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Behavioral choice by presynaptic inhibition of tactile sensory terminals

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When presented with multiple stimuli, animals generally choose to respond only to one input. The neuronal mechanisms determining such behavioral choices are poorly understood. We found that the medicinal leech had greatly diminished responses to moderate mechanosensory input as it fed. Feeding dominated other responses by suppressing transmitter release from mechanosensory neurons onto all of their neuronal targets. The effects of feeding on synaptic transmission could be mimicked by serotonin. Furthermore, the serotonin antagonist mianserin blocked feeding-induced decreases in synaptic transmission. These results indicate that feeding predominates behaviors by using serotonin at an early stage of sensory processing, namely on presynaptic terminals of mechanosensory neurons.

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An animal's behavioral state strongly influences decision-making. For example, attention, sleep, territorial status, reproductive state and hunger all heavily modulate neural responses to auditory[1], olfactory[2], tactile and nociceptive inputs[3]. This sensory gating allows animals to execute appropriate behaviors. Clearly, behavioral choice and behavioral state are inextricably linked, but the neural substrates for this association are not well understood.

Such interactions of behaviors are commonly studied using choice competition. Because animals typically perform only one behavior at a time[4], an animal that is presented with two stimuli, each of which elicits a different behavior when presented alone, will usually only perform one of them. By presenting different stimuli pairs, one can rank any number of behaviors into a hierarchy, from most likely to least likely[5]. Behavioral choice at the network level has been studied using animals such as the sea slug *Pleurobranchaea*, the pond snail *Lymnaea* and the leech *Hirudo*[6]. Such studies have found that one mechanism for making choices is for behaviorally relevant neurons to recruit or silence interneurons involved in another behavior's generation[7,8]. We found that leech feeding suppresses all touch-induced behaviors by employing a different neuronal mechanism: presynaptic inhibition of sensory terminals. Although presynaptic inhibition is a common mechanism for sensory gating[9,10], it has never been implicated in decision-making between qualitatively different behaviors.

In leeches, feeding tops the behavioral hierarchy[11]. Stimuli eliciting swimming, crawling, shortening or local bending in a quiescent leech have no effect while a leech feeds. Because this feeding is so robust, to the marked exclusion of responding to other sensory stimulation, we investigated the neuronal mechanisms by which feeding suppresses other behaviors in the leech (**Fig. 1a**). We specifically studied how feeding inhibits the local bend reflex (**Fig. 1b**) that is activated by the P cells, a small collection of segmentally iterated pressure-sensitive mechanosensory neurons that innervate the leech's skin. Feeding presynaptically inhibited the synaptic transmission from the P cells to many of their postsynaptic targets, and this presynaptic inhibition was accomplished largely via serotonin.

RESULTS

Feeding decreased motor responses to nerve stimulation

To study how feeding inhibits mechanically elicited behaviors, we employed a semi-intact preparation that has previously been used to study feeding in leeches[12]. We removed the body wall and inner organs from the posterior half of a leech, leaving only the nervous system intact (**Fig. 1c,d**). The anterior end of the leech was able to behave while we recorded from posterior segmental ganglia. Leeches typically remained at rest until stimulated. We presented them with a tube of blood covered by a membrane of sheep intestine and recorded from dorsal posterior nerves in the exposed nerve cord. Because each dorsal posterior nerve contains the afferent axon of a P cell and the motor efferent of a dorsal longitudinal excitatory motor neuron, cell DE-3, we could elicit fictive local bending by stimulating one dorsal posterior nerve while recording motorneuron output from the contralateral dorsal posterior nerve of the same ganglion (**Fig. 2a**).

Consistent with previous findings that feeding suppresses mechanically elicited behaviors in intact leeches[11], feeding in our semi-intact preparations decreased the firing rate of cell DE-3 (**Fig. 2b**). Integrating the spike count within 5 s of the stimulus revealed a 45% decrease in the spike number elicited during feeding compared with the number in the nonfeeding state ($P < 0.001$ and $P < 0.01$, repeated-measures ANOVA, $n = 11$ and 5 for the first and second feeding sessions, respectively; **Fig. 2c**).

Feeding decreased sensory input into the leech ganglion

To determine how feeding suppresses local bending behavior, we looked for changes at the earliest level of the local bend circuit, the synapse between the P cells and a representative local bend interneuron, cell 212 (ref. 13). In semi-intact preparations, we

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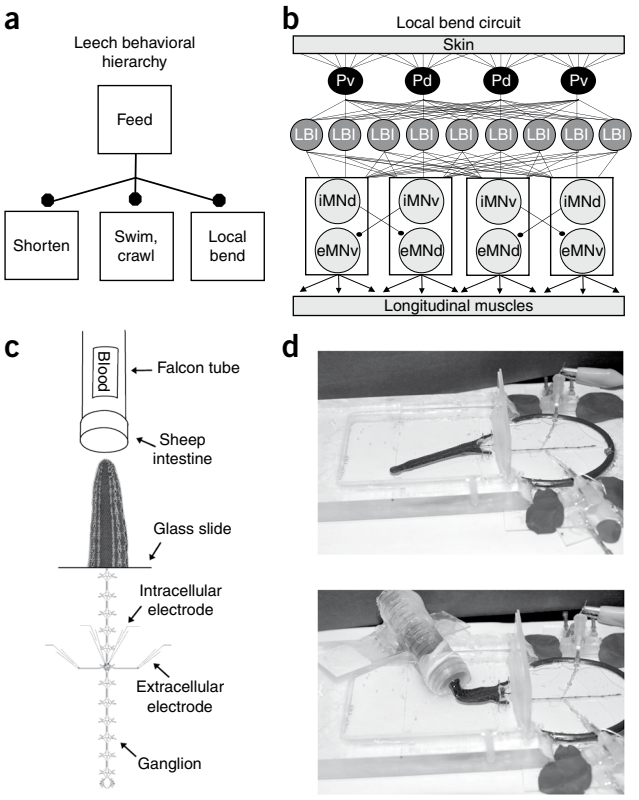

Figure 1 Leech behavior and the semi-intact preparation. (**a**) Feeding suppresses all motor behaviors in the medicinal leech. Black circles represent suppression. (**b**) Schematic diagram showing the connections in the local bend circuit, consisting of P cells, local bend interneurons (LBI), inhibitory motor neurons (iMN) and excitatory motor neurons (eMN) (v, ventral; d, dorsal). (**c**) Drawing of a semi-intact leech and the feeding apparatus with stimulating and recording suction electrodes on dorsal posterior nerves of ganglion 15. Two intracellular electrodes are also shown directed at neurons in ganglion 15. A plastic divider separated the anterior behavioral chamber from the posterior physiology chamber. The front end was fully intact and pinned down in denervated pieces of skin at the cut posterior end. The exposed nerve cord traversed the chambers through a slit in the plastic divider. Petroleum jelly was used to fill any gaps in the barrier between the chambers. We presented blood in a 15-mL Falcon tube with its open end covered by a piece of sheep intestine purchased as sausage casing. (**d**) Pictures of a semi-intact preparation attached to the bottom of its recording chamber by its front sucker (top) and attached to the sheep intestinal membrane as it feeds (bottom).

stimulated P cells to fire an action potential every 20 s while recording excitatory postsynaptic potential (EPSP) amplitudes from the postsynaptic neurons, as well as simultaneously recording from the dorsal posterior nerves to monitor motor output (**Supplementary Fig. 1**). Every 3 min, we produced a train of action potentials in the P cell that mimicked a natural stimulus; this burst of spikes elicited a fictive local bend response in the dorsal posterior nerves. We also monitored each cell's intrinsic excitability by determining either its spike threshold or the number of spikes elicited in it by a standard current injection.

