peptide-modulated locus for internal state control of threat-reward decision making. RIM expresses both the neuropeptide PDF-2 and its G protein-coupled receptor PDFR-1.

(B) Decision balance of wild-type and *pdf-2* null-mutant worms encountering a 2 M, 3 M, or 4 M fructose ring in the presence or absence of food odor. Rescue of *pdf-2* null-mutant phenotype by re-expression of a *pdf-2* transgene under the control of its own promoter.

(C) Exiting of the osmotic ring in the absence of food odor in wild-type and *pdf-2* null-mutant worms.

(D) Chemotaxis to various concentrations of diacetyl, measured as the fraction of worms that move to a test spot of diacetyl dilution versus a control spot of water after a fifteen minute trial on a standard assay plate, in wild-type and *pdf-2* null-mutant worms.

(E) Representative five minute trajectories of wild-type and *pdf-2* null-mutant worms inside a 3 M ring with food odor outside. The outer red circle represents the osmotic ring, and the shading indicates 1mm annular zones.

(F) Time spent by wild-type and *pdf-2* null-mutant worms in each of the radial zones ( $n=7-10$  worms for each genotype,  $F = 1.688$ ,  $p = 0.160$ ).

**Figure 3. PDF-2 signaling to PDFR-1 in RIM controls the multisensory decision balance by modulating tyramine secretion**

(A) Re-expression of PDF-2 in RIM and RIC in *pdf-2* null-mutant worms using the *tdc-1* promoter.

(B) Membrane-tethered peptide (t-peptide) system. Bioactive peptides can be expressed as chimeric fusion proteins with N-terminal secretory signal sequences and C-terminal glycolipid anchor targeting signals. t-peptides are secreted, but remain covalently anchored to the plasma membrane, and thus only activate their cognate receptors cell-autonomously. Adapted from Choi et al., 2009.

(C) Expression in *pdf-2* null-mutant worms of t-PDF-2 using either of two promoters whose activity overlaps only in RIM; expression of t-PDF-2 in RIC alone; or expression of sequence-scrambled t-SCR in RIM and RIC. The *tdc-1*, *nmr-2*, and *tbh-1* promoters are used to drive expression in RIM and RIC; RIM, multiple AV neurons, and PVC; and RIC alone, respectively.

(D) Tyramine is synthesized from tyrosine by tyrosine decarboxylase (TDC), encoded by the *tdc-1* gene expressed in RIM and RIC. Octopamine is synthesized from tyramine by tyramine- $\beta$ -hydroxylase (TBH), encoded by the *tbh-1* gene expressed in RIC.

(E) Multisensory and unisensory decision balance of *tdc-1* null-mutant worms, which lack both tyramine and octopamine, and *tbh-1* null-mutant worms, which lack only octopamine. Decision balance of *pdf-2*; *tdc-1* double-mutant is also shown.

(F) Re-expression of *tdc-1* in RIM and RIC, but not RIC alone, in *tdc-1* null-mutant worms.

**Figure 4. Tyramine acts on its receptor TYRA-2 in ASH osmosensory neuron to regulate the multisensory decision balance**

(A) Multisensory and unisensory decision balance of tyramine receptor mutants. Tyramine GPCR *ser-2* null-mutant worms exhibit increased exiting in both multisensory ( $p$  vs wild-type = 0.136) and unisensory contexts, though only the increased exiting in the unisensory context is statistically significant compared to wild-type (\*\*,  $p = 0.009$ ). Decision balance of the *pdf-2*; *tyra-2* double-mutant is also shown.

(B) Re-expression of *tyra-2* in *tyra-2* null-mutant worms under the control of the *sra-6* or *gpa-13* promoters, whose activity overlaps solely in ASH. Re-expression of *tyra-2* using the *mec-17* promoter in touch receptor neurons (TRN), some of which also normally express *tyra-2*.

(C-E) Representative images (C), time course (D), and peak (E)  $\text{Ca}^{2+}$  responses of ASH to 180 mM fructose (unless otherwise indicated) in wild-type worms with or without 50 mM tyramine pre-treatment imaged using GCaMP3.

(F-H) Representative images (F), time course (G), and peak (H)  $\text{Ca}^{2+}$  responses of ASH to 180 mM fructose in *tyra-2* null-mutant worms with or without 50 mM tyramine pre-treatment imaged using GCaMP3.

(Arrows indicate ASH cell body, magnified in the insets. Dashed lines outline the worm. mean  $\pm$  s.e.m; circles represent peak responses of individual animals;  $n > 12$  animals per genotype and treatment condition.)

**Figure 5. Computational modeling predicts non-linear slow tyramine signaling by RIM to ASH**

(A) Schematic of the simplified nervous system used for computational modeling. AWA and ASH provide direct inhibitory and excitatory inputs onto RIM, respectively. RIM integrates these sensory inputs and directionally biases forward locomotion via inhibition of steering and pirouette modulation. Tyraminerpic positive feedback from RIM to ASH increases ASH sensitivity to osmotic stimuli. Simulated *tyra-2* null-mutant worms lack the tyraminerpic RIM-ASH signal.

(B) Decision balance of simulated wild-type and *tyra-2* null-mutant worms encountering a 2 M, 3 M, or 4 M fructose ring in the presence or absence of food odor.  $n=1000$  single worm simulations per genotype and condition.

(C) Sample fifteen-minute trajectories of simulated wild-type and *tyra-2* null-mutant worms inside a 3 M ring with food odor outside. Trajectories are magnified in the insets.

(D) Neural activity profiles of AWA, ASH, and RIM during the simulated trajectories in (C). Activity of individual neurons and of tyramine signals are expressed in arbitrary units, plotted to the same scale for the simulated wild-type and *tyra-2* null-mutant worms.

**Figure 6. One-hour food deprivation fails to increase threat tolerance of *tyra-2* null-mutant worms**

(A-C) Effect of one hour or five hours of food deprivation on wild-type and *tyra-2* null-mutant worm multisensory decision balance (A), exiting of a 3 M fructose ring in the absence of food odor (B), and chemotaxis to 1:1000 diacetyl (C). (\*,  $p<0.05$ .)

(D and E) Effect of increasing RIM inhibition on simulated wild-type and *tyra-2* null-mutant worm multisensory (D) or unisensory (E) decision balance. Vertical dashed lines indicate the

degree of RIM inhibition that results in exiting rates for simulated wild-type worms that match those of real worms deprived of food for the indicated durations (see panel A).  $n=250$  simulated worms for each genotype and strength of RIM inhibition.

**Figure 7. One-hour food deprivation increases threat tolerance by inhibiting RIM activity and suppressing RIM-ASH tyraminerbic potentiation**

(A) Schematic depicting prediction that exogenous tyramine increases threat sensitivity.

Exogenous tyramine is predicted to reverse the effects of suppression of the RIM-ASH positive feedback loop on threat sensitivity. Yellow represents strong activity, while blue represents weak activity. Thickness of solid lines represents strength of signals, and dashed lines represent inactive signals.

(B) Effect of exogenous tyramine on multisensory decisions of wild-type, *tdc-1* null-mutant, and *tyra-2* null-mutant worms.

(C) Effect of exogenous tyramine on multisensory decisions of one-hour food-deprived wild-type and *tyra-2* null-mutant worms.

(D) Schematic depicting prediction that inhibition of RIM increases threat tolerance. Inhibition of RIM expressing His-C1 with exogenous histamine is predicted to increase threat tolerance and mimic one hour of food deprivation.

(E) Effect of 0, 10 mM, and 30 mM histamine on multisensory decision balance of worms expressing RIM::His-C1.

(F) Effect of 0 and 10 mM histamine on multisensory decision balance of one-hour food-deprived worms expressing RIM::His-Cl.

