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Tyramine Functions Independently of Octopamine in the *Caenorhabditis elegans* Nervous System

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Summary

Octopamine biosynthesis requires tyrosine decarboxylase to convert tyrosine into tyramine and tyramine β-hydroxylase to convert tyramine into octopamine. We identified and characterized a *Caenorhabditis elegans* tyrosine decarboxylase gene, *tdc-1*, and a tyramine β-hydroxylase gene, *tbh-1*. The TBH-1 protein is expressed in a subset of TDC-1-expressing cells, indicating that *C. elegans* has tyraminergic cells that are distinct from its octopaminergic cells. *tdc-1* mutants have behavioral defects not shared by *tbh-1* mutants. We show that tyramine plays a specific role in the inhibition of egg laying, the modulation of reversal behavior, and the suppression of head oscillations in response to anterior touch. We propose a model for the neural circuit that coordinates locomotion and head oscillations in response to anterior touch. Our findings establish tyramine as a neurotransmitter in *C. elegans*, and we suggest that tyramine is a genuine neurotransmitter in other invertebrates and possibly in vertebrates as well.

Introduction

Biogenic amines play pivotal roles in the control of animal behavior. The biogenic amine octopamine can act as a neurotransmitter in invertebrates and is considered the invertebrate counterpart to norepinephrine (Roeder et al., 2003). Octopamine has been implicated in several physiological processes, including light emission by fireflies (Nathanson, 1979), subordinate behavior by lobsters (Kravitz, 1988), foraging behavior (Barron et al., 2002) and the sting response of honeybees (Burrell and Smith, 1995), the fight or flight response of locusts (Orchard et al., 1993), associative learning by fruit flies and honeybees (Hammer and Menzel, 1998; Schwaerzel et al., 2003), and ovary muscle contraction in locusts and fruit flies (Orchard and Lange, 1985; Lee et al., 2003; Monastirioti, 2003). Although tyramine, the precursor for octopamine biosynthesis, has been suggested to play a role in invertebrate behavior, its physiological role is unknown (Roeder et al., 2003).

Octopamine is present in extracts of the nematode *Caenorhabditis elegans* (Horvitz et al., 1982). Exogenous octopamine inhibits egg laying and pharyngeal pumping and acts antagonistically to serotonin, which stimulates pharyngeal pumping and egg laying (Horvitz et al., 1982). Serotonin and octopamine have opposite effects on the firing rate of pharyngeal muscle and the duration of the pharyngeal muscle action potential (Rogers et al., 2001; Niacaris and Avery, 2003).

To study the physiological roles of octopamine and its precursor, tyramine, in *C. elegans*, we identified and characterized mutants that lack octopamine. Octopamine biosynthesis requires tyrosine decarboxylase to convert tyrosine into tyramine and tyramine β-hydroxylase to convert tyramine to octopamine (see Figure 1A). A tyramine β-hydroxylase (*Tβh*) gene has been described in *Drosophila melanogaster*, and *Tβh* mutants lack octopamine (Monastirioti et al., 1996). Although studies of *Drosophila* have shown that tyrosine decarboxylase activity is present in extracts from fly brains (Livingstone and Tempel, 1983), no animal tyrosine decarboxylase gene has been reported. In this study, we identify a *C. elegans* tyrosine decarboxylase gene, *tdc-1*, and a tyramine β-hydroxylase gene, *tbh-1*. We show that *tdc-1* is required for tyramine biosynthesis and that both *tdc-1* and *tbh-1* are required for octopamine biosynthesis. Our data indicate that there are tyraminergic cells that are distinct from octopaminergic cells and that tyramine has a distinct role in *C. elegans* behavior.

Results

H13N06.6 Encodes Tyramine β-Hydroxylase

The *C. elegans* genome sequence contains a single gene, H13N06.6, that encodes a protein with significant similarity to *Drosophila* tyramine β-hydroxylase (TBH) (Monastirioti et al., 1996) and mammalian dopamine β-hydroxylase (DBH) (Lamouroux et al., 1987). We named this gene *tbh-1* (tyramine β-hydroxylase) and obtained a 1.9 kb full-length complementary DNA (cDNA) clone for *tbh-1* (Figure 1B). The open reading frame of the *tbh-1* cDNA encodes a 561 amino acid protein that shares 32% identity with both *Drosophila* TBH and human DBH (Figure 1C). We isolated two *tbh-1* deletion alleles by screening libraries of mutagenized animals using PCR (Jansen et al., 1997). Both *tbh-1* deletions remove regions that encode domains conserved between the *Drosophila* and human β-hydroxylases.

