Mechanosensory Inputs Influence *Caenorhabditis elegans* Pharyngeal Activity via Ivermectin Sensitivity Genes

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ABSTRACT

Mechanical stimulation induces opposite behavioral responses in the adult and dauer pharynx. Tail tap of adults inhibits pharyngeal pumping via a pathway involving the innexin gene *unc-7* and components of the glutamatergic pathway encoded by the genes *avr-14* and *avr-15*. Tail tap of dauers stimulates pumping through a mechanism involving Gαo and Gαq. The nematocidal drug ivermectin is believed to kill worms by opening a glutamate-gated chloride channel (AVR-15) on pharyngeal muscle, causing complete pumping inhibition. However, ivermectin can also inhibit pumping in the absence of this channel. We propose that one of the ways ivermectin could prevent pumping, in the absence of the AVR-15 ivermectin-binding channel on pharynx muscle, is to target AVR-14 and AVR-15, which are expressed in the inhibitory pathway linking mechanosensation and pumping activity.

NEMATODE feeding is essential for survival. Knowledge of mechanisms that inhibit feeding is important for the development of drugs that can destroy populations of these medically and agriculturally important organisms. The relative simplicity of its body and ease of genetic manipulation in *Caenorhabditis elegans* have provided insights into the genetics of development and nervous system structure and function. *C. elegans* is related to pest nematodes and discovery of mechanisms used to inhibit feeding in this species should generalize to other species.

C. elegans is a small bacteria-eating soil nematode. Feeding is achieved by a pumping motion of the pharynx. Muscle contraction sucks fluid and bacteria into the lumen of the pharynx and relaxation expels the water and captures the bacteria. Captured bacteria are passed from the corpus in the anterior of the pharynx through the isthmus in a swallowing motion and into the terminal bulb where they are crushed by a grinder and passed into the intestine. The pharyngeal nervous system consists of 20 neurons. It is connected to the extrapharyngeal nervous system via gap junctions between the bilaterally symmetric RIP and I1 neurons. Although pumping can occur after complete ablation of the pharyngeal nervous system (Avery and Horvitz 1989), ablation studies have identified 4 neurons that are important for the regulation of the pump motion.

Genetic (Raizen *et al.* 1995) and laser ablation studies (Avery and Horvitz 1989) show that the pair of MC motor neurons controls the rate of excitation of pharyngeal muscle and positively influences adult pumping rates. Acetylcholine is implicated as the excitatory neurotransmitter released from MC onto pharyngeal muscle and nicotinic receptors on the muscle generate the excitatory postsynaptic potential. Immunological examination failed to show choline acetyltransferase in the MCs (Rand and Nonet 1997) but four observations suggest that they are nevertheless cholinergic. First, the behavioral effects of MC loss by laser ablation are mimicked by mutants of acetylcholine synthesis (*cha-1*, choline acetyltransferase) and packaging (*unc-17*, vesicle-associated acetylcholine transporter; Avery and Horvitz 1990) and by lesions in a gene encoding a pharyngeal muscle nicotinic acetylcholine receptor (nAchR) subunit (*eat-2*; J. McKay, personal communication). Second, contraction of pharyngeal muscle can be induced by application of nicotine, a nAchR agonist (Raizen *et al.* 1995). Third, an *unc-17*::GFP reporter transgene is expressed in the MCs (data not shown). Fourth, pumping rates can be reduced by application of D-tubocurarine, a nAchR antagonist (Raizen *et al.* 1995). The pair of inhibitory M3 motor neurons controls the timing of repolarization of pharyngeal muscle, which signals the end of a pump. M3 is believed to be glutamatergic on the basis of observations that glutamate pulses onto pharyngeal muscle imitate the effect of M3s and mutants of a pharyngeal muscle glutamate receptor subunit gene (*avr-15*) resemble M3-ablated worms (Dent *et al.* 1997). The NSM neurons are serotonergic (Horvitz *et al.* 1982). Although ablation of these neurons has little measurable effect on behavior, exogenous serotonin accelerates pumping and suppresses locomotion. Serotonin from the NSMs may signal to the body that food is abundant and alter behavior appropriately. Neuron M4 regulates the swallowing motion of the isthmus, and it is the only pharyngeal neuron essential for worm

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survival. Details of pharyngeal anatomy are described in Albertson and Thomson (1976) and its behavior is summarized in Avery and Thomas (1997).

Pumping is suppressed in wild-type worms during the dauer larval stage and in response to a touch stimulus in adults. Despite being a robust behavior, little is known about the pharynx/touch response circuit other than the fact that the RIP/I1 connection between the pharyngeal and extrapharyngeal nervous systems is required (Chalfie *et al.* 1985). Inhibition of pumping during the dauer stage is biologically significant. Dauer larvae are a special developmental stage that forms in response to adverse environmental and nutritional conditions. Dauers exhibit numerous morphological and developmental modifications (Cassada and Russell 1975) that are designed to aid survival and possibly also dispersal to new food sources. Perhaps inhibition of pharyngeal activity could contribute to survival by reducing energy loss due to pumping when there is no food to eat or by reducing the risk of ingesting environmental toxins under conditions where nothing is to be gained by feeding anyway. A cuticle plug forms over the dauer mouth and may help to prevent desiccation and would also prevent feeding even if pumping was not suppressed (Riddle and Albert 1997).

To understand how pumping is inhibited we observed the effect of a touch stimulus on pharyngeal activity in two developmental stages: adults, which normally pump continuously in the presence of food, and dauers, which do not. We have looked at a number of mutants to identify genetic components required for this behavior. Our results indicate that (1) dauers and adults exhibit opposite behavioral output in the pharynx/touch response, (2) signaling via gap junctions and glutamate is important in adult and dauer pumping inhibition, and (3) a stimulatory pathway involving Gαq/Gαo competes with the inhibitory pathway in both developmental stages.

MATERIALS AND METHODS

Worm handling and nomenclature: Unless otherwise stated, worms were grown at 20° using NGMSR plates seeded with *Escherichia coli* DA837 (Davis *et al.* 1995). Conventional *C. elegans* nomenclature is used (Horvitz *et al.* 1979). Bristol N2 (Brenner 1974) were used as wild type.

Mutations: The following mutations were used.