Figure 1

Figure 1

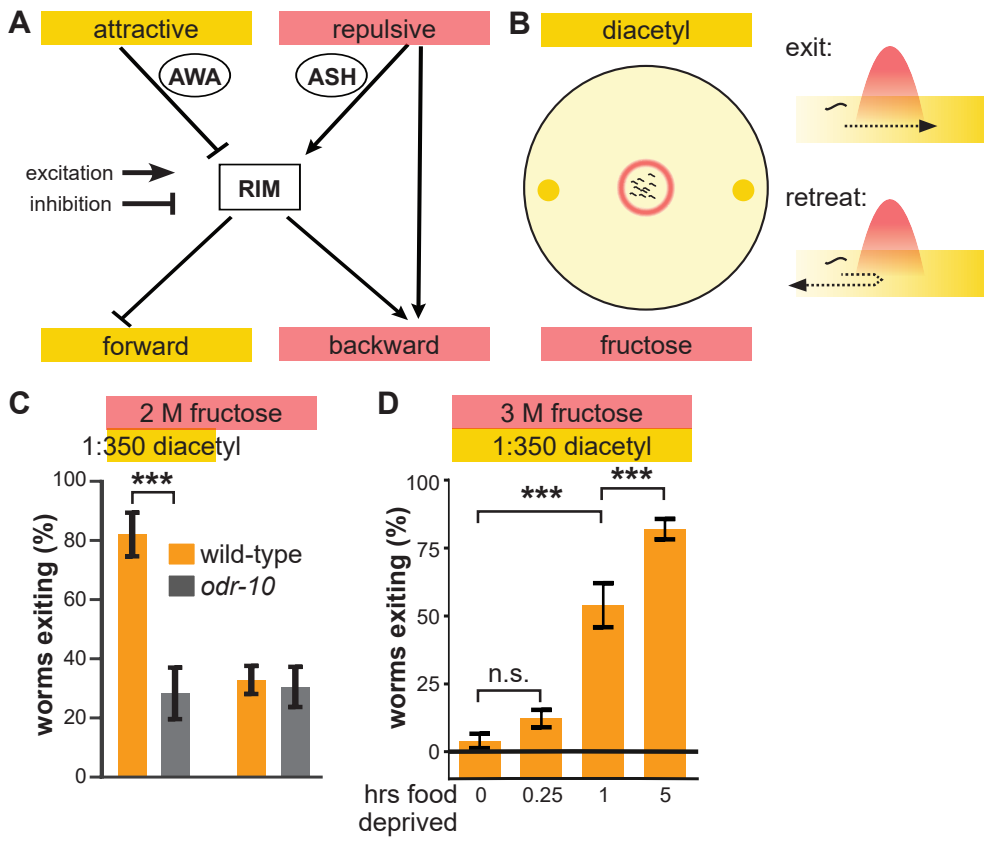


Figure 2

Figure 2

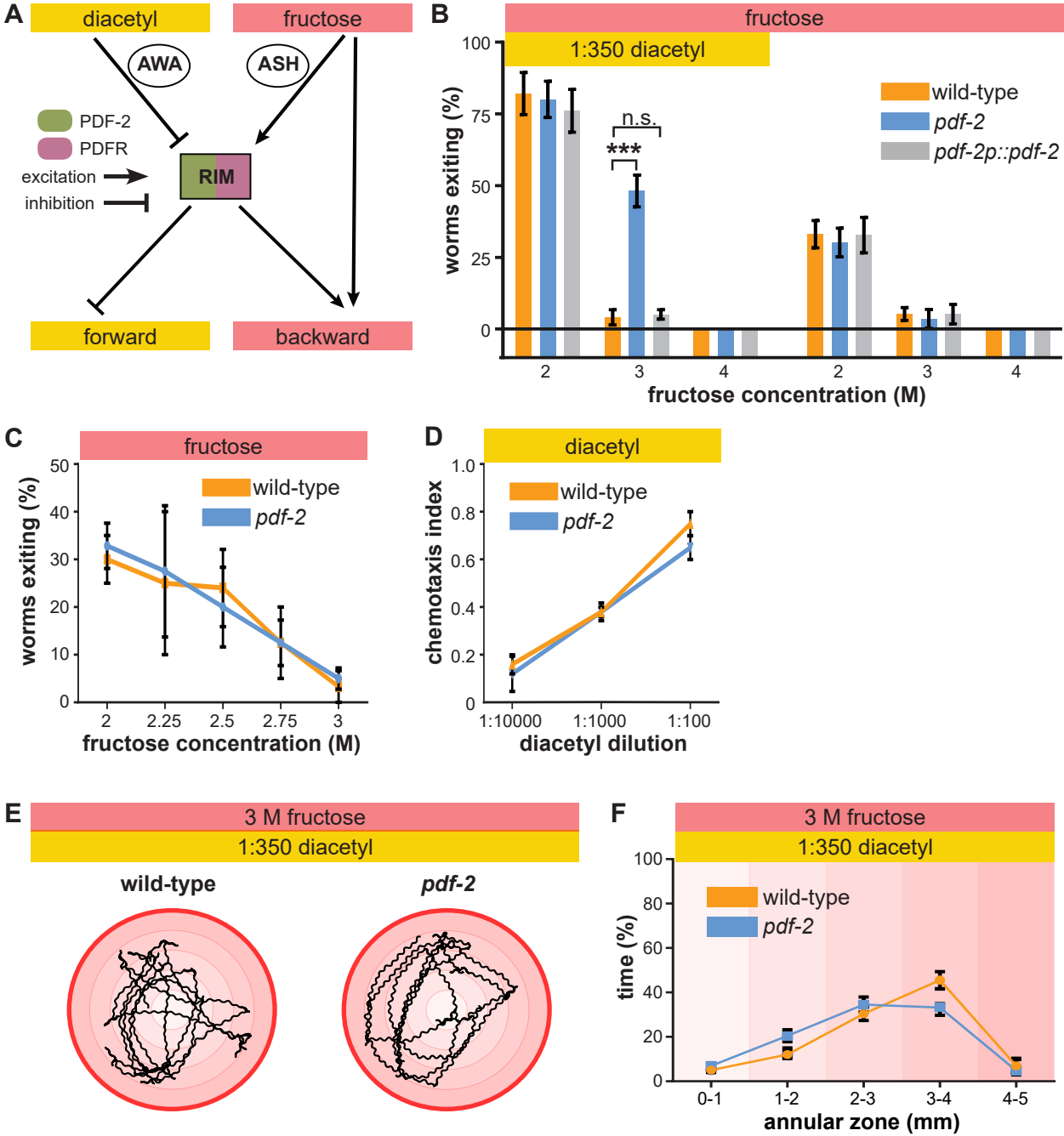


Figure 3

Figure 3

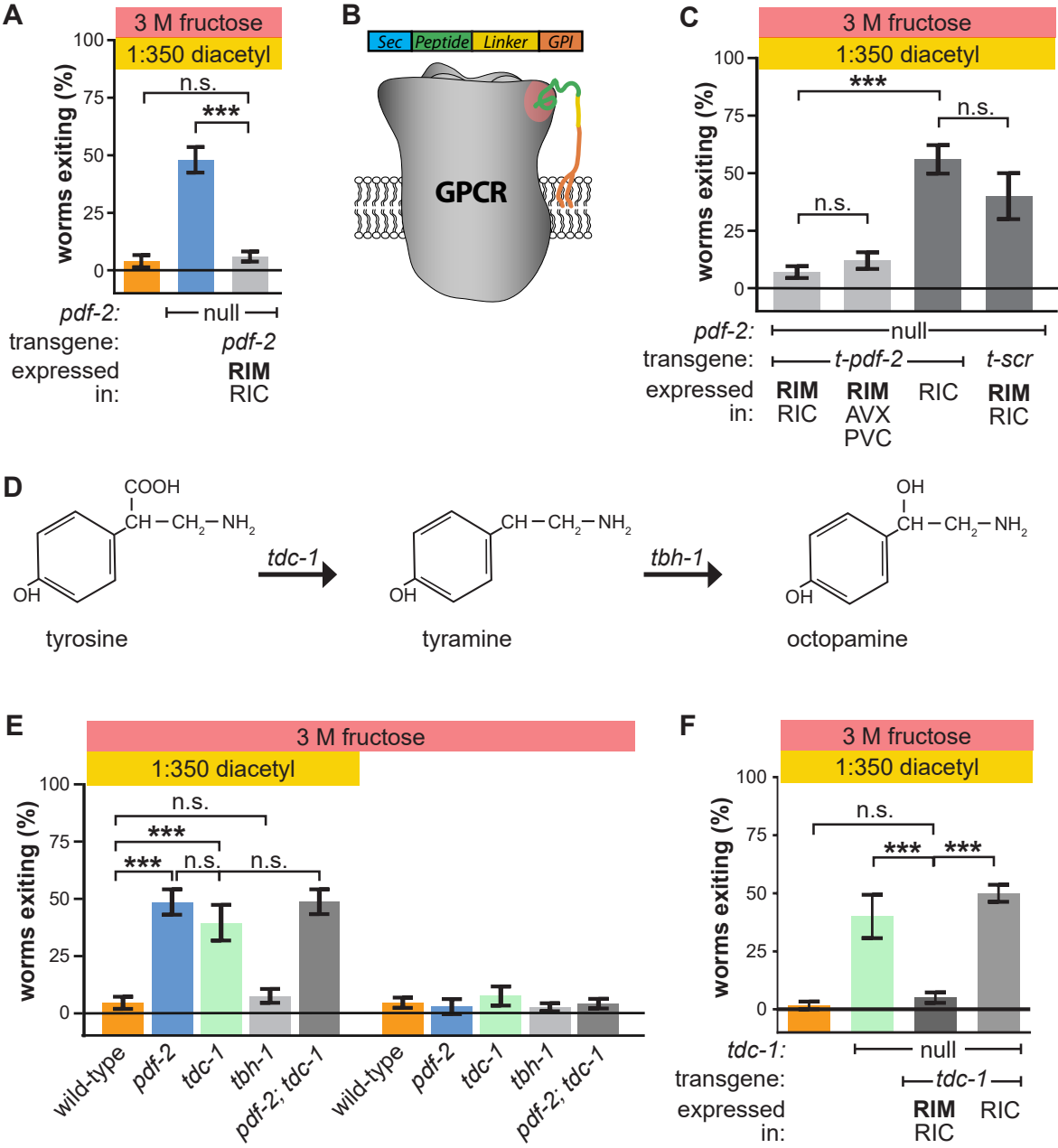


Figure 4

Figure 4

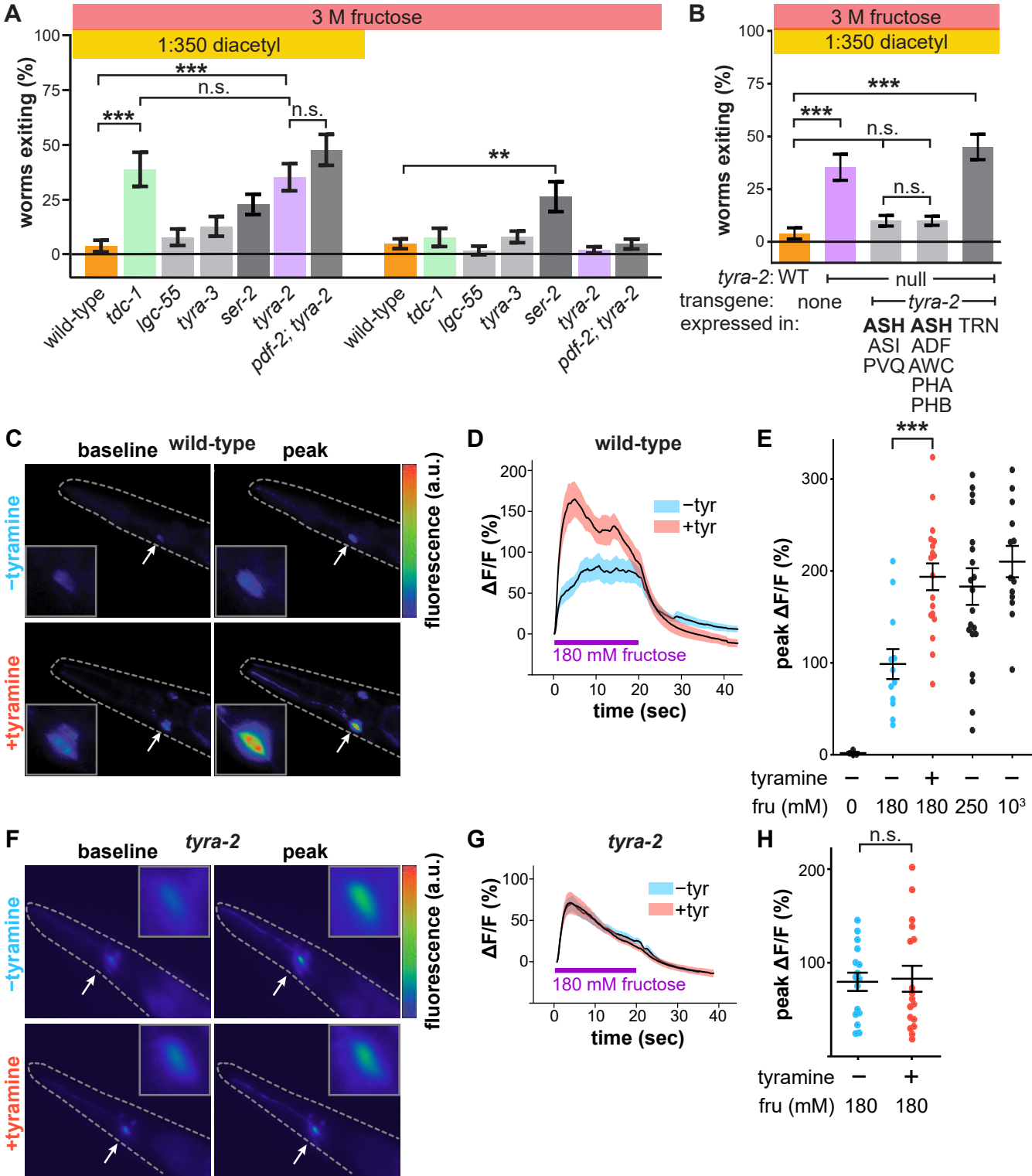


Figure 5

Figure 5

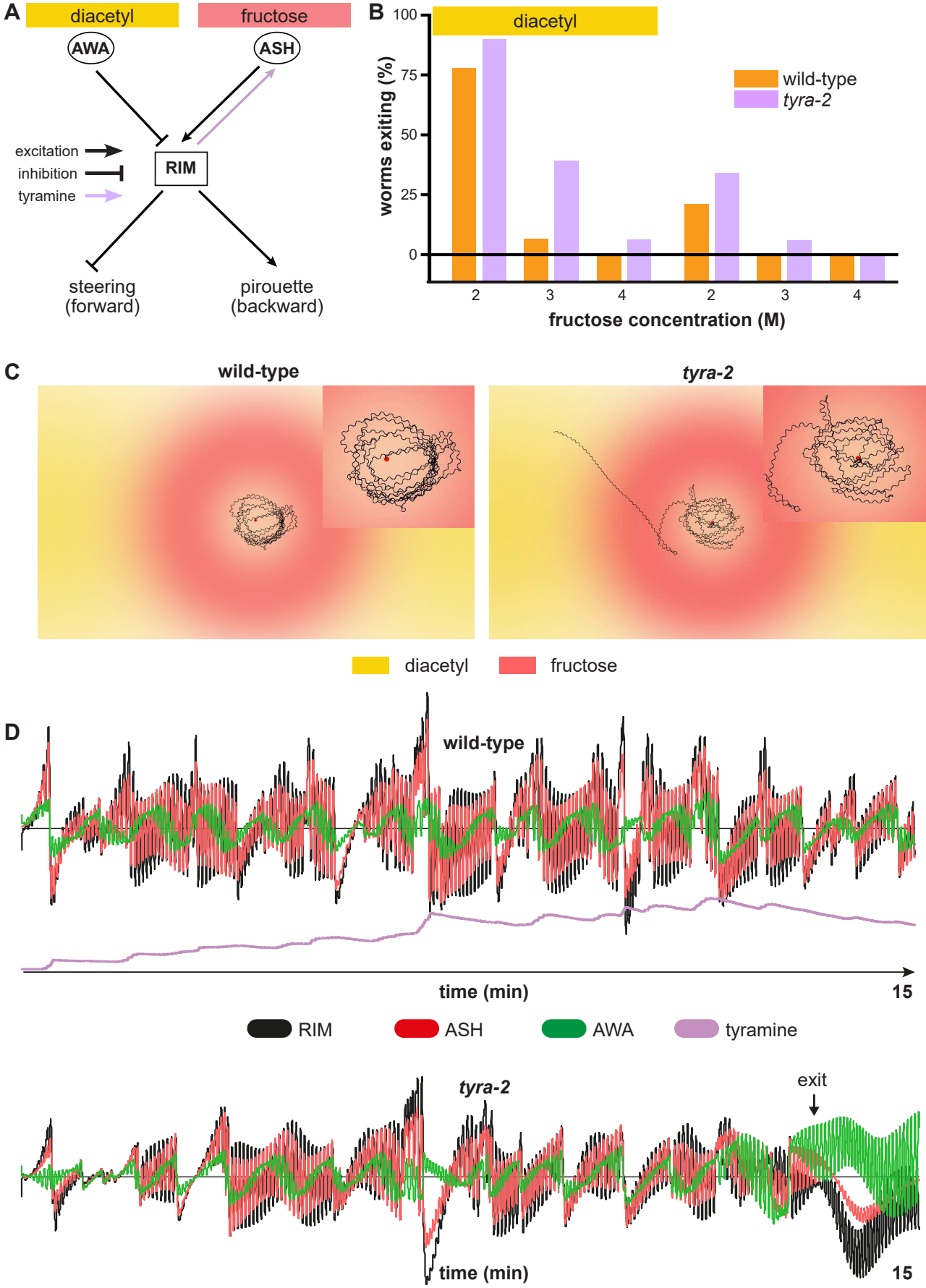


Figure 6

Figure 6

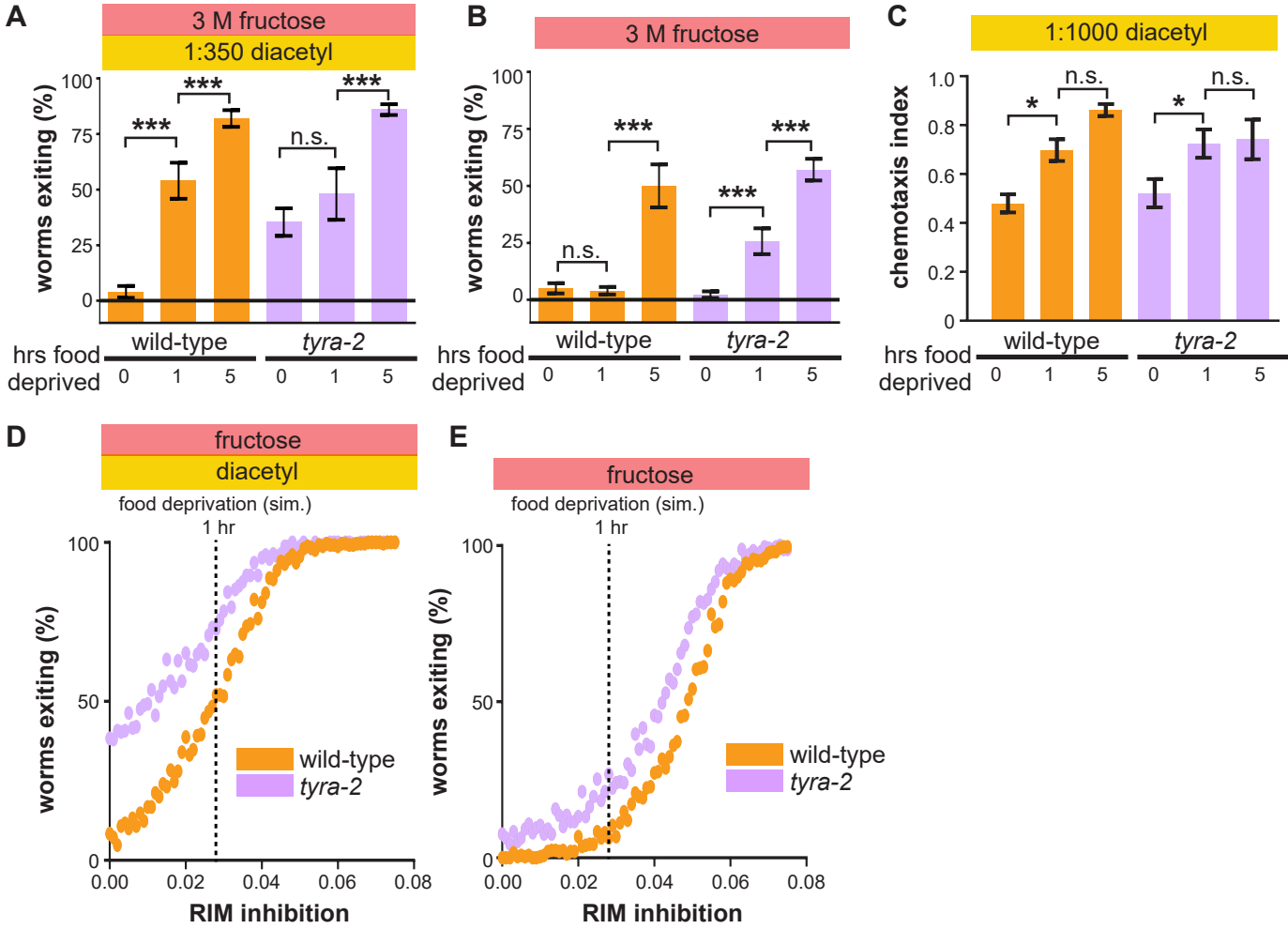


