Reciprocal modulation of 5-HT and octopamine regulates pumping via feedforward and feedback circuits in *C. elegans*

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Feeding is vital for animal survival and is tightly regulated by the endocrine, sensory, and central nervous systems. Averaging feeding behaviors are associated with body weight changes and a range of metabolic diseases (1, 2). Despite the evolutionary distance between the nematode *Caenorhabditis elegans* and mammals, numerous similarities exist in food-related behaviors and molecular mechanisms of feeding and fat regulation between these species (3, 4). *C. elegans* is widely used to study conserved feeding-regulatory mechanisms (refs. 5–9; reviewed in refs. 3 and 10). There has emerged a third wave of detailed functional studies on the pharyngeal nervous system aiming to elucidate the neural basis of feeding behavior in worms (commented in ref. 11). *C. elegans* uses pharyngeal pumping to ingest its food, bacteria (12). Pumping is governed by the pharyngeal nervous system and is regulated by the extrapharyngeal nervous system, which uses pharyngeal pumping to ingest bacteria into the gut. We reveal that a cross-modulation mechanism between 5-HT and OA, which convey feeding and fasting signals, respectively, mainly functions in regulating the pumping and secretion of both neuro-modulators via ADF/RIC/SIA feedforward neurcircuit (consisting of ADF, RIC, and SIA neurons) and ADF/RIC/AWB/ADF feedback neurcircuit (consisting of ADF, RIC, AWB, and ADF neurons) under conditions of food supply and food deprivation, respectively. Food supply stimulates food-sensing ADFs to release more 5-HT, which augments pumping via inhibiting OA secretion by RIC interneurons and, thus, alleviates pumping suppression by OA-activated SIA interneurons/motoneurons. In contrast, nutrient deprivation stimulates RICs to secrete OA, which suppresses pumping via activating SIA and maintains basal pumping and 5-HT production activity through excitation of ADFs relayed by AWB sensory neurons. Notably, the feedforward and feedback circuits employ distinct modalities of neurosignal integration, namely, disinhibition and disexcitation, respectively.

*Results*

**ADFs Modulate Pumping via Two Circuits.** 5-HT mediates the feeding signal and is necessary for enhancing pumping in *C. elegans*. 5-HT released from ADFs enhances pumping by acting on AVJs via SER-5 signaling in well-fed worms (7). 5-HT from NSM neurons inhibits tyraminergic RIM interneurons via the MOD-1 receptor to increase the pumping rate (9) and regulates pumping dynamics cross-inhibition between signaling of 5-HT and TA that integrates contradictory sensory cues and functions to fine-tune and hasten feeding decisions (9). However, whether feeding signal-conveying 5-HT and fasting signal-conveying OA functionally interact and whether cross-modulation exists in the secretion of 5-HT and OA remain unclear, as do the potential underlying mechanisms.

Here, using multiple methods, we investigated neurocircuit-related mechanisms of pumping regulation by 5-HT and OA. We discovered that serotoninergic ADFs modulate *C. elegans* pumping through two neurocircuits: a feedforward ADF/RIC/SIA circuit (consisting of ADF, RIC, and SIA neurons) that functions mainly under conditions of food supply to enhance pharyngeal pumping and a feedback ADF/RIC/AWB/ADF circuit (consisting of ADF, RIC, AWB, and ADF neurons) that maintains the homeostasis of pumping and 5-HT production by ADFs under various food concentrations and especially maintains the basal levels of both activities under fasting conditions. Notably, the feedforward and feedback circuits employ distinct modalities of neurosignal integration: disinhibition and disexcitation, respectively. Disexcitation, a modality of neuronal computation suggested by this study, has not been reported previously.