LG I: *unc-29(e1072am)*; *egl-30(n686sd)*; *avr-14(nr781, ad1305, nr391)*; *daf-8(e1393ts)*; *eat-18(ad1110)*; *goa-1(n1134, n363)*.
LG II: *tph-1(mg280)*; *eat-2(ad465, ad570)*; *cat-2(e1112); eat-2(ad570)*; *adEx1818[rol-6(d) myo-2::eat-2$^+$]*.
LG III: *daf-7(e1372ts)*; *eat-4(ad572, ky5)*; *unc-25(e156)*; *glr-1(n2461)*.
LG IV: *osm-3(p802)*; *mec-3(e1338)*; *cha-1(p1186ts, md39ts)*.
LG V: *avr-15(ad1051, nr785, nr395)*; *glc-1(pk54::Tc1)*.
LG X: *unc-7(e5, ad1565)*; *unc-1(e719)*; *mec-7(n434, e1343)*.

Adult pump rate assays: One day prior to testing, L4 hermaphrodites were picked from an uncrowded nonstarved plate to a fresh seeded plate where they developed into adults at 20°. On the day of testing, adults were singly picked to an unseeded NGMSR plate briefly to remove excess *E. coli* from their cuticle. A number of clean adults were then transferred to another unseeded NGMSR plate and allowed to recover for 5 min at room temperature. The pump rate of a single adult was counted for 20 sec. Single pumps were scored as a complete backward movement of the terminal bulb grinder. The worm was then tapped once on the posterior one-third of its body (subsequently referred to as a tail tap) and the pump rate was recorded for another 20 sec. The worm was then discarded. A given individual therefore gave rise to two pump rate scores, one in which the worm is not tapped and the other where it is tapped on its posterior. Adults go through cycles of pumping and not pumping on plates in the absence of food. Pump rates were scored only from adults that were actively pumping at the start of the recorded period. Adults were scored within 40 min of being placed on an unseeded plate; after this time the plate was discarded. Pumps were counted at room temperature (22–25°).

Dauer pump rate assays: Dauers for testing were identified according to these criteria: thin and dense body, presence of alae, and radial constriction of isthmus and terminal bulb (Riddle and Albert 1997). Except where specified, dauers were picked from starved plates grown at 20° and pump rates were scored within 2 days after the appearance of dauers on the plate. A platinum pick was used to transfer individual dauers from the starved plate to a fresh NGMSR plate whose surface was completely covered with *E. coli* (dauers leave tracks in the food, making them easier to find). The surface of the plate was immersed in light white oil (Sigma, St. Louis; M3516) to aid visual inspection of the pharynx. After 10–15 min recovery at room temperature, the pump rate of a single dauer was counted over 2 min. The same dauer was then heavily tapped once on the posterior one-third of its length with a pick and its pump rate was scored over 2 min. The dauer was then discarded. Plates were discarded when dauers had been on them for 40 min. Pumps were counted using a Wild M410 dissecting microscope (magnification, ×64 and ×20 eyepieces) at room temperature. Some special conditions were employed when testing the conditional *cha-1(ts)* mutants.

cha-1(ts) were grown at the permissive temperature of 15° until plates starved. Dauers were picked from plates for testing within 1 month of inoculating the plate. To study *cha-1(ts)* dauers under restrictive conditions, starved plates were transferred from 15° to 25° and left overnight. Dauers were then treated in the same way as any other strain. When *cha-1(ts)* dauers left at 25° overnight are returned to 15° on food plates they resume normal development.

Recording pumping activity per second: *Adults: unc-29(e1072am)* was used instead of wild type, which move too fast to score using this approach. *unc-29* encodes a levamisole-sensitive nicotinic acetylcholine receptor (Fleming *et al.* 1993) expressed in body wall muscles and has no effect on adult pumping activity (Raizen *et al.* 1995). Adults were prepared and tested as described above with the following modification. Single worms were scored for 20 sec pretapped and posttapped. Worms were observed by use of a dissecting microscope placed next to a computer. Depressing a single key on the computer keyboard when the worm pumped recorded the time of pumping events over the course of the observation. After a population of worms was tested the average number of pumps was calculated for each consecutive second.

Dauers: Dauers were prepared as in *Dauer pump rate assays* and tested as in *Recording pumping activity per second* described above. These recordings could be carried out only on slow moving/uncoordinated dauers. In cases where a genotype to be tested was not slow it was combined with *unc-29(e1072am)*

TABLE 1

Adult worms pump slower after tail tap

ID no.	Genotype	*N*	Pre-tail tapped (average ± SEM)	Post-tail tapped (average ± SEM)
1	*wt*	120	24.9 ± 1.41	4.1 ± 0.90
2	*unc-29(e1072am)*	120	26.4 ± 1.17	4.3 ± 0.59
3	*mec-7 (n434)*	10	31.3 ± 4.12	2 ± 1.39
4	*mec-7(e1343)*	10	20.6 ± 4.32	2.4 ± 1.97
5	*mec-3(e1338)*	42	24.1 ± 1.99	5.9 ± 1.20
6	*unc-7(e5)*	120	32.4 ± 1.35	19.5 ± 1.30
7	*unc-7(ad1565)*	90	30.6 ± 1.53	15.8 ± 1.44
8	*eat-4(ky5)*	30	30.7 ± 1.58	10.2 ± 1.60
9	*unc-29(e1072am); eat-4(ad572)*	120	37.6 ± 0.94	15.6 ± 1.09
10	*avr-14(nr391)*	90	51.7 ± 1.05	12.6 ± 1.49
11	*avr-15(ad1051)*	100	30.6 ± 1.46	8 ± 1.12
12	*glc-1(pk54::Tc1)*	6	34.8 ± 3.09	2.5 ± 1.97
13	*glr-1(n2461)*	10	21.5 ± 4.72	0.9 ± 2.18
14	*avr-14(ad1305); avr-15(ad1051)*	120	45.2 ± 1.10	21.2 ± 1.60
15	*avr-14(nr391); avr-15(nr395)*	60	38.6 ± 1.56	18.6 ± 2.10
16	*avr-14(nr781); avr-15(nr785)*	120	46.0 ± 0.88	22.4 ± 1.46
17	*goa-1(n363)*	40	18.9 ± 1.25	3.5 ± 0.69
18	*goa-1(n1134) unc-29(e1072am)*	90	32.5 ± 1.51	4.5 ± 0.84
19	*unc-7(e5); goa-1(n1134)*	100	30.1 ± 0.98	22.8 ± 1.00
20	*unc25(e156)*	6	29.2 ± 1.92	0.2 ± 0.17

