

Monoamines activate neuropeptide signaling cascades to modulate nociception in *C. elegans*: a useful model for the modulation of chronic pain?

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Abstract Monoamines and neuropeptides interact to modulate key behaviors in most organisms. This review is focused on the interaction between octopamine (OA) and an array of neuropeptides in the inhibition of a simple, sensory-mediated aversive behavior in the *C. elegans* model system and describes the role of monoamines in the activation of global peptidergic signaling cascades. OA has been often considered the invertebrate counterpart of norepinephrine, and the review also highlights the similarities between OA inhibition in *C. elegans* and the noradrenergic modulation of pain in higher organisms.

Keywords Monoamines · Neuropeptides · Nociception · Octopamine · Adrenergic

Introduction

The monoaminergic/peptidergic modulation of “fast” neurotransmission mediated by an array of ion channels is well documented at the level of individual neurons and simple circuits, but given the complexity of most nervous systems and the training of most neuroscientists as

“reductionists,” few studies have been stratified horizontally to examine the roles of these ligands in the global modulation of individual sensory-mediated behavioral circuits. *C. elegans* has a simple, well-annotated nervous system with only 302 neurons, and most sensory input is processed by 14 pairs of sensory neurons that lie in anterior and posterior sense organs. In this review, we have summarized the effects of two key monoamines, octopamine (OA) and its biosynthetic precursor, tyramine (TA), in the modulation of a simple, sensory-mediated aversive behavior in *C. elegans*, avoidance of the volatile repellent, 1-octanol, as a potential model for the noradrenergic modulation of pain in higher organisms. Although protostomes, including nematodes and insects, do not synthesize epinephrine/norepinephrine, OA has been often considered the invertebrate counterpart of norepinephrine, given the structural similarities of the two signaling molecules and the observation that OA receptors in invertebrates are most similar to α -adrenergic receptors in mammals (Evans and Maqueira 2005; Roeder 1999, 2005). As noted below, OA and TA appear to function independently to modulate many key behaviors.

Aversive responses to 1-octanol are mediated, in large part, by a single pair of polymodal, nociceptive sensory neurons, the ASHs, and are dramatically delayed or “inhibited” by both TA and OA (Chao et al. 2004; Mills et al. 2011; Wragg et al. 2007). This inhibition involves distinct subsets of TA and OA receptors and requires the release of multiple neuropeptides from a number of additional neurons. These neuropeptides appear to activate peptide receptors within the ASH-mediated circuit itself and on sensory neurons outside the circuit, suggesting that these global, monoamine-initiated peptidergic signaling cascades have the potential to not only amplify TA/OA signaling, but also integrate a variety of sensory inputs to

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modulate individual behaviors (Alkema et al. 2005; Hapiak et al. 2009; Hobson et al. 2006; Horvitz et al. 1982; Mills et al. 2011).

Monoamines modulate aversive responses mediated by the ASH sensory neurons

OA is formed by the β -hydroxylation of TA and both ligands function independently to modulate a host of behaviors in insects and nematodes (Alkema et al. 2005; Lange 2009; Roeder 2005). In *C. elegans*, the expression of *tdc-1* (tyrosine decarboxylase) and *tbh-1* (tyramine- β -hydroxylase) that encodes the rate-limiting enzymes for the synthesis of TA and OA, respectively, increases during starvation (and decreases in the presence of food), through a *daf-7/daf-1*-dependent signaling pathway, suggesting that TA and OA release may be involved in transmitting the “starvation signal” (Alkema et al. 2005; Greer et al. 2008; Suo et al. 2006). However, although supported by a wealth of genetic data, this hypothesis has never been confirmed directly by the measurement of TA and OA levels in different nutritional states. In the *C. elegans* nervous system, TA is released from two ring motor neurons (RIMs) and potentially two RIC interneurons and OA from only two RICs, based on the patterns of *tdc-1* and *tbh-1* expression, suggesting that although these monoamines have global effects on behavior, they are released from a very limited number of neurons (Alkema et al. 2005). In general, TA and OA oppose the action of 5-HT that is released in the presence of food, including humoral 5-HT release from the two serotonergic neurosecretory motor neurons (Harris et al. 2011). For example, food and 5-HT stimulate egg-laying, pharyngeal pumping, and feeding behavior, and this 5-HT stimulation is inhibited independently by both TA and OA.