Figure 7

**Figure 7**

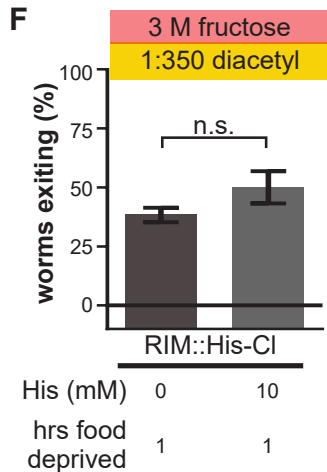
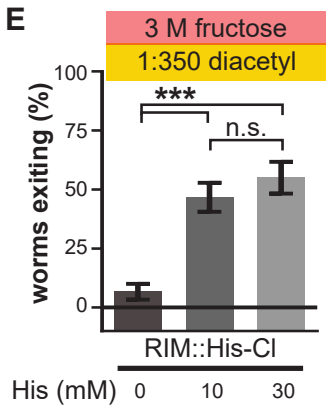
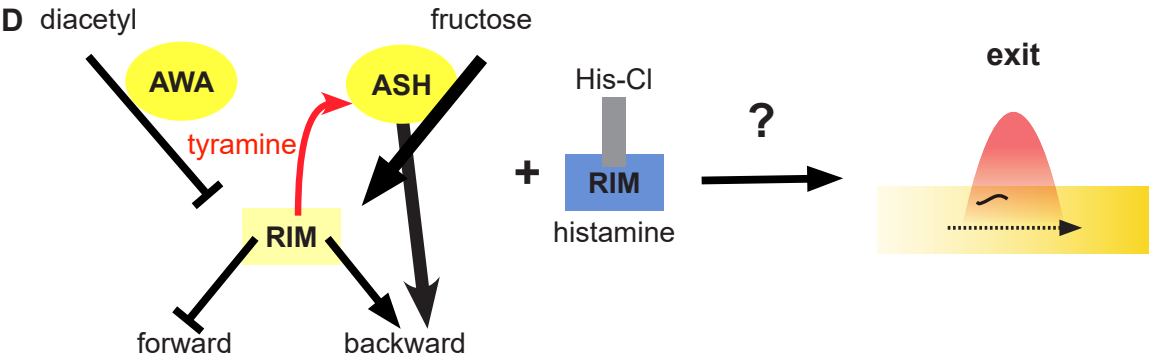
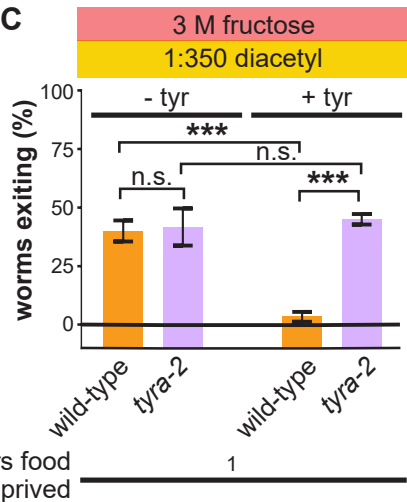
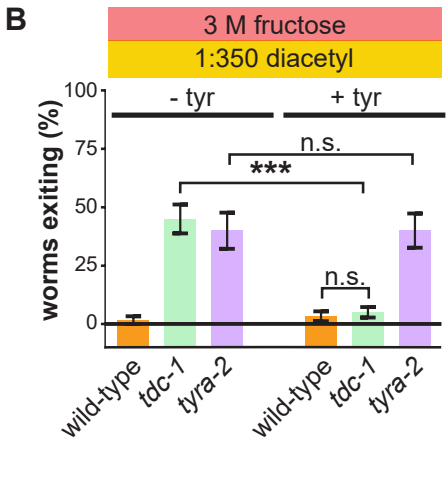
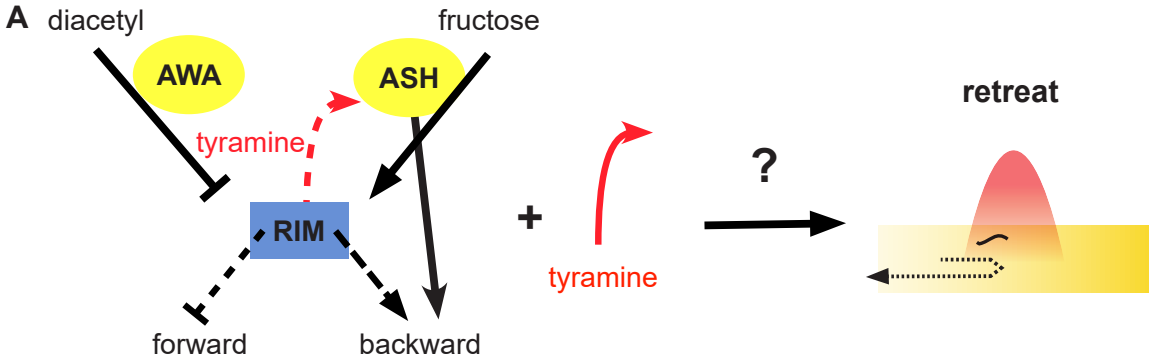


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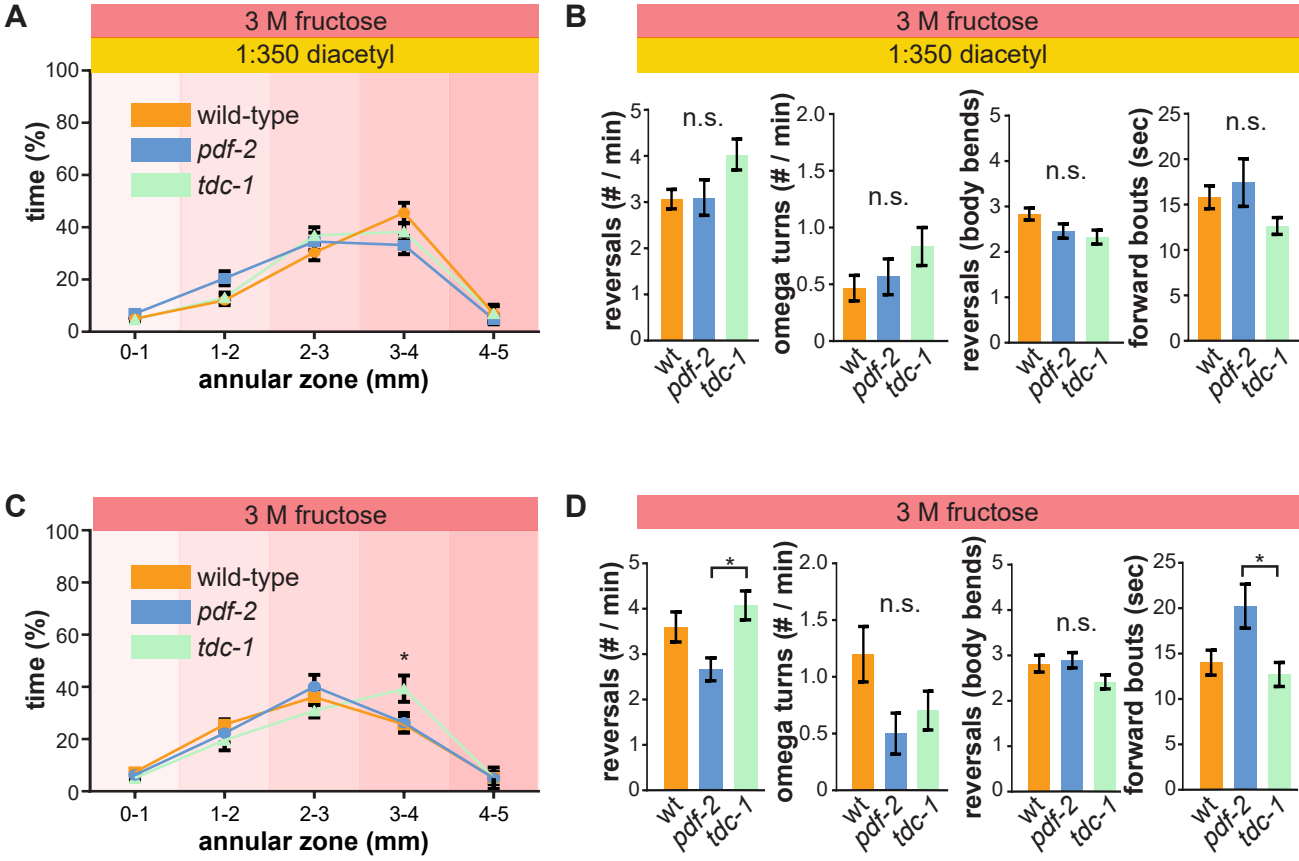