**Significance**

Physiological regulation and behavior depend less on neurons than on neuronal circuits. Neurosignal integration is the basis of neurocircuit function. The modalities of neuroinformation integration are evolutionarily conserved in animals and humans. Here, we identified two modalities of neurosignal integration in two different circuits by which serotoninergic ADFs regulate pharyngeal pumping in *Caenorhabditis elegans*: disinhibition in a feedforward circuit consisting of ADF, RIC, and SIA neurons and disexcitation, a modality of neurosignal integration suggested by this study, in a feedback circuit consisting of ADF, RIC, AWB, and ADF neurons.

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to elicit a burst of fast pumping via SER-1 and SER-4 signaling (13). Whether there are other mechanisms or circuits by which 5-HT modulates pumping remains unknown. We first examined the 5-HT source(s) in pumping (as indicated by average pumping rates) regulation under normal culture conditions. ADFs exhibited robust Ca\textsuperscript{2+} transients in response to the food-derived sensory cue Escherichia coli (OP50) supernatant as suggested (26), as measured by GCaMP5p Ca\textsuperscript{2+} imaging combined with the use of a microfluidic chip (27–29), and enhanced pumping in well-fed worms (SI Appendix, Fig. S1 A and B). We next genetically blocked vesicle fusion with the plasma membrane and, thus, neurotransmission by ADFs using neuron-specific expression of the light chain of tetanus toxin (TeTx), a specific protease of synaptobrevin that has been successfully used to inhibit chemical synaptic transmission in tested neurons (neuron:TeTx in short) in C. elegans (27, 28, 30, 31). Continual inhibition of neurotransmission by TeTx beginning in embryonic periods may interfere with nervous system development. We thus used chemogenetics to acutely inhibit the tested neurons using HisC11 channels and 10 mM histamine (neuron chemogenetic inhibition in short). HisC11 is a histamine-gated chloride channel subunit from Drosophila that is effective for silencing neurons when activated by exogenous histamine (32). As expected, ADF::TeTx and ADF chemogenetic inhibition resulted in similar reductions in pumping rates in well-fed animals (Fig. 1A). We used multiple methods to identify the 5-HT receptors and the downstream effector neurons of ADFs that may be involved in pumping regulation. Our results indicated that 5-HT from ADFs regulated pumping by inhibiting RICs via MOD-1 signaling (Fig. 1B and SI Appendix, Fig. S1).

OA, which mediates the fasting signal (24, 33–36), counteracts the effects of 5-HT in pumping regulation, and administration of exogenous OA inhibits worm pumping (16, 20). In C. elegans, RICs are the only octopaminergic neurons that synthesize and release OA (37). Which OA receptor(s) and downstream effector neuron(s) of RICs function in pumping regulation? As shown in Fig. 1 C and D and SI Appendix, Fig. S2 A–D, our data indicated that ser-3 null mutants displayed increased pumping rates under well-fed conditions, while ser-6 loss mutants exhibited decreased pumping rates under food deprivation conditions (8 h). Extra-chromosomal expression of ser-3 and ser-6 in SIAs and AWBs alone, respectively, restored wild-type pumping; furthermore, the results of Ca\textsuperscript{2+} imaging and neuron-specific manipulation with TeTx and HisC11 confirmed the genetic results. These data suggest that OA from RICs acts on SIAs and AWBs via SER-3 and SER-6 signaling and functions to suppress and augment worm pumping under well-fed and fasting conditions, respectively.

