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# Serotonin regulates repolarization of the *C. elegans* pharyngeal muscle

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**Summary**

***Caenorhabditis elegans*** **feeds by rhythmically contracting its pharynx to ingest bacteria. The rate of pharyngeal contraction is increased by serotonin and suppressed by octopamine. Using an electrophysiological assay, we show that serotonin and octopamine regulate two additional aspects of pharyngeal behavior. Serotonin decreases the duration of the pharyngeal action potential and enhances activity of the pharyngeal M3 motor neurons. Gramine, a competitive serotonin antagonist, and octopamine have effects opposite to those of serotonin: gramine and octopamine increase action potential duration and suppress M3 activity. The effects of serotonin, gramine and octopamine on action potential duration are dependent on the pharyngeal motor neurons MC and M3. When the MC and M3 motor neurons are functionally defective, serotonin and octopamine do not regulate the action potential. Our data suggest that serotonin alters pharyngeal physiology to allow for rapid contraction–relaxation cycles. Reciprocal regulation of pharyngeal behavior by serotonin and octopamine provides a mechanism for adapting to the presence and absence of food, respectively.**

Key words: serotonin, nematode, *Caenorhabditis elegans*, gramine, octopamine, action potential, motor neuron, feeding, pharynx.

## Introduction

The *C. elegans* pharynx is a neuromuscular organ responsible for ingestion of bacteria, the worm's food. Rhythmic contraction and relaxation of the pharyngeal muscle transports bacteria from the mouth of the pharynx posteriorly to the intestine. The rate of pharyngeal contraction varies, depending on the worm's environment. When bacteria are abundant, pharyngeal pumping rates can exceed 250 pumps $min^{-1}$. However, when food is scarce, the rate of pharyngeal pumping decreases (Avery and Horvitz, 1990; Croll, 1978; Horvitz et al., 1982).

Proper timing of the pharyngeal contraction–relaxation cycle is important for efficient feeding (Avery, 1993b). A pair of neurons, the M3s, modulates the timing of pharyngeal relaxation. When the M3s are ablated with a laser, or decoupled from the pharyngeal muscle by mutating the M3-post synaptic receptor, the duration of pharyngeal contraction increases (Avery, 1993b; Dent et al., 1997; Raizen and Avery, 1994). In addition to regulating pump length, the M3s are important for maintaining coordinated pharyngeal motions that are required for efficient feeding. When the M3s are killed along with other pharyngeal neurons, feeding is inefficient and the pharyngeal lumen becomes clogged with bacteria that are not efficiently transported to the intestine (Avery, 1993b).

Serotonin regulates pharyngeal pumping rate. Exogenous serotonin stimulates pharyngeal pumping (Avery and Horvitz, 1990; Croll, 1975; Horvitz et al., 1982), while chronic depletion of endogenous serotonin suppresses pumping (Duerr et al., 1999; Sze et al., 2000). Gramine, a competitive serotonin antagonist (Evans and O'Shea, 1978), and octopamine decrease pharyngeal pumping rate (Avery and Horvitz, 1990; Horvitz et al., 1982). In addition to regulating pharyngeal behavior, serotonin and octopamine also regulate other behaviors that are influenced by the presence of food, such as locomotion and egg laying (Horvitz et al., 1982; Segalat et al., 1995). Serotonin suppresses locomotion and enhances egg laying, mimicking the behaviors displayed by worms in the presence of abundant food. Conversely, octopamine enhances locomotion and suppresses egg laying. These observations suggest that serotonin and octopamine may be important for signaling the presence and absence, respectively, of food.

To take maximal advantage of abundant food, worms must increase their pharyngeal pumping rate while preserving muscle coordination and efficiency. While it is clear that serotonin and octopamine regulate the rate of pumping, little is known about the effects of these transmitters on pharyngeal physiology. We have used an electrophysiological approach to show that serotonin and octopamine regulate the effective activity of the M3 motor neurons and timing of pharyngeal muscle repolarization. These observations suggest a mechanism for maintaining proper pharyngeal coordination in the context of varied pumping rates.

## Materials and methods

### *General methods and strains*

Worms were cultured and handled as described by Sulston and Hodgkin (1988) with minor modifications as described by Avery (1993a). All worms were grown at 20°C on *E. coli* strain HB101 (Boyer and Roulland-Dussoix, 1969). The wild type was *C. elegans* variety Bristol, strain N2. Mutant strains used were DA1110 *eat-18*(*ad1110*) I, DA1051 *avr-15*(*ad1051*) V, DA1697 *eat-18*(*ad1110*) I; *avr-15*(*ad1051*) V, DA1696 *eat-18*(*ad1110*) I; *tph-1*(*mg280*) II, and DA1775 *eat-2*(*ad465*) II; *avr-15*(*ad1051*) V.

### *Chemicals*

Chemicals used were the creatinine sulfate complex of serotonin (5-hydroxytryptamine) from (Sigma; cat. no. H-7752), gramine (3-[dimethylaminomethyl]indole) (Sigma; cat. no. G$^{-1}$647) and octopamine (1-[p-hydroxyphenyl]-2-amino-ethanol) (Research Biochemicals International; cat. no. O-101).

### *Preparation of worms for electrophysiological analysis*

Eggs of each genotype tested were collected and synchronized as previously described (Lewis and Fleming, 1995). Approximately 500 synchronized larvae were transferred to fresh 10 cm agarose plates seeded with HB101 and grown to adulthood. Adult hermaphrodites were washed from the plate with 5 ml M9 into a 15 ml conical tube and spun briefly at 800 ***g***. We removed the M9 and washed the worms twice with 2 ml of fresh M9 to remove any bacteria present in the M9-worm suspension. For the single-point data (Figs 3, 6 and 7) as well as the gramine and octopamine titrations (Fig. 5B,C), worms were placed on a rocker at 20°C for 30 min. For the serotonin titration experiment (Fig. 5A) worms were placed on a rocker at 20°C for 3 h. The longer starvation period in the serotonin titration experiment was used to promote further decrease of endogenous serotonin levels.

### *Extracellular recordings (electropharyngeograms)*

Extracellular recordings were made using methods based on those previously described (Davis et al., 1995; Raizen and Avery, 1994). Synchronized adult hermaphrodites were transferred into 100 µl Dent's saline (Avery et al., 1995) on a 35 mm×50 mm glass coverslip (Fisher Scientific) and cut just posterior to the terminal bulb of the pharynx with a #11 surgical blade. Recordings were done at ambient temperature (21–26°C). Current measurements were done with an Axopatch-1D amplifier from Axon Instruments (Foster City, CA, USA) in voltage-clamp mode. The data were low-pass filtered at 1 kHz using a 4-pole Bessel filter in the amplifier. The electrical signal from the amplifier was sampled and recorded using an AT-MIO-16X ADC board from National Instruments (Austin, TX, USA) and Accbin, our own acquisition software written by M. W. Davis in the National Instruments Labview development environment. The sampling rate was 2 kHz. Further filtering and analysis were done with Igor Pro 4.01 from Wave Metrics (Lake Oswego, OR, USA). Each pharynx was assayed approximately 1 min after dissection and recorded for 3 min.