Figure S2

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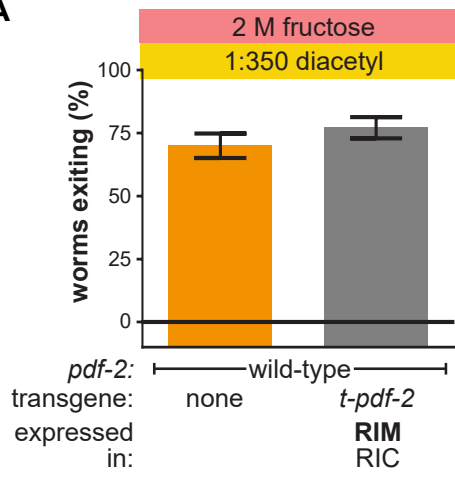
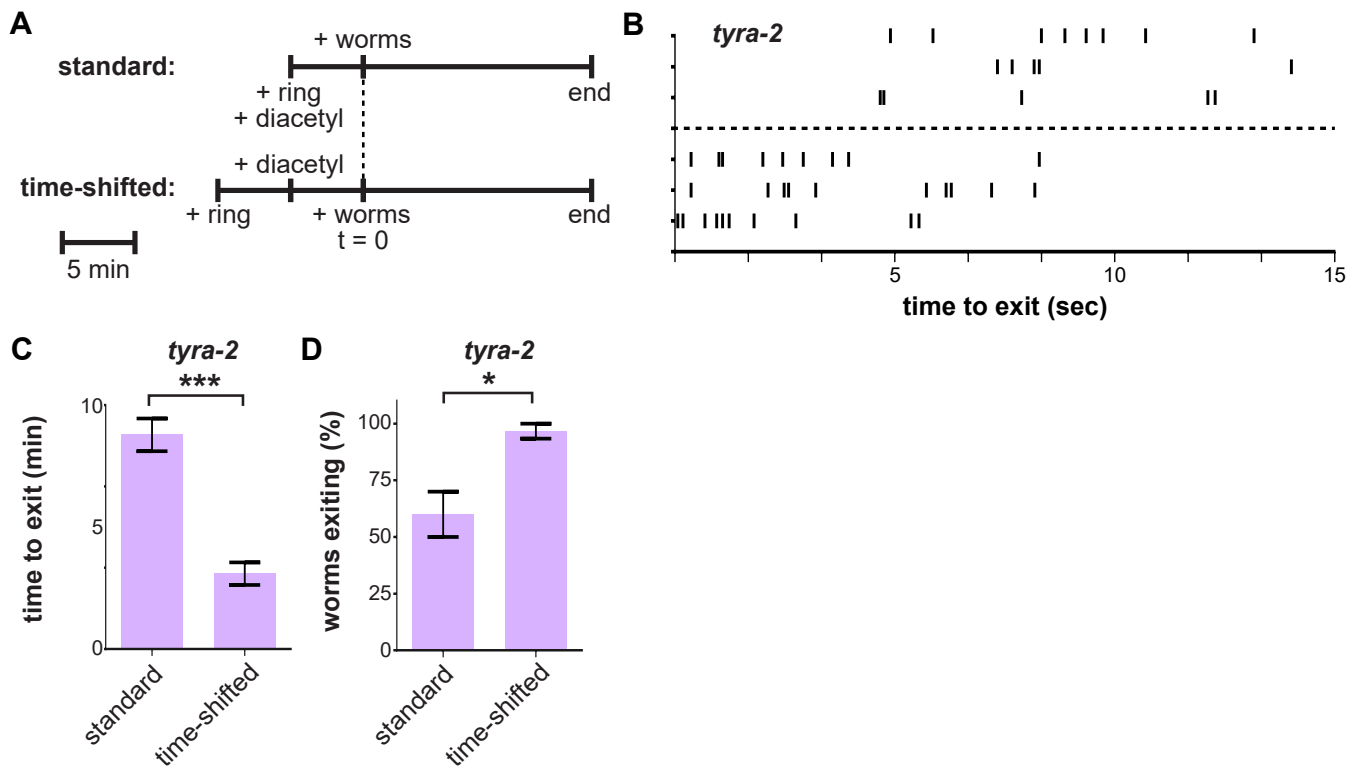
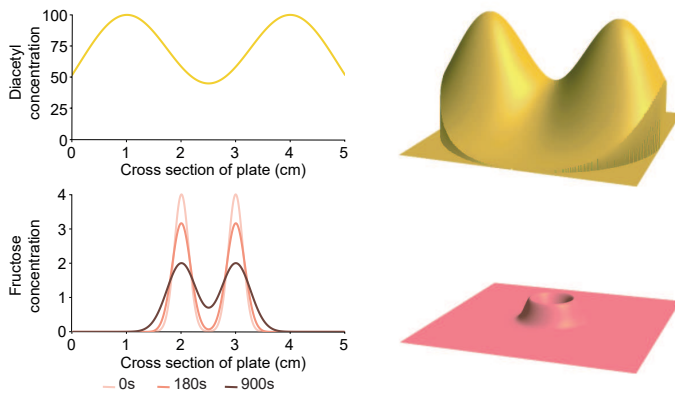


Figure S3

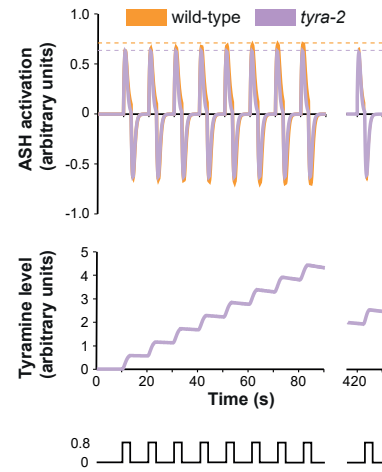


**Figure S4**

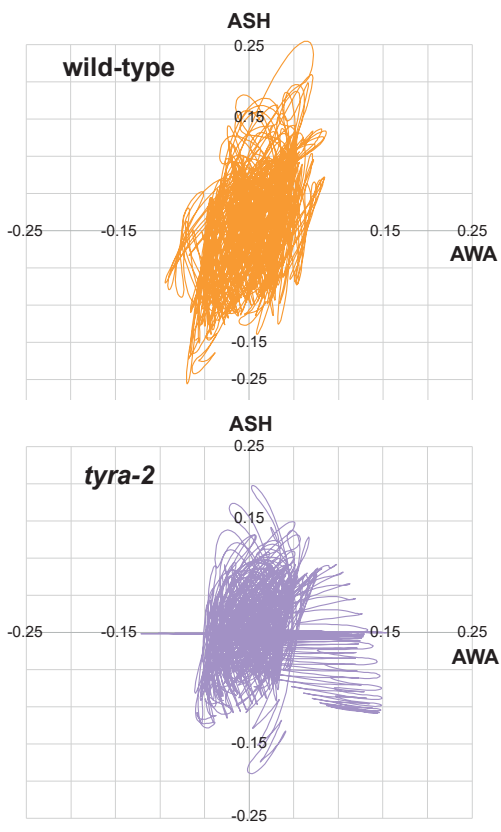
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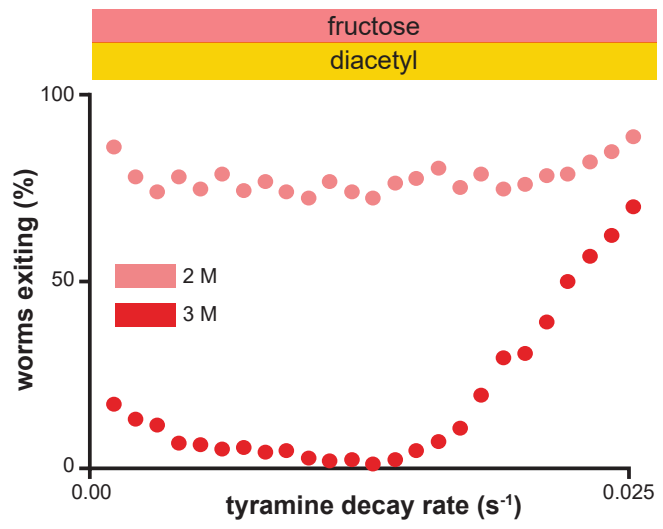
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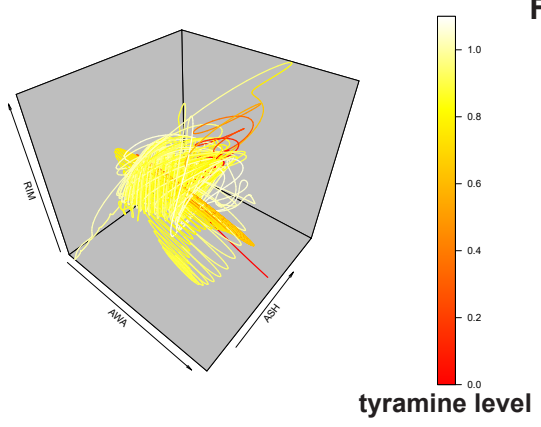
**C**