The pump rates of untapped and tapped adult worms of various genotypes are shown. Tail tap always results in a decreased pump rate. Tail-tapped columns show the arithmetic average number of pumps over a 20-sec period. *N*, number of individuals tested; SEM, standard error of the mean.

to produce a slow-moving double mutant. It is possible to measure pumping activity as a function of time using electropharyngeograms (EPGs; Raizen and Avery 1994). Two factors prevented the use of EPGs for this purpose in this study. First, recorded EPG pump rates for any given strain were extremely variable, possibly influenced by random disruption of the dauer buccal plug due to suction of the dauer head. Second, we found that serotonin used to induce pumping also induced a rhythmic electrical activity in dauer EPG traces, which we call B-phase activity. This hindered identification of authentic pumps in a trace. On the basis of simultaneous EPG and video recording of dauers, B-phase activity appears to arise from pulsing of the excretory/secretory pore (Nelson and Riddle 1983).

Statistics: The average percentage reductions in adult pump rates after tail tap shown in Figure 2 were normalized to take into account differences in initial pump rates between lines and were calculated as follows for each worm in each population:

$$\%\ \text{reduction} = ((\text{tapped pump rate} - \text{untapped pump rate})/\text{untapped pump rate}) \times 100.$$

In certain mutant strains, some individuals pumped faster after tail tap. When this occurred, the normalized increase in pump rate was given a negative sign. The nonparametric Wilcoxon two-sample test was used to compare pairs of data sets (Avery 1993; Sokal and Rohlf 1995).

RESULTS

Pharyngeal activity responds to mechanical stimulus and is developmentally modified: Chalfie *et al.* (1985) found that light touch briefly inhibits pumping activity in adult worms. We have quantified the effect of harsh touch on pumping in an attempt to identify genetic components of the pathway in both adults and dauers. The pump rates of adult worms over a 20-sec period before and after a harsh tail tap are shown in Table 1. Wild-type (Table 1, no. 1) activity was 24.9 ± 1.41 pumps prior to tail tap and 4.1 ± 0.90 pumps after tail tap, which corresponds to a relative decrease in pump rate of 88.7 ± 1.98%. Under the conditions employed in this study, adult worms were found to have a relatively stable pump rate over the 20-sec period in the absence of mechanical stimuli. However, application of a tail tap almost completely inhibits pumping for a short time (~3 sec) and then pumping recovers extremely slowly. These observations are presented in Figure 1 for *unc-29* mutant adults, which behave similarly to wild-type N2 (Table 1, nos. 1 and 2). This behavior is extremely robust and we have used it to screen for mutants that resist pumping inhibition following tail tap (Figure 2 and Table 1).

The adult locomotory response to touch has been studied in detail in *C. elegans* (Huang and Chalfie 1994; Wicks and Rankin 1995) and can be genetically and anatomically divided into separate neural circuits that mediate response to light and harsh touch. We tested two types of touch-response mutants to identify which circuit is involved in the pharynx/touch response. *mec-7* mutants are defective in a β-tubulin gene that is expressed in neurons responsible for gentle touch

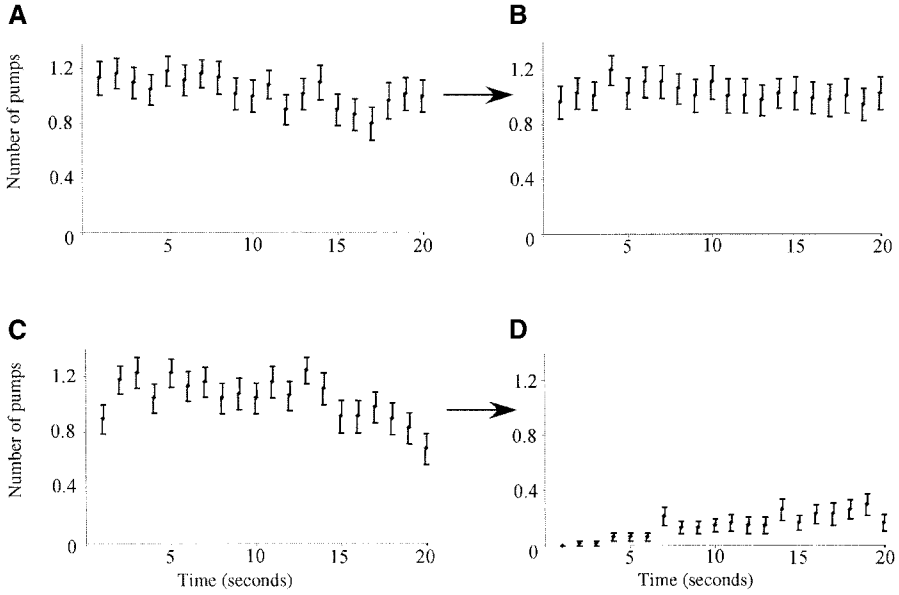

FIGURE 1.—In the absence of food, tail tap reduces the relatively constant pump rate of adult worms. The effect of tail tap on adult *unc-29* worms is shown. (A and C) Number of pumps per second over 20 sec before tail tap. (B) Pump rate of worms for subsequent 20 sec without tail tap. (D) Pump rate for subsequent 20 sec following tail tap. The average numbers of pumps during each 20-sec period are (A) 20.7 ± 2.08, (B) 20.8 ± 2.16, (C) 20.9 ± 1.91, and (D) 3.0 ± 0.81. Sixty individuals were tested in each case. D is significantly different from A–C ($P < 0.001$).

(CHALFIE and SULSTON 1981). *mec-7* worms are insensitive to gentle touch. The two alleles of *mec-7* that were tested (Table 1 and Figure 2, nos. 3 and 4) were not tap resistant. The gentle-touch neurons are not essential for this behavior but it is possible that they contribute to it redundantly with other circuits. WAY and CHALFIE (1989) discovered that response to harsh touch could be eliminated when the PVD neurons were killed in addition to the gentle-touch neurons. *mec-3* encodes a homeodomain protein that is expressed in all of the touch neurons and in PVD. *mec-3* mutants fail to express touch cell-specific structural genes, resulting in loss of light and harsh touch response. *mec-3* is only slightly resistant to tail tap (Table 1 and Figure 2, no. 5), suggesting that the neural circuits underlying the locomotion/touch and pharynx/touch behaviors may not overlap. PVDs detect harsh touch to the central region of the body but adults lacking the PVDs can respond to harsh head and tail tap (WAY and CHALFIE 1989). The mechanosensory neurons involved are unknown.