### *Single-point drug assays*

For single-point drug assays (Figs 3, 6 and 7), the 3 min recordings were split into two segments. The first 90 s were recorded either in the absence of drug or in 1 µmol l$^{-1}$ serotonin, 100 µmol l$^{-1}$ gramine or 100 µmol l$^{-1}$ octopamine. The remaining 90 s were recorded in the presence of 1 µmol l$^{-1}$ serotonin, 100 µmol l$^{-1}$ gramine, 100 µmol l$^{-1}$ octopamine, 100 µmol l$^{-1}$ gramine+1 µmol l$^{-1}$ serotonin, or 100 µmol l$^{-1}$ octopamine+1 µmol l$^{-1}$ serotonin. The pattern of drug addition was varied to control for temporal effects. For each strain tested, two separate synchronized preparations were made. 15 dissected pharynxes were assayed from each synchronized worm preparation using the drug exposure pattern shown in Table 1. All recordings were completed less than 2.5 h after transferring the synchronized worm population to M9.

### *Dose–response assays*

For dose–response assays (Fig. 5), dissected pharynxes were exposed to a single drug condition over the entire 3 min recording. For serotonin dose–response data (Fig. 5A) at least ten pharynxes were assayed for each data point. For gramine and octopamine dose–response data (Fig. 5B,C) 100 nmol l$^{-1}$ serotonin was present in addition to the indicated gramine and octopamine concentrations. Each point in the serotonin and gramine dose–response curves represents data from a minimum of four pharynxes. Data from the final 150 s of each recording were used to determine the mean action potential duration and M3 activity for the trial.

Table 1. *Pattern of drug exposure used in the experiments*

| | Drug treatment | | | |
|---|---|---|---|---|
| | First synchronized preparation | | Second synchronized preparation | |
| | First 90 s | Second 90 s | First 90 s | Second 90 s |
| First five pharynxes | No drug | Gramine | Serotonin | Serotonin+Octopamine |
| Second five pharynxes | Gramine | Gramine+Serotonin | Octopamine | Octopamine+Serotonin |
| Third five pharynxes | Serotonin | Serotonin+Gramine | No drug | Octopamine |

Drug concentrations were Serotonin, 1 µmol l$^{-1}$; Gramine, 100 µmol l$^{-1}$; Octopamine, 100 µmol l$^{-1}$.
15 dissected pharynxes were assayed from each synchronized worm preparation.

*Analysis and quantification of electropharyngeograms*

Data from each recording were analyzed using Igor Pro 4.01 from Wave Metrics. We developed an Igor Procedure (available on request) for quantifying the length and M3 activity of each action potential generated during the recording. We defined action potential duration as the time between the peak of the depolarization (E) spike and the peak of the repolarization (R) spike in the electropharyngeogram (EPG). We fit the EPG to a fourth-order polynomial equation to correct for baseline drift that was not eliminated by high-pass filtering. We calculated M3 activity as the mean square deviation about the drift-corrected baseline during the portion of the plateau phase of the action potential not affected by current due to the E and R spikes (the interval of the EPG beginning 10 ms after the E spike and ending 10 ms before the R spike; Fig. 2, pink box). This value was corrected for random noise by subtracting the mean variance about the drift-corrected baseline in regions of the EPG not affected by pharyngeal currents. M3 activity is reported in units of $pA^2$. The Student's *t*-test was used for statistical analysis of both M3 activity and action potential duration.

*Pumping rate assays*

Unsynchronized adult worms were dissected in 200 µl Dent's saline containing 1 µmol $l^{-1}$ levamisole. EPGs were performed as described above, except that individual trials were separated into three segments and lasted approximately 8 min. The first segment of the recording was performed in the absence of serotonin and gramine, the second was performed in the presence of 1 µmol $l^{-1}$ serotonin, and the final segment was performed in the presence of 1 µmol $l^{-1}$ serotonin and 100 µmol $l^{-1}$ gramine. Pumping rate was quantified by calculating the number of pumps (defined by the presence of an R spike in the EPG) during the last 2 min of each segment of the recording. Data shown are the results of three independent trials. The Student's *t*-test was used for statistical analysis of pumping rate.

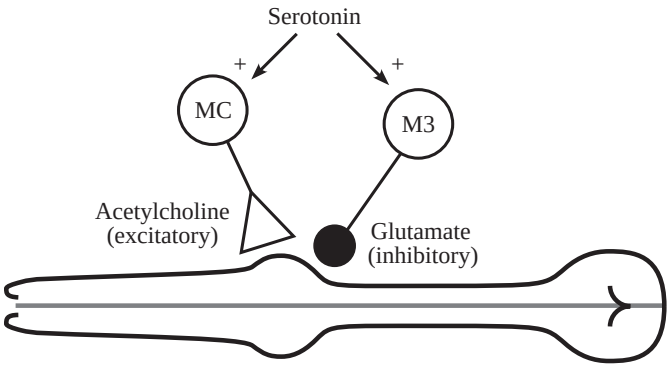


Fig. 1. Motor neurons MC and M3 control the timing of pharyngeal muscle action potentials. MC is an excitatory cholinergic neuron (Raizen et al., 1995; T. Niacaris and L. Avery, unpublished observations). Its firing triggers a pharyngeal muscle action potential *via* the release of acetylcholine, which acts on a muscle nicotinic receptor (Raizen et al., 1995; J. McKay, personal communication). MC thus controls the frequency of pharyngeal pumping. M3 is an inhibitory glutamatergic neuron. It fires during the action potential, releasing glutamate, which causes inhibitory postsynaptic potentials that can end the muscle action potential (Avery, 1993b; Dent et al., 1997; Li et al., 1997). M3 thus acts to decrease action potential duration. Serotonin acts to increase the activity or effect of both of these neurons on pharyngeal muscle, resulting in decreased action potential duration. Note that MC and M3 are actually located within the pharynx although they are shown here as external for clarity.