**D**



**E**



**F**

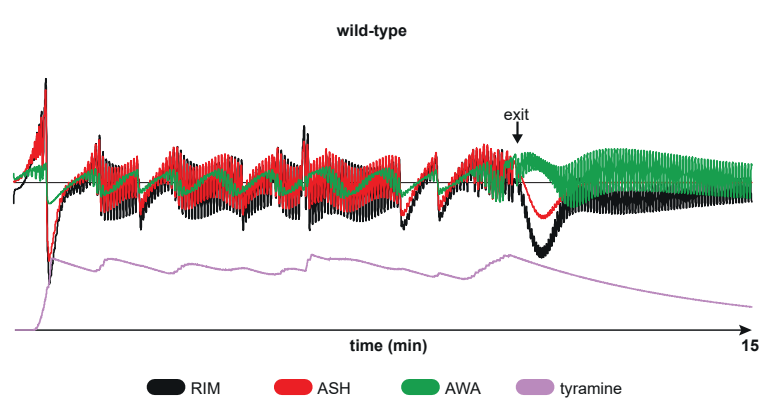


Figure S5

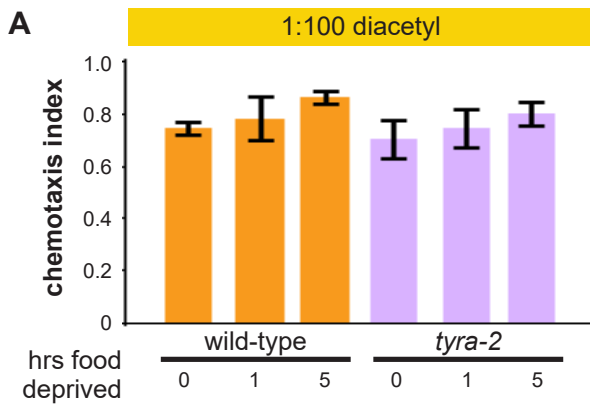
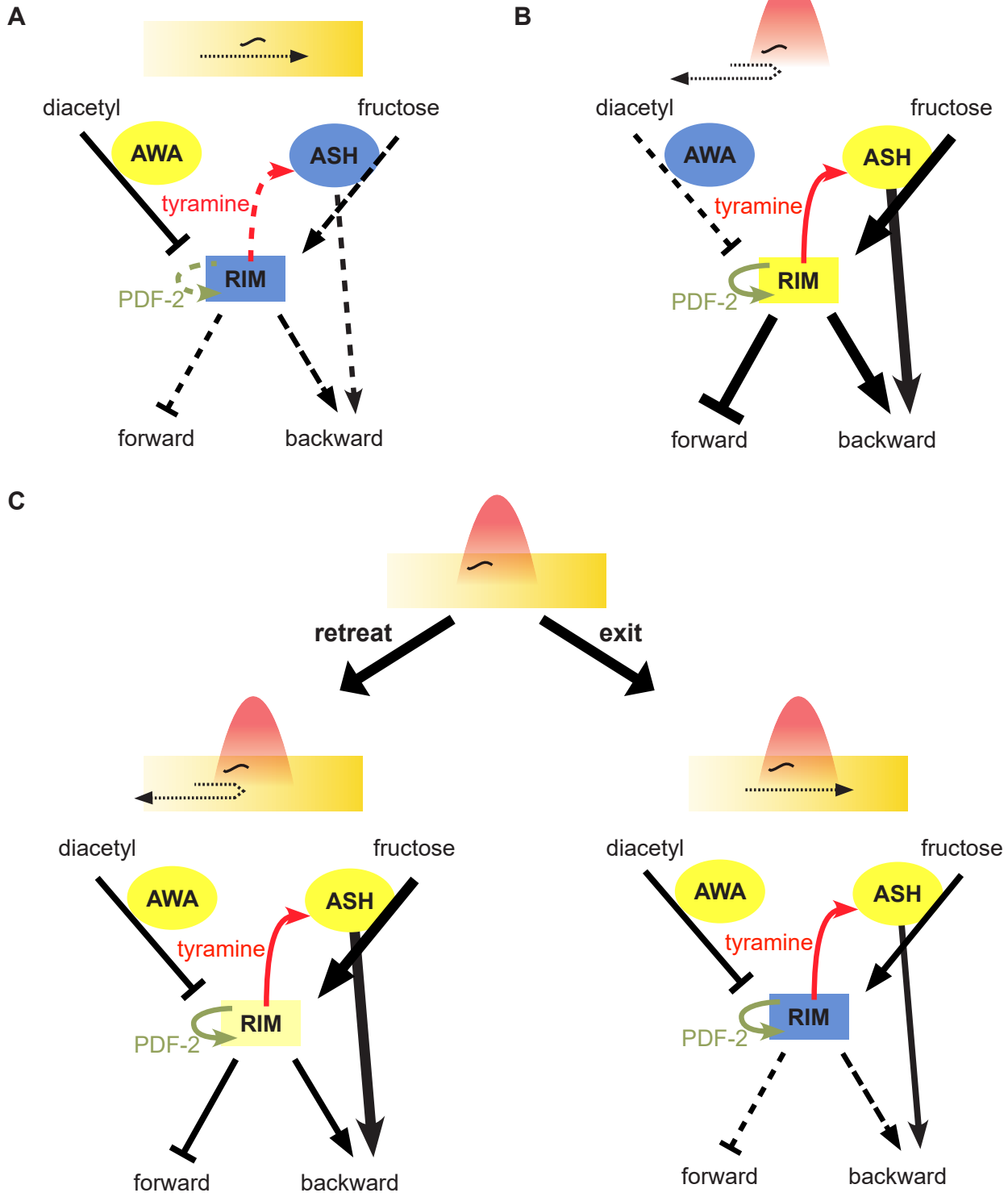


Figure S6



**Figure S1, related to Figures 2,3. Kinematic analysis of *pdf-2* and *tdc-1* null mutants**

(A) Wild-type, *pdf-2*, and *tdc-1* null-mutant worms spend indistinguishable fractions of time in each of the radial zones of the osmotic ring in the presence of food odor. This excludes the potential confound that wild-type and mutant worms spend different amounts of time near the barrier, and thus have an altered opportunity to interact with it. ( $n=7-10$  worms for each genotype,  $F = 1.273$ ,  $p = 0.265$ )

(B) General defects in locomotion are not involved in aberrant multisensory decision making by *pdf-2* and *tdc-1* null-mutant worms. Kinematic parameters are indistinguishable between wild-type, *pdf-2* and *tdc-1* null-mutant worms as they navigate within the ring in the multisensory context.

(C) Wild-type and *pdf-2*, but not *tdc-1* null-mutant worms, spend indistinguishable fractions of time in each of the radial zones of the fructose ring in the absence of food odor ( $N=7-10$  worms for each genotype,  $F = 2.510$ ,  $p = 0.015$ ). *tdc-1* null-mutant worms spend increased time in the 3-4 mm radial zone (\*,  $p$  vs wild-type = 0.009).

(D) *pdf-2* and *tdc-1* null-mutant worm locomotor kinematics differ from one another as they navigate within the ring in the absence of food odor.

**Figure S2, related to Figure 3. PDF-2-PDFR-1 autocrine loop is maximally active**

(A) Expression of t-PDF-2 in RIM and RIC in a wild-type background did not suppress exiting of a 2 M fructose ring in the presence of diacetyl.

**Figure S3, related to Figure 4. Diffusion of osmotic ring determines time to exit**

(A) Schematic depicting protocols for determining effect of diffusion of osmotic ring on time to exit. Worms were either confronted with standard or time-shifted osmotic rings. Time-shifted rings are allowed five more minutes to diffuse than standard rings.

(B) Ethogram of time-to-exit reveals that *tyra-2* null-mutant worms exit time-shifted rings earlier than standard rings.

(C) Average time-to-exit of *tyra-2* null-mutant worms. Worms exit time-shifted rings approximately five minutes before standard rings.

(D) More worms exit time-shifted rings than standard rings in the 15 minute trial.

**Figure S4, related to Figure 5. Time constants of accumulation and decay of tyramine signals determine multisensory decision balance**

(A) Profiles of diacetyl and fructose concentration in simulated decision arena. While the bilobed diacetyl gradient is modeled as invariant over the time course of the decision, the simulated fructose gradient becomes shallower over time.

(B) Model ASH responses to repetitive fructose stimulation. In wild-type worms, the ASH response increases with every consecutive stimulus presentation due to increasing tyramine levels. This effect is not observed in *tyra-2* null-mutants. That tyramine potentiates ASH osmosensitivity by acting on TYRA-2 is somewhat surprising given that TYRA-2 has been characterized as a  $G_{i/o}$ -coupled receptor (Rex et al., 2005). However, invertebrate tyramine receptors have demonstrated the capacity to signal through  $G_{i/o}$  while still increasing intracellular calcium (Rex et al., 2005; Rex and Komuniecki, 2002; Rex et al., 2004; Wragg et al., 2007).

(C) 2D phase space profile of ASH (y-axis) and AWA (x-axis) neuronal activity in a virtual wild-type worm (top) that remains inside the ring for the duration of the assay and a *tyra-2* null-mutant worm (bottom) that exits the ring. These phase space profiles demonstrate how the magnitude of ASH oscillatory activity is greater in wild-type

worms than in *tyra-2* null-mutant worms; in contrast, in *tyra-2* null-mutant worms, the AWA signals are larger, before dominating as the worm exits.

**(D)** Slow tyramine signals (decay constants of  $0.001\text{s}^{-1}$  to  $0.025\text{s}^{-1}$ ) in the model are necessary for wild-type worms to remain confined in the 3 M fructose ring, as observed experimentally. As tyramine decay becomes faster, wild-type worms begin exiting in substantial numbers.  $n=250$  simulated worms per genotype and condition.

**(E)** 3D phase space profile of neuronal activity and tyramine level in a virtual wild-type worm that exits a 3 M fructose ring in the presence of diacetyl, with RIM inhibited to  $\sim 0.03$  to simulate one-hour of food deprivation. This analysis (shown dynamically in **Supplemental Movie 1**) demonstrates several distinct phases in neuronal activity over the course of the decision. Progression through the phases depends on accumulation of tyramine signals that reinforce ASH activity upon repeated encounters with the ring. Initial oscillations are primarily in the ASH-RIM plane (red) as the animal nears and retreats from the fructose ring, leading to a slow accumulation of tyramine (white). The gradual diffusion of the fructose ring eventually results in the decay of tyramine levels and activity in RIM and ASH responses (yellow, then orange). Phase space oscillations get smaller, and tilt toward the AWA-RIM plane, indicating a desensitization of ASH and potentiation of AWA as the worm continues to approach the ring. Exiting of the ring follows a dip in tyramine levels after nine minutes (yellow-orange). AWA activity begins to dominate, despite the subsequent rapid increase in tyramine and strong RIM-ASH oscillations as the animal exits the ring (white). As the worm crosses the peak of the fructose ring, it encounters the steepest diacetyl gradient, as indicated by the significant increase in AWA activity. Once outside the ring, AWA then decreases again as the worm navigates towards the diacetyl spot. Once the animal reaches the diacetyl spot, both ASH and AWA activity levels decay and ASH activity is nearly abolished (red).

**(F)** Neural activity and tyramine signal profiles of AWA, ASH, and RIM in a virtual wild-type worm that exits a 3 M fructose ring in the presence of diacetyl, with RIM inhibited to  $\sim 0.03$  to simulate one-hour of food deprivation, corresponding to phase space profile depicted in **(D)**.

#### **Figure S5, related to Figure 6. Chemotaxis to 1:100 diacetyl after food deprivation**

**(A)** Chemotaxis to 1:100 diacetyl is unaffected by food deprivation.

#### **Figure S6, related to Figure 7. Model for sensorimotor network control of multisensory threat-reward decision making**

**(A)** In the unisensory attraction-only context, AWA inhibits RIM and RIM neither inhibits forward command nor excites backward command interneurons. Thus, the worm proceeds forward up the diacetyl odor gradient. Yellow represents strong activity, while blue represents weak activity. Thickness of solid lines represents strength of signals, and dashed lines represent inactive signals. Other synaptic routes by which AWA and ASH indirectly communicate with forward and backward command locomotor interneurons are not shown.

**(B)** In the unisensory aversion-only context, ASH activates RIM, triggering tyramineric RIM-ASH positive feedback and further potentiating ASH osmosensitivity. This combined strong ASH and RIM activation strongly inhibits forward command and strongly excites backward command interneurons. Thus, the worm reverses and retreats from the osmotic barrier. However, because of the absence of AWA-mediated inhibition of RIM in the unisensory aversion-only context, RIM-ASH feedback is not required for retreat from the osmotic barrier.

**(C)** In the multisensory context where RIM is both inhibited by AWA and activated by ASH, the worm makes a probabilistic choice between retreat and exit based on the instantaneous stochastic state of the network as it interacts with the osmotic barrier. An increase in RIM activity state leads to reversal behaviors that promote retreat from threats; however, these reversal behaviors are probabilistic because instantaneous activity state of RIM itself is stochastic (Gordus et al., 2015). When instantaneous network activity is strong, combined RIM and ASH activity inhibits forward command and activates backward command interneurons to trigger a reversal and retreat from the osmotic barrier. When instantaneous network activity is weak, combined RIM and ASH activity is insufficient to inhibit forward command and excite backward command interneurons, and the worm proceeds forward through the barrier to exit the ring. Autocrine PDF-2 signaling to RIM increases the gain of the RIM-ASH positive feedback

loop, and is suppressed by food deprivation to increase threat tolerance. Longer time-scale tyraminerpic feedback increases the likelihood that instantaneous RIM and ASH network activity is strong. Disruptions that break the positive feedback loop increase threat tolerance in the multisensory context by allowing AWA-mediated inhibition of RIM to dominate and prevent reversals.

**Supplemental Movie, related to Figures 5,6 (Online-only)**

(A) Phase space profile of neuronal activity and tyramine levels in a virtual wild-type worm that exits a 3 M fructose ring in the presence of diacetyl, with RIM inhibited to  $\sim 0.03$  to simulate one-hour of food deprivation. Left: 10 second sliding window of AWA, ASH, and RIM activity levels, plotted in phase space. Color represents tyramine level. Top right: Corresponding time traces of AWA and ASH. Bottom right: simulated worm trajectory over the course of the decision. Note that the concentration gradient of fructose changes over time.