To determine whether TBH-1 is required for octopamine biosynthesis, we measured the octopamine content of *tbh-1* mutants using HPLC coupled to electrochemical detection. HPLC analysis of extracts of wild-type animals showed a peak with the same retention time as octopamine (Figure 2A). Spiking the sample with octopamine increased the peak area, indicating that this peak represents octopamine (data not shown). The octopamine content was 5 ± 2 pmol per mg of wet weight, similar to estimates obtained from radioenzymatic assays (Horvitz et al., 1982). In extracts from *tbh-1* mutant animals, the octopamine peak was absent (Figure 2B).

We examined tyramine levels using thin-layer chromatography (TLC) of dansylated *C. elegans* extracts (Figure 2B). TLC of wild-type animal extracts showed a

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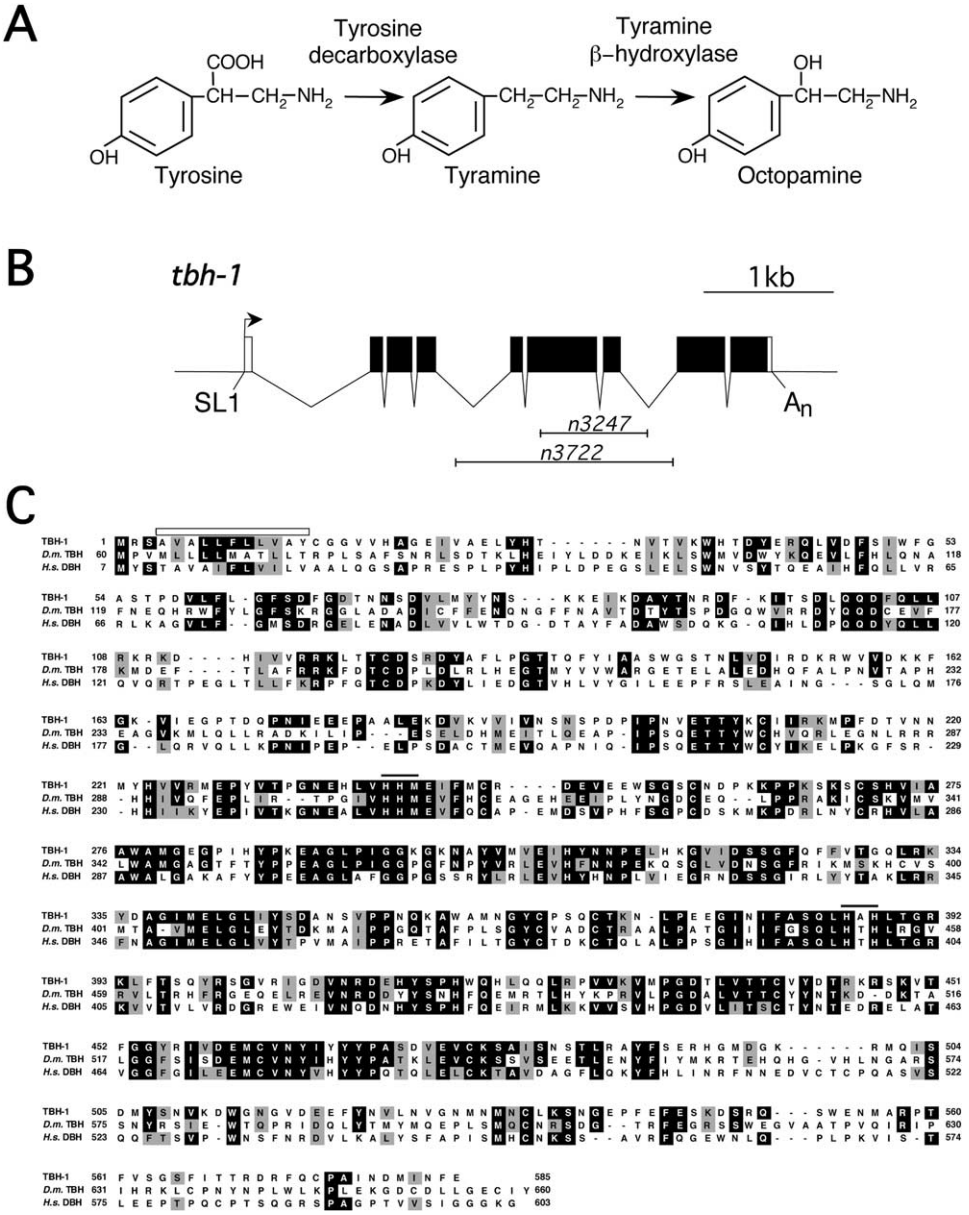