In contrast to adult behavior, dauer larvae show an increase in pump rate after tail tap, as illustrated in Figure 3. Although the increase in tapped dauer pump rate is generally small it has been observed in every strain tested with a few notable exceptions. Mutation of *eat-2* and *eat-18* results in slow-pumping adults because of defective cholinergic signaling between motor neuron MC and the pharynx (RAIZEN *et al.* 1995). The *eat-2*-encoded nAchR subunit is expressed in pharyngeal muscle (J. MCKAY, personal communication) but the pharynx of *eat-2* mutants remains sensitive to exogenous nicotine. *eat-18* genetically interacts with *eat-2* but *eat-18* mutants are insensitive to exogenous nicotine. *eat-2(ad570)* and *eat-18(ad1110)* dauers (Table 2, nos. 24 and 25) exhibit slower untapped pumping rates than those of *wt*, suggesting that acetylcholine signaling persists at the pharynx even during the dauer stage. We do not know why the two *eat-2* alleles exhibit different untapped pump rates in dauers (Table 2, nos. 24 and 26) despite having almost identical effects on adult pump rates. Expression of EAT-2 in pharyngeal muscle under the transcriptional control of the myogenic promoter *myo-2* rescued *eat-2(ad570)* dauer pumping rates to wild-type levels (*eat-2(ad570); adEx1818[rol-6(d) myo-2::eat-2$^+$]*, untapped pump rate = 0.433 ± 0.128 pumps/min). Comparison of untapped and tapped dauer pumping rates (Table 2, nos. 24–26) reveals that the ability to pump faster after tail tap is lost in *eat* mutants. There may even be a tendency toward a reduced pump rate. We interpret these results to mean that cholinergic signaling from MC to the pharynx via nAchRs is necessary for the basal pumping rate in dauers and for the increase in pump rate following tail tap.

***unc-7* is required for normal stimulus/response in adults and dauers:** Information about tail tap detected by the extrapharyngeal nervous system must pass to the pharyngeal nervous system either via connections made between the RIPs and I1s, which is the only direct neural link, or via a long-distance humoral pathway. The RIPs are coupled to the I1s by gap junctions. *unc-7* encodes a gap junction channel subunit (STARICH *et al.* 1993, 1996) and *unc-7* mutants are defective in both adult and dauer tail tap response. Tail tap does not inhibit adult pumping in *unc-7* mutants to the same extent as *wt* (Figure 2 and Table 1, nos. 6 and 7). Some individual *unc-7* adults actually pumped faster after tail tap, a response never seen in *wt*. *unc-7* mutants also tend to have a faster untapped pump rate than that of controls (Table 1, no. 1 *vs.* nos. 6 and 7) but it is not commensurate with their resistance to tail tap pumping inhibition.

The *unc-7* inhibitory pathway is also functional in dauers. The response of *unc-7* dauers to tail tap is ap-

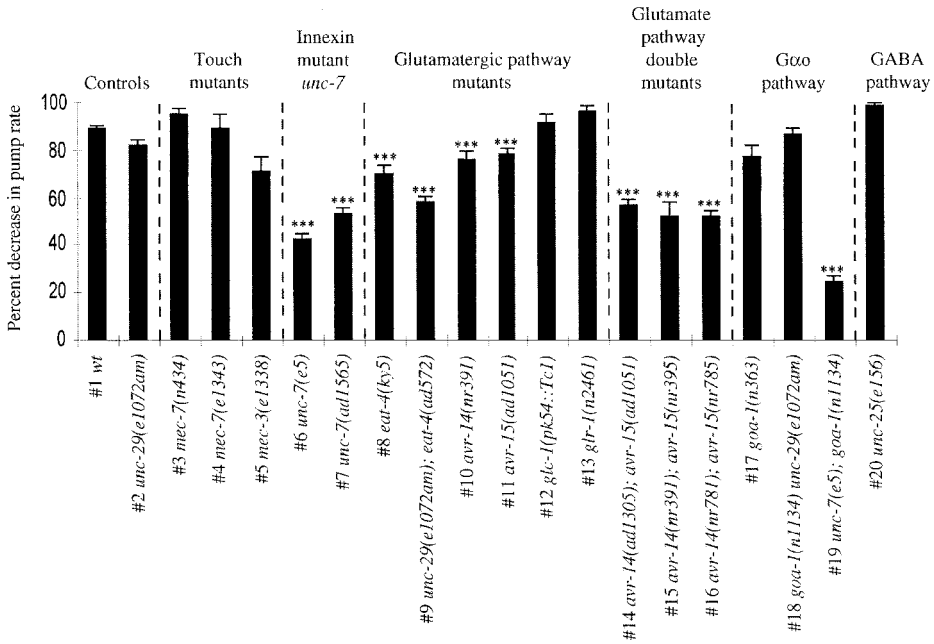

FIGURE 2.—Mutations in a number of neural signaling pathways confer resistance to the inhibitory effect of tail tap on adult pharyngeal activity. The average normalized percentage decrease in pump rate of mutant adults following harsh tail tap is shown. The pathways being examined are as follows: nos. 1 and 2, controls; 3–5, touch mutants; 6 and 7, Innexin *unc-7*; 8–16, glutamatergic pathway; 17–19, Gαo pathway; 20, GABA. *** indicates significant difference from control ($P < 0.001$). Wild type is the control in all cases except for nos. 9 and 18, which are compared to no. 2 (*unc-29*). No. 19 (*unc-7; goa-1*) is significantly different from no. 6 (*unc-7*; $P < 0.001$).

proximately six times greater than that of *wt* (Table 2, nos. 27 and 28). *unc-7* allele *ad1565* was originally identified in a screen for dauers that can pump fast. The *unc-7* increase in pump rate is dependent, at least in part, on normal cholinergic communication between MC and pharyngeal muscle. The double mutants *unc-7; eat-2* and *unc-7; eat-18* (Table 2, nos. 29–31) do not show much of an increase in pump rate following tail tap. In fact, tail tap can actually reduce pump rates in these mutants. This indicates that the pathway in which *unc-7* functions is upstream of, and inhibitory to, MC. *unc-7; eat-2* and *unc-7; eat-18* pump faster than *eat-2* and *eat-18* single mutants, suggesting that *unc-7* also functions in an MC independent pathway. DENT *et al.* (2000) previously presented a model and some supporting data in which *unc-7* is required for the flow of ivermectin-induced hyperpolarization from extrapharyngeal cells into the pharynx. Our results indicate that gap junctions are present in the pathway that transduces the mechanical stimulus from the tail to the pharynx. *unc-7* may be required at a number of points along the pathway.