## Supplemental Experimental Procedures

### Strains

*C. elegans* strains were maintained on Nematode Growth Medium (NGM) agar plates with *E. coli* OP50 as a food source. All strains were derived from the Bristol N2 wild-type strain. Each strain was backcrossed to the wild-type N2 at least 3 times. At least three independent lines for all transgenic strains were generated and tested, and presented results are aggregates of the independent lines. Strains used for each figure are detailed below:

Figure 1: N2, *odr-10* (*ky225*)

Figure 2: N2, CX14484 *pdf-2(tm4393)*, MNN1 *pdf-2(tm4393); lin-15(n765ts); mnnEx1 [pdf-2p::pdf-2; lin-15<sup>+</sup>]*

Figure 3: N2, CX14484 *pdf-2(tm4393)*, MNN2 *pdf-2(tm4393); lin-15(n765ts); mnnEx2 [tdc-1p::pdf-2; lin-15<sup>+</sup>]*, MNN3 *pdf-2(tm4393); lin-15(n765ts); mnnEx3 [tdc-1p::t-pdf-2; lin-15<sup>+</sup>]*, MNN4 *pdf-2(tm4393); lin-15(n765ts); mnnEx4 [nmr-2p::t-pdf-2; lin-15<sup>+</sup>]*, MNN5 *pdf-2(tm4393); lin-15(n765ts); mnnEx5 [tbh-1p::t-pdf-2; lin-15<sup>+</sup>]*, MNN6 *pdf-2(tm4393); lin-15 (n765ts); mnnEx6 [tdc-1p::t-scr; lin-15<sup>+</sup>]*, MT13113 *tdc-1(n3419)*, MT9455 *tbh-1(n3247)*, MNN10 *pdf-2 (tm4393); tdc-1 (n3419)*

Figure 4: N2, MT13113 *tdc-1(n3419)*, QW245 *lgc-55(tm2913)*, VC125 *tyra-3(ok325)*, OH313 *ser-2 (pk1357)*, QW42 *tyra-2(tm1815)*, MNN7 *tyra-2(tm1815); mnnEx7 [sra-6p::tyra-2; myo-3p::gfp]*, MNN8 *tyra-2(tm1815); mnnEx8 [gpa-13p::tyra-2; myo-3p::gfp]*, MNN9 *tyra-2(tm1815); mnnEx9 [mec-17p::tyra-2; myo-3p::gfp]*, XP631 *lite-1(ce314); kyEx2865 [sra-6p::GCaMP3, unc-122p::gfp]*, XP649 *lite-1(ce314); tyra-2(tm1846); kyEx2865 [sra-6p::GCaMP3, unc-122p::GFP]*, MNN11 *pdf-2 (tm4393); tyra-2(tm1815)*

Figure 6: N2, QW42 *tyra-2(tm1815)*

Figure 7: N2, MT13113 *tdc-1(n3419)*, QW42 *tyra-2(tm1815)*, CX16632 *kyIs693 [pNP502(tdc-1::HisCl1::sl2::mcherry)]*

Supplemental Figure 1: N2, CX14484 *pdf-2(tm4393)*, MT13113 *tdc-1(n3419)*

Supplemental Figure 2: N2, MNN12 N2; *mnnEx9 [tdc-1p::t-pdf-2; myo-3p::gfp]*

Supplemental Figure 3: N2, QW42 *tyra-2(tm1815)*

Supplemental Figure 5: N2, QW42 *tyra-2(tm1815)*

Tethered peptides were designed as described previously (Choi et al., 2012; Choi et al., 2009; Choi and Nitabach, 2013; Fortin et al., 2009). Briefly, tethered peptides are chimeric fusion proteins with an N-terminal secretory signal sequence, followed by the sequence of the peptide of interest, a linker, and a C-terminal glycolipid anchor targeting signal. Tethered peptide transgenes were synthesized by Entelechon. Transgenes were synthesized with optimal *C. elegans* codon usage and optimal upstream Kozak sequences.

Amino acid sequences for construction of tethered peptides are as follows (peptide is **bold**, \* indicates stop codon):

t-PDF-2:

MSALLLILALVGA AVANNAEVVN**HILKNF**GALDRLG**DV**GNEQKLISEEDLGNGAGFATPVTLALVPALLA  
TFWSLL\*

t-SCR:

MSALLLILALVGA AVAVIALDN**KRNLNF**ENGAV**GVH**LDGNEQKLISEEDLGNGAGFATPVTLALVPALLA  
TFWSLL\*

Transgenic strains were constructed by injection of plasmid DNA into the germline according to standard practice. Plasmid DNA was engineered using Multisite Gateway Cloning technology (Invitrogen, <http://www.lifetechnologies.com/us/en/home.html>). *pdf-2*, *t-pdf-2*, *t-scr*, and *tyra-2* protein coding sequences were cloned into pDONR-221 vectors. These were linked with cell-specific promoters *pdf-2*, *tdc-1*, *nmr-2*, *tbh-1*, *sra-6*, *gpa-13*, and *mec-17*. Promoters were generated by amplifying genomic DNA using Promoterome sequences (<http://wormfdb.dfc.harvard.edu/promoteromedb/>) or published primer sequences and cloning them into pDONR-P4-P1R vectors. These were then integrated into final vectors, and injected at concentrations of 20-50 ng/ $\mu$ L. A *lin-15* rescue construct pL15EK or *myo-3::GFP* construct was co-injected to mark transformation.

### *Behavioral assays*

For unisensory and multisensory assays, the fructose ring was constructed essentially as described (Culotti and Russell, 1978). Briefly, 10 $\mu$ L of fructose solution is applied in a 1cm diameter ring centered on a standard NGM plate. For multisensory assays, 1 $\mu$ L diluted diacetyl (1:350 in water) was applied to each side of the plate, at least 1cm from the ring border. Four minutes after construction of the ring, ten (unless otherwise indicated) 18-24 hours post-L4 stage adult worms were removed from a food plate, placed on a plate without food to remove attached bacteria, and then quickly transferred inside the ring. Fifteen minutes later, the number of worms outside the ring were counted. For the food deprivation assays, worms were transferred to a plate without food to remove attached bacteria, then quickly transferred to another plate without food for the indicated time interval. 10 mM or 30 mM histamine and 30 mM tyramine plates were prepared exactly as described previously (Jin et al., 2016; Pokala et al., 2014).

Chemotaxis assays were performed essentially as described, with minor changes from (Sengupta et al., 1996), as described below. First, a standard sized (5 cm) NGM plate was used. Second, similar to the multisensory assay, ten staged adult worms were placed at a location equidistant to the two spots, control and test, applied to either side of the plate. To remain consistent with the multisensory assay, chemotaxis was measured after 15 minutes. One-way ANOVA with Tukey-Kramer *post-hoc* test was used for statistical comparison with wild-type worms.

For kinematic analysis, assays prepared as detailed above were recorded using a Point Grey Grasshopper camera (GRAS-50S5C-C) filming at 7.5 fps. Dishes were illuminated with a red LED ring obtained from Moritex Schott (Model RLA-75X46-00R). These videos were then analyzed using commercially available WormLab software from MBF Bioscience (<http://www.mbfbioscience.com/wormlab>). Software provided *x-y* position information. A picture of the ring, taken immediately after the assay, was used to determine the center of the ring. Distance from the center of the ring was then calculated and sorted into annular zones. Two-way ANOVA with a *post-hoc* Tukey-Kramer test was used to determine statistically significant differences from wild-type. Other locomotor parameters were manually scored as previously described (Alkema et al., 2005; Donnelly et al., 2013; Gray et al., 2005; Pirri et al., 2009).

### *Imaging*

Calcium imaging of ASH neurons was performed on young adult *lite-1 (ce314)* worms immobilized in an olfactory chamber as described previously (Chronis et al., 2007). Imaged worms expressed the genetically-encoded calcium sensor GCaMP3 from the *sra-6* promoter (Tian et al., 2009). Prior to loading into the olfactory chamber worms were bathed in neuronal buffer (10mM HEPES pH 7.1, 40mM NaCl, 1mM MgSO<sub>4</sub>, 1mM tetramisole) for 10 min either in the presence or absence of 50mM tyramine. Once in the chamber worms were exposed to a laminar flow of neuronal buffer (with or without tyramine) for at least 10 sec before switching flow in the chamber to direct the test stimulus across the animal's nose (diluted in neuronal buffer containing 1mg/ml bromophenol blue). Bromophenol blue was added to monitor test stimulus flow and had no effect on ASH activity. Worms were exposed to the test stimulus for 20 sec. All imaging experiments were performed using a Nikon FN1 microscope equipped with a 40x water immersion objective. Images were captured at ~10Hz using a Photometrics CoolSnap EZ camera with a single frame exposure time of 100ms. Averaged fluorescence was calculated for a region of interest (ROI) surrounding the ASH soma and was background corrected using NIS Elements AR software. The percent change in fluorescence for each frame was calculated relative to the corrected fluorescence of the ROI just prior to stimulus onset.

### Computational model

Model animals possess a simplified nervous system comprising sensory neurons AWA and ASH, interneuron RIM, and two reciprocally inhibited motor neurons denoted DMN and VMN, which activate dorsal and ventral body bends, respectively. Neurons are modeled as “leaky integrators,” such that each neuron integrates its input over time, while subject to continuous decay (“leak”) of activity. AWA and ASH sensory neurons respond with transient activation to changes in diacetyl or fructose stimuli, respectively. These transients were modeled with a fast depolarizing signal, which drives a delayed rectifying counter-signal. This sensory response was adapted from chemotaxis models in unicellular eukaryotes, in which the delayed signal modeled intracellular diffusion (Cohen and Sanders, 2014; Levine and Rappel, 2013). Neuronal time scales were set in accordance with calcium imaging data (Kato et al., 2014), and were fast enough to mediate steering responses on time scales of an undulation. Activation of model sensory neurons AWA and ASH instantaneously provides differentially weighted inhibitory and excitatory inputs onto RIM, respectively. RIM integrates these sensory inputs and inhibits motorneurons DMN and VMN to bias dorsal versus ventral bends, thereby inducing gradual steering of the worm. Additionally, RIM activity positively increases the likelihood of pirouettes, which are modeled as instantaneous step changes in angular heading. In the absence of sensory input, the model worm’s dorsoventral undulatory locomotion is driven by stable oscillations of a simplified central pattern generating circuit consisting of DMN and VMN. The animals move forward along an undulating path at a fixed speed of 0.11 mm/s, interrupted by occasional stochastic turning events (average pirouette rate of 2.1 turns/min). Sensory input allows both steering (klinotaxis), by biasing the oscillator, and a “tumble-and-run” strategy (klinokinesis), by varying the pirouette probability. Importantly, in addition to the above previously established feedforward sensorimotor pathways, the simulation includes RIM-ASH tyramineric positive feedback. Tyramineric potentiation of ASH in the model is only engaged above a threshold level of RIM activation (see **Equation M3**). This non-linear relationship ensures that the RIM-ASH positive feedback loop is activated only in response to sufficiently aversive stimuli, a feature of the model that is necessary to successfully reproduce experimental exiting rates. The TYRA-2 receptor in ASH promotes ASH sensitivity to osmotic stimuli in the presence of tyramine, but with no direct effect on the ASH membrane potential. *tyra-2* null-mutant worms are modeled as lacking this tyramineric feedback to ASH. Food deprivation was modeled as direct inhibition of RIM activity.

Model parameters were manually calibrated until exiting rates of simulated wild-type and *tyra-2* null-mutant worms matched experimental exiting rates in multisensory and unisensory contexts for 2 M, 3 M, and 4 M fructose (compare **Figure 2B** with **Figure 5B**). Specifically, model parameters subject to calibration comprise: the strengths of the AWA-RIM and ASH-RIM synapses, the tyramine accumulation rate, the tyramine decay rate, and the threshold activity value above which tyramine is secreted from RIM. Of these, the first two were constrained by the experimental exiting rates of *tyra-2* null-mutant worms in the multisensory and unisensory contexts; the latter three were then calibrated to match the experimental exiting rates of wild-type worms in those same contexts.

The virtual decision arena comprises a continuously diffusing fructose gradient ring and time-invariant diacetyl gradients originating from two spots outside the ring (**Figure S4A**). Modeling the fructose gradient as dynamically changing was essential, as continued diffusion of the fructose ring influences the time and probability of exit in our experiments (**Figure S3**), and static fructose gradients failed to reproduce experimental exiting rates in the model. Each simulation begins with a single worm in the center of the virtual arena with a randomly selected initial heading, and the simulation is allowed to proceed for fifteen virtual minutes.

See accompanying **Supplemental Experimental Procedures** section for more details.