Figure 1. *tbh-1* Encodes a Tyramine β-Hydroxylase

(A) Octopamine biosynthetic pathway. Octopamine biosynthesis requires two steps: a tyrosine decarboxylase converts tyrosine into tyramine, and a tyramine β-hydroxylase converts tyramine into octopamine.

(B) Gene structure of *tbh-1* as derived by comparing genomic and cDNA sequences. Coding sequences are represented by black boxes; untranslated regions are represented by white boxes. The SL1 *trans*-spliced leader and the poly(A) tail are indicated. The *tbh-1(n3247)* mutant allele removes 791 bp, deleting parts of exons 6 and 7, and causes a frameshift that leads to a premature truncation of the TBH-1 protein. The *tbh-1(n3722)* mutant allele has a 1610 bp in-frame deletion of exons 5, 6, and 7 and causes a 242 amino acid deletion. The deleted regions are indicated by bars.

(C) Alignment of TBH-1 with *Drosophila* TBH and human DBH. Solid boxes indicate identities, and shaded boxes indicate similarities with TBH-1. The conserved histidine-rich copper binding regions (Pettingill et al., 1991) are denoted by black bars. The open box indicates a putative signal sequence.

spot that comigrates with the dansylated derivative of tyramine. Wild-type animals contained 0.3 ± 0.1 pmol tyramine per mg of wet weight. Tyramine levels were approximately 20-fold increased in *tbh-1* mutant animals (6 ± 2 pmol tyramine per mg of wet weight), presumably because tyramine can no longer be converted to octopamine in the absence of TBH activity. A similar increase in tyramine was found in *Drosophila Tβh* mutants (Monastirioti et al., 1996). These data indicate that *tbh-1* encodes a tyramine β-hydroxylase required for octopamine biosynthesis.

K01C8.3 Encodes Tyrosine Decarboxylase

We sought to identify a *C. elegans* L-aromatic amino acid decarboxylase (AADC) gene required for the decarboxylation of tyrosine, the first step in octopamine