Although feeding did not affect any intrinsic property of the P cells or cell 212 (**Supplementary Fig. 2**), it caused large decreases in the EPSPs from P cells onto cell 212 (**Fig. 3a**). The average decrease was nearly 65% ($P < 0.001$, repeated-measures ANOVA, $n = 10$; **Fig. 3b**). Postfeeding values were recorded once EPSP amplitudes stabilized after feeding was terminated, at 6.6 ± 1.0 min.

To determine whether the synaptic depression at P-cell synapses was restricted to the local bend circuit, we repeated our experiments on several neurons that are postsynaptic to the P cell, but are known to not be involved in the local bend circuit, including the S cell, cell Tr1 and the AP neuron. The S cell is activated during shortening[14], cell Tr1 triggers bouts of swimming[15], and although the function of the AP neuron is unknown, it does not participate in these behaviors[13]. The AP neuron showed minimal changes in intrinsic excitability (**Supplementary Fig. 1**), but substantial decreases in EPSP amplitude during feeding (**Fig. 3a**), by an average 57% ($P < 0.001$, repeated-measures ANOVA, $n = 10$, recovery time = 7.5 ± 1.1 min; **Fig. 3c**). We also tested the synapse between the P and S cells. This synapse underwent similar depression ($n = 5$, $P < 0.001$; **Fig. 3d**). We wanted to test the P-cell synaptic input onto the swim trigger neuron, cell Tr1,

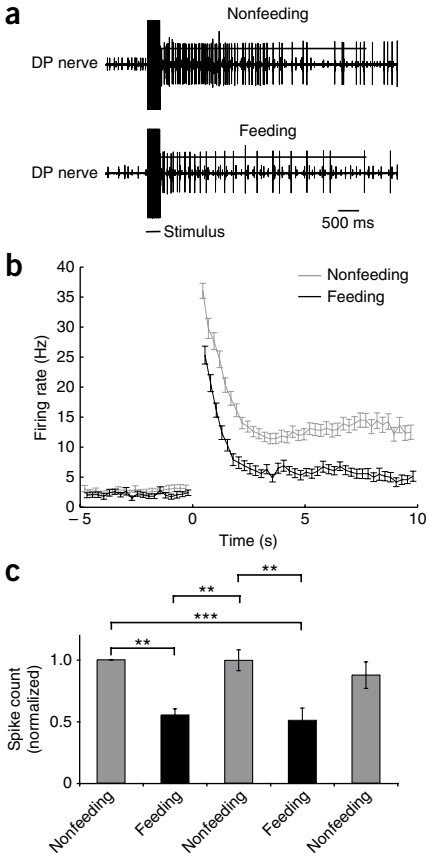

Figure 2 Local bend motor responses during feeding and nonfeeding. (**a**) Sample traces of dorsal posterior (DP) nerve responses to contralateral nerve stimulation before and during feeding. Any spike that crossed the threshold (horizontal lines) was counted. The stimulus lasted 250 ms and was delivered every 3 min. (**b**) Firing frequency of the dorsal posterior motor units during feeding and nonfeeding. Nerve stimulation occurred at 0 s. Bin duration was 250 ms. (**c**) Histogram of normalized spike counts in three sessions of nonfeeding and two sessions of feeding. The responses in each leech were normalized to the mean of the three responses during the first nonfeeding trial for that leech. One of the dorsal posterior nerves in each leech was shocked three times in each condition and the mean of these trials are shown (*** $P < 0.001$, ** $P < 0.01$, repeated-measures ANOVA comparing all nonfeeding trials to all feeding trials; $n = 11$ for the first feeding session and $n = 5$ for the second feeding session). The only significant differences were between the feeding and nonfeeding conditions. The last nonfeeding condition was not statistically different from either feeding bout ($P > 0.05$, repeated-measures ANOVA).

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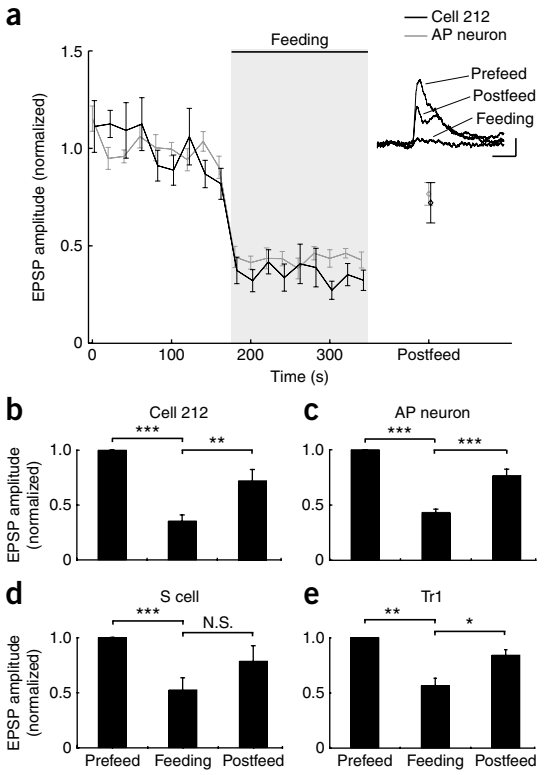

