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 and nervous systems. To study the mechanisms of humoral regulation of feeding behavior, we investigated serotonin (5-HT) and octopamine (OA) signaling in *Caenorhabditis elegans*, 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 neuromodulators via ADF/RIC/SIA feedforward neurocircuit (consisting of ADF, RIC, and SIA neurons) and ADF/RIC/AWB/ADF feedback neurocircuit (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 SIAs 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.

C. elegans | pharyngeal pumping | serotonin | octopamine | disexcitation

Feeding is a fundamental behavior for animal survival and is tightly regulated by the sensory, endocrine, and central nervous systems. Aberrant 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, humoral factors, internal nutrition status, and external cues (refs. 5, 9, 13, and 14; commented and reviewed in refs. 3, 10, and 11). Neuromodulatory signals play important roles in pumping regulation. Two neuroendocrine factors, serotonin (5-hydroxytryptamine, 5-HT) and tyramine (TA) [and its metabolite octopamine (OA, an invertebrate counterpart of noradrenaline)], act contrarily to modulate pumping (7, 9, 13, 15–20). 5-HT, a putative food signal, promotes pumping, whereas OA and TA inhibit this behavior. 5-HT acts directly on MC and M3 pharyngeal motor neurons to enhance pumping (18, 21–23). OA released from RICs during food deprivation activates cholinergic SIAs through SER-3 and SER-6 receptors (24, 25), suggesting that OA possibly acts on SIAs to regulate pumping. 5-HT secretion by food-sensing ADFs is regulated by FLP-18/NPR-5 signaling, which conveys the internal nutrient state (14). Previous evidence suggests the existence of 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 serotonergic 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.

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

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 serotonergic 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.

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^{2+} transients in response to the food-derived sensory cue *Escherichia coli* OP50 supernatant as suggested (26), as measured by GCaMP3 Ca^{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 HisCl1 channels and 10 mM histamine (neuron chemogenetic inhibition in short). HisCl1 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. 1*A*). 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. 1*B* 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* lof mutants exhibited decreased pumping rates under food deprivation conditions (8 h). Extrachromosomal expression of *ser-3* and *ser-6* in SIAs and AWBs alone, respectively, restored wild-type pumping; furthermore, the results of Ca^{2+} imaging and neuron-specific manipulation with TeTx and HisCl1 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* lof mutation eliminated the effects of 5-HT and OA on the food cue-elicited Ca^{2+} transients of off responses in AWBs (*SI Appendix*, Fig. S3 *A* and *B*). In addition, RIC chemogenetic inhibition reduced the AWB Ca^{2+} signals (Fig. 2*A*). However, unexpectedly, chemogenetically activating RICs with TRPV1 and 50 μM exogenous capsaicin (neuron chemogenetic activation in short) did not significantly affect the AWB Ca^{2+} signals (Fig. 2*B*). AWBs have a chemosynaptic 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^{2+} signals but also blocked the effect of exogenous OA on ADF Ca^{2+} signals (Fig. 2*C*). AWB chemogenetic inhibition reduced the ADF Ca^{2+} signals (Fig. 2*D*), while AWB chemogenetic activation increased the ADF Ca^{2+} signals (Fig. 2*E*). These results indicate that
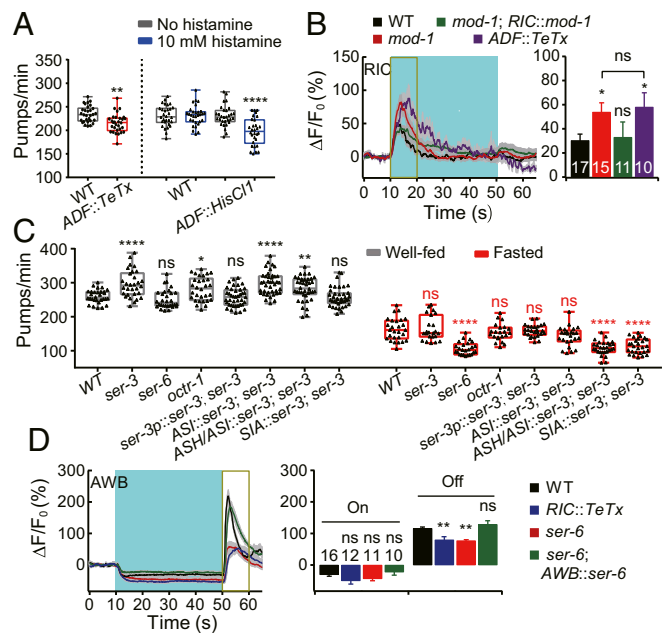