## Results

*Motor neurons MC and M3 control the timing of pharyngeal muscle action potentials*

Two motor neurons, MC and M3, control the timing of pharyngeal muscle contractions (Fig. 1). MC is an excitatory cholinergic neuron (Raizen et al., 1995; L. Avery, unpublished observations). Its firing triggers a pharyngeal

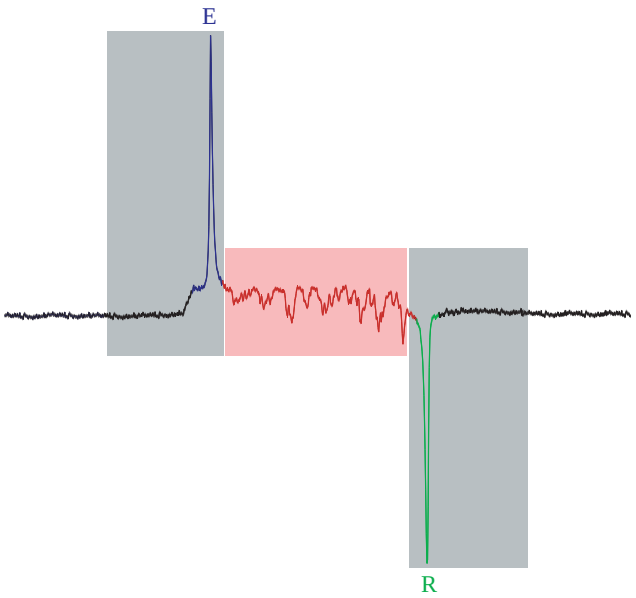


Fig. 2. The electropharyngeogram (EPG) reflects current movement across the pharyngeal muscle membrane. In the EPG, the large positive transient (E, blue) corresponds to depolarization of the pharyngeal muscle and onset of contraction. The large negative transient (R, green) corresponds to repolarization of the pharyngeal muscle and the end of the action potential. We defined action potential duration as the time difference between the peaks of the E and R spikes. The interval between the E and R spikes represents the plateau phase of the action potential (red portion of the trace). The negative transients during the plateau phase are pharyngeal muscle inhibitory postsynaptic potentials (IPSPs) caused by firing of the M3 motor neurons. We calculated the activity of the M3 motor neurons as the mean-square deviation about the baseline during the portion of the action potential affected only by M3-induced currents (pink box). Regions of the EPG trace not affected by pharyngeal currents (regions outside the gray and pink boxes) were used to determine the portion of baseline deviation due to random noise. This value is subtracted from the preliminary measurement of M3 activity to determine a noise-corrected value, which we report as M3 activity.

muscle action potential *via* the release of acetylcholine, which acts on a muscle nicotinic receptor (Raizen et al., 1995; J. McKay, personal communication). MC thus regulates activation of pharyngeal muscle and the frequency of pharyngeal pumping. EAT-18 is necessary for the function of the pharyngeal nicotinic receptor (Raizen et al., 1995). In *eat-18* mutant worms, MC is decoupled from pharyngeal muscle and is functionally defective.

M3 is an inhibitory glutamatergic neuron. It fires during the pharyngeal action potential, releasing glutamate, which acts on a glutamate-gated chloride channel encoded in part by *avr-15*. Activation of this channel causes inhibitory postsynaptic potentials that can end the muscle action potential (Avery, 1993b; Dent et al., 1997; Li et al., 1997). M3 thus decreases action potential duration, promoting rapid relaxation of the pharyngeal muscle following contraction (Avery, 1993b; Dent et al., 1997; Raizen and Avery, 1994).