Figure 3 Feeding decreases the amplitudes of EPSPs at P cell synapses. (**a**) Plot of EPSP amplitude in cell 212 and the AP neuron produced by individual P-cell spikes. We recorded for 3 min in both the prefeeding and feeding states (gray shaded box) while eliciting a P-cell spike every 20 s. Postfeeding values were the average of the last nine recordings made after the EPSP amplitudes stabilized, which was 6.6 ± 1.0 min and 7.5 ± 1.1 min after feeding was interrupted for cell 212 and the AP neuron, respectively. Insert, sample traces of the EPSP in cell 212 elicited by P-cell action potentials during prefeeding, feeding and postfeeding periods. Scale bars represent 2 mV and 100 ms. We only used the initial peaks were used to calculate EPSP amplitudes. (**b**–**e**) Summary data for changes in EPSP amplitudes measured in cell 212 (a local bend interneuron), AP neuron (of unknown function), S cell (related to shortening) and swim-trigger neuron Tr1 in the prefeeding, feeding and postfeeding periods (*** $P < 0.001$, ** $P < 0.01$, $n = 10$ for cell 212 and AP neuron and $n = 5$ for S cell and Tr1). N.S., not significant ($P > 0.05$, repeated-measures ANOVA comparing prefeeding to feeding and feeding to postfeeding values).

but, as this neuron's soma is located in the leech head brain, it was inaccessible using our standard preparation. Therefore, we used a more reduced preparation in which only the leech's dorsal lip was intact and the entire nervous system, including the head brain, was isolated (**Supplementary Fig. 3**)[16]. Applying blood serum to the preparations' lips decreased EPSP amplitudes between P cells and Tr1 neurons by 44% ($n = 5$, $P < 0.001$; **Fig. 3e**). Synaptic depressions at these synapses paralleled decreases in dorsal posterior nerve spikes in response to single P-cell spikes (for ipsilateral nerve, $P < 0.05$) and trains of P-cell spikes taken simultaneously during AP neuron recordings ($P < 0.01$ for ipsilateral and contralateral nerves).

To test for presynaptic contributions to the synaptic depression observed during feeding, we measured paired-pulse ratios (PPRs) at two P-cell synapses. Any change in PPR is a strong indication that presynaptic transmitter release has been modified[17] (Online Methods and **Supplementary Fig. 4**). We used cells AP and Tr1 because their soma are much larger than those of cell 212 and the S cell, and they stand up well to the long-lasting recordings that these tests required. In semi-intact leeches, feeding caused the PPR to rise during synaptic depression at the P cell–to–AP neuron synapse (**Fig. 4a**). We calculated PPRs by measuring the EPSP amplitudes in response to pairs of P-cell spikes at 500-ms interpulse intervals, each minute. Both synaptic potentials decreased during feeding, but the first decreased more than the second, increasing the PPR. The EPSP amplitude decreased ($P < 0.001$, $n = 9$; **Fig. 4b**) and the PPR increased ($P < 0.01$, $n = 9$; **Fig. 4c**) during feeding. At the P cell–to–Tr1 cell synapse, serum application to the isolated-lip preparation produced a significant decrease in the EPSP amplitude (**Fig. 4d**) and an increase in the PPR ($P < 0.001$, $n = 5$) (**Fig. 4e**). We conclude that feeding produces presynaptic inhibition of all P-cell terminals, reducing the amplitude of the EPSPs that P cells make onto a variety of target neurons.

Downstream interneurons remained unaffected during feeding

To test whether feeding affects only the pressure mechanosensory P cells and leaves downstream neurons unaffected, we stimulated

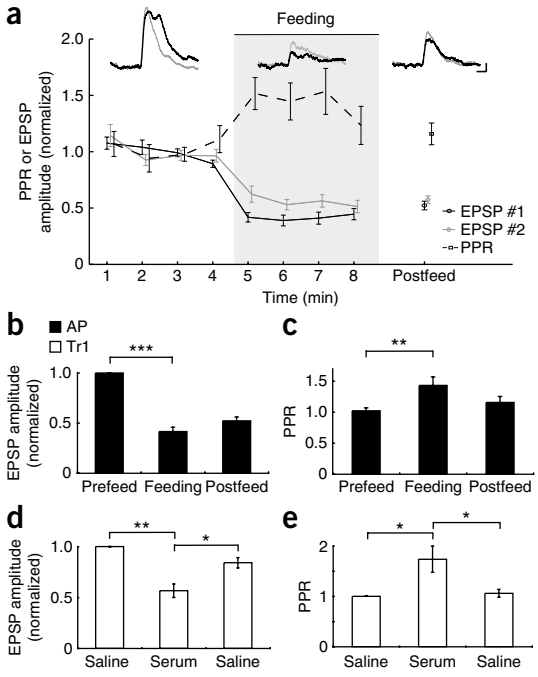

Figure 4 Increased PPR during feeding suggests a decrease in the probability of transmitter release at P-cell terminals. (**a**) Effects of feeding on EPSP amplitudes and PPR at the P cell–to–AP neuron synapse. First and second EPSP amplitudes of each pair of pulses are connected by solid black and gray lines, respectively. The data points connected by the dashed line correspond to the ratios between the responses to the two pulses (second/first). Inserts show an overlap of sample traces from one such experiment, with the same conventions. Scale bars represent 2 mV and 50 ms. We used an interspike interval of 500 ms for each pair of pulses. Postfeeding values are the means of the four recordings made after EPSPs stabilized (5.3 ± 0.66 min after the termination of feeding). (**b**,**c**) Summary data for changes in first EPSP amplitude (**b**) and PPR (**c**) measured before, during and after feeding in the AP neuron. Postfeeding values are not statistically different from values obtained during feeding ($P > 0.05$, repeated-measures ANOVA comparing prefeeding to feeding and feeding to postfeeding values). (**d**,**e**) Summary data collected from Tr1 before, during and after application of blood serum to an isolated lip preparation showing changes in EPSP amplitude (**d**) and PPR (**e**). Data shown in **d** are the same as those shown in **Figure 3e** ($n = 5$). *** $P < 0.001$, ** $P < 0.01$ and * $P < 0.05$.

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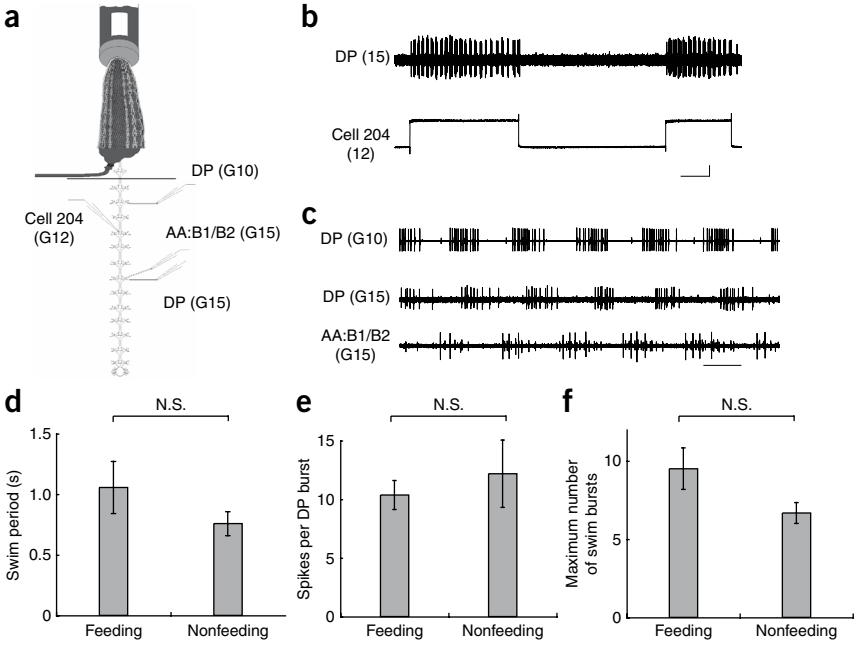