The ASH sensory neurons respond to a variety of aversive stimuli by initiating a rapid reversal response. For example, aversive responses to mechanical (nose touch), volatile (1-octanol), and soluble (copper, glycerol and primaquine) stimuli are mediated, at least in part, by the ASHs (Hart et al. 1999; Hilliard et al. 2002, 2005; Kaplan and Horvitz 1993; Troemel et al. 1997). The mechanism of ASH signaling has been reviewed recently (de Bono and Maricq 2005; Hart and Chao 2010). The ASHs synapse directly on both the forward and backward command interneurons, and activation of the ASHs potentiates the activation of the backward command interneurons and initiates reversal, most probably by shifting the balance between forward and backward locomotion (see Fig. 1 for the ASH-mediated circuit). For example, ASH activation either by nose touch or by light after the transgenic expression of channel rhodopsin is accompanied by the

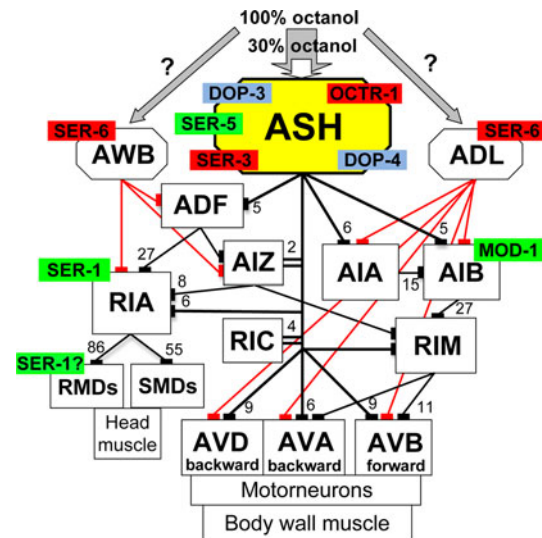


Fig. 1 ASH wiring diagram and localization of key monoamine receptors modulating ASH-mediated aversive responses. Green 5-HT receptors; red OA receptors; blue DA receptors (color figure online)

transient downstream activation of the AVA backward command interneurons through an AMPA-like glutamate receptor subunit, GLR-1, based on the direct electrophysiological recording or increases in Ca^{++} signaling after the transgenic expression of fluorescent calcium indicators, such as cameleon or GCaMP in the AVAs (Guo et al. 2009; Lindsay et al. 2011; Mellem et al. 2002). The command interneurons integrate signals from a variety of sensory-modulated interneurons, and it is the sum of these inputs that ultimately appear to dictate locomotory behavior.

The food-dependent monoaminergic modulation of the ASHs is surprisingly complex. Most of the predicted *C. elegans* monoamine G-protein-coupled receptors (GPCRs) have been heterologously expressed and at least partially characterized and many play a role in modulating octanol avoidance (GPCR ligand specificity summarized in Table 1). For example, ASH-mediated aversive responses are enhanced by food or 5-HT and can be modulated by dopamine (DA) and inhibited by TA and OA. Receptors for 5-HT (SER-5), DA (DOP-3, DOP-4), and OA (OCTR-1, SER-3) appear to function directly in the ASHs to differentially modulate aversive behavior, although the role of these receptors in modulating ASH signaling is still only cursorily understood (Ezak and Ferkey 2010; Ezcurra et al. 2011; Harris et al. 2009; Mills et al. 2011; Wragg et al. 2007). In addition, 5-HT (SER-1, MOD-1), OA (SER-6), and TA (TYRA-3) receptors also function downstream in the ASH circuit directly or outside the circuit to modulate aversive behavior, as described more fully below (Hapiak, unpublished; Harris et al. 2009, 2010; Mills et al. 2011). The ASHs are “on” neurons, i.e., calcium signaling increases after ligand addition, and, as noted above, ASH

Table 1 Predicted ligand specificity and G-protein coupling of *C. elegans* monoamine receptors

Receptor	Name	Preferred ligand/coupling	Mammalian subtype	References
F59C12.2	SER-1	5-HT $G\alpha_q$	5-HT ₂ -like	Hamdan et al. (1999)
Y22D7AR.13	SER-4	5-HT $G\alpha_o$	5-HT ₁ -like	Olde and McCombie (1997)
C09B7.1	SER-7	5-HT $G\alpha_s$	5-HT ₇ -like	Hobson et al. (2006)
F16D3.7	SER-5	5-HT $G\alpha_7$	5-HT ₆ -like?	Komuniecki (unpublished)
C02D4.2	SER-2	Tyramine $G\alpha_o$	Invertebrate-specific TA/OA receptors	Rex et al. (2004)
F01E11.5	TYRA-2	Tyramine $G\alpha_o$	Invertebrate-specific TA/OA receptors	Rex et al. (2005)
M03F4.3	TYRA-3	Tyramine $G\alpha_q$	Invertebrate-specific TA/OA receptors	Hapiak (unpublished)
KO2F2.6	SER-3	Octopamine $G\alpha_q$	α_2 -adrenergic-like	Petrascheck et al. (2007)
Y54G2A.35	SER-6	Octopamine $G\alpha_{s/q}$	α_1 -adrenergic-like?	Mills et al. (2011)
F14D12.6	OCTR-1	Octopamine $G\alpha_o$	α_2 -adrenergic-like	Wragg et al. (2007)
F15A8.5	DOP-1	Dopamine $G\alpha_s$	D1-like	Suo et al. (2002)
K09G1.4	DOP-2	Dopamine $G\alpha_o$	D2-like	Suo et al. (2003)
T14E8.3	DOP-3	Dopamine $G\alpha_{s/q}$	D2-like	Sugiura et al. (2005)
C52B11.3	DOP-4	Dopamine $G\alpha_q$	DAMB, invert-specific	Suo et al. (2004)
T02E9.3		?	?	
C24A8.1		?	?	