Fig. 1. ADFs augment pharyngeal pumping possibly via two neuronal circuits. (*A*) Pumping rates in worms of different genotypes as indicated treated with or without the indicated chemicals. (*B*) Curves (*Left*) and average peak intensities (*Right*) of the somal Ca^{2+} transients in RIC interneurons 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^{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.

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^{2+} signals (Fig. 2 *F* and *G*). However, RIC chemogenetic activation did not significantly affect ADFs (Fig. 2*H*), similar to the case for AWBs (Fig. 2*B*). 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^{2+} transients (Fig. 2*I*). Moreover, *RIC::TeTx* or *AWB::TeTx* not only decreased the ADF Ca^{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. S3*C*) and that the *lim-4* lof mutation resulted in a lack of AWB neurons (41), we dosed the *RIC::TeTx* and *AWB::TeTx* transgenic worms and *ser-6(tm2146)* and *lim-4(ky403)* mutants with 4 μM 5-HT and examined the effects on pumping. As expected, either the *lim-4* or *ser-6* mutation and *AWB::TeTx* or *RIC::TeTx* eliminated the inhibitory effect of 4 μM 5-HT on pumping (Fig. 2*L*). 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 neurosignal transmission 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
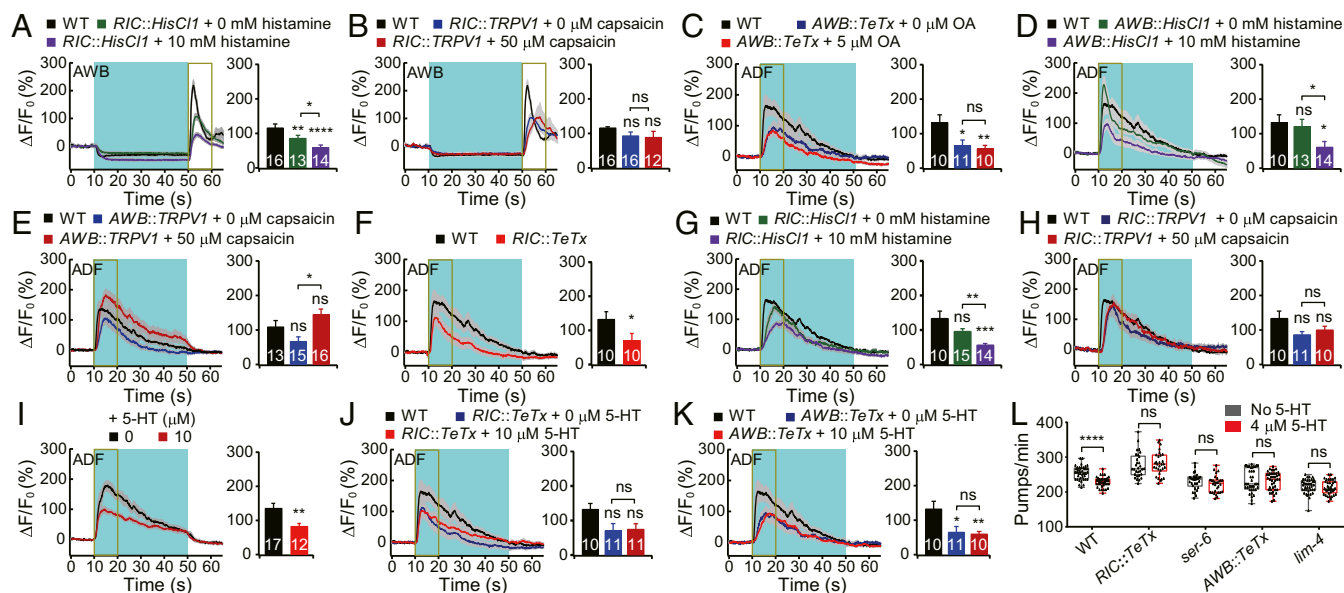
Fig. 2. Top-down feedback circuit consisting of RIC, AWB, and ADF neurons. (*A–K*) Curves (*Left*) and average peak intensities (*Right*) of the food cue-evoked somal Ca²⁺ signals in AWBs (*A* and *B*) and ADFs (*C–K*) in worms of the indicated genotypes treated with or without chemicals as indicated. (*L*) Pumping rates in the indicated worms treated with or without exogenous 5-HT (4 µM). 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. S4*A*). 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 disexcitatory feedback regulation. We herein provide data to support this hypothesis. First, ADFs displayed graded Ca²⁺ responses to *E. coli* OP50 at various concentrations (indicated by the optical densities). The peak intensities of ADF Ca²⁺ signals were well correlated with the bacterial concentrations (Pearson *r* = 0.9949, Fig. 3*A*). Second, RICs also exhibited graded Ca²⁺ responses to bacteria at various concentrations (Fig. 3*B*). The activation of RICs may be a result of neurotransmission from other neurons because the RIC Ca²⁺ signals were significantly reduced in both *unc-13(e1091)* and *unc-31(e928)* mutants (*SI Appendix*, Fig. S4*B*). *ADF::TeTx* augmented the RIC Ca²⁺ responses to food at various concentrations. The peak intensities of RIC Ca²⁺ 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. 