Figure 5 Stimulation of swim-gating neurons elicits bouts of swimming during feeding. (**a**) A schematic diagram of semi-intact feeding preparation showing the sites of intracellular stimulation and extracellular recordings. Leeches were fed on warmed bovine serum. Cell 204 was impaled in one ganglion between ganglia 11 and 14. Dorsal posterior nerve recordings were made anterior and posterior to the impaled cell 204 to assure that swimming propagated throughout the CNS. Dorsal posterior nerves contained the axon of a dorsal excitor motorneuron (DE-3) and spiking indicated the dorsal contraction phase of each swim cycle, whereas the largest spikes in AA:B1/B2 nerve were from the axon of a ventral excitatory motor neuron (cell 108) and indicate the ventral contraction phase of each swim cycle. (**b**) Depolarization of cell 204 with a 2-nA current resulted in swim pattern generation in the dorsal posterior nerve recorded in ganglion 15. Traces were recorded while the anterior end of the leech was feeding from the serum tube. Four leeches stimulated in this manner showed swimming activity during feeding. The vertical scale bar represents 50 mV and the horizontal scale bar represents 5 s. (**c**) Sample traces of three nerves taken during a swimming bout initiated by stimulation of cell 204 in ganglion 12 during feeding. We found an anterior-to-posterior propagation of the dorsal contractions down the nerve cord of the leech in dorsal posterior nerves 10 and 15. The antiphase spike bursts seen between dorsal posterior (ganglion 15) and AA:B1/B2 (ganglion 15) revealed the alternation between dorsal and ventral contractions seen in a segment in intact swimming leeches. Scale bar represents 500 ms. (**d**) Period of swimming as measured in dorsal posterior nerve recordings in feeding and control nonfeeding leeches. (**e**) The number of spikes per burst in the dorsal posterior nerve in feeding and nonfeeding preparations are shown. (**f**) The maximum numbers of swim bursts that could be induced by cell 204 stimulation in feeding and nonfeeding preparations are shown ($n = 4$ feeding leeches and $n = 3$ nonfeeding leeches for **d**, **e** and **f**). N.S., not significant ($P > 0.05$, Student's t test).

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interneurons that are responsible for the initiation of behaviors during feeding. We used the well-characterized swim circuit[18,19], as swimming is readily initiated by single-interneuron activation[15,20], unlike the local bend circuit, which requires the activation of a pool of interneurons. Cell 204 (which is directly postsynaptic to Tr1) is readily found in ganglia 10 through 16 and provides tonic excitation that drives swimming bursts in isolated nerve cords and semi-intact preparations[20]. We tested whether cell 204's ability to initiate swimming was affected by feeding (**Fig. 5a**). We impaled a single cell 204 in segments 11–14 while the anterior end of the leech fed and monitored swimming activity using nerve recordings from ganglia both anterior and posterior to the impaled cell 204 to ensure that swimming propagated along the nerve cord in both directions. As in nonfeeding preparations, stimulating a cell 204 initiated the swimming motor program and maintained it as the cell 204 remained depolarized (**Fig. 5b**). Swimming was elicited in all four of the preparations during feeding, and the same level of current injection (1–2 nA, 204 firing rate = ~64 Hz) was sufficient to elicit swimming in the feeding ($n = 4$) and the nonfeeding conditions ($n = 3$).

The swimming activity was normal as measured by anterior-posterior delays (compare dorsal motor neuronal bursts in the dorsal posterior nerve from ganglion 10 to the dorsal bursts in the DP nerve from ganglion 15), as well as by the alternation between dorsal and ventral contraction in a ganglion (compare dorsal motor neuronal bursts in the dorsal posterior nerve from ganglion 15 with the ventral bursts from the AA:B1/B2 nerve in the same ganglion) (**Fig. 5c**). No difference was found in the swim period (**Fig. 5d**) or the number of cell DE-3 spikes per burst in the dorsal posterior nerve (**Fig. 5e**). Swimming was maintained for

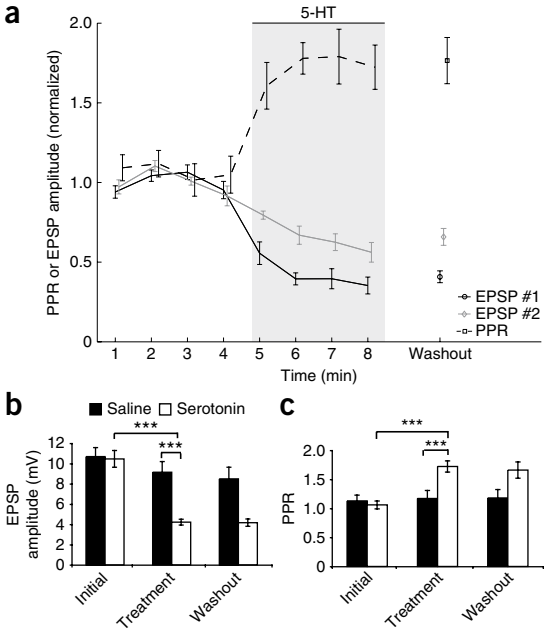

Figure 6 Serotonin decreases the P cell–to–AP neuron synaptic potential amplitudes. (**a**) EPSP amplitude and PPR, measured every minute in isolated ganglia. Application of serotonin (gray area) decreased the EPSP amplitude and increased the PPR. (**b**,**c**) Graphs comparing EPSP amplitudes (**b**) and PPR (**c**) in isolated ganglia bathed in saline or saline plus serotonin ($n = 8$ for both). Washout values were not statistically significant from treatment values ($P > 0.05$, repeated-measures ANOVA comparing prefeeding to feeding and feeding to postfeeding values). *** $P < 0.001$, ** $P < 0.01$.