activation by a variety of different stimuli, including nose touch and soluble repellents, initiates significant increases in intracellular calcium in vivo (Ezcurra et al. 2011; Hilliard et al. 2005; Mills et al. 2011). However, the monoaminergic modulation of ASH signaling appears to be both stimulus modality and intensity dependent. For example, food or 5-HT accelerates aversive responses to nose touch or dilute 1-octanol, but has no effect on responses to soluble repellents or 100% 1-octanol (Chao et al. 2004; Ezcurra et al. 2011). Three different 5-HT receptors (SER-5, MOD-1, and SER-1) operating at different levels within the ASH-mediated locomotory circuit appear to be essential for food or 5-HT stimulation of aversive responses to dilute 1-octanol (see Fig. 1; Harris et al. 2009, 2010). SER-5 functions in the ASHs directly, and SER-5 signaling is essential for the stimulation of aversive responses by peptides encoded by *nlp-3* (Harris et al. 2010). Genetic analyses suggest that the activation of ASH $G\alpha_s$ signaling is required for the stimulation of aversive responses to dilute 1-octanol by ASH *nlp-3*-encoded peptides, but that SER-5 does not couple directly to $G\alpha_s$ in vivo (Harris et al. 2010). Interestingly, aversive responses to dilute 1-octanol are abolished in (1) wild-type animals after laser ablation of the ASHs, (2) *eat-4* null animals that lack a vesicular glutamate transporter essential for glutamatergic transmission in many sensory neurons or (3) animals expressing ASH: *eat-4* RNAi (Chao et al. 2004; Harris et al. 2010).

Interestingly, although food and 5-HT also stimulate nose touch, they do not appear to alter ASH calcium dynamics in response to nose touch, in contrast to previous reports (Hilliard et al. 2005), suggesting that 5-HT acts downstream of ASH calcium signaling, perhaps by

modulating SER-5 dependent SV/DCV release (Ezcurra et al. 2011). It will be important to determine whether the 5-HT stimulation of octanol avoidance is accompanied by alterations in ASH calcium dynamics. In contrast to SER-5, SER-1 and MOD-1 appear to operate in the RIA and AIB interneurons, respectively, downstream in the ASH-mediated circuit and are essential not only for the food/5-HT stimulation of aversive responses to dilute 1-octanol, but also for the post-initiation behaviors described below (Harris et al. 2009, 2011). However, although the ASHs synapse on both the AIBs and the RIAs, it is not clear whether ASH synaptic input is essential for SER-1 and/or MOD-1 modulation or whether these receptors/neurons modulate tonic release and/or input from other sensory neurons to ultimately dictate reversal behavior. Similarly, the activation of the mechanosensory dopaminergic neurons by food or incubation in exogenous DA directly enhances aversive responses and increases the magnitude and duration of ASH calcium transients in response to soluble repellents through a DA receptor, DOP-4, expressed on the ASHs, but delays aversive responses to dilute 1-octanol through DOP-3, also expressed on the ASHs (Ezak and Ferkey 2010; Ezcurra et al. 2011). Whether these differential responses to DA are modality specific and hard wired in the ASHs or are modulated by the stimulus-dependent signaling from other neurons remains to be determined. Clearly, it will be important to determine the role of all ASH-expressed monoamine receptors in the presence of a broad range of ASH ligands to determine whether they modulate ASH calcium signaling directly, as described for DOP-4, or function downstream to modulate ligand release or other aspects of neuronal signaling. In

fact, recent reports suggest the modality-specific activation of different signaling pathways may operate in other sensory neurons. For example, olfactory signals from the AWCs appear to inhibit the AIY interneurons and require the glutamate-gated chloride channel subunit, GLC-3 in the AIYs, while thermal signals sensed by the AWCs appear to activate the AIYs through as yet unidentified glutamate receptors (Chalasanani et al. 2007, 2010; Ohnishi et al. 2011). However, the Ca^{++} transients observed in these experiments appear to be temporally delayed relative to the initiation of the behavior and may be translated through additional neurons.