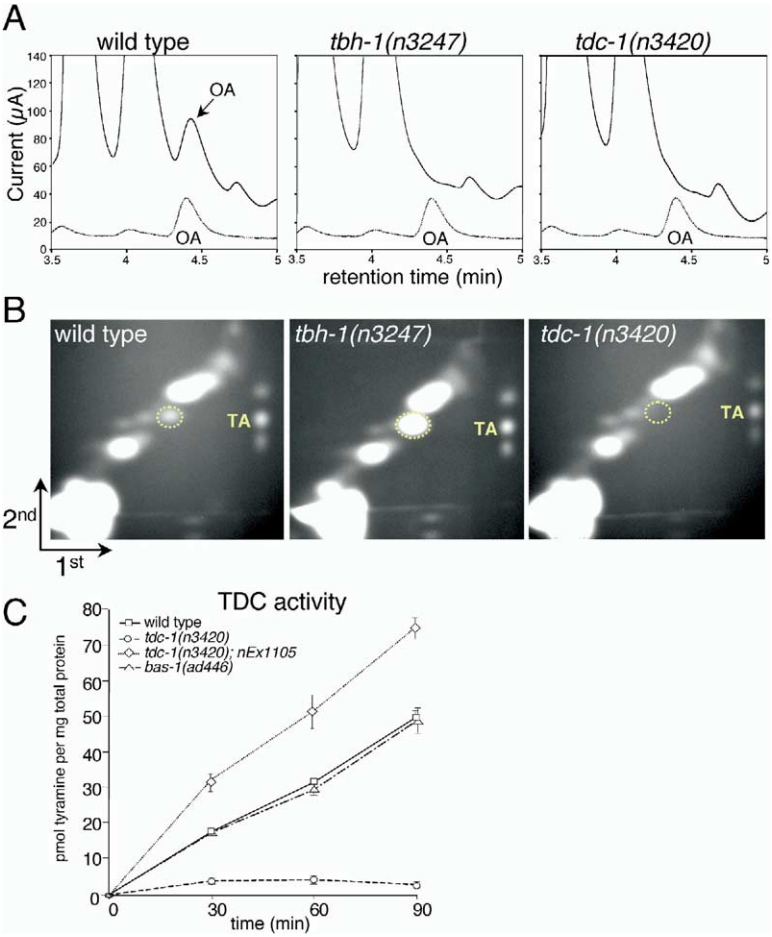


Figure 2. *tbh-1* Mutants Lack Octopamine and Have Increased Tyramine Levels, While *tdc-1* Mutants Lack Both Octopamine and Tyramine

(A) HPLC traces of wild-type, *tbh-1(n3247)*, and *tdc-1(n3420)* extracts. Octopamine (2 pmol) was used as a standard. Wild-type animals contained 5 ± 2 pmol octopamine per mg of wet weight.

(B) Thin-layer chromatography of dansylated derivatives of wild-type, *tbh-1(n3247)*, and *tdc-1(n3420)* extracts. Dansylated tyramine (50 pmol) was used as a standard (TA). The dansylated tyramine spot from worm extracts is circled by a dotted line. Wild-type animals contained 0.3 ± 0.1 pmol tyramine per mg of wet weight. Dansylated tyramine is absent in extracts from *tdc-1(n3420)* mutants.

(C) TDC activity is absent in *tdc-1(n3420)* mutants. TDC activity was measured by monitoring the conversion of [^{3}H]tyrosine to [^{3}H]tyramine in extracts of wild-type, *tdc-1(n3420)*, *tdc-1(n3420); Ex1105*, and *bas-1(ad446)* animals at 30, 60, and 90 min. Tyrosine and tyramine were separated by organic phase extraction (McClung and Hirsh, 1999). TDC activity is virtually absent in extracts of *tdc-1(n3420)* mutants and is rescued by a *tdc-1* genomic clone [*tdc-1(n3420); nEx1105*]. Error bars indicate standard deviations.

biosynthesis. AADCs are homodimeric pyridoxal 5′-phosphate (PLP) enzymes that can decarboxylate many naturally occurring L-aromatic amino acids. We identified five putative *C. elegans* AADC genes, C05D2.4/*bas-1* (Hare and Loer, 2004), C05D2.3, F12A10.3, K01C8.3, and ZK829.2 (Figure 3A), on the basis of similarity to mammalian and insect DOPA decarboxylases (DDCs). To determine whether any of these genes is required for tyramine biosynthesis, we obtained deletion alleles of the corresponding putative decarboxylases genes and assayed tyramine content using TLC. C05D2.3, C05D2.4/*bas-1*, F12A10.3, and ZK829.2 deletion mutants had normal tyramine levels (data not shown), whereas K01C8.3 deletion mutants lacked tyramine (Figure 2B). K01C8.3 deletion mutants had normal dopamine and serotonin levels, as judged by formaldehyde-induced fluorescence (Sulston et al., 1975) and serotonin immunohistochemistry (Desai et al., 1988) (data not shown), but lacked octopamine, as shown by HPLC analysis (Figure 2A). These observations indicate that K01C8.3 encodes a tyrosine decarboxylase required for the first step in octopamine biosynthesis in *C. elegans*. We named this gene *tdc-1* (tyrosine decarboxylase).