A glutamatergic pathway is involved in normal stimulus/response in adults and dauers: Glutamate inhibits pharyngeal activity (LI *et al.* 1997) and glutamatergic neurons are present in the pharyngeal nervous system (DENT *et al.* 1997). We examined whether this transmitter is responsible for pump inhibition in tapped adults and untapped dauers. The *eat-4* gene encodes a vesicular glutamate transporter (LEE *et al.* 1999) and *eat-4* mutants exhibit no detectable glutamate signaling between M3 and pharynx muscle. Tail tap-induced pumping inhibition in *eat-4* adults is not as pronounced as in controls (Figure 2, nos. 8 and 9 *vs.* nos. 1 and 2). The small effect of *eat-4* on this behavior could indicate that glutamatergic signaling has a minor or mostly redundant role in this pathway. Another possibility is that glutamate

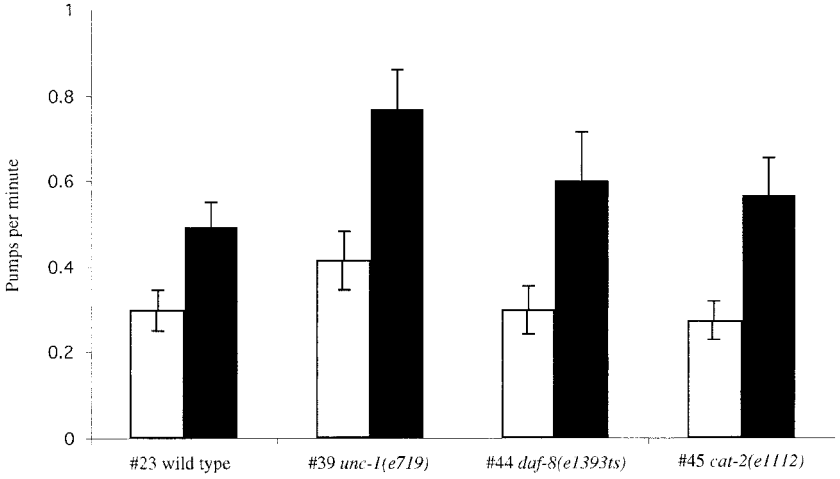

FIGURE 3.—Tail tap stimulates pharyngeal activity in dauers. Increased pump rate following harsh tail tap in dauers of four different genotypes is shown. Open bars, pump rate before tail tap; solid bars, pump rate after tail tap. Pretapped and posttapped pump rates are significantly different for nos. 23, 39, 45 ($P < 0.05$), and 44 ($P < 0.1$).

TABLE 2

The effect of tail tap on dauer pumping rates

ID no.	Genotype	*N*	Pre-tail tapped (average ± SEM)	Post-tail tapped (average ± SEM)
23	*wt*	60	0.30 ± 0.049	0.49 ± 0.060
24	*eat-2(ad570)*[a]	60	0.11 ± 0.032	0.07 ± 0.040
25	*eat-18(ad1110)*	30	0.03 ± 0.023	0.10 ± 0.056
26	*eat-2(ad465)*	60	0.39 ± 0.057	0.18 ± 0.035
27	*unc-7(ad1565)****	30	2.03 ± 0.348	7.83 ± 1.513
28	*unc-7(e5)****	60	1.08 ± 0.167	6.49 ± 0.992
29	*unc-7(e5); eat-2(ad465)*	30	0.87 ± 0.195	1.75 ± 0.255
30	*unc-7(ad1565); eat-2(ad570)*	30	1.58 ± 0.247	1.53 ± 0.232
31	*unc-7(ad1565); eat-18(ad1110)*	30	0.52 ± 0.121	0.21 ± 0.071
32	*unc-29(e1072am); eat-4(ad572)****	30	0.70 ± 0.095	2.20 ± 0.493
33	*goa-1(n1134)*	30	0.32 ± 0.051	ND
34	*goa-1(n1134) unc-29(e1072am); daf-7(e1372ts)****	60	2.31 ± 0.505	16.63 ± 1.380
35	*unc-29(e1072am); daf-7(e1372ts)****	30	0.38 ± 0.052	1.53 ± 0.195
36	*unc-7(e5); goa-1(n1134)****	30	5.18 ± 0.873	13.72 ± 1.713
37	*goa-1(n1134) unc-29(e1072am); eat-2(ad465); daf-7(e1372ts)****	30	0.22 ± 0.128	1.73 ± 0.427
38	*egl-30(n686sd)*	30	0.32 ± 0.056	0.30 ± 0.051
39	*unc-1(e719)*	30	0.42 ± 0.068	0.77 ± 0.098
40	*tph-1(mg280)****	30	0.18 ± 0.051	0.65 ± 0.102
41	*unc-7(e5); tph-1(mg280)****	30	0.93 ± 0.147	2.70 ± 0.814
42	*goa-1(n1134) unc-29 (e1072am); tph-1 (mg280); daf-7(e1372ts)****	60	3.85 ± 0.743	21.49 ± 1.671
43	*daf-8(e1393ts); osm-3 (p802)*[b]	30	0.30 ± 0.056	0.60 ± 0.115
44	*daf-8(e1393ts)*[b]	60	0.28 ± 0.045	0.57 ± 0.089
45	*cat-2 (e1112)*	30	0.65 ± 0.120	0.90 ± 0.139

The pump rates of untapped and tapped dauers of various genotypes are shown. Tail-tapped columns show the arithmetic average number of pumps per minute. *N*, number of individuals tested; SEM, standard error of the mean; ND, not done, because worms moved too rapidly to score after tail tap. ***Pretapped and post-tapped pump rates were significantly different ($P < 0.001$) for these genotypes.

[a] Only 30 of the 60 dauers were tail tapped.