ASH-mediated aversive responses to 1-octanol

The intensity of ASH-mediated aversive responses to 1-octanol is stimulus dependent, with the time to initiate reversal dependent on the concentration of 1-octanol, i.e., after octanol presentation in front of forward-moving animals, reversal occurs after about 15, 10, and 5 s in response to 15, 30, and 100% 1-octanol, respectively (Chao et al. 2004; Harris et al. 2009). Whether these differences result from the differential activation of the sensory neurons, differential processing by downstream interneurons and/or the time taken for octanol to diffuse is unclear. At submaximal octanol concentrations, both food and exogenous 5-HT dramatically accelerate the initiation of reversal, i.e., animals respond to 30% 1-octanol by reversing in about 10-s off food and in about 5-s on food or exogenous 5-HT. In contrast, at 100% 1-octanol, food or 5-HT appears to have no effect on the initiation of reversal. TA and OA abolish the 5-HT stimulation of submaximal responses to dilute octanol and inhibit maximal aversive responses at higher octanol concentrations (Mills et al. 2011; Wragg et al. 2007). This octanol-initiated reversal behavior is complex, and although food and 5-HT have no effect on the initiation of reversal to 100% 1-octanol, they both have dramatic effects on post-initiation reversal behaviors, regardless of the intensity of the initiating stimulus (Harris et al. 2011). For example, on food reversals are short (<1 head swing/reversal) and after reversal is complete, most animals continue forward along their previous path (<45° from initial trajectory). In contrast, off food animals back up more extensively (>2 head swings/reversal) and turn significantly away from their previous trajectory (>45° from initial trajectory). Surprisingly, these post-initiation phenotypes are independent of the intensity of the initiating stimulus, i.e., even though animals initiate reversal more rapidly to 100% than 30% 1-octanol, food has identical effects on post-initiation responses, suggesting that nutritional state and not intensity of the noxious stimulus dictates these post-initiation responses. Interestingly, the three

5-HT receptors that are essential for the stimulation of aversive responses to dilute 1-octanol are also responsible for food-dependent alterations in post-initiation behaviors to both 30 and 100% 1-octanol (Harris et al. 2011).

Genetic manipulations of the food or 5-HT stimulation of aversive responses to dilute octanol either have (1) no effect, (2) stimulate wild-type animals off food to respond in about 5 s, as if they were on food, or (3) abolish food or 5-HT sensitization to levels observed off food (10 s). Intermediary responses (7–8 s) are only rarely observed, suggesting that some aspect of this modulatory circuit may exist in two activity states with the sum of excitatory and inhibitory inputs pushing this “bistable switch” from one activity state to the other, much as has been suggested for the command interneurons in the decision to move forward or backward.

The OA and TA modulation of ASH-mediated aversive responses is also surprisingly complex and involves multiple OA and TA receptors (Mills et al. 2011; Wragg et al. 2007). *C. elegans* contains three GPCRs with a marked preference for TA over OA, with TYRA-2 and SER-2 coupling to $G\alpha_o$ and TYRA-3 coupling to $G\alpha_q$ in vitro, and three GPCRs with a marked preference for OA over TA with OCTR-1 coupling to $G\alpha_o$ and SER-3 and SER-6 coupling to $G\alpha_{q/s}$ (see Table 1). In addition, a TA-gated chloride channel, LGC-55, has also been identified that operates in AVB forward command interneurons and neck muscles to coordinate locomotion and head movement (Pirri et al. 2009; Ringstad et al. 2009). As described more fully below, OA modulates the ASHs directly, and both OA and TA independently modulate the release of an array of “inhibitory” neuropeptides from additional sensory neurons that (1) may not respond directly to the “sensory signal” and (2) activate an array of neuropeptide receptors expressed throughout the sensory-mediated locomotory system (see Fig. 2). Much of the data supporting these observations has been generated by neuron-selective RNAi knockdown and overexpression in wild-type animals and neuron-selective rescue in null animals. Neuron-selective RNAi knockdown has been confirmed using multiple promoters and RNAi constructs and any potential spreading examined by the expression of off-target RNAi that is known to either inhibit or stimulate aversive responses when expressed in other neurons (Esposito et al. 2007; Harris et al. 2010). Wherever possible, null mutants have also been analyzed, but many of these subtle modulatory phenotypes are often masked in null mutants that abolish both excitatory and inhibitory inputs, i.e., individual monoamines, such as 5-HT or OA, potentially have both excitatory and inhibitory inputs into most behaviors, regardless of the directionality of their ultimate effect. For example, although 5-HT stimulates both pharyngeal pumping and egg-laying, inhibitory serotonergic inputs