3 *C* and *D*). Furthermore, treatment of *ADF::TeTx* transgenic worms with exogenous 5-HT at various concentrations dose-dependently decreased the OP50 supernatant-elicited RIC Ca²⁺ signals (well-fitted by a Hill function with EC₅₀ = 0.31 µM, Fig. 3*E*), 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 AWB Ca²⁺ signals with a dose–response relationship well-fitted by a Hill function with an EC₅₀ of 0.87 µM (Fig. 3*F*). In addition, graded down-regulation of RICs with HisCl1 channels and histamine at various doses dose-dependently decreased the AWB Ca²⁺ transients (Hill function EC₅₀ = 0.67 mM, Fig. 3*G*). Moreover, application of exogenous 5-HT at various concentrations to the *ADF::TeTx* transgenic worms also dose-dependently reduced the AWB Ca²⁺ signals according to a Hill function with an EC₅₀ of 0.38 µM (Fig. 3*H*). Finally, dosing N2 worms and *tph-1(mg280)* mutants with exogenous 5-HT at various concentrations diminished the ADF Ca²⁺ signals in a graded manner, with EC₅₀ values of 0.29 µM and 0.39 µM, respectively (Fig. 3 *I* 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 disexcites the tonic activation of ADFs by RICs, suggesting disexcitatory neurosignal 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, *AWB::TeTx* transgenic, and *lim-4* mutant worms, respectively, Fig. 4*A*). Both the *lim-4* lof mutation and *AWB::TeTx* similarly reduced pumping rates under various food concentrations, especially in the absence of food (Fig. 4 *A* 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 [*P* < 0.0001 for all genotypes except *tph-1* mutants; *P* < 0.05 for *tph-1(mg280)*]. Only the AWB-impaired animals, *lim-4* and *ser-6* mutants and *AWB::TeTx* transgenic worms, showed marked decreases in off-food pumping rates. In contrast, *ADF::TeTx* transgenic worms and *thp-1(mg280)* mutants displayed no changes or mild decreases in off-food pumping rates, and RIC-impaired animals [e.g., *tbh-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. 4*C*). 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 Ca²⁺ signals without feedback under
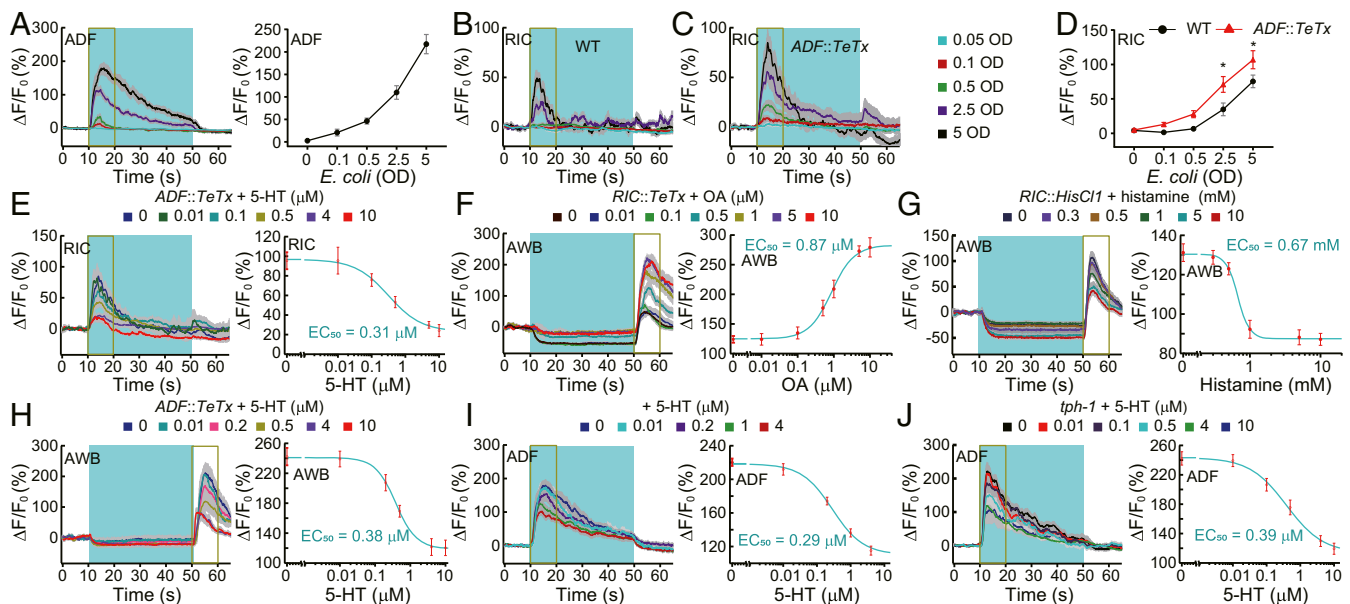
Fig. 3. Identification of the neurotransmission modalities between neurons in the ADF-RIC-AWB-ADF feedback circuit. (*A*) Curves (*Left*) and average peak intensities (*Right*) of the somal Ca²⁺ signals in ADFs in WT worms under the administration of various concentrations (in OD) of *E. coli* OP50. (*B*–*D*) Curves (*B* and *C*) and average peak intensities (*D*) of the somal Ca²⁺ 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 Ca²⁺ signals (peak values in the first 10 s of the on or 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 ± SEM as indicated by solid traces or bars ± gray shading or error bars. The number of tested worms for each genotype is indicated.