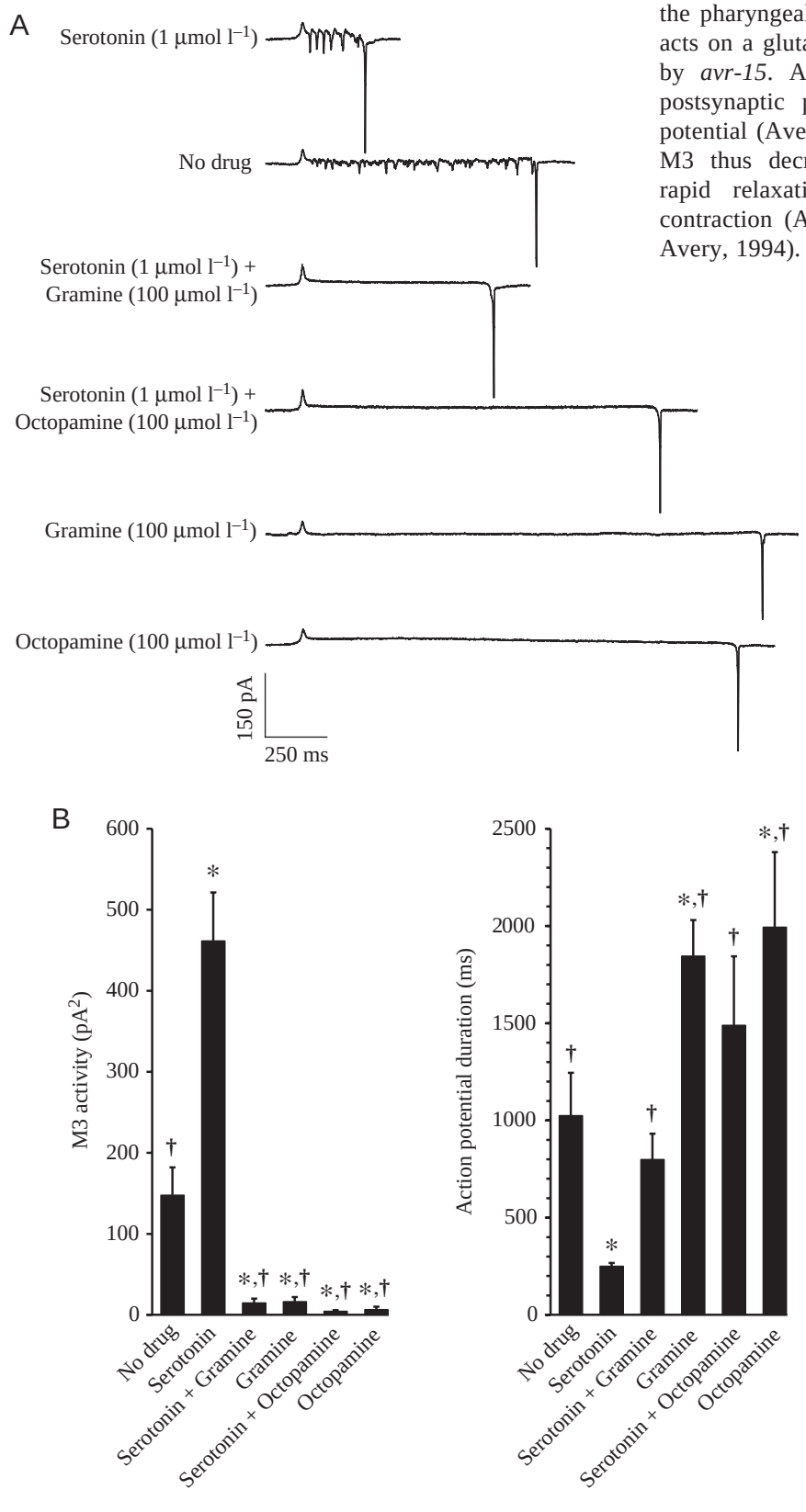


Fig. 3. (A) Serotonin, gramine and octopamine affect the electropharyngeograms (EPGs) of *eat-18* mutants. Serotonin (1 μmol l$^{-1}$) enhances M3 activity and decreases the action potential duration of *eat-18* mutant pharynxes. Gramine (100 μmol l$^{-1}$) and octopamine (100 μmol l$^{-1}$) increase action potential duration and suppress M3 activity in the presence and absence of exogenous serotonin. EPGs shown for each drug condition are statistically representative of the average action potential duration and M3 activity of all recordings. (B) Quantitative analysis of M3 activity and action potential duration in *eat-18* mutants. Serotonin (1 μmol l$^{-1}$) significantly decreases action potential duration and enhances M3 activity ($P$<0.005). Gramine (100 μmol l$^{-1}$) and octopamine (100 μmol l$^{-1}$) block the serotonin-stimulated decrease in action potential duration and enhancement of M3 activity ($P$<0.001). Gramine and octopamine also increase action potential duration and suppress M3 activity in the absence of exogenous serotonin ($P$<0.05). Values are means + S.E.M. Significant differences between the indicated measurement and data recorded * in the absence of drug or † in the presence of 1 μmol l$^{-1}$ serotonin.

### *Serotonin, gramine and octopamine affect the pharyngeal action potential*

We examined the electrophysiological properties of pharyngeal muscle to determine whether serotonin affects the pharyngeal action potential. The EPG allows us to determine the start (Fig. 2, E spike) and end (Fig. 2, R spike) of the pharyngeal action potential. In addition, we can measure M3 activity (Fig. 2, red portion of trace). To determine whether serotonin can affect these properties of the pharyngeal action potential we examined EPGs from *eat-18* mutants (Raizen et al., 1995). In the absence of MC function, pharyngeal pumping rate decreases

to approximately one-fifth the wild-type rate (Avery and Horvitz, 1989). Thus, *eat-18* mutants allow us to examine the effects of serotonin without inducing rapid pumping. In *eat-18* mutants, serotonin enhances the activity of the M3 motor neurons and decreases pharyngeal action potential duration (Fig. 3A,B). Gramine, a competitive serotonin antagonist, decreases pumping rate in the presence of food (Avery and Horvitz, 1990) and blocks serotonin-stimulated increases in pumping rate (Fig. 4). We found that gramine increases action potential duration and suppresses M3 activity both in the presence and absence of exogenous serotonin (Fig. 3A,B). The effects of gramine in the absence of added serotonin are probably due to antagonism of endogenous serotonin activity. Serotonin has a residual effect in the presence of gramine (compare the effects of 1 μmol $l^{-1}$ serotonin+100 μmol $l^{-1}$ gramine with 100 μmol $l^{-1}$ gramine; Fig. 3), indicating that the gramine block of serotonin action is incomplete. Octopamine also affects the pharyngeal action potential in a manner opposite to that of serotonin: octopamine suppresses M3 activity and increases action potential duration both in the presence and absence of serotonin (Fig. 3A,B).

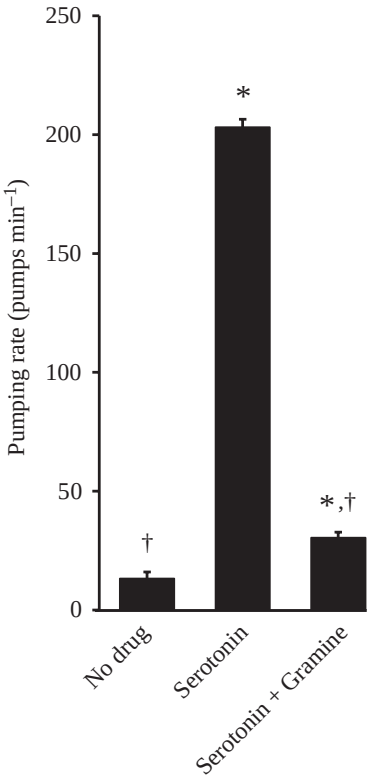


Fig. 4. Gramine suppresses serotonin-stimulated pumping in wild-type worms. 1 μmol $l^{-1}$ serotonin induces rapid pharyngeal pumping ($P$<0.0001). Addition of 100 μmol $l^{-1}$ gramine suppresses serotonin-stimulated pumping ($P$<0.0001). However, serotonin still has residual effects on pumping rate in the presence of 100 μmol $l^{-1}$ gramine ($P$<0.005). Values are means + S.E.M. Significant differences between the indicated measurement and data recorded * in the absence of drug or † in the presence of 1 μmol $l^{-1}$ serotonin.

To determine whether the effects of serotonin, gramine and octopamine on the pharyngeal action potential are dosage sensitive, we measured action potential duration at several drug concentrations. 10 nmol $l^{-1}$ serotonin decreases action potential duration and enhances M3 activity (Fig. 5A). Higher concentrations of serotonin further decrease action potential duration and enhance M3 activity. Gramine and octopamine block the effects of serotonin in a dosage-sensitive manner (Fig. 5B,C).

### *The M3 and MC motor neurons mediate regulation of the pharyngeal action potential by serotonin, gramine and octopamine*

The M3s promote repolarization of pharyngeal muscle (Avery, 1993b; Dent et al., 1997). To determine whether serotonin decreases action potential duration solely by enhancing the activity of the M3s, we examined *avr-15* mutants. AVR-15 is a subunit of the postsynaptic receptor for the M3s and is required for the pharyngeal muscle to respond to the M3 motor neurons (Dent et al., 1997). In the absence of AVR-15, M3 activity is absent from EPGs (Fig. 6A–C). We found that serotonin, gramine and octopamine do not affect action potential duration in *eat-18*; *avr-15* double mutants (Fig. 6A). Thus, the regulation of action potential duration by serotonin, gramine and octopamine in *eat-18* mutants is largely due to regulation of M3 activity. [It is a formal possibility that AVR-15 regulates action potential duration in response to serotonin by activity at synapses other than the M3 neuromuscular junction. Since no other such synapse is known within the pharynx, and since M3 has been shown previously to affect action potential duration (Avery, 1993b; Dent et al., 1997), we believe that the effect of the *avr-15* mutation is mostly or entirely caused by blocking M3 action.] The *avr-15* single mutant behaves differently than the *eat-18*; *avr-15* double mutant: serotonin decreases and gramine increases the action potential duration of *avr-15* mutant worms (Fig. 6B). These observations suggest that EAT-18 also mediates the effect of serotonin on action potential duration.