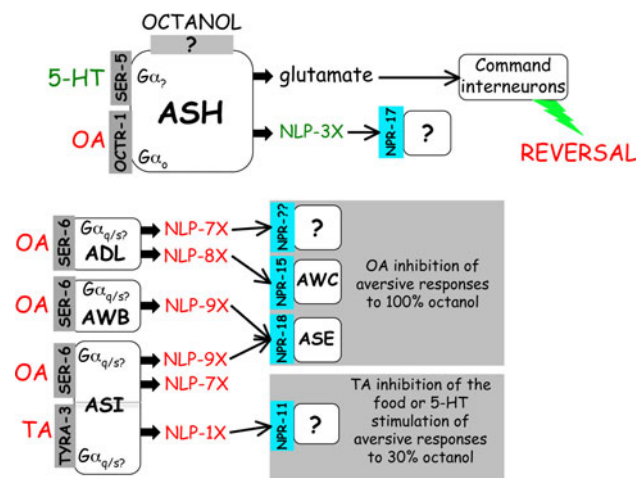


Fig. 2 OA and TA activate global peptidergic signaling cascades that delay ASH-mediated aversive responses to 1-octanol. *Green* stimulates aversive responses to 30% 1-octanol (*nlp-3*); *red* inhibits the food or 5-HT stimulation of aversive responses to 30% 1-octanol (*nlp-1*) or 100% 1-octanol (*nlp-7*, *nlp-8*, or *nlp-9*); *blue* neuropeptide receptors (color figure online)

into both processes also have been demonstrated (Hapiak et al. 2009; Hobson et al. 2006).

OA inhibition of aversive responses to dilute 1-octanol Exogenous OA (at 4 mM) abolishes the food or 5-HT stimulation of aversive responses to dilute 1-octanol (from about 5 to 10 s, the time taken to reverse in the absence of 5-HT), and both OTR-1 and $G\alpha_o$ signaling in the ASHs are required for this OA-dependent delay (Harris et al. 2010; Mills et al. 2011; Wragg et al. 2007). In contrast, exogenous OA (at 10 mM) has no effect on food or 5-HT stimulation in wild-type animals, but inhibits 5-HT stimulation in *ser-3* null animals or in wild-type animals with ASH *ser-3* expression compromised by ASH selective *ser-3* RNAi knockdown, suggesting that at increased OA concentrations, SER-3 is differentially activated in the ASHs (Mills et al. 2011). Together, these data suggest that the octopaminergic modulation of the ASHs is complex with the $G\alpha_q$ -coupled SER-3 differentially antagonizing the action of the $G\alpha_o$ -coupled OTR-1, potentially depending on the intensity or site of OA release.

OA inhibition of aversive responses to 100% 1-octanol OA also dramatically inhibits reversal in response to 100% 1-octanol (from about 4–5 s to 10 s), but surprisingly at these higher octanol concentrations, OTR-1 is not directly involved (Mills et al. 2011; Wragg et al. 2007). Instead, this OA inhibition is absolutely dependent on another OA receptor, SER-6 (Mills et al. 2011). Surprisingly, SER-6 does not function directly in the ASH-mediated locomotory circuit, but instead in a diverse group of peptidergic sensory neurons, including the paired AWBs, ADLs, and ASIs, that have previously been implicated in a

range of sensory behaviors, including a poorly defined role for the AWBs and ADLs in octanol avoidance (Bargmann et al. 1993; Beverly et al. 2011; Chao et al. 2004; Ha et al. 2010; Kimura et al. 2010; Mills et al. 2011; Troemel et al. 1997). For example, laser ablation of the ASHs reduces, but does not abolish, aversive responses 100% 1-octanol off food (Chao et al. 2004). In contrast, aversive responses are almost completely abolished by the co-ablation of the ASHs, AWBs, and ADLs (Chao et al. 2004). Interestingly, glued animals expressing the calcium indicator, G-CaMP3, in their ASHs respond to octanol with a robust increase in calcium, as has been observed for other ASH ligands (Mills et al. 2011). In contrast, animals expressing G-CaMP3 in their ADLs or AWBs do not respond to octanol, suggesting that the AWBs and ADLs may not respond to octanol directly, at least with alterations in calcium dynamics (Mills et al. 2011). This observation suggests that the ADLs and AWBs may instead function downstream of as yet unidentified octanol-sensing neurons, or perhaps that ablation of the ASHs causes compensatory changes in the octanol responsiveness of the ADLs and AWBs. Surprisingly, the addition of food or 5-HT dramatically inhibits aversive responses to 100% 1-octanol in animals with compromised ASH function, although the mechanism of this inhibition is unclear (Chao et al. 2004; Harris, unpublished).