tdc-1 encodes two splice variants, *tdc-1a* and *tdc-1b*, which differ at their 3′ ends (Figure 3B). The *tdc-1a* transcript encodes a 651 amino acid protein; the *tdc-1b* transcript uses a cryptic splice donor site in exon 8 and encodes a 706 amino acid protein. The TDC-1A/B predicted proteins contain several regions conserved in PLP-dependent decarboxylases, including a lysine residue important for PLP binding (Figure 3C). All three *tdc-1* deletion alleles (Figure 3B) remove *tdc-1* regions that encode domains highly conserved in PLP-dependent decarboxylases. A BLAST search against TDC-1 identified the *Drosophila* protein CG30446 and the predicted orthologous proteins XP_394424 of the honeybee *Apis mellifera* and XP_308519 of the mosquito *Anopheles gambiae* as the closest homologs of TDC-1. TDC-1 shares 40% identity with *Drosophila* DDC compared to 50% identity with the *Drosophila* CG30446-honeybee XP_394424, and mosquito XP_308519 proteins (Figure 3C), suggesting that CG30446, XP_394424, and XP_308519 are insect tyrosine decarboxylases.

We examined tyrosine decarboxylase activity in worm extracts by measuring the conversion of [^{3}H]tyrosine to [^{3}H]tyramine. Tyrosine decarboxylase activity was present in wild-type extracts but was almost undetectable in extracts from *tdc-1* mutants (Figure 2C). Tyrosine decarboxylase activity was rescued in transgenic *tdc-1* mutants carrying a genomic *tdc-1* fragment (Figure 2C). Since the *Drosophila* DDC can decarboxylate tyrosine in vitro, albeit with much lower affinity (Livingstone and Tempel, 1983), we also assayed tyrosine decarboxylase activity in C05D2.4/*bas-1* mutant extracts, which lack DOPA decarboxylase (Hare and Loer,