**AWBs Act as Interneurons To Relay RIC Action in a RIC/AWB/ADF Top-Down Feedback Circuit.** Our above results indicate that ADFs inhibit RICs via 5-HT/MOD-1 signaling and that RICs neuroendocrinally activate AWBs through OA/SER-6 signaling, blocking RIC activity should remove the impacts of exogenous 5-HT and OA on AWB activity. Indeed, RIC::TeTx and ser-6 loss mutation eliminated the effects of 5-HT and OA on the food cue-elicited Ca\textsuperscript{2+} transients of off responses in AWBs (SI Appendix, Fig. S3 A and B). In addition, RIC chemogenetic inhibition reduced the AWB Ca\textsuperscript{2+} signals (Fig. 2A). However, unexpectedly, chemogenetically activating RICs with TRPV1 and 50 μM exogenous capsaicin (neuron chemogenetic activation in short) did not significantly affect the AWB Ca\textsuperscript{2+} signals (Fig. 2B). AWBs have a chemosensory connection with ADFs (38). Activation of AWBs up-regulates the expression of TPH-1 (a tryptophan hydroxylase) and the production of 5-HT in ADFs (39, 40). We then tested whether AWBs regulate ADF activity utilizing the above methods. AWB::TeTx not only reduced the ADF Ca\textsuperscript{2+} signals but also blocked the effect of exogenous OA on ADF Ca\textsuperscript{2+} signals (Fig. 2C). AWB chemogenetic inhibition reduced the ADF Ca\textsuperscript{2+} signals (Fig. 2D), while AWB chemogenetic activation increased the ADF Ca\textsuperscript{2+} signals (Fig. 2E). These results indicate that signal transmission in the RIC/AWB/ADF circuit is excitatory. If this neuronal chain is linear, RICs should have an impact on ADFs similar to that on AWBs. The results of our tests using TeTx and chemogenetic manipulation supported this hypothesis. RIC::TeTx or RIC chemogenetic inhibition reduced the ADF Ca\textsuperscript{2+} signals (Fig. 2 F and G). However, RIC chemogenetic activation did not significantly affect ADFs (Fig. 2H), similar to the case for AWBs (Fig. 2B). Exposure to exogenous 5-HT should inhibit RICs and, thus, reduce ADF activity. Indeed, treatment with 5-HT (10 μM) significantly reduced the ADF Ca\textsuperscript{2+} transients (Fig. 2I). Moreover, RIC::TeTx or AWB::TeTx not only decreased the ADF Ca\textsuperscript{2+} signals but also eliminated the effects of 5-HT treatment on ADFs (Fig. 2 J and K). We next introduced behavioral tests to examine the effects of exogenous 5-HT on pumping during inhibition of the feedback circuit. Given that treatment with 4 μM exogenous 5-HT decreased the pumping rates in well-fed animals (SI Appendix, Fig. S3C) and that the lmr-4 loss mutation resulted in a lack of AWB neurons (41), we dosed the RIC::TeTx and AWB::TeTx transgenic worms and ser-6(km2140) and lmr-4(ky403) mutants with 4 μM 5-HT and examined the effects on pumping. As expected, either the lmr-4 or ser-6 mutation and AWB::TeTx or RIC::TeTx eliminated the inhibitory effect of 4 μM 5-HT on pumping (Fig. 2L). Thus, our results support the existence of a feedback neurocircuit consisting of ADFs, RICs, and AWBs.

**The Feedback Circuit Employs Disexcitation for Neuronal Computation.** In the ADF/RIC/AWB/ADF feedback circuit, the neurotransmission among RICs, AWBs, and ADFs was found to be excitatory, while that between ADFs and RICs was found to be inhibitory. RICs transmitted their chemogenetic inhibition, but not their chemogenetic activation, to AWBs (Fig. 2 A and B), and treatment with 5 μM exogenous OA did not significantly affect