various food concentrations. *AWB::TeTx* only significantly inhibited ADF Ca²⁺ signals in comparison with those in intact N2 worms (Fig. 3*A*) under a food concentration of 5 OD (Fig. 4 *D* and *E*). The peak ADF Ca²⁺ 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 Ca²⁺ 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 *mgIs42; AWB::TeTx* and *mgIs42; lim-4* worms under all food concentrations compared with that in the intact *mgIs42* 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 *mgIs42*, *mgIs42; AWB::TeTx*, and *mgIs42; lim-4* (*ky403*) worms, respectively, Fig. 4*F*]. 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 neurohumorally 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* lof mutation and *ADF::TeTx* manipulation (Fig. 4*C*). 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. S1*A*) 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
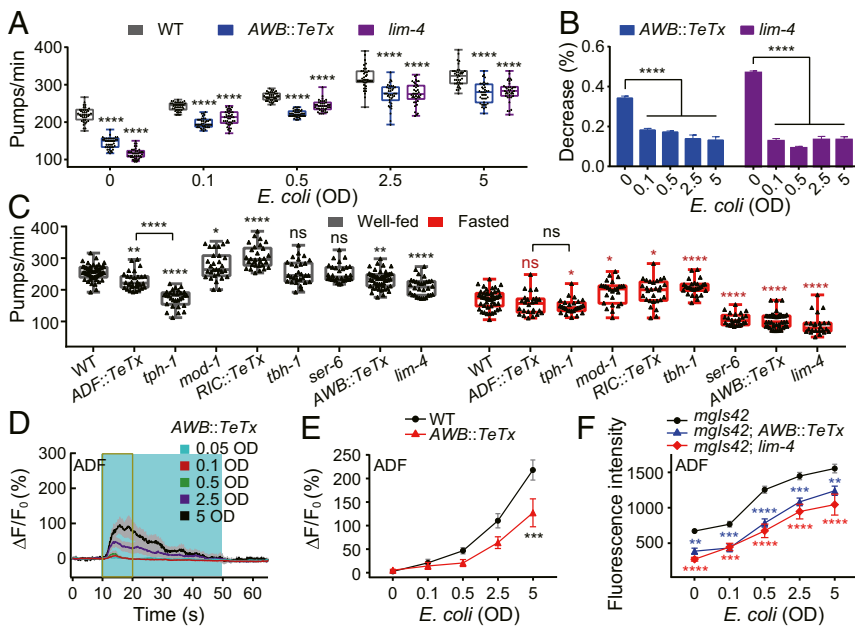
Fig. 4. Roles of the feedback circuit in the regulation of pharyngeal pumping and ADF activity. (*A* and *B*) Pumping rates (*A*) and percent decreases in pumping rates (*B*) in worms of the indicated genotypes cultured in solutions with various *E. coli* OP50 concentrations. (*C*) Pumping rates in the 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. 3*A*) of the somal Ca^{2+} signals in ADFs in the indicated worms under various concentrations of *E. coli* OP50. (*F*) Fluorescence intensity of GFP in ADFs 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 *mgIs42* worms (*D*), as indicated (*B*), or within a feeding condition (*C*).