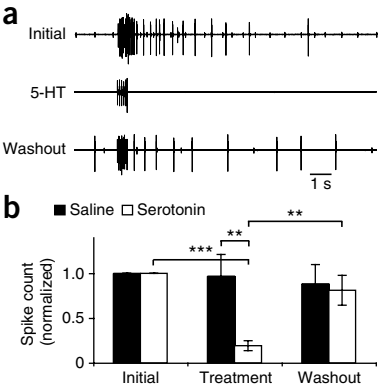

Figure 7 Serotonin mimics feeding by decreasing local bending. (**a**) A train of five spikes elicited in the P cell caused a fictive local bend response in the ipsilateral dorsal posterior nerve (top trace). Bath application of serotonin to the saline reduced the amount of motor activity generated (middle trace), an effect that was reversed by reapplication of saline (bottom trace). (**b**) Average spike counts in fictive local bending before, during and after the application of 50 μM serotonin. In the control treatment, we replaced the saline solution ($n = 12$ for the serotonin treatment and $n = 4$ for the saline control). In this case, washing out the serotonin solution with leech saline returned the spike count to a level that was not different from initial conditions, but was different from treatment values ($P < 0.01$). *** $P < 0.001$, ** $P < 0.01$.

an equal number of cycles in feeding and nonfeeding leeches (**Fig. 5f**). These data indicate that feeding has no measurable effect on the swim circuitry from swim gating neurons through central pattern-generating interneurons. These results imply that feeding suppresses other behaviors mainly by decreasing synaptic transmission from the mechanosensory neurons onto their targets.

Serotonin mimicked feeding effects at P-cell synapses

We tested several neuromodulators to determine whether they depressed the P cell–to–AP neuron synapse while increasing the PPR and suppressed fictive local bending. These modulators were dopamine[21] (50 μM), myomodulin[22] (50 μM), GABA[9] (50 μM) and serotonin[23] (50 μM). Of these, only serotonin reliably met both criteria.

Serotonin presynaptically depressed EPSP amplitudes at the P cell–to–AP neuron synapse (**Fig. 6a**), decreasing the mean first EPSP amplitude to 42% of its original size ($P < 0.001$, $n = 8$). This serotonin effect was long-lasting and difficult to reverse by washout. Because washout previously restored EPSP amplitudes in semi-intact feeding preparations (**Figs. 3** and **4**), we believe that this prolonged effect of applied serotonin may have resulted from the combination of serotonin and the PPR protocol. We observed that the higher frequencies of stimulation that we used to test PPR could produce more pronounced synaptic depression than did single pulses. Pilot studies using single pulses (as in **Fig. 3**) showed a more complete return to baseline (**Supplementary Fig. 5**). Nevertheless, control ganglia were employed to rule out the possibility that a loss of preparation viability was responsible for the synaptic depression. These ganglia were matched for initial EPSP amplitudes (10.6 ± 0.88 mV in control preparations versus 10.4 ± 0.82 in experiments) and they received the same P-cell stimulation protocol, but were never exposed to serotonin. Ganglia exposed to serotonin had significantly smaller EPSPs than control ganglia ($P < 0.001$, $n = 8$; **Fig. 6b**), and their PPRs were significantly increased ($P < 0.001$, $n = 8$; **Fig. 6c**).

To measure the effect of serotonin on the local bend motor output, we recorded extracellularly from dorsal posterior nerves of isolated ganglia (**Fig. 7a**). Serotonin decreased the number of spikes generated in the nerves ipsilateral to the P cell by 78% compared with control ganglia ($P < 0.001$; serotonin application, $n = 12$; control saline application, $n = 4$; **Fig. 7b**).

Mianserin blocked P-cell synaptic depression in feeding

We tested several serotonin antagonists (methysergide, methiothepin, mianserin, granisetron, ondansetron and tropisetron) for their ability to block serotonin's effects on the local bending response in isolated ganglia and found that only mianserin had this effect. Mianserin has not previously been shown to affect synaptic transmission in the leech, although it does block serotonin receptors in other invertebrate nervous systems[24,25]. Mianserin blocked the serotonin-induced depression of the P cell–to–AP neuron synapse and reduced the suppression of

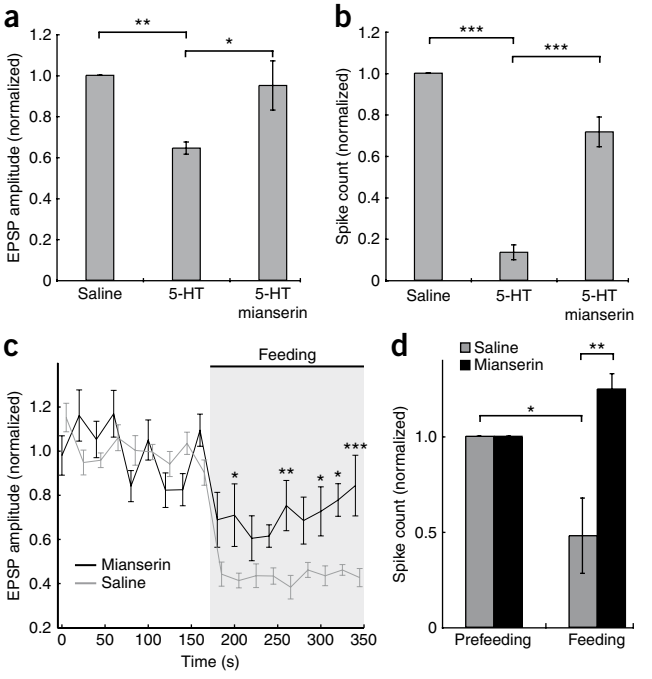

Figure 8 Mianserin blocks feeding-induced depression of P-cell EPSPs and reduces local bending. (**a**) The serotonin antagonist mianserin (200 μM) reversed the depression of the P cell–to–AP neuron synapse in isolated ganglia caused by serotonin (50 μM). We recorded EPSP amplitudes once per minute for 3 min in each solution ($n = 5$). (**b**) Mianserin (200 μM) also restored fictive local bending in isolated ganglia exposed to serotonin (50 μM, $n = 6$). We recorded spike responses in the dorsal posterior nerves once per minute for 3 min in each solution; the solutions were exchanged via perfusion. (**c**) Mianserin blocked feeding-induced synaptic depression. We added mianserin (200 μM) to the recording chamber around three midbody ganglia in semi-intact leeches immediately before recordings. The longer the exposure to mianserin, the greater the block of the synaptic depression that we observed during feeding (*** $P < 0.001$, ** $P < 0.01$ and * $P < 0.05$, mianserin exposed preparations compared with control preparations at the same time points during experiments, $n = 5$). Control data were taken from **Figure 4**. (**d**) Mianserin blocked the effects of feeding on local bending motor activity. We elicited bursts of P-cell action potentials just before feeding semi-intact leeches in control conditions (with exposed ganglia in saline) and in preparations bathed in mianserin (200 μM) for 3 min. We measured the spike counts (normalized to prefeeding conditions, with the preparation in saline) that represented local bending responses. ($n = 9$ and 7 for control and mianserin, respectively.)