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**Fig. 1.** ADFs augment pharyngeal pumping possibly via two neuronal circuits. (A) Pumping rates in worms of different genotypes as indicated treated with or without the indicated chemicals. (B) Curves (Left) and average peak intensities (Right) of the somal Ca\textsuperscript{2+} transients in RIC interneurons in the indicated worms under the administration of OP50 supernatant. (C) Pumping rates in worms of different genotypes as indicated. (D) Curves (Left) and average peak intensities (Right) of the somal Ca\textsuperscript{2+} transients in AWBs in the indicated worms under the administration of OP50 supernatant. Statistical significance is indicated as follows: ns, not significant, *p < 0.05, **p < 0.01, and ****p < 0.0001 in comparison with the value for WT worms or as indicated.
AWB activity (SI Appendix, Fig. S4A). What is the underlying mechanism? A reasonable explanation is that RICs have maximal tonic activity, at least under the conditions of our experiments, and that ADFs hierarchically inhibit RICs, thus providing disinhibitory feedback regulation. We herein provide data to support this hypothesis. First, ADFs displayed graded Ca\textsuperscript{2+} responses to E. coli OP50 at various concentrations (indicated by the optical densities). The peak intensities of ADF Ca\textsuperscript{2+} signals were well correlated with the bacterial concentrations (Pearson r = 0.9949, Fig. 3A). Second, RICs also exhibited graded Ca\textsuperscript{2+} responses to bacteria at various concentrations (Fig. 3B). The activation of RICs may be a result of neurotransmission from other neurons because the RIC Ca\textsuperscript{2+} signals were significantly reduced in both unc-13(e1091) and unc-31(e928) mutants (SI Appendix, Fig. 5A). ADF::TeTx augmented the RIC Ca\textsuperscript{2+} responses to food at various concentrations. The peak intensities of RIC Ca\textsuperscript{2+} signals were well correlated with the bacterial concentrations (Pearson r = 0.9971 and 0.9871 for the correlations of WT N2 and ADF::TeTx transgenic worms, Fig. 3C and D). Furthermore, treatment of ADF::TeTx transgenic worms with exogenous 5-HT at various concentrations dose-dependently decreased the OP50 supernatant-elicited RIC Ca\textsuperscript{2+} signals (well-fitted by a Hill function with EC\textsubscript{50} = 0.31 μM, Fig. 3E), mimicking the graded inhibition of ADFs under various bacterial concentrations. Third, treatment of RIC::TeTx transgenic worms with exogenous OA at various concentrations resulted in augmentation of ADF Ca\textsuperscript{2+} signals with a dose–response relationship well-fitted by a Hill function with an EC\textsubscript{50} of 0.87 μM (Fig. 3F). In addition, graded down-regulation of RICs with HisCl1 channels and histamine at various doses dose-dependently decreased the AWB Ca\textsuperscript{2+} transients (Hill function EC\textsubscript{50} = 0.67 mM, Fig. 3G). Moreover, application of exogenous 5-HT at various concentrations to the ADF::TeTx transgenic worms also dose-dependently reduced the AWB Ca\textsuperscript{2+} signals according to a Hill function with an EC\textsubscript{50} of 0.38 μM (Fig. 3H). Finally, dosing N2 worms and tph-1(mu280) mutants with exogenous 5-HT at various concentrations diminished the ADF Ca\textsuperscript{2+} signals in a graded manner, with EC\textsubscript{50} values of 0.29 μM and 0.39 μM, respectively (Fig. 3I and J). In summary, ADFs dose-dependently respond to food at various concentrations and inhibit RIC tonic excitation in a graded manner, and AWBs relay feedback regulation from RICs to ADFs by excitatory connections. Thus, inhibition of RICs by ADFs disinhibits the tonic activation of ADFs by RICs, suggesting disinhibitory neuronal signal integration in the feedback circuit.