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 be not able to activate AWBs. Thus, SER-3 signaling in the feedforward 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 ADFs. 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 ADFs (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* lof mutation 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. 1*C*). 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 ADFs 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. 4 *A* 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 *thp-1* mutants, and *RIC::TeTx* transgenic worms displayed more severe defects in on-food and off-food pumping rates than *tbh-1* mutants (Fig. 4*C*). Given that *mod-1* is expressed in multiple neurons including RICs and AIYs, serotoninergic MOD-1 signaling should act not only via RICs in pumping regulation. Our data show *AIY::TeTx*
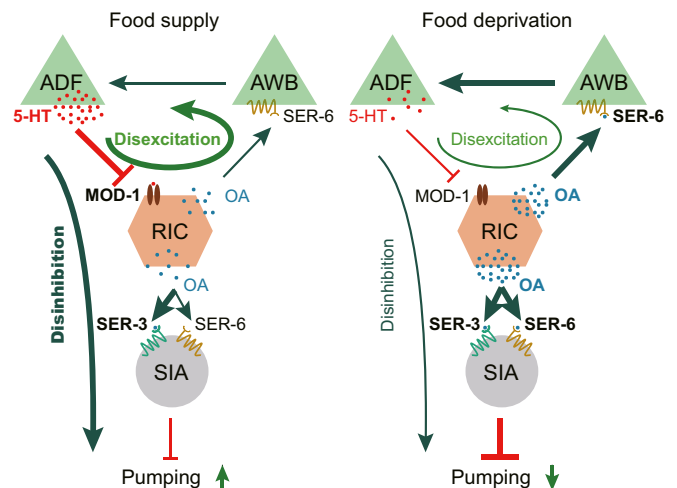

Fig. 5. Model of the neural circuits that regulate pumping and 5-HT production in food-sensing ADFs under conditions of food supply and food deprivation. In a well-fed state, increased 5-HT release from ADFs augments disinhibitory activity in the feedforward circuit to enhance pumping via suppression of OA secretion in RIC interneurons that results in reduced activity in SIA neurons. In a fasting state, reduced disexcitation in the feedback circuit maintains basal 5-HT production in ADFs, 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 unimpaired off-food pumping (*SI Appendix*, Fig. S2*A*), suggesting AIYs 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 impairs the production of OA. Although pharyngeal I1 interneurons are not essential for pharyngeal contraction when *C. elegans* is in the presence of food (22), they are required to maintain basal pumping in the absence of food (56). Whether the I1 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 Ca^{2+} signals were measured by epi-fluorescence imaging, and other fluorescent imaging was performed by using a confocal fluorescence imaging system.

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