### **Supplemental References**

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## Supplemental Experimental Procedures

### Computational model

A computational simulation framework was developed to study *in silico* the behavior of single model worms confronted with the decision making assay. Our model of the animal assumes that during locomotion, the body follows the head, allowing us to focus on sensory-motor control of a point worm. Thus, at each point in time, animals are represented by their point coordinates  $\vec{r}(t) = (x(t), y(t))$  and heading  $\theta(t)$ . Animals move at fixed speed and sense their environment using a simplified nervous system which dynamically controls their direction of locomotion.

Our computational model extends a previously developed *C. elegans* sensorimotor model that was extensively tested against a number of assays (Sanders T., PhD thesis 2016). To study sensory integration, our new model circuit contained two sensory neurons (AWA and ASH) and one interneuron (RIM) as well as a simplified motor system (Fig. M1). The motor output consists of undulations that are modulated by the sensory system to generate steering, and instant turning events representing pirouettes. All neuronal equations and parameters, including the sensory responses, interneuron and motor neuron dynamics were taken without modification from our previous model, in which they were constrained by calcium imaging data (Thiele et al., 2009; Larsch et al., 2013) and published behavioral data (Iino and Yoshida, 2009; Jansen et al., 2002; Hukema et al., 2006, 2008).

To extend the model to cope with sensory integration, we began by modeling the assay environment and specifically the fructose and diacetyl concentration profiles over time (Fig. M3). The addition of RIM to our model circuit required synaptic connections from ASH and from AWA onto RIM. The two synaptic weights were set by matching behavioral data from *tyra-2* animals (Fig. 5). Next, RIM's tyramine accumulation threshold and the tyramine accumulation and decay rates were set, based on behavioral results from wild type animals (Fig. 5). Thus, only five of the model parameters were free. One additional variable (the RIM rest potential) was needed to capture food deprivation results; the corresponding results are presented as a parameter sweep. This incremental modeling approach ensures the model is maximally constrained and is aimed at enhancing its predictive potential.

#### 1.1 Model neurons

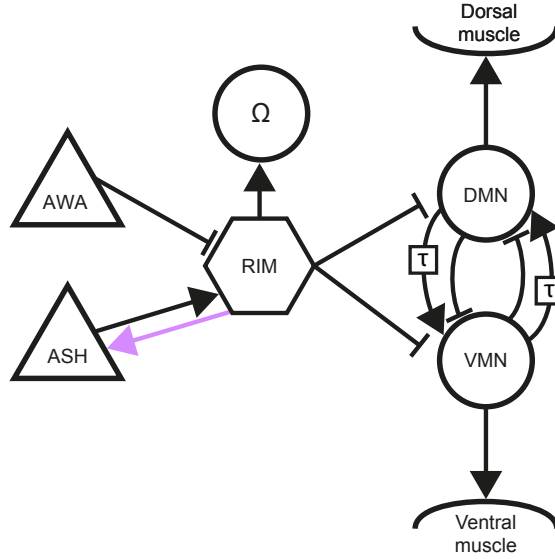
As the AWA, ASH and RIM Left/Right neuronal pairs are all coupled by gap junctions (White et al., 1986) and ASH Left and Right neurons are known to respond identically (Thiele et al., 2009), all three neuron pairs have been collapsed into single model neurons. We model all neurons as leaky integrators (with arbitrary units)

$$\tau_m \frac{dV_i}{dt} = -V_i + V_{0,i} + \sigma(I), \quad (\text{M1})$$

where  $V_i$  is a voltage like variable for neuron  $i$ , also referred to here as neuronal activity;  $\tau_m$  is a neuronal time constant; finally,  $V_{0,i}$  represents the resting potential (set at 0).  $\sigma(I)$  is a modified sigmoid function over the input

$$\sigma(I) = \tanh(bI), \quad (\text{M2})$$

where  $b$  is a gain parameter. Thus a neuron's activation ranges from -1 to 1 and converges to  $V_{0,i} + \sigma(I)$  with a timescale of  $\tau_m$ . This formulation is borrowed from rate neuron models



**Figure M1:** *The model sensorimotor system consists of two sensory neurons, AWA and ASH (triangles), a single interneuron, RIM (hexagon), and a motor circuit (circles). RIM integrates over inputs from the sensory neurons and projects to all three units of the motor circuit. The extrasynaptic neuropeptide tyramine mediates the feedback signal from RIM to ASH. A half-center oscillator (DMN, VMN) acts as a central pattern generator for ongoing undulations that is subject to a steering bias from the sensory inputs. The delay  $\tau$  is modeled as an implicit neuron. The muscles are included in the diagram for ease of visualization only. An abstract turn component  $\Omega$  drives pirouettes.*

and naturally leads to thresholding and saturation of activation, as observed in *C. elegans* sensory neurons (Suzuki et al., 2008; Thiele et al., 2009; Larsch et al., 2013).

The input term  $I$  sums over all synaptic and sensory contributions  $I = I_{\text{syn}}(t) + I_{\text{sens}}(t)$ , where  $I_{\text{syn}} = \sum_j w_{ij} V_j$  is a weighted sum over all presynaptic neuron activations. The sensory contributions  $I_{\text{sens}}$  are given in Equations (M5)–(M7). Note that since synapses are not rectified, hyperpolarizing a neuron will effectively reverse the sign of its synaptic outputs (with hyperpolarizing and depolarizing postsynaptic transmission across excitatory and inhibitory synapses respectively). Food deprivation is modeled as sustained inhibition of RIM, by suppressing the RIM resting potential,  $V_{0,\text{RIM}}$ . The tyramine signal, denoted here  $T(t)$ , is modeled according to

$$\frac{dT}{dt} = \alpha_{\text{tyr}} H(V - V_{\text{tyr}}) - \beta_{\text{tyr}} T, \quad (\text{M3})$$

where the function  $H(x) = x$  for  $x > 0$  and 0 otherwise. In other words, the tyramine level  $T \geq 0$  at all times and increases only when RIM activity is above an activity threshold  $V_{\text{tyr}}$ .

Neuron parameters	Value	Description
$\tau_m$	0.5 s	Neuronal time constant
$V_{0,i}$	0	Resting potential
$b$	2	Activation gain factor (used in sigmoid function)
$\alpha_{\text{TYT}}$	$0.4 \text{ s}^{-1}$	Tyramine integration rate
$\beta_{\text{TYT}}$	$0.004 \text{ s}^{-1}$	Tyramine decay rate
$V_{\text{TYT}}$	0.05	Tyramine accumulation threshold
Synaptic weights	Value	Description
$w_{\text{AWA,RIM}}$	-0.4	AWA onto RIM
$w_{\text{ASH,RIM}}$	0.75	ASH onto RIM
$w_{\text{RIM,DMN}}$	-0.5	RIM onto DMN and VMN
$w_{\text{RIM},\Omega}$	1	RIM onto $\Omega$
$w_{\text{D},\text{V}}^+$	0.88	DMN to VMN excitation (to, from hidden neuron)
$w_{\text{V},\text{D}}^+$	0.88	VMN to DMN excitation (to, from hidden neuron)
$w_{\text{D},\text{V}}^-$	-1.4	DMN to VMN inhibition
$w_{\text{V},\text{D}}^-$	-1.4	VMN to DMN inhibition

### 1.1.1 RIM inhibition

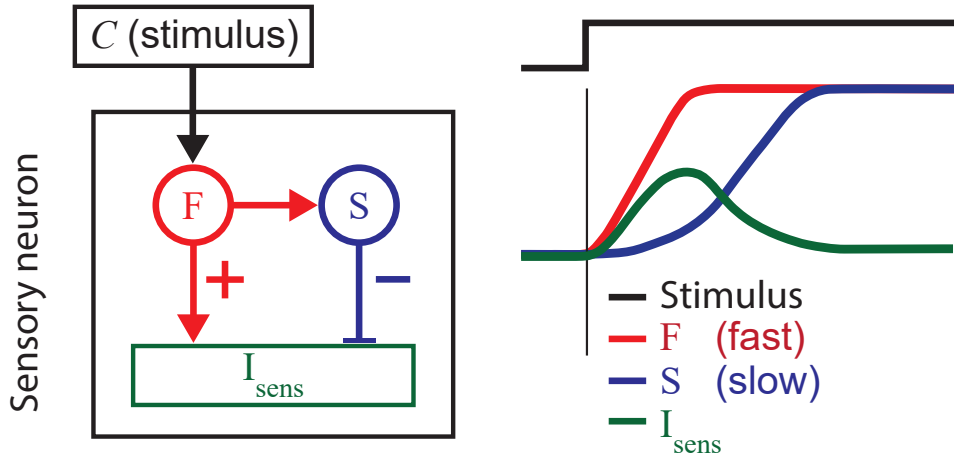
As mentioned above, food deprivation was mimicked in our model using a single pathway: sustained RIM inhibition, implemented as a modulated RIM resting potential  $V_{0,\text{RIM}} \leq 0$ . To parametrize the food deprivation effect on RIM, we fit the exit rate as function of the RIM inhibition by the logit link function. A maximum likelihood best fit was found using the glm function in the R statistical language (R Core Team, 2014). The resulting fit is the inverse-logit (logistic) function:

$$P[y(x)] = \frac{1}{1 + \exp[-y(x)]} = \frac{1}{1 + \exp(-\beta_0 - \beta_1 x)}, \quad (\text{M4})$$

where  $x$  is the RIM inhibition and  $y(x)$  is the exit rate. The parameters for the four fits, which we dub RIM inhibition coefficients, are given in the table below.

RIM inhibition coefficient	Value	Description
$\beta_0$	-3.022	Intercept, diacetyl and fructose, wild type
$\beta_1$	117.006	Coefficient, diacetyl and fructose, wild type
$\beta_0$	-0.988	Intercept, diacetyl and fructose, <i>tyra-2</i> null
$\beta_1$	87.403	Coefficient, diacetyl and fructose, <i>tyra-2</i> null
$\beta_0$	-6.393	Intercept, fructose, wild type
$\beta_1$	135.158	Coefficient, fructose, wild type
$\beta_0$	-3.830	Intercept, fructose, <i>tyra-2</i> null
$\beta_1$	99.109	Coefficient, fructose, <i>tyra-2</i> null

Experimental food deprivation data points were matched visually to RIM inhibition values by matching the percentage of wild type animals exiting the ring in the fructose only assay. If multiple RIM inhibition strengths provided an equal fit, virtual *tyra-2* null mutant data points were used in addition to pick the best matching RIM inhibition value.



**Figure M2:** *Left:* Model sensory neurons exhibit a transient response to changes in concentrations. The response is mediated by a fast component ( $F$ ) that drives a slow delayed-rectifier component ( $S$ ). *Right:* Schematic traces of the response to a concentration step change, showing the fast ( $F$ ) and slow ( $S$ ) components and the overall transient response ( $I_{\text{sens}}$ ).

For the assay with diacetyl and fructose, the estimates from the fructose only assay were used.

### Sensory Neurons

Calcium imaging data has shown that the responses of many sensory neurons in *C. elegans* roughly approximate a low-pass filtered time derivative over their respective inputs (Hilliard et al.; Suzuki et al., 2008; Thiele et al., 2009; Kato et al., 2014; Larsch et al., 2013). In many cases, changes in stimuli selectively produce a response with a characteristic rise time, followed by a slower decay to a baseline (i.e., rest). In particular, AWA and ASH respond with a transient depolarization to stimulus upsteps, and with a transient hyperpolarization to stimulus downsteps (Thiele et al., 2009; Larsch et al., 2013). The biphasic nature of the derivative-like responses seen in AWA and ASH suggests sensory neurons use two components with opposite effect and a separation of timescales (Kato et al., 2014). Accordingly, in our model the sensory neuron response  $I_{\text{sens}}$  is given by the difference between a fast component  $F$  and a slow (delayed-rectifying) component  $S$  (Figure M2)

$$I_{\text{sens}} = F - S. \quad (\text{M5})$$

The fast component  $F$  integrates over the sensory stimulus  $C = C(x, y, t)$  (here the concentration of the respective signal) with a characteristic rate  $\alpha_i$  and decays at rate  $\beta_i$ . The slow component  $S$  follows  $F$  with a delay, producing the characteristic biphasic response

Sensory neuron parameters	Value	Description
$\alpha_{\text{AWA}}$	$4 \text{ s}^{-1}$	AWA depolarization rate
$\beta_{\text{AWA}}$	$15 \text{ s}^{-1}$	AWA depolarization leak rate
$\gamma_{\text{AWA}}$	$2 \text{ s}^{-1}$	AWA rectification (repolarization) rate
$\bar{\alpha}_{\text{ASH}}$	$4 \text{ s}^{-1}$	ASH depolarization rate
$\beta_{\text{ASH}}$	$15 \text{ s}^{-1}$	ASH depolarization leak rate
$\gamma_{\text{ASH}}$	$2 \text{ s}^{-1}$	ASH rectification (repolarization) rate

(Figure M2):

$$\frac{dF}{dt} = \alpha_i C - \beta_i F, \quad (\text{M6})$$

$$\frac{dS}{dt} = \gamma_i (F - S). \quad (\text{M7})$$

The physiological mechanism by which TYRA-2 modulates ASH excitability is unknown. The application of tyramine did not appear to affect the ASH membrane potential. Instead, when a hyperosmotic stimulus was applied in the presence of tyramine, the ASH response appeared enhanced compared to the response in the absence of tyramine (main text Fig. 4). Consistent with these observations, here the effect of TYRA-2 on ASH excitability is modeled as a contribution to the neuronal activation rate  $\bar{\alpha}_{\text{ASH}}$

$$\alpha_{\text{ASH}}(t) = \bar{\alpha}_{\text{ASH}} + T(t). \quad (\text{M8})$$

Initially, the activity of the neurons, all neuron components and the tyramine level are set to 0.