*eat-18* mutants have MC motor neurons that are functionally decoupled from pharyngeal muscle (Raizen et al., 1995). To determine whether EAT-18 mediates serotonergic regulation of the action potential solely by affecting MC function, we examined *eat-2*; *avr-15* mutants. EAT-2 is a non-alpha nicotinic acetylcholine receptor subunit that localizes to the MC-pharyngeal muscle synapses and is required for MC transmission to pharyngeal muscle (J. McKay, personal communication). Thus, it is likely that absence of EAT-2 specifically affects MC function. Similar to *eat-18*; *avr-15* mutants, *eat-2*; *avr-15* mutant pharynxes do not modulate their action potential duration in response to serotonin, gramine or octopamine (Fig. 6C). These data suggest that EAT-18 and EAT-2 mediate the effects of serotonin, gramine and octopamine by an MC-dependent mechanism.

In wild-type worms that contain intact MC and M3 motor neurons, exogenous serotonin does not significantly enhance M3 activity or decrease action potential duration (Fig. 6D).

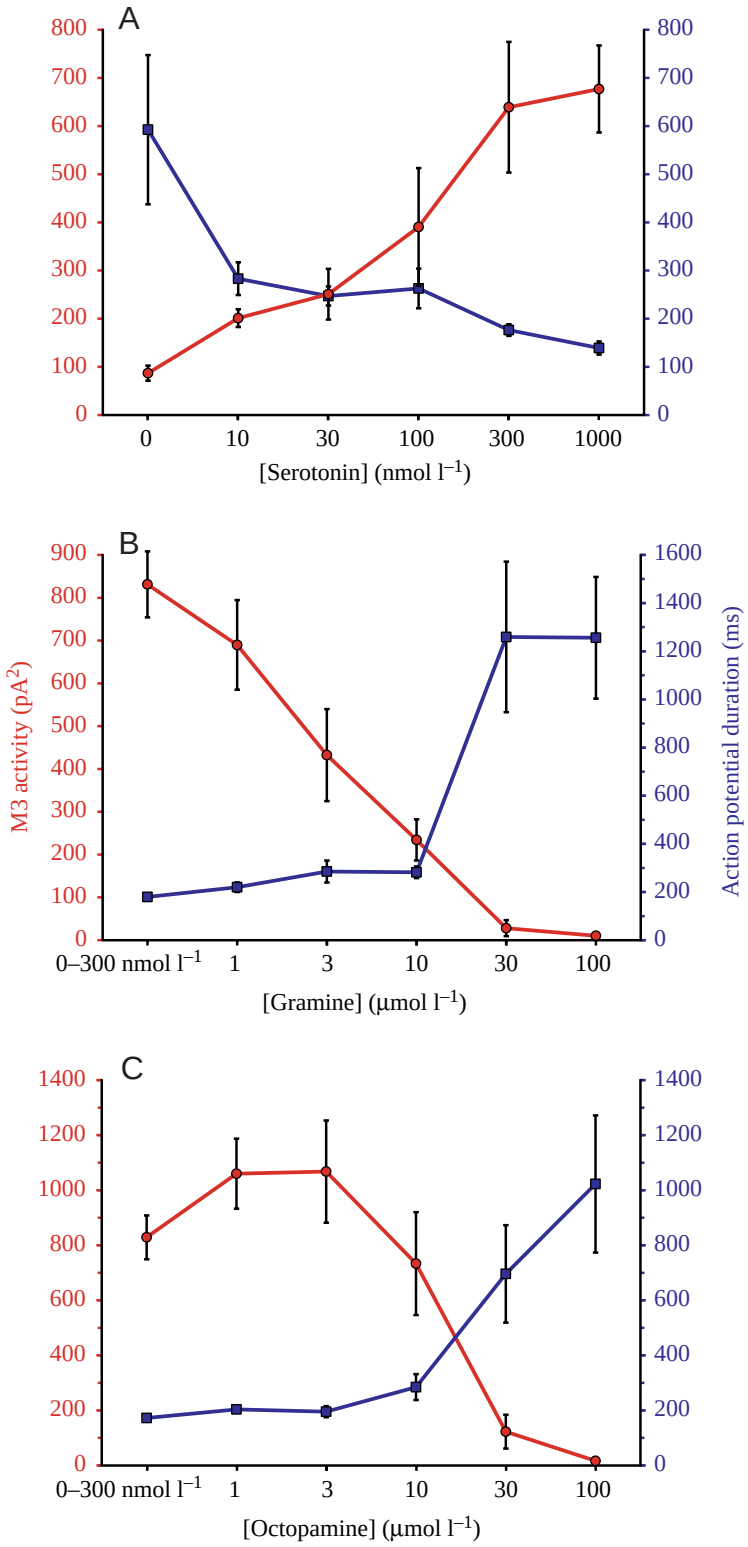


Fig. 5. Serotonin, gramine and octopamine affect the M3 activity (red lines) and action potential duration (blue lines) of *eat-18* mutant pharynxes in a dosage-sensitive manner. (A) Serotonin enhances M3 activity and decreases action potential duration with an $EC_{50}$ of approximately 30 nmol $l^{-1}$. In the presence of 100 nmol $l^{-1}$ exogenous serotonin, gramine (B) and octopamine (C) suppress M3 activity and increase action potential duration in a dosage-sensitive manner. Values are means ± S.E.M.

This is likely to be a consequence of the high levels of M3 activity and short action potentials we observe in the absence of added serotonin. However, gramine and octopamine can suppress M3 activity and increase the action potential duration of wild-type worms. This suggests that the serotonergic and octopaminergic regulatory mechanisms are involved in regulating the action potential of wild-type worms (Fig. 6D).

### *Chronic depletion of endogenous serotonin affects the pharyngeal action potential*

To determine whether chronic depletion of endogenous serotonin affects action potential duration and M3 activity we examined *eat-18*; *tph-1* double mutants. TPH-1 is the sole *C. elegans* ortholog of tryptophan hydroxylase. Tryptophan hydroxylases are required for converting tryptophan to the immediate precursor of serotonin, 5-hydroxytryptophan, but are not involved in the synthesis of other bioamines. Worms lacking TPH-1 have several defects consistent with reduced levels of endogenous serotonin (Sze et al., 2000). However, *tph-1* mutants retain some serotonin immunoreactivity, so may contain residual serotonin (C. Loer, personal communication).