[b] Dauer formation was induced at 25°.

signaling is relatively unperturbed at the required site. For instance, the observed loss of communication between M3 and pharyngeal muscle in *eat-4* may arise because glutamate cannot be packaged into vesicles fast enough for it to keep up with the rapid rate of pharyngeal activity (Rankin and Wicks 2000). However, neurons that are active only rarely, such as mechanosensory or interneurons, may be able to replenish their vesicular glutamate adequately to permit signaling with their postsynaptic partner. If glutamate is released from a synapse that is not frequently active in response to the single tail tap this could explain the small effect of *eat-4* in this behavior.

Adults with lesions in any of three glutamate-gated chloride channel α (GluClα) subunits, *avr-14*, *avr-15*, and *glc-1* (Dent *et al.* 2000), are not very resistant to tail tap (Figure 2, Table 1, nos. 10–12), and neither are mutants of the AMPA-type glutamate receptor GLR-1 (Hart *et al.* 1995; Figure 2, Table 1, no. 13). However, *avr-14; avr-15* double mutants are quite resistant (Figure 2, Table 1, nos. 14–16). *avr-14* is not expressed in the pharyngeal nervous system so it cannot be required for direct inhibition of pharynx muscle via M3. It is expressed in mechanosensory neurons, ring ganglia, and the ventral chord. *eat-4* is also expressed in mechanosensory neurons, suggesting that mutations in the glutamatergic pathway may effect detection of harsh touch. *eat-4* dauers, like wild type, exhibit a chronically suppressed pump rate, indicating that glutamate is not required for this long-term effect.

Gαo is required for normal response in dauers and adults: The role of Gαo in pump/touch response in dauers is unusual. Gαo is encoded by the *goa-1* gene (Mendel *et al.* 1995) and null mutants do not form dauers. A reduction-of-function mutant, *goa-1(n1134)* (Segalat *et al.* 1995), produces some dauers on starved plates but they pump at *wt* rates (Table 2, no. 33). However, when *goa-1(n1134)* is combined as a double mutant with an uncoordinated (*unc*) mutation it pumps faster than controls both before and after tail tap (Table 2, nos. 34 and 36). Temporal analysis of the *goa-1* response to tail tap shows an immediate rapid response

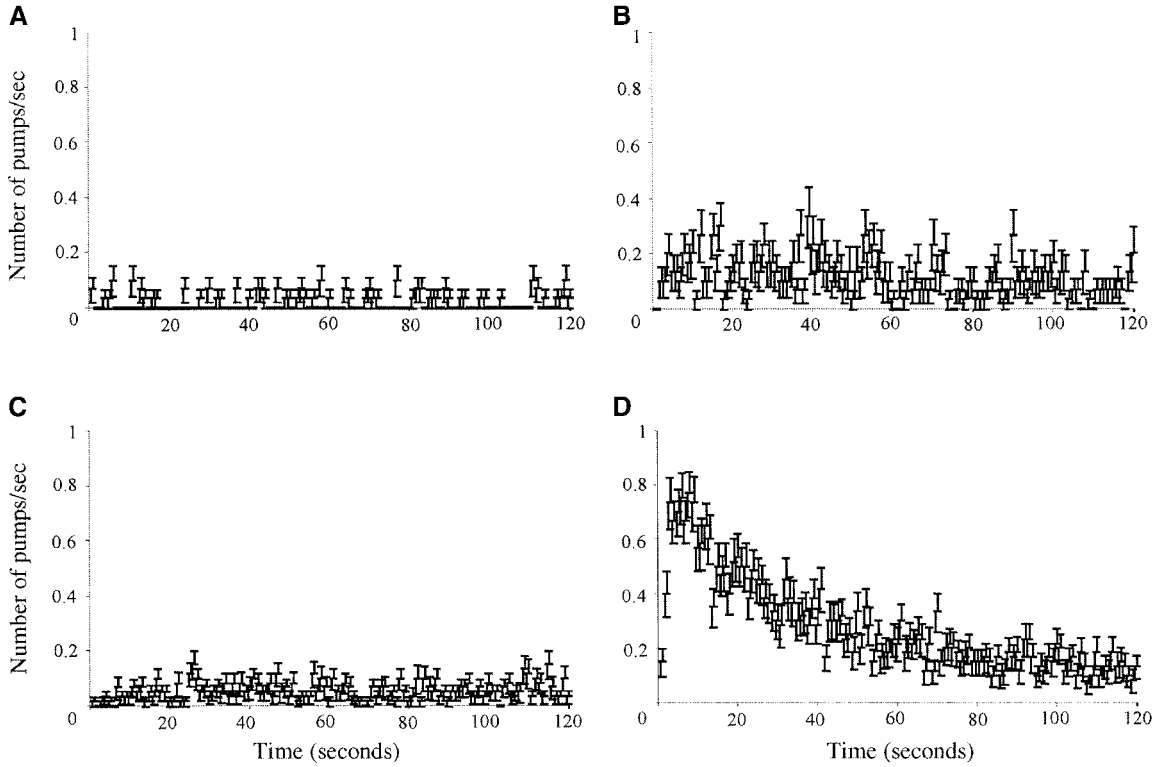

FIGURE 4.—Qualitative and quantitative differences in dauer tail tap response between *unc-7* (A and B) and *goa-1* (C and D) suggest that these genes function in different pathways. Graphs show pump rate per second over a 2-min period before (A and C) and after (B and D) tail tap. Thirty *unc-7* dauers were tested for A and B. Sixty *goa-1* dauers were tested for C and D. Bars show standard error of the mean. The strains used and average pump rates are *unc-7(e5); daf-8(e1393ts)*, (A) 1.37 ± 0.389 pumps/min and (B) 7.02 ± 1.365 pumps/min; *goa-1(n1134) unc-29(e1072am); daf-7(e1372ts)*, (C) 3.29 ± 0.704 pumps/min and (D) 16.76 ± 1.455 pumps/min.

in pump rate followed by a slow decay. The response is qualitatively and quantitatively different from *unc-7*, suggesting that separate pathways are involved (Figure 4). However, the *goa-1* pathway is similar to *unc-7* in its requirement for functional nAchRs (Table 2, no. 37 *vs.* no. 34). *goa-1* adults are not resistant to tail tap-evoked pumping inhibition (Table 1, Figure 2, nos. 17 and 18) but an *unc-7; goa-1* double mutant is more resistant than *unc-7* alone (Table 1, Figure 2, no. 19 *vs.* no. 6), indicating that the *goa-1*-mediated stimulation of pumping is active in adults as well as dauers.