The RNAi knockdown of SER-6 in the AWBs, ADLs, or ASIs reduces OA inhibition. In contrast, the overexpression of SER-6 in any one of these neuron pairs significantly delays aversive responses off food in the absence of exogenous OA (Mills et al. 2011). How does OA/SER-6-dependent signaling in these additional sensory neurons inhibit reversal in response to 100% 1-octanol? The first clues came from the observations that (1) OA did not inhibit aversive responses to 100% 1-octanol in *egl-3* null animals that lack a key proprotein convertase essential for the processing of many neuropeptides and (2) OA inhibition was also significantly reduced after *egl-3* RNAi knockdown in either the ADLs, AWBs or ASIs or, more importantly, by the RNAi knockdown of individual peptide-encoding genes in these neurons (Mills et al. 2011). These studies identified a group of peptide-encoding genes (*nlp-6*, *nlp-7*, *nlp-8*, and *nlp-9*) that were essential for maximal OA inhibition and suggested that OA/SER-6 stimulated $G\alpha_s$ -dependent peptide release in these neurons, at least in the ADLs (Mills et al. 2011). For example, the ADL RNAi knockdown of $G\alpha_s$ (*gsa-1*) also decreased OA inhibition, while the RNAi knockdown of *pde-4*, a 5' cAMP phosphodiesterase whose knockdown would be predicted to increase cAMP levels, slowed aversive responses in wild-type animals in the absence of OA (Mills et al. 2011). However, since peptide release was not assayed directly in these studies, potential developmental

effects cannot be ruled out. Similarly, the OA-dependent release of neuropeptides from *Drosophila* motorneurons also required $G\alpha_s$ signaling (Shakiryanova et al. 2011). Interestingly, this OA-dependent neuropeptide release, rather than relying on the activity dependent entry of external Ca^{++} , instead required signaling from both endoplasmic reticulum Ca^{++} and a cAMP-dependent protein kinase (Shakiryanova et al. 2011).

If the knockdown of the individual peptide-encoding genes decreased OA inhibition, then the knockdown of their cognate receptors would be expected to mimic this phenotype. Indeed, a survey of animals with putative null alleles for over 50 genes predicted to encode peptide receptors identified a subset of animals that were insensitive to OA in these aversive assays. To tentatively pair predicted neuropeptides with cognate receptors, individual peptide-encoding genes were overexpressed in receptor null backgrounds, on the assumption that the peptide overexpression phenotypes would be absent in the appropriate null background. Using this approach, peptides encoded by *nlp-9* were tentatively paired with NPR-18 and peptides encoded by *nlp-8* with NPR-15. However, in contrast with the monoamine receptors, the ligand specificity of these peptide receptors has not yet been confirmed by heterologous expression. The deorphanization of peptide receptors is complicated by the fact that many of the peptides (1) are only predicted and have not been confirmed by direct sequencing (2) require post-translational modification for bioactivity, and (3) bind to large extracellular N-terminal domains of these heptahelical peptide receptors whose folding may be compromised in heterologous systems and not in a binding pocket formed by the transmembrane domains as observed for monoamine receptors. Interestingly, NPR-15 and NPR-18 do not appear to function in the ASH-mediated circuit directly, but instead in the AWC or ASER sensory neurons, respectively, that appear to mediate either attraction (AWC) or repulsion (ASER) (Bargmann and Horvitz 1991; Bargmann et al. 1993; Bretscher et al. 2011; Suzuki et al. 2008). This observation suggests that the OA-dependent inhibition of reversal in response to 100% 1-octanol involves the peptidergic modulation of the pathways both favoring and opposing reversal/backward locomotion and that sensory-mediated locomotory decisions may require the integration of multiple sensory inputs. Stated simply, ASH-mediated aversive responses may be inhibited by modulating tonic signaling from other sensory neurons or perhaps interneurons mediating both forward and backward locomotion.

TA inhibition of ASH-mediated aversive responses TA delays the food or 5-HT stimulation of aversive responses to 30% 1-octanol from 5 to 10 s, and this TA-dependent

delay requires the $G\alpha_q$ -coupled TA receptor, *tyra-3* (Wragg et al. 2007). In contrast, TA also delays the initiation in reversal to 100% 1-octanol, but this TA-dependent delay requires not *tyra-3*, but instead *lgc-55* that encodes a TA-gated chloride channel (Hapiak, unpublished). These results, combined with those described above for OA, suggest that TA and OA can independently modulate both the circuit involved in the food or 5-HT enhancement of aversive responses to 30% 1-octanol through one set of TA/OA receptors and the circuit involved in aversive responses to 100% 1-octanol through a different subset of TA/OA receptors.