*eat-18*; *tph-1* double mutants have altered responses to serotonin, gramine and octopamine relative to *eat-18* single mutants (Fig. 7A,B). In the absence of exogenous serotonin, *eat-18*; *tph-1* mutants have reduced M3 activity and fail to respond to gramine and octopamine. The level of M3 activity in *eat-18*; *tph-1* in the absence of added drugs is not different from that of *eat-18* in the presence of gramine. Therefore, it is likely that chronic depletion of serotonin and acute blocking of the serotonin signal affect M3 activity similarly. However, action potential duration in the absence of added drug is not significantly different between *eat-18*; *tph-1* and *eat-18*, suggesting there is a serotonin and M3-independent mechanism for shortening the action potential.

Exogenous serotonin increases M3 activity and decreases action potential duration in *eat-18*; *tph-1* mutants. Serotonin also restores the ability of gramine to regulate action potential duration and M3 activity in *eat-18*; *tph-1* mutants. Indeed, gramine can suppress the M3 activity of serotonin-stimulated *eat-18*; *tph-1* worms to the same extent as in *eat-18* worms. Octopamine lengthens the serotonin-stimulated action potential duration of *eat-18*; *tph-1* mutants, but does not significantly suppress serotonin-stimulated M3 activity.

## Discussion

In the presence of food, the rate of pharyngeal pumping increases (Avery and Horvitz, 1990; Croll, 1978; Horvitz et al., 1982). This increase in pharyngeal pumping rate is mimicked by addition of serotonin (Avery and Horvitz, 1990; Croll, 1975; Horvitz et al.,

1982). Serotonin increases the contraction rate of many other rhythmically contracting neuromuscular organs including the *Ascaris* pharynx (Brownlee et al., 1995), and the *Drosophila* (Zornik et al., 1999), cockroach (Collins and Miller, 1977) and human hearts (Frishman and Grewall, 2000; Page, 1954). Our data suggest that serotonin modifies the electrophysiological properties of the pharynx to allow for rapid contraction–relaxation cycles. The rate of serotonin-stimulated

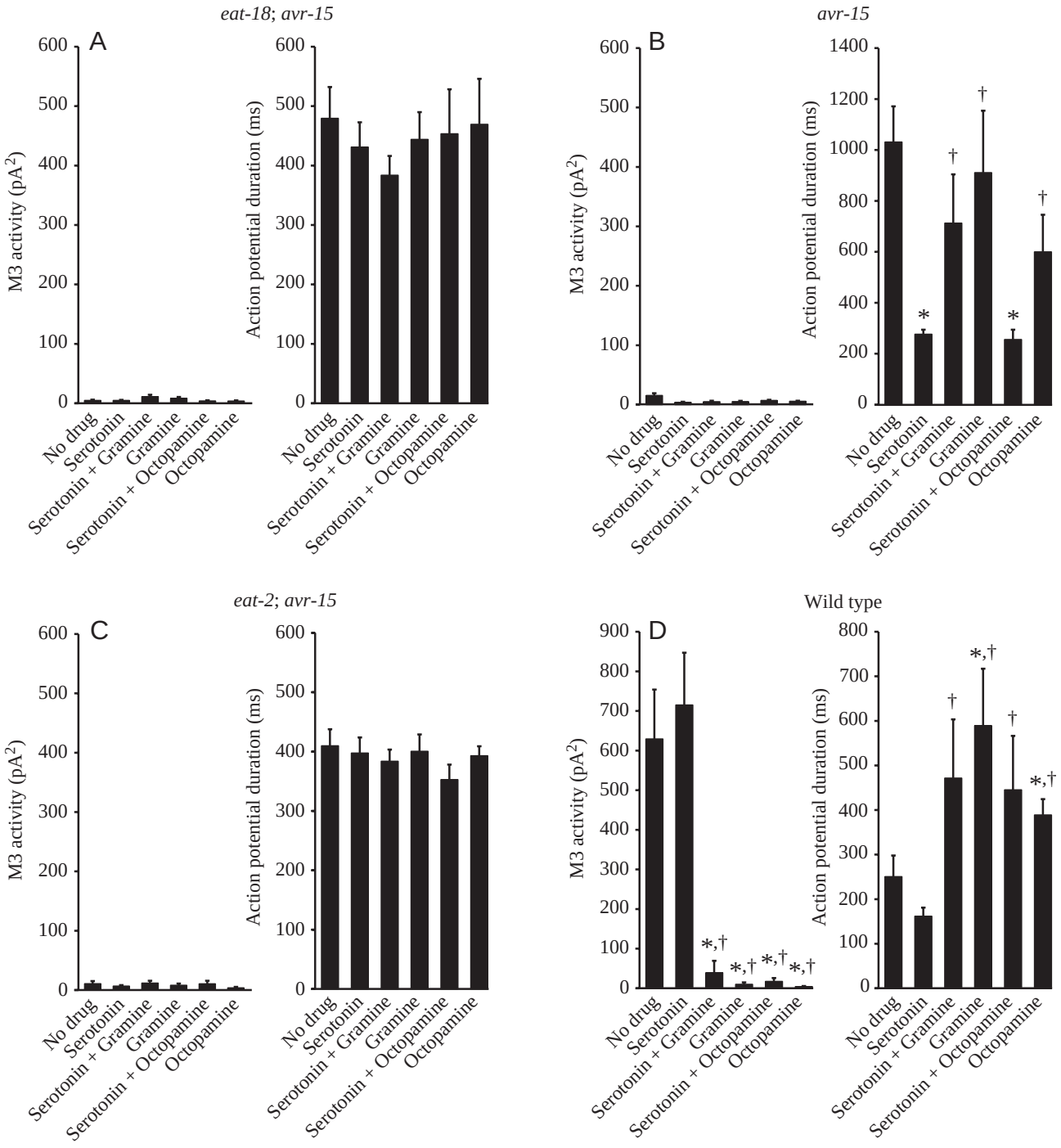


Fig. 6. (A) Serotonin (1 μmol l$^{-1}$), gramine (100 μmol l$^{-1}$) and octopamine (100 μmol l$^{-1}$) do not affect the action potential duration of *eat-18*; *avr-15* mutants ($P$>0.5). (B) Serotonin (1 μmol l$^{-1}$) significantly decreases the action potential duration of *avr-15* mutants ($P$<0.001). This effect is blocked by addition of 100 μmol l$^{-1}$ gramine ($P$<0.05), but not by 100 μmol l$^{-1}$ octopamine ($P$>0.5). (C) Serotonin (1 μmol l$^{-1}$), gramine (100 μmol l$^{-1}$) and octopamine (100 μmol l$^{-1}$) do not affect the action potential duration of *eat-2*; *avr-15* mutants ($P$>0.5). (D) Gramine (100 μmol l$^{-1}$) and octopamine (100 μmol l$^{-1}$) suppress the M3 activity ($P$<0.001) and increase the action potential duration of wild-type worms ($P$<0.05).

pharyngeal pumping can exceed 250 pumps min$^{-1}$ (Horvitz et al., 1982). To achieve this level of rapid pumping, the duration of individual pumps must be shorter than 240 ms on average. When serotonin signaling is blocked by the presence of gramine, action potentials can be greater than 1 s in duration. Action potentials of this length place a maximum limit of 60 pumps min$^{-1}$ on the rate of pharyngeal pumping. However, in the presence of serotonin, the average action potential duration decreases to less than 240 ms, which is consistent with the serotonin-stimulated pumping rates we observe. Our data suggest that serotonin acts not only to increase the rate of action potential generation, but also to modify the action potential to allow for rapid contraction–relaxation cycles.