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fictive local bending; serotonin (50 μM) decreased the EPSP amplitude by 35% and this synaptic depression disappeared when the perfusion solution contained both serotonin (50 μM) and mianserin (200 μM) ($P < 0.05$, $n = 5$; **Fig. 8a**). Mianserin also restored local bending during serotonin application. We elicited fictive local bending as previously described (**Fig. 7**), then added serotonin (50 μM), which decreased the spike count elicited in the dorsal posterior nerve (to 13% of initial values, in this case). Replacing the serotonin solution with a mixture of serotonin (50 μM) and mianserin (200 μM) reversed this spike-count decrease ($P < 0.001$, $n = 6$; **Fig. 8b**).

These results indicate that mianserin can antagonize the effects of bath-applied serotonin, but to more fully examine serotonin's role in the suppression of behavior, we tested whether mianserin could reverse feeding's effects in a semi-intact leech. We restricted mianserin to the recorded ganglion plus one ganglion anterior and posterior by constructing a vaseline dam around the three ganglia. Preliminary experiments indicated that mianserin took several minutes to permeate the ganglion and affect synaptic transmission. To allow for this permeation time, we added mianserin 3 min before presenting food and recorded synaptic potentials at the P cell–to–AP neuron synapse for 3 min after feeding onset. We observed stable EPSP amplitudes before feeding and less of a decrease in EPSP amplitudes after feeding began (**Fig. 8c**). EPSP amplitudes continued to increase with time, becoming significant ($P < 0.001$, $n = 5$) at 350 s. In fact, the EPSP amplitudes in mianserin-treated leeches 350 s after feeding began were not statistically different ($P > 0.05$, Student's t test) from the EPSP amplitudes before feeding (control data taken from **Fig. 4**).

We also tested whether mianserin could restore the local bend in feeding semi-intact preparations. We used bursts of P-cell spikes to elicit fictive local bending immediately prior and 3 min into a feeding episode. Pre-incubating preparations in mianserin for 6 min (similar time course as the final data point from **Fig. 8c**) produced an increase in dorsal posterior nerve activity in the ganglia exposed to mianserin. This increase in background activity probably results from mianserin's effects on several receptor types. To determine whether mianserin affected local bending, we measured activity increases in response to a P-cell burst (fictive local bending) by subtracting the number of action potentials 1 s before the P-cell burst from the number of dorsal posterior spikes elicited during the 1-s period following P-cell stimulation. The mianserin-treated ganglia showed significantly higher levels of dorsal posterior spikes compared with saline control leeches ($P < 0.01$, $n = 9$ control and $n = 7$ mianserin treated). Therefore, despite mianserin's broader effects, a significant percentage of the presynaptic inhibition produced by feeding appears to be blocked by mianserin.

DISCUSSION

Here, we found that leeches use a neuromodulator for presynaptic inhibition as it performs one behavior to suppress other behaviors (**Fig. 2**). Many touch-evoked behaviors are elicited by P-cell activity[18] and all these behaviors cannot be elicited in a feeding leech[11]. We focused on the synaptic contacts made by P cells involved in these behaviors. We found that feeding reduced synaptic potentials made by P cells onto cell 212, a local bend interneuron, the AP neuron, the shortening-related S cell, and the swim-trigger cell Tr1. (**Fig. 3**). We measured PPRs for synaptic connections from P cells onto AP and Tr1 neurons, both of which showed paired-pulse facilitation during feeding-induced depression (**Fig. 6**), implying that synaptic depression is mediated by a decrease in the release probability at the P-cell terminals[26]. Of the neuromodulators tested, only serotonin mimicked the effects of feeding (**Figs. 6** and **7**), suggesting that serotonin is probably the signal that the leech uses to produce feeding-induced presynaptic inhibition. This is supported by our finding that the serotonin antagonist mianserin blocked the synaptic depression at the P cell–to–AP neuron synapse (**Fig. 8c**) and restored fictive local bending during feeding (**Fig. 8d**).

Suppressing behaviors by presynaptic inhibition

In many invertebrate[27] and vertebrate[28] animals, one of the most common functions of presynaptic inhibition is the modulation of tactile sensory inputs, particularly input from proprioceptors and nociceptors. Presynaptic inhibition of proprioceptors can have a myriad of functions, from gain control of sensory feedback to influencing spatial discrimination, maintaining directional sensitivity and integrating sensory messages. In all these cases, presynaptic inhibition acts as a sensory gate, eliminating the influence of sensory input onto motor neurons or motor circuits[27]. In none of these instances, however, has presynaptic inhibition been implicated in behavioral choice, nor has it been shown to be directly activated by an animal's own behavior rather than by sensory feedback resulting from a behavioral act. Because the leech's nervous system is relatively simple and accessible during behaviors, and because many of the neurons in behavioral circuits have been identified[18], we found a strong, behaviorally driven presynaptic inhibition and the behavioral consequences of this inhibition on suppressing behaviors.

We found sensory gating to be a powerful, albeit blunt, tool for producing behavioral choice. Such a strategy is not suitable for more refined decision-making tasks. Tasks that depend on discriminating between visual stimuli[29] or vibrations to a finger tip[30] would be impossible if the information was inhibited at the earliest processing levels, in the retina or in the spinal cord. In fact, all of the models of such sensory discriminations focus on interactions among high-level sensory processing systems[29] or the transformation from sensory to motor processing areas[31]. Even previous studies of decision-making in invertebrates have only considered interactions among 'higher-order interneurons'[32], emphasizing the influence of command-like neurons[6]. It is likely that sensory gating is most suitable for the suppression of innate or reflexive behaviors when these motor patterns would interfere with an animal's current behavioral state.

How does sensory gating actually suppress behavioral responses? We believe that by decreasing the excitatory drive of sensory input onto critical behavior-initiating neurons, these behaviors are suppressed. This is supported by the observation that two qualitatively different behavior types are suppressed differently. All-or-none behaviors (for example, swimming are crawling) are entirely abolished during feeding. Consider how swimming is activated. P cells excite trigger neurons (such as cell Tr1) that turn on gating neurons (such as cell 204), which activate central pattern-generating neurons that produce the motor pattern for swimming. Because input onto the trigger neuron Tr1 is greatly reduced (**Fig. 4d**,**e**), cell 204 activity is not sufficient to elicit swimming (**Fig. 5**); thus, there is no vestige of the swim motor pattern seen in response to P-cell stimulation during feeding. Behaviorally, swimming and crawling are whole-body behaviors that would interfere with feeding. Local bending, however, is localized, graded and does not interfere greatly with feeding. It is possible that the reduction of the local bending amplitude is a reflection of the most parsimonious solution to turning off all other incompatible behaviors. Essentially, the leech depresses a small set of synapses in its nervous system to eliminate all conflicting behaviors and the local bend's amplitude is consequently reduced.