Tyra-3 appears to function in a number of neurons, including the ASI sensory neurons, to mediate the TA-dependent delay of food or 5-HT stimulation of aversive responses to dilute 1-octanol, based on ASI-specific *tyra-3* RNAi knockdown in wild-type animals and ASI *tyra-3* rescue in *tyra-3* null animals (Hapiak, unpublished). This TA/TYRA-3-dependent “delay” appears to function similarly to that observed for the OA-dependent inhibition of responses to 100% 1-octanol, i.e., ASI peptides are essential for both TA/OA-dependent modulation (Fig. 2; Hapiak, unpublished). Interestingly, the ASI peptides involved in OA inhibition of responses to 100% 1-octanol appear to be different from the ASI peptides involved in TA inhibition of 5-HT stimulation of responses to 30% 1-octanol (Fig. 2; Hapiak, unpublished). *Tyra-3* has also been implicated in the differential modulation of foraging behavior in N2 and HW strains (Bendesky et al. 2011). Interestingly, an apparent twofold change in *tyra-3* expression is sufficient to dramatically alter foraging rates, with *tyra-3*-dependent decreases in the activity of the ASK sensory neurons and increases in activity in the BAG sensory neurons acting synergistically to increase foraging in N2s. Since the BAGs are also primarily peptidergic, it will be interesting to determine whether BAG peptides are also involved in the *tyra-3*-dependent foraging phenotypes or the TA inhibition of 5-HT-stimulated, ASH-mediated aversive responses.

Role of TA and OA in the activation of global signaling cascades

How does the release of monoamines, such as OA and TA, from such a limited number of neurons have such global effects on most key aspects of *C. elegans* behavior? Based on the observations outlined above, it appears that OA and TA independently activate global signaling cascades that involve the release of an array of additional peptidergic modulators that activate receptors on many key neurons modulating locomotory transitions (Fig. 2). These OA- and

TA-dependent signaling cascades probably modulate most nutritionally dependent behaviors and would be expected to amplify the “hunger signal” and provide multiple sites for interaction with other sensory inputs. Interestingly, phenotypes demonstrated by RNAi knockdown of ligands or receptors in one pair of neurons in wild-type animals can often be rescued by expression (overexpression?) in a different pair of neurons in null animals. We have observed this phenomenon for a number of peptide-encoding genes and receptors, even after rigorously excluding the potential for RNAi spreading. Monoamines or neuropeptides are released both tonically and acutely in response to internal and environmental cues and activate a variety of extra synaptic receptors on multiple neurons, suggesting that a changing humoral “soup” of monoamines/neuropeptides may, at least in part, define “behavioral state.” The composition of this “soup” is dependent on contributions from multiple neurons, suggesting that small increases or decreases in ligand release from any one neuron pair have the potential to alter signaling. Indeed, the Bargmann laboratory has recently demonstrated that twofold changes in the expression level of *tyra-3* can have profound effects on locomotory behavior (Bendesky et al. 2011). These observations suggest that any overexpression in rescued animals has the potential to compensate for an absence of release in other neurons. This philosophy can also be extrapolated to neural circuits that are modulated by multiple inputs, i.e., the loss of one modulator can be masked by the overexpression of another, i.e., neural circuits are not necessarily analogous to enzymatic pathways with one rate-limiting step operating at saturation. In the past, cell-specific rescue has been the “gold standard” for functional localization, and for structural proteins, this is certainly true. However, we have observed potential “off-target” phenotypes with the expression of many *C. elegans* G-protein-coupled receptors, presumably because ligands for the receptors are either tonically released or the receptors themselves exhibit constitutive activity in the absence of ligand. The effects of G-protein signaling on neurotransmitter release in *C. elegans* are well documented, and we have observed that the expression of many Gs-, Go-, and Gq-coupled receptors in interneurons or motorneurons modulating locomotion often yield artifactual locomotory phenotypes, just as the expression of the gain-of-function G-proteins themselves. If neurotransmitter release from any neuron in a circuit has the potential to increase the output of the circuit, then neuron-specific RNAi knockdown may be more diagnostic than rescue, highlighting the need for both techniques in defining circuit modulation.