The Feedback Circuit Functions To Maintain Homeostasis of Pharyngeal Pumping and ADF Activity Under Various Degrees of Food Supply. What is the function of the feedback circuit in the modulation of pharyngeal pumping and ADF activity? C. elegans responds to various degrees of food availability and exhibits graded pumping rates depending on food concentrations (13). We first examined the role of the feedback circuit in worm pumping regulation with various concentrations of E. coli OP50 bacteria using methods described in SI Appendix, SI Materials and Methods. The pumping rates in the worms of all of the different genotypes were positively correlated with food concentrations (Pearson r = 0.8491, 0.7945, and 0.6886 for the correlations of N2, ADB::TeTx transgenic, and lim-4 mutant worms, respectively, Fig. 4A). Both the lim-4 lorf mutation and ADF::TeTx similarly reduced pumping rates under various food concentrations, especially in the absence of food (Fig. 4A and B). We then investigated the effects of interrupting functional connections in the feedback circuit on pumping under both well-fed and 8-h fasting conditions. The animals of various genotypes showed lower off-food than on-food pumping rates [r < 0.001 for all genotypes except tph-1 mutants; P < 0.05 for tph-1(mu280)]. Only the ADF-impaired animals, lim-4 and ser-6 mutants and ADF::TeTx transgenic worms, showed marked decreases in off-food pumping rates. In contrast, ADF::TeTx transgenic worms and tph-1(mu280) mutants displayed no changes or mild decreases in off-food pumping rates, and RIC-impaired animals [e.g., dbh-1 (encoding a tyramine β-hydroxylase that converts TA to OA) mutants and RIC::TeTx transgenic worms] showed significant increases in off-food pumping rates (Fig. 4C). RIC activation of ADFs relayed by AWBs is required for normal 5-HT synthesis and secretion in ADFs and for 5-HT-mediated fat loss (39). 5-HT production in ADFs is regulated not only by food sensory cues and feeding experience but also by internal nutrition status conveyed by kynurenic acid (14). We then tested the effects of feedback on ADF activity. First, we examined ADF Ca\textsuperscript{2+} signals without feedback under
various food concentrations. AWB::TeTx only significantly inhibited ADF Ca\(^{2+}\) signals in comparison with those in intact N2 worms (Fig. 3A) under a food concentration of 0.1 OD (Fig. 4D and E). The peak ADF Ca\(^{2+}\) signal intensities were well correlated with food concentrations (Pearson \(r = 0.9949\) and 0.9983 for the correlations of WT N2 and AWB::TeTx worms). Second, given that the ADF Ca\(^{2+}\) signals may represent only the excitatory responses to food, we used GFP fluorescence driven by the tph-1 promoter as an indicator of 5-HT production in ADFs, as suggested (39). We measured the GFP fluorescence in ADFs as described in SI Appendix, SI Materials and Methods. As expected, the ADF fluorescence intensity was significantly reduced in the mgs42; AWB::TeTx and mgs42; lim-4 worms under all food concentrations compared with that in the intact mgs42 animals. The intensities of GFP fluorescence in ADFs were well correlated with the bacterial concentrations [Pearson \(r = 0.8368, 0.9138, \) and 0.8907 for the correlations of mgs42, mgs42; AWB::TeTx, and mgs42; lim-4 (ky-403) worms, respectively, Fig. 4F]. All these results suggest that the feedback circuit is required for the homeostasis of pharyngeal pumping and 5-HT production in ADFs under various food concentrations and especially under conditions of starvation.

Discussion

In the present study, we dissected two neuronal circuits, a feedforward circuit and a feedback circuit, that are involved in pumping regulation in C. elegans (Fig. 5). ADFs are activated in a graded manner by E. coli OP50 bacteria at various concentrations. 5-HT released from ADFs inhibits RICs via MOD-1 signaling. RICs release OA to neurohormonally excite SIAs and AWBs via SER-3/SER-6 and SER-6 signaling, respectively. Interestingly, the feedforward and feedback circuits employ two distinct modalities of neurosignal integration or neuronal computation: disinhibition and disexcitation, respectively. Individual neurons of various types are the basic units of the nervous system, yet brain functions depend less on the variety of neurons than on their organization into anatomical and functional neurocircuits. Neurosignal transmission and integration are the basis for the functions of neurocircuits

(42). The identified modalities include excitatory and inhibitory connections, disinhibition or unmasking processes (43–46), reciprocal inhibition (9, 27, 47), and gate control (48, 49). Disexcitation, a modality suggested by the present study, has not been previously reported. Whether this paradigm works widely in the animal kingdom requires further study. Despite the great diversity from simple to complex species, animals, including humans, employ only a few evolutionarily conserved modalities of neural information integration (50, 51). We believe that disexcitation may play a common role and have general importance in animals.