## Motor system

Model animals move at a constant speed while the direction of motion is modulated by two separate motor programs: a stochastic pirouette command unit and a central pattern generating undulation circuit consisting of two motor neurons, denoted VMN and DMN (Figure M1). Reciprocal inhibition between VMN and DMN supports a half-center oscillator that generates and maintains stable oscillations (representing the animal’s undulatory locomotion). This reciprocal connectivity pattern is reminiscent of connectivity found in several classes of head motor neurons in *C. elegans*.

The oscillator subcircuit includes delayed excitatory connections (Figure M1). To simplify our model, we used ‘hidden’ interneurons to create a delayed connection from VMN to DMN and vice versa. Thus, the delayed excitatory connection from VMN to DMN is implemented as two connections, one from VMN to the hidden interneuron, and another from the hidden interneuron to DMN, using the neuronal time constant of the hidden interneuron ( $\tau_m$ ) as a synaptic delay.

In the absence of a sensory input, this subcircuit will produce stable oscillations, facilitated by fast reciprocal inhibition and delayed reciprocal excitation (Figure M1). Any activity in one of the oscillating neurons will cause fast inhibition, followed by slower excitation of the other which in turn leads to recurrent inhibition. Thus the frequency and amplitude of the oscillations are determined by the timescales of the neurons ( $\tau_m$ ), the

connection strengths of the reciprocal inhibition, and delay in the reciprocal excitatory connections. When both neurons are equally active, the circuit does not oscillate. However, any small difference in activity is amplified by the mutual inhibition. Thus neuronal fluctuations would ensure stable oscillations even in a symmetric circuit. For simplicity, in the model, simulations are initiated with brief, equal and opposite input pulses into the two motor neurons VMN and DMN. Input from RIM into VMN and DMN biases the undulations to mediate steering.

Similar to other computational models of *C. elegans* (Bryden and Cohen, 2004, 2008; Izquierdo and Lockery, 2010), the direction of locomotion ( $\theta$ ) evolves as a function of the difference of the dorsal and ventral activation:

$$\frac{d\theta}{dt} = \omega (V_{\text{VMN}} - V_{\text{DMN}}), \quad (\text{M9})$$

where  $\omega$  sets the undulation amplitude. Point worms move with constant velocity  $v = 0.11$  mm/s along a direction vector  $\theta$  according to

$$\frac{d(x, y)}{dt} = (v \sin \theta, v \cos \theta). \quad (\text{M10})$$

Pirouettes are executed by resetting the orientation of movement of the point worm  $\theta$ , to a random value between 0 and  $2\pi$  drawn from a uniform distribution. The probability rate of a pirouette is given by the activation of the pirouette command unit ( $\Omega$ ) and given by a piece-wise linear monotonic function:

$$P_{\Omega}(V_{\Omega}) = \begin{cases} \bar{V}_{\Omega} + \epsilon & \bar{V}_{\Omega} + \epsilon \leq \bar{V}_{\Omega} + w_{\Omega} V_{\Omega} \\ \bar{V}_{\Omega} + w_{\Omega} V_{\Omega} & \bar{V}_{\Omega} - \epsilon \leq \bar{V}_{\Omega} + w_{\Omega} V_{\Omega} < \bar{V}_{\Omega} + \epsilon \\ \bar{V}_{\Omega} - \epsilon & \bar{V}_{\Omega} + w_{\Omega} V_{\Omega} < \bar{V}_{\Omega} - \epsilon \end{cases} \quad (\text{M11})$$

where  $V_{\Omega}$  denotes the activity of the abstract pirouette neuron,  $\bar{V}_{\Omega}$  is the mean pirouette rate (over the domain), and the proportionality constant is set to  $w_{\Omega} = 2.1$ .

Motor output	Value	Description
$v$	$0.11 \text{ mm s}^{-1}$	Forward speed
$V_{\text{start}}$	0.001	Oscillator start signal
$\tau_{\text{start}}$	0.01 s	Duration of oscillator start signal
$w_{\Omega}$	2.1	$P_{\Omega}(V_{\Omega})$ proportionality constant
$\bar{V}_{\Omega}$	$0.035 \text{ s}^{-1}$	Mean pirouette rate
$\epsilon$	$0.035 \text{ s}^{-1}$	Pirouette rate variation amplitude

All assays are simulated on a virtual two dimensional 5 cm plate, which animals cannot leave. All coordinates are given relative to an origin at the center of the arena.

## 1.2 Decision making assay

A virtual decision making assay was implemented to reproduce our experimental assay. The fructose ring,  $\text{FR}(\vec{r}, t)$  was modeled by continuous diffusion from an initial condition of a 1 cm diameter, placed in the center of the arena. Due to the radial symmetry of the

Assay parameter	Value	Description
$\tau_{\text{end}}$	900 s	Duration of the assay
$\delta t$	0.0001 s	Integration time step of the simulation
$\vec{r}(0) = (x(0), y(0))$	(0, 0) cm	Initial worm positions
$\theta(0)$	U(0, $2\pi$ )	initial direction drawn from uniform distribution
$V_i$	0	Initial activity for all neurons
$F_i$	0	Initial value: fast component, all sensory neurons
$S_i$	0	Initial value: slow component, all sensory neurons
$T(0)$	0	Initial value of Tyramine
$t_{\text{FR}}$	300 s	Fructose diffusion time prior to assay start
$\vec{r}_{\text{FR}} = (x_{\text{FR}}, y_{\text{FR}})$	(0, 0) cm	Center of fructose ring
$R$	0.5 cm	Fructose ring radius
$D_{\text{FR}}$	$3 \times 10^{-5}$ cm <sup>2</sup> /s	Fructose ring diffusion coefficient
$C_{\text{FR}}$	20, 30, 40	Fructose coefficients for 2, 3 and 4 M respectively
$t_{\text{DA}}$	60 s	Diacetyl diffusion time prior to assay start
$\vec{r}_{\text{DA}}^{1,2} = (x_{\text{DA}}^{1,2}, y_{\text{DA}}^{1,2})$	( $\pm 1.5, 0$ ) cm	Center of diacetyl spots
$D_{\text{DA}}$	$2.52 \times 10^{-4}$ cm <sup>2</sup> /s	Diacetyl diffusion coefficient
$C_{\text{DA}}$	100	Diacetyl amplitude

problem, assuming an initial narrow ring at  $R$  and neglecting the boundary conditions at the edge of the plate, the exact solution to the fructose diffusion in two dimensions is given by

$$C(\vec{r}, t) = M_0 \int_0^\infty d\lambda \lambda e^{-\lambda^2 D_{\text{FR}} t} J_0(\lambda R) J_0(\lambda r), \quad (\text{M12})$$

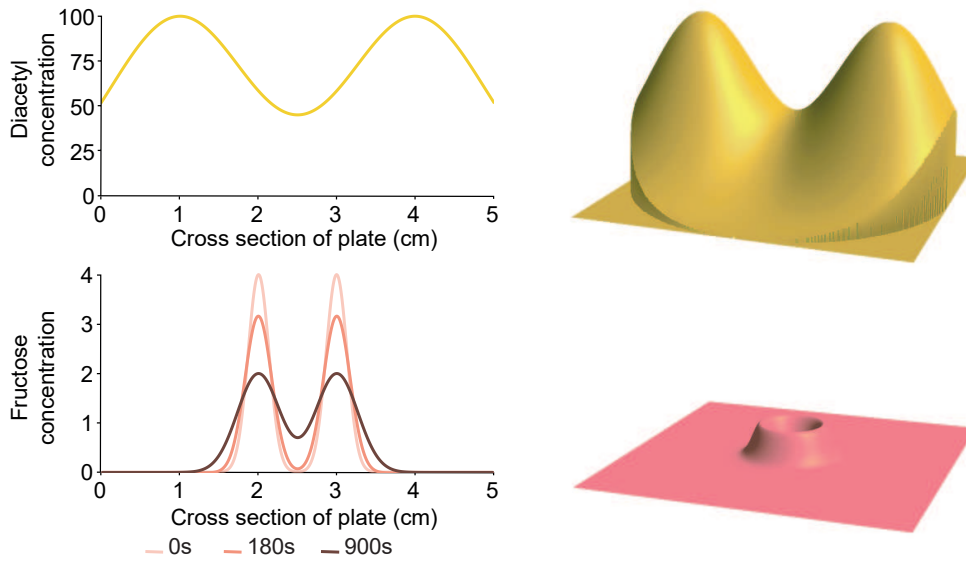
where  $r = |\vec{r}|$ ,  $J_0$  is the zeroth regular Bessel function and  $M_0$  is a normalization constant. Sufficiently close to the ring, the solution is well approximated by a one dimensional diffusion along the polar coordinate  $r$ :

$$\text{FR}(r, t) = C_{\text{FR}} \left( \frac{t_{\text{FR}}}{t_{\text{FR}} + t} \right)^{\frac{1}{2}} \left( \exp \left[ -\frac{(r - R)^2}{4D_{\text{FR}}(t_{\text{FR}} + t)} \right] + \exp \left[ -\frac{(r + R)^2}{4D_{\text{FR}}(t_{\text{FR}} + t)} \right] \right). \quad (\text{M13})$$

Here  $t_{\text{FR}}$  denotes the time of fructose diffusion prior to the start of the experiment at  $t = 0$ , when virtual worms are added to the simulation. The coefficient  $C_{\text{FR}}$  incorporates the concentration, diffusion coefficient factor and sensory neuron receptor strengths, and is referred to for simplicity as a concentration. The two exponentials correspond to contributions from the two edges of the ring: The closer edge at a distance  $r - R$  from the worm and the farther edge at a distance  $r + R$ . The concentration and diffusion coefficient were set to match experimental results with different durations of fructose diffusion  $t_{\text{FR}}$ .

Visual inspection of experimental worm traces showed animals moving towards diacetyl peaks even after 15 minutes. This is consistent with continuing evaporation of diacetyl, leading to an sustained concentration gradient throughout the assay. To capture this, we approximated the diacetyl concentration profile  $\text{DA}(x, y, t)$ , by a two phased process: an initial rapid diffusion (to establish the concentration profile from two initial spots), followed by a static field:

$$\text{DA}(x, y, t) = C_{\text{DA}} \sum_{i=1}^2 \exp \left( -\frac{(x - x_{\text{DA}}^i)^2 + (y - y_{\text{DA}}^i)^2}{4D_{\text{DA}} t_{\text{DA}}} \right), \quad (\text{M14})$$



**Figure M3:** Concentration profiles (cross sections and corresponding top-down views) used in the in silico decision making assay. **Top:** Steady state diacetyl profile resulting from two diacetyl sources at  $\pm 1.5$  cm from the center. **Bottom:** Snapshots of fructose concentration profile due to diffusion from a 1 cm diameter ring.

where  $(x_{DA}^i, y_{DA}^i)$  are the coordinates for the  $i^{\text{th}}$  diacetyl spot and  $t_{DA}$  denotes the duration of diacetyl diffusion.

At  $t = 0$  a single worm is placed at the center of the arena. Numerical integration of its nervous system and motor control is implemented with Euler integration, with a fixed integration time step (0.1 ms). As in the experimental assay, the balance of attraction and repulsion was quantified by the percentage of independently simulated worms that have exited the ring by 900 seconds.

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