Locomotory decision making is binary, i.e., do I move forward or backward? and is ultimately integrated at the level of the command interneurons that have been

suggested to function as a “bistable switch” (Zheng et al. 1999). The decision to move forward or backward is probably dependent, at least in part, on the integration of both positive and negative sensory inputs, i.e., reversal can be inhibited by inhibiting sensory inputs favoring reversal or stimulating sensory inputs opposing reversal, as suggested by the sites of peptidergic interaction described above for the AWC and ASER sensory neurons in the OA inhibition of aversive responses to 1-octanol.

These results also confirm the selectivity of TA and OA signaling, with each monoamine activating a distinct subset of monoamine receptors and the release of different downstream peptides. Interestingly, the TA-mediated peptidergic signaling cascade appears to modulate the food and 5-HT stimulation of submaximal ASH-mediated aversive responses to dilute 1-octanol, but has no effect on responses in the absence of food or with increased ASH activation. In contrast, the OA-mediated peptidergic cascade appears to modulate responses to 100% 1-octanol directly, but has no effect on food or 5-HT stimulation of aversive responses to dilute 1-octanol. Both TA and OA appear to independently stimulate neuropeptide release from the ASIs, with peptides encoded by *nlp-1* required for TA/TYRA-3-mediated responses and peptides encoded by *nlp-7*, *nlp-8*, and *nlp-9* required for OA/SER-6-mediated responses (see Fig. 2; Hapiak, unpublished; Mills et al. 2011). It will be important to determine whether these monoamine receptors stimulate the bulk release of neuropeptides from the ASIs or whether the individual receptors differentially modulate the release of distinct ASI peptide pools.

***C. elegans* as a model to understand noradrenergic signaling in mammals**

As noted above, OA has been often considered the nematode counterpart of norepinephrine, given the structural similarities of the two signaling molecules and the observation that nematode OA receptors are most similar to mammalian α -adrenergic receptors (Evans and Maqueira 2005; Mills et al. 2011; Roeder 1999, 2005). Interestingly, the network of OA receptors modulating aversive responses in *C. elegans* mimics the noradrenergic inhibition of nociception in mammals, where norepinephrine released from descending pathways suppresses pain through inhibitory α_2 -adrenoreceptors (G α_i -coupled) on afferent nociceptors and by the activation of α_1 -receptors on inhibitory peptidergic interneurons (Millan 2002; Seybold 2009). Under basal conditions, the noradrenergic system has little effect on nociception, but sustained pain induces noradrenergic feedback inhibition (Monhemius et al. 2001; Obata et al. 2005). Similarly, in *C. elegans*, OA has no

effect on basal aversive responses to submaximal ASH stimulation, but as the intensity of ASH stimulation increases, OA inhibition increases, with the SER-6-dependent release of “inhibitory” peptides only apparent at increased levels of noxious stimulation. Interestingly, the ASHs form multiple gap junctions with the octopaminergic RICs, suggesting that ASH-dependent OA release from the RICs may prevent ASH “overstimulation,” i.e., reduce gain and maintain the ability to sense further increases. Pronociceptive adrenergic pathways have also been identified, with norepinephrine apparently increasing excitability of dorsal root ganglion neurons in nerve injured animals through the α_2 -adrenergic receptor-mediated blockade of N-type Ca^{2+} channels and subsequent inhibition of Ca^{2+} -activated K^+ channels (Honma et al. 1999). Whether the antagonistic effects of SER-3 on OCTR-1-mediated OA inhibition in the ASHs mimics the pronociceptive action of α_2 -adrenoreceptors on Ca^{2+} dynamics remains to be determined.

TA and OA also have direct effects on neurotransmission in mammals that appear to be mediated by both GPCRs and alterations in monoamine reuptake/vesicle loading. In fact, a subset of mammalian $\text{G}\alpha_s$ -coupled trace amine receptors have been identified that are activated by TA and other trace amines. However, the mammalian trace amine receptors do not appear to be evolutionarily related to the invertebrate TA/OA receptors, and it is still unclear whether TA is a bona fide signaling molecule in mammals or whether changes in TA levels are modulated by diet or pathophysiological alterations in monoamine metabolism. In fact, clear orthologous of the mammalian $\text{G}\alpha_s$ -coupled trace amine receptors are not apparent in the *C. elegans* genome (Komuniecki, unpublished).

In summary, *C. elegans* should provide an excellent model to study the role of monoamines in the modulation of peptidergic signaling. New examples of monoaminergic/peptidergic interactions in mammals are being described almost daily, and the relatively simple *C. elegans* nervous system should permit the dissection of potentially orthologous interactions at the level of individual circuits. For example, do monoamines modulate peptide release by directly activating DCV release or indirectly by modulating neuronal input or excitability? With the wealth of cell-based assays available in *C. elegans*, it is certainly an exciting time to be a *C. elegans* neuroscientist.

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