5-HT released under conditions of food supply and OA secreted under conditions of food deprivation mediate feeding and fasting signals, respectively, which function in the regulation of behaviors, metabolism, life span, and development of the nervous system (14, 24, 33–36, 52, 53). 5-HT and OA mediate feeding and fasting signals to enhance and reduce C. elegans pumping activity, respectively, as supported by the results obtained tph-1 and tbh-1 loss of function and ADF::TeTx manipulation (Fig. 4C). Notably, the tph-1 mutation had a more severe effect on pumping than ADF::TeTx. This result and the full rescue effect of ADF::tph-1 (SI Appendix, Fig. S1A) suggest that ADF is a major source but likely not the only source of 5-HT that mediates feeding signals.

5-HT from different sources may be engaged in modulation of pharyngeal pumping and pumping dynamics under different environmental conditions (7, 9, 13). The discrepancies regarding the 5-HT sources acting in pumping regulation, for instance, ADFs (ref. 7 and this study) and NSMs (9, 13), might also be an artifact of the extrachromosomal overexpression of the tph-1 gene. 5-HT and OA functionally interact to regulate pumping through both feedforward and feedback circuits. Fascinatingly, these two circuits use the same upstream neurons and modulatory molecules. How do these two circuits perform different roles? This open question needs further study. One of the reasonable hypotheses is that SER-3 and SER-6 receptors may have high and low affinity for OA, respectively. SIAs express both SER-6 and SER-3, while AWBs only express SER-6. Under conditions of food supply, OA secretion by RICs is inhibited by ADFs. OA

Fig. 3. Identification of the neurotransmission modalities between neurons in the ADF-RIC-AWB-ADF feedback circuit. (A) Curves (Left) and average peak intensities (Right) of the somal Ca\(^{2+}\) signals in ADFs in WT worms under the administration of various concentrations (in OD) of E. coli OP50. (B–D) Curves (B and C) and average peak intensities (D) of the somal Ca\(^{2+}\) signals in the indicated worms in RICs under different concentrations of E. coli OP50. (E–J) Curves (Left) and Hill plots (Right) of the somal Ca\(^{2+}\) signals (peak values in the first 10 s of the on and off responses as indicated by the frame) in neurons in the indicated worms treated with the indicated chemicals at various concentrations. All data are expressed as the mean \(\pm\) SEM as indicated by solid traces or bars \(\pm\) gray shading or error bars. The number of tested worms for each genotype is indicated.
at low concentrations mainly binds with high-affinity SER-3 and mildly activates SIAs to maintain basal SIA activity that may suppress hyperactive pumping but may not be able to activate AWBs. Thus, SER-3 signaling in the feedback circuit enhances pumping via disinhibition of pumping suppression by SIAs under conditions of food supply (Fig. 5). The basal activity of SIAs should explain why RIC:TeTx transgenic worms showed increases in both on-food and off-food pumping rates. In contrast, under fasting conditions, RIC release of OA increases due to starvation and reduced inhibition of RICs by ADTs. OA at elevated concentrations strongly activates AWBs and SIAs via acting on SER-6 and on both SER-6 and SER-3, respectively. Thus, the functions of the feedback circuit via SER-6 signaling emerge and dominate in the modulation of off-food pumping and 5-HT production in ADTs (and perhaps also in other serotoninergic cells) under conditions of food deprivation (Fig. 5). This hypothetical explanation is supported by several findings. The ser-3 loss of function significantly reduced on-food pumping but had no obvious impact on off-food pumping, whereas SIA::TeTx greatly and mildly augmented on-food and off-food pumping, respectively (Fig. 1C). These results also suggest that SER-3 signaling may be maximized and that SER-6 signaling in the feedforward circuit is sufficient for pumping regulation under conditions of food deprivation. A recent report supports this hypothesis; that study indicated that AIB neurons use different receptors, namely, a low-threshold glutamate receptor with fast inactivation, GLR-1, and a high-threshold glutamate receptor with slow inactivation, GLR-5, to decode the different sensory signals of low and high concentrations of quinine from presynaptic ASHs (54).