Sensory gating is probably a universal model that is also present in mammals. Nociceptive input is particularly susceptible to gating

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mechanisms. A variety of external stimuli and behavioral states can inhibit first-order nociceptive inputs presynaptically and postsynaptically to profoundly affect a mammal's response to painful stimuli[3,33]. A stimulus as mild as ingesting a sucrose solution can activate anti-nociceptive pathways in rats[34]. Similarly, human infants show reduced pain reactions to heel lancing while feeding on a sucrose solution[35]. More intense stimuli (for example, predator presence) can also produce a serotonin-mediated analgesia in mice[36]. The 'do not respond to pain' decision may activate OFF cells of the rostral ventromedial medulla (RVM) that lead to pain suppression at the spinal cord level[33]. These decisions to ignore pain allow animals to perform other behaviors that may then be more important. These behavioral consequences of sensory gating have been recently cast into the framework of behavioral choice[3]: complex animals (that is, mammals) decide how to respond to a stimulus on the basis of their behavioral state. This explains why we, for example, do not realize that we have injured ourselves during an intense emotional or physical experience until after the event is over. We choose, at some level, to ignore the pain of the injury. Notably, serotonin modulation of sensory inflow is also a major contributor to these choices by mammals. One difference between mammals and leeches is that the inhibition in mammals appears to occur at many levels of sensory inflow, rather than just at the sensory terminals, as we have found in the leech. This, however, may be a difference in complexity rather than the basic logic of the behavioral-choice mechanism.

In addition to being an efficient mechanism for suppressing responses to tactile stimuli, presynaptic inhibition also enables interneurons to become available for other behaviors. In a cat's spinal cord, the same interneuron can be active in locomotion, reflexive movements and voluntary leg movements[37]. The cat also uses a subset of multifunctional neurons to generate breathing, coughing and sneezing[38]. Gating out sensory input may keep the interneurons available for higher-order commands. In leeches, motor neurons used in local bending are also used to drive peristaltic movements along the leech's length during feeding[39], a behavior that would be compromised if motor neurons were affected by tactile input. In fact, there is increasing evidence that many leech interneurons contribute to multiple behaviors, such as swimming, crawling and shortening[8,40,41]. Thus, it would be difficult to bias one behavior without affecting others.

Mechanisms of presynaptic inhibition

We propose that serotonin released during feeding acts on the P cells' presynaptic terminals. Serotonin has been implicated as being sufficient and necessary for the expression of feeding behavior in the leech[42]. Globally depleting serotonin using 5,7-DHT causes hungry leeches to lose interest in food and applying serotonin to the leech restores its appetitive behaviors[23]. During feeding, serotonin is secreted from the serotonin-containing neurons of the cephalic ganglion and midbody ganglia, causing a global depletion in 5-HT[43]. The serotonergic pair of giant Retzius neurons initially increase their firing rates when the leech's lip touches an appetitive or warm solution[42,44].

Leech P cells have ionotropic[45] and metabotropic[46] serotonin receptors. Both receptor types may be involved in different aspects of the behavioral suppression that we observed. The ionotropic receptor inhibits by fluxing chloride. This receptor could provide an inhibitory shunt at presynaptic terminals. A similar serotonin receptor has been described in *C. elegans*[25]; this channel (named MOD-1) is permeable to chloride and is blocked by mianserin, but not by antagonists (granisetron and ondansetron) of 5-HT_3 receptors, the class of ionotropic receptors that has previously been reported[47]. The faster time course of an ionotropic receptor such as MOD-1 makes it more suitable for the rapid onset seen as the leech starts feeding (**Figs. 3** and **4**). The offset, or return of EPSP amplitudes, was often much slower and incomplete in some cases (**Figs. 3**, **4** and **6**). One explanation for this may be that the leech remains aroused when feeding terminates because the serotonin levels remain elevated. Uninterrupted feeding bouts generally last upwards of 45 min in the leech. Alternatively, long-lasting metabotropic effects may be activated and render the leech less responsive following a meal, a phenomenon that has been well documented[48]. Mianserin is known to target metabotropic 5-HT receptors and, as described above, affects a specific class of ionotropic 5-HT receptors.

In summary, leeches use sensory gating to turn off their responses to mechanosensory stimulation during feeding by releasing serotonin onto the presynaptic terminals of pressure-sensitive mechanosensory neurons. This is an extremely powerful mechanism for establishing the priority of feeding over other behaviors in the medicinal leech (**Fig. 1a**).

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/natureneuroscience/.

Note: Supplementary information is available on the Nature Neuroscience website.

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AUTHOR CONTRIBUTIONS

Q.G. and W.B.K. designed the experiments and wrote the manuscript. Q.G. performed the experiments.

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ONLINE METHODS

Preparations. We performed all experiments on the European medicinal leech, *Hirudo*, acquired from Carolina Biological. We maintained the leeches at 15 °C in artificial pond water; they weighed 0.5–1.0 g. We used only hungry leeches, defined by their exploratory behavior: raising of the head and flaring the dorsal lip in response to agitating the water surface in their aquaria. To dissect the leeches, we first anesthetized them by immersion in ice-cold leech saline solution consisting of 115 mM NaCl, 4 mM KCl, 1.8 mM $CaCl_2$, 1.5 mM $MgCl_2$ and 10 mM glucose, adjusted to a pH of 7.4 with 10 mM HEPES buffer. During the dissection, we confirmed that the leech did not have blood stored in its crop to ensure that it was hungry. We used three types of preparations: single isolated ganglia from the midbody of the leech, which we pinned to a layer of Sylgard on the bottom of a recording chamber, isolated lip preparations, and semi-intact leeches, in which the posterior nervous system was exposed. For the latter preparation, we made a longitudinal incision from the tenth segment to the tail sucker along the dorsal midline and then removed the body wall, viscera and muscles from the posterior half of the body. To record extracellular motor spikes, we dissected the muscle and connective tissue from the pair of dorsal posterior nerves connected to ganglia in one or more segments. We then pinned the intact anterior portion of the leech to a layer of Sylgard on the bottom of the chamber (**Fig. 1c**). We pulled the nerve cord through a slit in the Sylgard and pinned it down in the posterior portion of the chamber. We inserted a plastic slide to divide the chamber in two. We fed the anterior end of the preparation thawed bovine serum made by centrifuging fresh whole bovine blood (Animal Technologies) at 1,000 *g* for 60 min. We delivered the serum from a Falcon tube fitted with a preserved piece of sheep intestine (sausage casing).