Gαo and Gαq often act antagonistically (HAJDU-CRONIN *et al.* 1999) and we found that a Gαq mutant, *egl-30 (n686sd)* (BRUNDAGE *et al.* 1996), eliminates the increase in pumping rate caused by tail tap in dauers (Table 2, no. 38). On the basis of this result we propose the following model. Harsh touch in adults and dauers causes release of an unknown neuromodulator that stimulates pumping and movement away from the stimulus via a G-protein-coupled receptor linked to Gαq. Activation of Gαq renders target tissues more responsive to acetylcholine signaling via nAchRs. In the absence of Gαq there is no response, but in the absence of Gαo the response is large because nothing opposes the stimulation mediated by Gαq. The response eventually decays due to receptor adaptation. Why do untapped *unc-7; goa-1* and *unc-29 goa-1* double mutants pump faster than the *goa-1* single mutant? A possible explanation is that the presence of an *unc* causes the dauer body to bend and move and the worm perceives these as a light touch resulting in a small release of neuromodulator. We observe that *unc; goa-1* double mutants move more often than controls and there is a positive correlation between movement and pumping (data not shown). All *unc* dauers tested (*unc-1*, *unc-7*, and *unc-29*) appear to pump faster than *wt* (Table 2, nos. 39, 28, and 35 *vs.* no. 23). Only *unc-7* pumps significantly faster. The molecular products of these three loci are different and it is unlikely that all of them affect the dauer touch/pharynx response pathway.

Analysis of neurotransmitters potentially involved in the tail tap response: Having ascertained that glutamate is unlikely to act directly on the pharynx to inhibit pumping in adults and dauers we wished to identify the neurotransmitter that did. We also wanted to identify the ligand responsible for signaling via the proposed Gαq/Gαo pathway. A prime candidate for stimulating pumping in tapped dauers is serotonin because it stimulates

pumping in adults (Horvitz *et al.* 1982). *tph-1(mg280)* is a null mutation of the single tryptophan hydroxylase homolog of *C. elegans*, a key enzyme involved in serotonin biosynthesis, and *tph-1* mutants exhibit defects in serotonin-dependent behaviors (Sze *et al.* 2000). *tph-1* dauers pump slower than *wt* but show an increase in pump rate after tail tap (Table 2, no. 40 *vs.* no. 23). The untapped pump rate *of unc-7; tph-1* dauers is the same as *unc-7* alone, but the tapped pump rate is lower than that of *unc-7* (Table 2, no. 41 *vs.* no. 28). The presence of *tph-1* has a negligible effect on *goa-1* (Table 2, no. 42 *vs.* no. 34). This could be interpreted in two ways: (1) that serotonin does increase pump rates in response to tap but it is not the agonist (or at least is not the only agonist) for the Gαq/Gαo pathway or (2) that some serotonin remains in *tph-1* mutants. If the former interpretation is true then an unknown neuromodulator must increase pump rates in *tph-1* and *unc-7; tph-1* mutants. Another good candidate is acetylcholine. A significant amount of data implicate it as the neurotransmitter at the MC/pharynx neuromuscular junction but it could also act elsewhere in the touch/pharynx pathway. Also, many of the existing *egl-30* (Gαq) alleles were identified in a screen for mutants that suppress the effects of muscarinic acetylcholine signaling in the pharynx (Brundage *et al.* 1996). Acetylcholine is synthesized by choline acetyltransferase, an enzyme encoded by the *cha-1* gene. We tested two conditional alleles of *cha-1* and found that pumping prior to tail tap is completely absent after dauers are left at the restrictive temperature overnight. However, *cha-1* mutants can pump after tail tap at rates similar to *eat-2* and *eat-18* (*cha-1 (md39ts)*, 0.13 + 0.063 pumps/min; *cha-1(p1186ts)*, 0.07 ± 0.046 pumps/min), indicating that acetylcholine is probably not responsible for increasing pumping rate via Gαq.

Exogenous application of octopamine (Horvitz *et al.* 1982), dopamine (our personal observation), and γ-aminobutyric acid (GABA; Avery and Horvitz 1990) can suppress pharyngeal activity. Mutants reportedly deficient in the neuromodulators octopamine (*osm-3*; Horvitz *et al.* 1982; Table 2, no. 43 *vs.* no. 44) and dopamine (*cat-2*; Sulston *et al.* 1975; Table 2, no. 45 *vs.* no. 23) exhibit dauer behavior similar to control levels. Therefore, they are not individually responsible for pumping suppression throughout the dauer stage and are not agonists of the Gαq/Gαo pathway. GABA does not mediate pumping inhibition in adults as tail tap of the *unc-25* GABA-deficient mutant (McIntire *et al.* 1993) effectively inhibits pumping (Table 1, Figure 2, no. 20). We cannot exclude the possibility that respective neurotransmitter levels are insufficiently low in some of these mutants to cause an observable defect. It is also possible that these neurotransmitters play a redundant role in pumping inhibition and individual roles cannot be detected using a single-mutant approach.

DISCUSSION

Inhibitory and stimulatory pathways control pharyngeal output after tail tap: The behavioral output of harsh tail tap is opposite in adults and dauers. However, the inhibitory pathway identified in adults is active in dauers, as *unc-7* and glutamatergic pathway mutant dauers can pump faster than controls. The stimulatory pathway identified in dauers involving Gαq/Gαo is similarly active in adults, as *unc-7; goa-1* adults are more resistant to tail tap than is *unc-7* alone. The behavioral output observed in these developmental stages appears to reflect a shift in dominance between the two competing pathways (Table 3). Despite testing many individually and multiply mutant worms we failed to completely eliminate the effects of tail tap in adults and dauers, suggesting that many redundant pathways underlie these behaviors.