5-HT is essential for animals. In C. elegans, as in mammals, 5-HT signaling modulates a wide array of behaviors and physiological activities, including foraging, mating, egg-laying, decision-making, cellular stress responses, and changes in metabolism and food intake behavior (reviewed in refs. 3 and 55). Survival of starvation in C. elegans requires the preservation of pharyngeal function under fasting conditions for subsequent feeding (3). Homeostasis of ADF activity is important. Our present study shows that the feedback circuit, which mainly functions under conditions of food deprivation, is required to maintain homeostasis of ADF 5-HT production and basal pumping activity. Inhibition of AWB neurotransmission by TeTx and developmental disruption of AWB by lim-4 mutation decreased pumping and 5-HT production in ADTs under various food concentrations. In particular, these inhibitory effects were much stronger under conditions of long-term food deprivation than under conditions of food supply at various concentrations (Fig. 4A and B). Interruption at other points of connection in the feedback circuit elicited complicated effects. For example, mod-1 mutants showed increased on-food and off-food pumping rates, which differed from the phenotype in tph-1 mutants, and RIC:TeTx transgenic worms displayed more severe defects in on- and off-food pumping rates than tbl-1 mutants (Fig. 4C). Given that mod-1 is expressed in multiple neurons including RICs and AYIs, serotoninergic MOD-1 signaling should act not only via RICs in pumping regulation. Our data show AYI::TeTx

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**Fig. 4.** Roles of the feedback circuit in the regulation of pharyngeal pumping and ADT activity. (A and B) Pumping rates (A) and percent decreases in pumping rates (B) in worms of the indicated genotypes cultured in solutions with various E. coli OP50 concentrations. (C) Pumping rates in the indicated worms under well-fed conditions and after 8 h of food deprivation. (D and E) Curves (D) and average peak intensities (E; WT data from Fig. 3A) of the somal Ca++ signals in ADTs in the indicated worms under various concentrations of E. coli OP50. (F) Fluorescence intensity of GFP in ADTs driven by the tph-1 promoter in the indicated worms. Statistical significance is indicated as follows: ns, not significant, *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001 compared with the value for WT N2 (A) or mgk42 worms (B), as indicated (B), or within a feeding condition (C).

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**Fig. 5.** Model of the neural circuits that regulate pumping and 5-HT production in food-sensing ADTs under conditions of food supply and food deprivation. In a well-fed state, increased 5-HT release from ADTs augments disinhibitory activity in the feedforward circuit to enhance pumping via suppression of OA secretion in RIC interneurons that results in reduced activity in SIA neurons. In a fasting state, reduced disinhibition in the feedback circuit maintains basal 5-HT production in ADTs, and reduced disinhibition in the feedforward circuit mediates pumping inhibition due to food deprivation signals conveyed by OA.
transgenic animals displayed augmented on-food bumping and unpaired off-food bumping (SI Appendix, Fig. S2A), suggesting AYs suppress pumping under feed conditions and are possibly inhibited by 5-HT. Why did RIC::TeTx and the tbh-1 ·LoF mutation affected pumping differently? One explanation is that RIC neurons may have released not only OA but also TA that inhibited serotoninergic NSM neurons. TeTx blocks the release of both OA and TA, while tbh-1 ·LoF mutation only impaired the production of OA. Although pharyngeal II interneurons are not essential for pharyngeal contraction when C. elegans is in the presence of food (22), they are required to maintain bustral pumping in the absence of food (56). Whether the II circuit and the feedback circuit identified in this study functionally interact or function independently is an open question.

Materials and Methods

For full details, see SI Appendix, SI Materials and Methods. The worms of various genotypes used in this study were day one adults. All pumping assays were performed at room temperature between 20 and 22 °C. The Ca²⁺ signals were measured by epi-fluorescence imaging, and other fluorescent imaging was performed by using a confocal fluorescence imaging system.

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