We coaxed the semi-intact leeches to feed by mechanically detaching their front sucker from the Sylgard surface and directing it toward the feeding apparatus. Typically, they began feeding as soon as their front sucker contacted the sheep intestine. During long bouts of feeding, blood was actively removed by leaving the severed portion of the gut open and washing away the blood that seeped into the anterior half of the chamber. In some cases, we removed the blood by cannulating the gut with polyethylene tubing. For shorter feeding bouts, we sutured the gut shut to prevent seepage. To terminate a bout of feeding, we peeled the sucker from the feeding tube by inserting the tips of a set of coarse forceps between the sucker and the sheep intestine.

We made isolated lip preparations as described previously[16,44]. In short, we dissected the entire body away from the CNS, leaving only the dorsal lip attached by cephalic nerves to the subesophageal ganglion. We pinned this preparation to clear Sylgard over the bottom of a chamber, then constructed a vaseline dam to separate the solutions surrounding the lip from those bathing the CNS.

Having tested several serotonin antagonists (methysergide, methiothepin, mianserin, granisetron, ondansetron and tropisetron) for their ability to block the effects of serotonin in isolated ganglia, we found that only mianserin showed depressed local bending. To apply mianserin to semi-intact feeding preparations, we made a vaseline well around three adjacent ganglia (typically G14–16) to restrict the drug to those ganglia and used the middle ganglion for recording. Mianserin was applied immediately before impaling neurons or 3 min before delivering our first P-cell burst in fictive bending preparations because preliminary experiments on isolated ganglia showed that bath-applied mianserin solutions took 3–6 min to exert their full effects.

Solutions. All electrophysiology was conducted in our normal leech ringer solution described above unless otherwise noted. For our modified Ca^{2+}/Mg^{2+} solution, we compensated for changes in osmolarity by adjusting the NaCl concentrations. We prepared all modulator solutions immediately before use and delivered them through a Harvard Apparatus perfusion manifold. We used serotonin hydrochloride, dopamine hydrochloride, GABA (all from Sigma Scientific) and molluscan myomodulin (SynPep) in 50 μM solutions. We used mianserin HCl (Sigma Scientific) at a concentration of 200 μM. To facilitate diffusion of the modulators into the ganglionic neuropil, we removed the sheath surrounding the ganglion, except in semi-intact preparations. Only hungry leeches were used for all preparations.

Electrophysiological recordings. We acquired and analyzed all data using custom Matlab software (Data Acquisition Toolbox, MathWorks). We recorded extracellularly from the dorsal posterior nerves with suction electrodes fed into a differential amplifier (model 1700, A-M Systems). We stimulated the nerves with 10-ms pulses at 20 Hz for 250 ms using a Grass S88 stimulator. We monitored the activity of the longitudinal motor neuron cell DE-3 in the dorsal posterior recordings by setting the trigger high enough to capture only the largest units[49]. We recorded intracellularly from neuronal somata with sharp glass microelectrodes (30–45 MΩ filled with 2 M potassium acetate and 20 mM KCl). We sampled the recordings from neuronal somata at 10 kHz and delivered current-clamp pulses using an Axoclamp 2B amplifier (Molecular Devices) controlled with a 16-bit analog/digital board (BNC-2090, National Instruments). We identified each P cell and AP neuron on the basis of its unmistakable soma size, soma location and electrophysiological properties. We identified cell 212 by its soma position in the posterior corner of the central packet and by the unique branching pattern of its primary neurite: it bifurcates contralaterally and the anterior-going branch projects out of the ganglion[13]. To determine the neuritic morphology of cell 212, we re-impaled it at the end of each experiment with a microelectrode containing Alexa 488 and injected the dye into the soma using 1-nA depolarizing pulses for 500 ms at a 50% duty cycle for a total of 10–30 min. To view the dye-filled neurons, we fixed the preparation in 2% paraformaldehyde for 30 min, dehydrated it in ethanol solutions and cleared it in methyl salicylate. Alternatively, we visualized the unfixed ganglion using a Ziess Axioscop II microscope. The S cell was identified by its location in the middle on the central packet and by its large over-shooting action potential. Tr1 was identified by its location, size, ability to elicit swimming and the morphology of its primary neurite[15].

We calculated input resistances by hyperpolarizing each cell with 0.5 nA for 500 ms (**Supplementary Fig. 1**). We determined the threshold for generating a spike in P cells (that is, their excitability) by delivering 6-ms pulses at amplitudes from 1–3.6-nA in increments of 0.2 nA (**Supplementary Fig. 1**). To be able to detect both increases and decreases in excitability after feeding, we excluded those P cells with prefeeding thresholds of 0.2 nA because decreases in excitability could not be detected. For cell 212 and AP neurons, we calculated excitability as the number of spikes elicited during the 500-ms depolarization minus the number of spikes during the 500 ms preceding the depolarizing pulse (**Supplementary Fig. 1**). We normalized the values by dividing the excitability during and after feeding by the prefeeding excitability. To determine EPSP amplitudes, we hyperpolarized the neurons to prevent their spiking during the evoked excitatory synaptic inputs (**Supplementary Fig. 1**). We measured the EPSP amplitudes as the maximal voltage measured within 50 ms of the sensory cell spike. If there were polysynaptic components, we used only the initial peak. EPSP amplitudes in the AP neuron averaged between 8–20 mV in the prefeeding condition.

PPRs. Changes in PPRs are generally believed to reflect presynaptic changes in the probability of release at presynaptic terminals[29]. If internalization or desensitization of postsynaptic receptors mediates a given depression, we would expect both EPSPs of the paired pulses to decrease identically and thus result in no change in the EPSP ratio. However, if the synaptic depression is caused by a decrease in neurotransmitter release, we would expect a larger readily available vesicle pool at the time of the second pulse, resulting in a proportionally larger second EPSP and an increase in the PPR. To calculate the optimal paired-pulse interstimulus interval for revealing changes in probability of releases, we used a modified leech saline that contained half the normal level of calcium (0.9 mM) and twice the normal level of magnesium (3.0 mM). We measured PPRs, the amplitude of the second evoked EPSP in the pair divided by the amplitude of the first one, only once per minute to prevent synaptic depression. We used an interspike interval of 500 ms, which we determined experimentally to be the optimal interval to reveal changes in the probability of release at the P cell–to–AP neuron synapse (**Supplementary Fig. 5**).

Statistics. We used GraphPad Instat software to perform statistical tests. We used repeated-measures ANOVA with Tukey *post hoc* test to compare prefeeding, feeding and postfeeding data. During extracellular stimulation experiments, we stimulated each leech three times in each condition, then averaged the data to generate a single value for that leech in that condition. When comparing ganglia exposed to serotonin versus control ganglia, we used a repeated-measures ANOVA with Bonferroni *post hoc* test between relevant columns. We used paired Student's *t* tests at each time interval to compare the PPR measured in normal versus modified leech saline for that interval.

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