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.

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, olfactory, tactile and nociceptive inputs. 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, 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. Behavioral choice at the network level has been studied using animals such as the sea slug, the pond snail and the leech Hirudo. 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. 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, it has never been implicated in decision-making between qualitatively different behaviors.

In leeches, feeding tops the behavioral hierarchy. 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 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. 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, 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...
Nonfeeding conditions. The last nonfeeding condition was not statistically different from either feeding bout (∼0.05, repeated-measures ANOVA).

**Figure 3** Spike count (normalized) in three sessions of nonfeeding and two sessions of feeding. The average decrease was nearly 65% (∼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.

**Figure 4** 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).

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\(^6\), cell Tr1 triggers bouts of swimming\(^1\), and although the function of the AP neuron is unknown, it does not participate in these behaviors\(^2\). 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% (∼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 (∼0.001; Fig. 3d). We wanted to test the P-cell synaptic input onto the swim trigger neuron, cell Tr1,
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)\(^1\). 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\(^1\) (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).

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...
interneurons that are responsible for the initiation of behaviors during feeding. We used the well-characterized swim circuit\(^1\)\(^2\)\(^3\)\(^4\)\(^5\)\(^6\)\(^7\), as swimming is readily initiated by single‐interneuron activation\(^5\)\(^6\)\(^7\), 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\(^8\). 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 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 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).

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 50 mV and the horizontal scale bar represents 5 s. (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).

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.
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 dopamine21
(50 µM), myomodulin22 (50 µM), GABA9 (50 µM) and serotonin23
(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. Mianserin blocked the serotonin-induced depression of the
P cell–to–AP neuron synapse and reduced the suppression of

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 reaplication 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
control saline treatments (\(P < 0.01\)). ** \(P < 0.001\), \(^* P < 0.01\).
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 activity18 and all these behaviors cannot be elicited in a feeding leech11. 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 terminals26. 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 invertebrate27 and vertebrate28 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 circuits27. 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 identified18, 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 stimuli29 or vibrations to a finger tip30 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 systems29 or the transformation from sensory to motor processing areas31. Even previous studies of decision-making in invertebrates have only considered interactions among higher-order interneurons32, emphasizing the influence of command-like neurons6. 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. 3); 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
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. 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. During feeding, serotonin is secreted from the serotonin-containing neurons of the cephalic ganglion and mbdody ganglia, causing a global depletion in 5-HT. The serotonergic pair of giant Retzius neurons initially increase their firing rates when the leech’s lip touches an appetitive or warm solution. L eech P cells have ionotropic and metabotropic 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; this channel (named MOD-1) is permeable to chloride and is blocked by mianserin, but not by antagonists (granisetron and ondansetron) of 5-HT receptors, the class of ionotropic receptors that has previously been reported. 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 metabolic effects may be activated and render the leech less responsive following a meal, a phenomenon that has been well documented. 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).
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 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, visera 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 for 60 min. We delivered the serum from a Falcon tube fitted with a preserved piece of sheep intestine (sawing 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. When feeding was complete, we removed the gut back into the leech 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. We used serotonin hydrochloride, purchased from Sigma Scientific, in 50 µM solutions. We used mianserin HCl (Sigma Scientific) at a concentration of 200–3,000 nM. We measured EPSP amplitudes 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. 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 Student’s t tests at each time interval to compare the PPR measured in normal versus modified leech saline for that interval.

PPRs. Changes in PPRs are generally believed to reflect presynaptic changes in the probability of release at presynaptic terminals. 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 intrasynaptic interval for revealing changes in probability of release, 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 Student’s t tests at each time interval to compare the PPR measured in normal versus modified leech saline for that interval.

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. We recorded intracellularly from neuronal somata with sharp glass micropipettes (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 (BNCO-1900, 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. To determine the neuritic morphology of cell 212, we re-imaged it at the end of each experiment with a microscope 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 Zeiss Axioskop 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.

PPRs. Changes in PPRs are generally believed to reflect presynaptic changes in the probability of release at presynaptic terminals. 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 intrasynaptic interval for revealing changes in probability of release, 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.