Two classes of pharyngeal motor neurons, MC and M3, are required for serotonergic regulation of the action potential. In the absence of MC and M3 function, serotonin does not affect action potential duration. However, when either MC or M3 activity is restored, serotonin decreases the length of the action potential. Thus, both MC and M3 dependent-mechanisms are required for normal regulation of the action potential by serotonin. Since the M3s act to promote pharyngeal muscle repolarization, it is clear that serotonergic enhancement of M3 function can directly influence action potential duration. However, it is less clear how serotonin affects action potential duration in an MC-dependent fashion. Since MC acts to depolarize pharyngeal muscle, the effect of MC on action potential duration is likely to be indirect. One possibility is that serotonin enhances MC-dependent stimulation of pharyngeal muscle causing elevation of intracellular calcium, which could activate calcium-sensitive repolarization mechanisms.

The MC and M3-dependent effects of serotonin could be the result of direct stimulation of the activity of the MC and M3 motor neurons or, alternatively, serotonin may regulate the action potential by modulating postsynaptic receptors for MC and M3 that are located in the pharyngeal muscle. Consistent with the latter model, we have identified a serotonin receptor, SER-1, that is expressed in pharyngeal muscle, but have not identified any candidate serotonin receptors within the *C. elegans* genomic sequence that express in MC or M3 (Hamdan et al., 1999; T. Niacaris and L. Avery, unpublished observations). While this suggests that serotonin acts within the pharyngeal muscle to regulate action potential duration, it is possible that serotonin affects the activity of MC and M3 by an unidentified serotonin receptor.

Chronic depletion of endogenous serotonin suppresses M3 activity, similar to the acute effects of the serotonin antagonist gramine. However, the chronic absence of serotonin does not significantly affect action potential duration, despite the suppression of M3 activity. This suggests the presence of a serotonin and M3-independent mechanism for shortening the action potential. Since long action potentials limit the rate of pharyngeal pumping and slow pumping inhibits growth, it is likely that worms have an adaptive mechanism for shortening the action potential under these conditions. Our observation that *eat-18*; *tph-1* worms have significantly shorter action potentials in the presence of gramine than those of *eat-18*

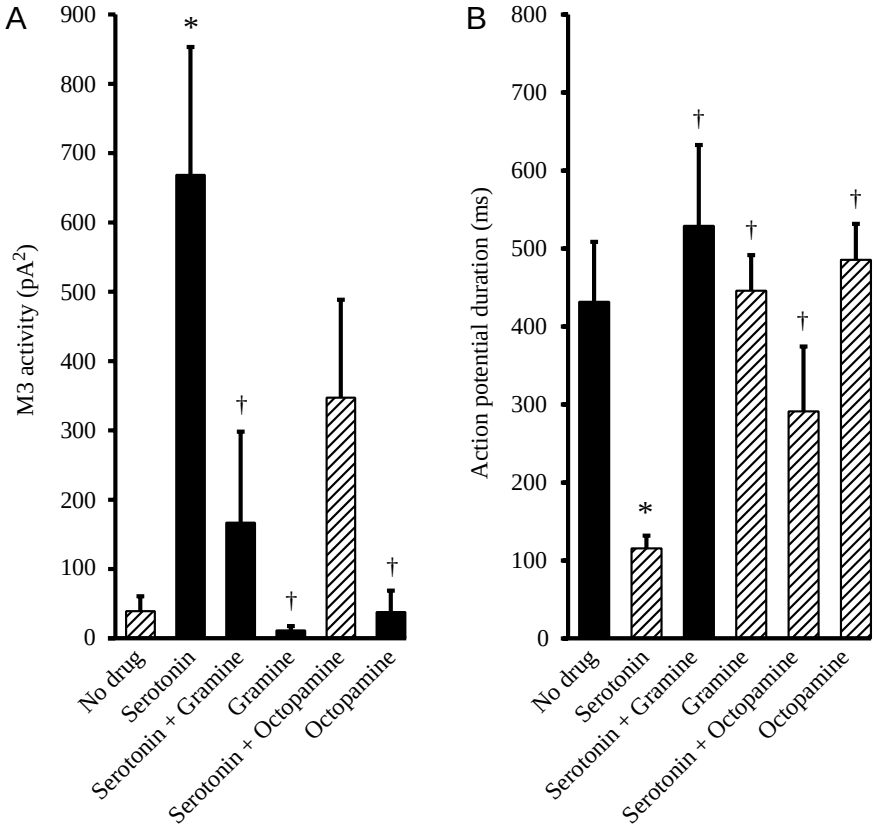


Fig. 7. Chronic depletion of endogenous serotonin affects pharyngeal behavior. (A) In the absence of exogenous serotonin (1 μmol l$^{-1}$), *eat-18*; *tph-1* worms have decreased M3 activity relative to *eat-18* worms (Fig. 3B) ($P$<0.05), and are unresponsive to gramine (100 μmol l$^{-1}$) and octopamine (100 μmol l$^{-1}$) ($P$>0.2). Exogenous serotonin enhances M3 activity to levels comparable to serotonin-stimulated *eat-18* worms ($P$<0.05). Gramine can suppress the serotonin-stimulated enhancement of M3 activity in *eat-18*; *tph-1* worms ($P$<0.05). (B) In the absence of exogenous serotonin, *eat-18*; *tph-1* worms have action potentials similar in length to those of *eat-18* worms ($P$>0.05). Gramine and octopamine have no effect on action potential duration in the absence of added serotonin ($P$>0.6). Exogenous serotonin shortens the action potential of *eat-18*; *tph-1* worms ($P$<0.05). This effect is blocked by gramine and octopamine ($P$<0.05). Values are means + S.E.M. Significant differences between the indicated measurement and data recorded * in the absence of drug or † in the presence of 1 μmol l$^{-1}$ serotonin. Hatched bars represent significant differences between measurements from *eat-18*; *tph-1* (this figure) and those from *eat-18* (Fig. 3B).

worms further supports the existence of a serotonin-independent adaptive mechanism that regulates pump length.