***unc-7* and glutamate may function in the same pathway:** We have identified genetic components of an inhibitory circuit connecting harsh touch receptors in the posterior body to the pharynx. Mutants of the innexin gene *unc-7* and various combined mutants involved in glutamate signaling are resistant to pumping inhibition to a similar extent (Figure 2, nos. 6 and 7 and 14–16). We propose that these mutants work in the same pathway but communicate the inhibitory signal at different points within the circuit. Glutamate and gap junctions communicate the same signal at the same mechanosensory synapse in other touch-response circuits (Lee *et al.* 1999). This is unlikely to be the case here because *unc-7* and *avr* genes do not both have to be mutant to observe a large behavioral effect. Glutamate signaling may be required in the sensory neurons and *unc-7* may form the gap junction linking the pharyngeal to the extrapharyngeal nervous system. If *unc-7* is essential for coupling RIP to I1 we would expect complete loss of adult tail tap inhibition, but this is not observed. Either the *unc-7* lesion does not completely uncouple RIP and I1 or other inhibitory systems exist.

What is the ligand for the Gαq/Gαo stimulatory pathway? Receptors for the neuromodulators serotonin and dopamine can signal via Gαq in vertebrates (Watson and Arkinstall 1994); however, neither of these chemicals appears to be an agonist of the Gαq/Gαo system in the dauer pharynx. Muscarinic acetylcholine receptors can also signal through Gαq. Our analysis of *cha-1(ts)* mutants suggests that this may not be the ligand for the Gαq-coupled receptor. However, this result is not conclusive as there is a chance that some acetylcholine remains within a cholinergic neuron belonging to the touch/pharynx-response pathway even after worms have remained at the restrictive temperature overnight. When *cha-1(ts)* adults are placed under conditions conducive to rapid pumping, at the restrictive temperature, conditions that should rapidly deplete any available acetylcholine, some individuals can still pump very slowly

TABLE 3

Summary of observations

Genetic lesion	Pharyngeal output in tapped dauers	Pharyngeal output in tapped adults	Reason
wt	+	− − −	Following tail tap a stimulatory pathway is dominant in dauers. An inhibitory pathway is dominant in adults.
unc-7 inhibitory pathway	++	− −	Stimulatory pathway has greater effect due to reduction of inhibitory pathway function.
goa-1 inhibitory pathway	+++	− − −	Increase in *egl-30* stimulatory pathway function in dauers. *unc-7* inhibitory pathway is dominant over *egl-30* pathway in adults.
unc-7 and *goa-1*	+++	−	Maximum elevation of pump rate in dauers is achieved by loss of Gαo. Additional loss of *unc-7* cannot increase pump rate. In adults, reduction of *unc-7* inhibitory pathway and increase in *egl-30* stimulatory pathway result in only a small overall decrease in pump rate after tail tap.

The behavioral observations arising from mutation in the *unc-7* and *goa-1* inhibitory pathways in dauers and adults are summarized. +, increase in pump rate; −, decrease in pump rate. Number of + or − corresponds to magnitude of effect.

after 5 hr. In addition, dauers kept at the restrictive temperature overnight can still move away from a touch stimulus, indicating that acetylcholine remains in locomotory motor neurons. This is not unexpected as dauers tend to remain motionless when left undisturbed, conserving available neurotransmitter. As acetylcholine is released at body wall neuromuscular synapses, permitting dauers to move away from the tail tap stimulus, it is possible that this distant release is detected by the pharynx and contributes to the increase in pump rates.

Suppressed pumping during the dauer stage is not due (solely) to constitutive activation of the inhibitory *unc-7*/glutamate pathway: *unc-7* and glutamate pathway mutant dauers exhibit the same long-term suppression of pharyngeal activity observed in *wt* dauers. In the presence of exogenous serotonin (50 mM) some *wt* dauers are capable of pumping at rates of 75 pumps/minute when measured by EPG recording. This is significantly faster than rates observed in the absence of drug when dauers are on NGMSR plates (Table 2, no. 23). The dauer pharynx is therefore capable of pumping quite fast but is prevented from doing so by a mechanism that does not involve the *unc-7* or the glutamatergic pathways discussed here.

Why does touch affect pharyngeal activity? We know of no reason, at least under laboratory conditions, why it would be beneficial for adult worms to cease pumping, or for dauers to start pumping, following harsh touch. One could propose that it may be in the adult worms' interest to briefly shut down any nonessential energy-consuming distractions following a shock such as harsh tail tap to focus on escape from the stimulus. However, in many cases using this stimulus the adult worm completely freezes both locomotion and pumping. So perhaps both of these phenotypes are the result of a general shock response capable of overriding all other activities. The Gαq/Gαo-mediated pathway is required for efficient pharyngeal activity during nondauer stages of development and for normal locomotion; *egl-30* mutants pump and move slowly and *goa-1* mutants move rapidly. We speculate that the small increase in pump rate observed in dauers is due to reception of a signal primarily intended to activate the locomotory circuit.

Ivermectin may kill worms by hijacking the *unc-7*/glutamatergic inhibitory pathway: The inhibitory pharynx/touch pathway discussed in this article has been studied using mutants previously reported to confer resistance to the nematocidal drug ivermectin (combinations of *avr-14*, *avr-15*, and *unc-7*). The most direct mechanism by which ivermectin kills worms is to open AVR-15-containing glutamate-gated channels in pharyngeal muscle, hyperpolarizing the organ, and thus preventing pumping and feeding. Dent *et al.* (2000) have proposed another model in which ivermectin also kills worms by inhibiting pumping. In this model ivermectin irreversibly opens a glutamate-gated chloride channel composed of *avr-14* and/or *avr-15* in an extrapharyngeal neuron. This results in chloride influx and neuron hyperpolarization. Hyperpolarization descends to the pharynx via gap junctions encoded by *unc-7* and inhibits pumping. This proposal has been tested by laser ablation of neuron I1 thereby uncoupling the pharynx from neurons containing ivermectin-sensitive GluCls in the extrapharyngeal nervous system. Laser-operated worms were resistant to ivermectin (Dent *et al.* 2000). This model is intriguing because it suggests that I1 is coupled directly to pharyngeal muscle via gap junctions. *unc-7; eat-2* dauers pump faster than *eat-2* single-mutant dauers,

suggesting that *unc-7*-mediated pumping inhibition is partly MC independent. Perhaps this inhibition is due to *unc-7*-dependent coupling of I1, or a connected neuron, to pharyngeal muscle.

eat-2(ad570) adEx1818 [rol-6(d) myo-2::eat-2$^+$] was provided courtesy of Dr. Jim McKay. The Caenorhabditis Genetics Center (CGC) provided some of the strains described in this article. This work was supported by research grant HL46154 from the U.S. Public Health Service to Leon Avery.

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