We have recently shown that GPB-2, a G-protein $\beta_5$ subunit, also affects M3 activity and action potential duration (Robatzek et al., 2001). In the absence of GPB-2 function, M3 activity is greatly reduced; however, action potential duration is not significantly affected. This is the same phenotype that we observe in worms chronically deprived of serotonin. Since chronic deprivation of serotonin mimics loss of GPB-2 function, GPB-2 may be required for pharyngeal muscle to respond to serotonin. In addition, the GPB-2 phenotype provides further evidence that there is an adaptive mechanism for shortening the action potential when M3 is chronically suppressed.

Reciprocal regulation of pharyngeal behavior by serotonin and octopamine provides a mechanism for adapting to a range of food availability. In the presence of abundant food, serotonin acts to increase the speed and efficiency at which worms feed. When the food source is exhausted, falling levels of serotonin and, possibly, rising levels of octopamine switch the pharynx to an inactive state characterized by slow pumping. Thus, serotonin and octopamine may function to adapt pharyngeal behavior to the presence or absence, respectively, of food. Since reciprocal chronotropic effects of serotonin and octopamine have been identified for several rhythmically contracting organs (Collins and Miller, 1977; Zornik et al., 1999), it will be interesting to determine whether serotonin and octopamine also modify action potential duration in these systems similarly to their effects in *C. elegans*.

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## References

**Avery, L.** (1993a). The genetics of feeding in *Caenorhabditis elegans*. *Genetics* **133**, 897-917.

**Avery, L.** (1993b). Motor neuron M3 controls pharyngeal muscle relaxation timing in *Caenorhabditis elegans*. *J. Exp. Biol.* **175**, 283-297.

**Avery, L. and Horvitz, H. R.** (1989). Pharyngeal pumping continues after laser killing of the pharyngeal nervous system of *C. elegans*. *Neuron* **3**, 473-485.

**Avery, L. and Horvitz, H. R.** (1990). Effects of starvation and neuroactive drugs on feeding in *Caenorhabditis elegans*. *J. Exp. Zool.* **253**, 263-270.

**Avery, L., Raizen, D. and Lockery, S.** (1995). Electrophysiological methods. *Meth. Cell Biol.* **48**, 251-269.

**Boyer, H. W. and Roulland-Dussoix, D.** (1969). A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. *J. Mol. Biol.* **41**, 459-472.

**Brownlee, D. J., Holden-Dye, L., Walker, R. J. and Fairweather, I.** (1995). The pharynx of the nematode *Ascaris suum*: structure and function. *Acta. Biol. Hung.* **46**, 195-204.

**Collins, C. and Miller, T.** (1977). Studies on the action of biogenic amines on cockroach heart. *J. Exp. Biol.* **67**, 1-15.

**Croll, N. A.** (1975). Indolealkylamines in the coordination of nematode behavioral activities. *Can. J. Zool.* **53**, 894-903.

**Croll, N. A.** (1978). Integrated behavior in the feeding phase of *Caenohrabditis elegans* (Nematoda). *J. Zool.* **184**, 507-517.

**Davis, M. W., Somerville, D., Lee, R. Y., Lockery, S., Avery, L. and Fambrough, D. M.** (1995). Mutations in the *Caenorhabditis elegans* Na,K-ATPase alpha-subunit gene, *eat-6*, disrupt excitable cell function. *J. Neurosci.* **15**, 8408-8418.

**Dent, J. A., Davis, M. W. and Avery, L.** (1997). *avr-15* encodes a chloride channel subunit that mediates inhibitory glutamatergic neurotransmission and ivermectin sensitivity in *Caenorhabditis elegans*. *EMBO J.* **16**, 5867-5879.

**Duerr, J. S., Frisby, D. L., Gaskin, J., Duke, A., Asermely, K., Huddleston, D., Eiden, L. E. and Rand, J. B.** (1999). The *cat-1* gene of *Caenorhabditis elegans* encodes a vesicular monoamine transporter required for specific monoamine-dependent behaviors. *J. Neurosci.* **19**, 72-84.

**Evans, P. D. and O'Shea, M.** (1978). The identification of an octopaminergic neurone and the modulation of a myogenic rhythm in the locust. *J. Exp. Biol.* **73**, 235-260.

**Frishman, W. H. and Grewall, P.** (2000). Serotonin and the heart. *Ann. Med.* **32**, 195-209.

**Hamdan, F. F., Ungrin, M. D., Abramovitz, M. and Ribeiro, P.** (1999). Characterization of a novel serotonin receptor from *Caenorhabditis elegans*: cloning and expression of two splice variants. *J. Neurochem.* **72**, 1372-1383.

**Horvitz, H. R., Chalfie, M., Trent, C., Sulston, J. E. and Evans, P. D.** (1982). Serotonin and octopamine in the nematode *Caenorhabditis elegans*. *Science* **216**, 1012-1014.

**Lewis, J. A. and Fleming, J. T.** (1995). Basic culture methods. *Meth. Cell Biol.* **48**, 3-29.

**Li, H., Avery, L., Denk, W. and Hess, G. P.** (1997). Identification of chemical synapses in the pharynx of *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* **94**, 5912-5916.

**Page, I. H.** (1954). Serotonin (5-hydroxytryptamine). *Physiol. Rev.* **14**, 563-588.

**Raizen, D. M. and Avery, L.** (1994). Electrical activity and behavior in the pharynx of *Caenorhabditis elegans*. *Neuron* **12**, 483-495.

**Raizen, D. M., Lee, R. Y. and Avery, L.** (1995). Interacting genes required for pharyngeal excitation by motor neuron MC in *Caenorhabditis elegans*. *Genetics* **141**, 1365-1382.

**Robatzek, M., Niacaris, T., Steger, K., Avery, L. and Thomas, J. H.** (2001). *eat-11* encodes GPB-2, a Gbeta(5) ortholog that interacts with G(o)alpha and G(q)alpha to regulate *C. elegans* behavior. *Curr. Biol.* **11**, 288-293.

**Segalat, L., Elkes, D. A. and Kaplan, J. M.** (1995). Modulation of serotonin-controlled behaviors by Go in *Caenorhabditis elegans*. *Science* **267**, 1648-1651.

**Sulston, J. E. and Hodgkin, J. A.** (1988). Methods. In *The Nematode* Caenorhabditis elegans (ed. W. B. Wood), pp. 587-606. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

**Sze, J. Y., Victor, M., Loer, C., Shi, Y. and Ruvkun, G.** (2000). Food and metabolic signalling defects in a *Caenorhabditis elegans* serotonin-synthesis mutant. *Nature* **403**, 560-564.

**Zornik, E., Paisley, K. and Nichols, R.** (1999). Neural transmitters and a peptide modulate *Drosophila* heart rate. *Peptides* **20**, 45-51.