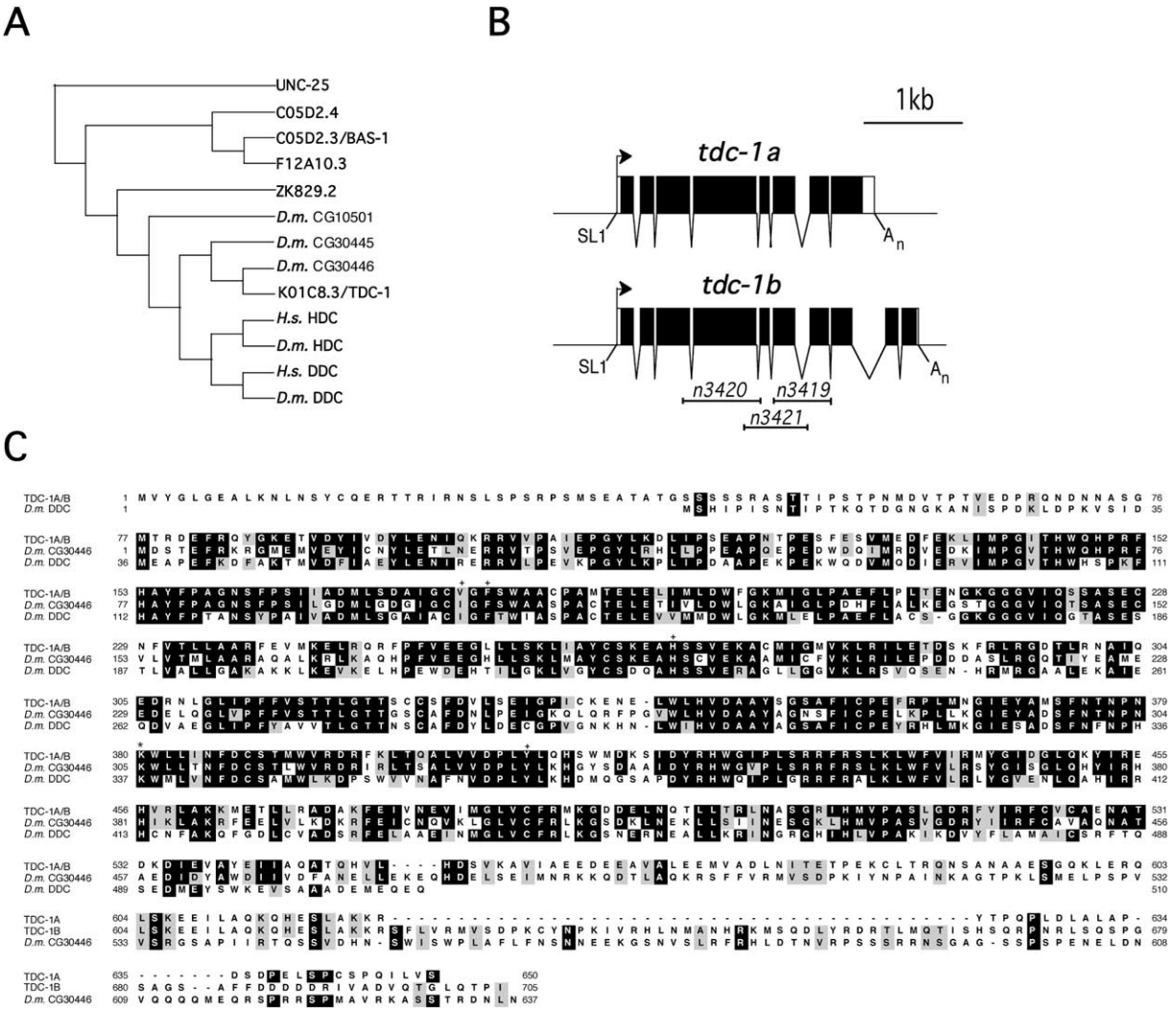


Figure 3. *tdc-1* Encodes a Tyrosine Decarboxylase

(A) Phylogenetic analysis of all predicted *C. elegans*, *Drosophila melanogaster* (*D.m.*), and human (*H.s.*) aromatic amino acid decarboxylases; UNC-25, a *C. elegans* glutamate decarboxylase, was used as an outgroup. HDC, histidine decarboxylase; DDC, DOPA decarboxylase. A phylogenetic tree of decarboxylase-conserved regions was determined by bootstrap analysis and the unweighted pair group method with arithmetic mean (UPGMA) using MacVector software (Accelrys).

(B) Gene structures of *tdc-1a* and *tdc-1b* as derived by comparing genomic and cDNA sequences. The *tdc-1b* transcript uses a cryptic splice donor site in exon 8. Coding sequences are represented by black boxes; untranslated regions are represented by white boxes. The SL1 *trans*-spliced leader and the poly(A) tail are indicated. The *tdc-1(n3419)* allele has a 578 bp deletion that removes part of exon 6 and the entire exon 7; the *tdc-1(n3420)* allele has an 803 bp deletion that removes part of exons 3 and 5 and all of exon 4; the *tdc-1(n3421)* allele has a 585 bp deletion that removes part of exon 4 and exons 5 and 6 in their entirety. The deleted regions are indicated by bars.

(C) Alignment of TDC-1A/B with *Drosophila* DDC and the *Drosophila* predicted protein CG30446. TDC-1A and TDC-1B differ at their C termini. Solid boxes indicate identities and shaded boxes indicate similarities with TDC-1. Amino acids that form part of the catalytic core of the enzyme and are essential for decarboxylase function are denoted by plusses (Burkhard et al., 2001). The lysine residue required for pyridoxal phosphate binding is denoted by an asterisk.

2004). We found that TDC activity in C05D2.4/*bas-1* mutant extracts was similar to that in the wild-type. Our data indicate that *tdc-1* encodes the major tyrosine decarboxylase in *C. elegans*.

TBH-1 Is Expressed in a Subset of Cells that Express TDC-1

To analyze the expression patterns of *tbh-1* and *tdc-1*, we generated rabbit polyclonal antibodies against the TBH-1 and TDC-1 proteins. TBH-1 antibodies recognized a single band of approximately 70 kDa in wild-type protein extracts, in accordance with the TBH-1 predicted size of 67 kDa. The 70 kDa band was absent in extracts from *tbh-1* mutants (Figure 4A). TDC-1 antibodies recognized a band (which may be a doublet) around 75 kDa, in agreement with the predicted sizes of 73.2 kDa and 79.7 kDa of TDC-1A and TDC-1B, respectively; these bands were absent in the *tdc-1* mutants (Figure 4B).

C. elegans whole-mount staining with TBH-1 antibodies labeled a single pair of head interneurons in the lateral ganglion: we identified these neurons as the

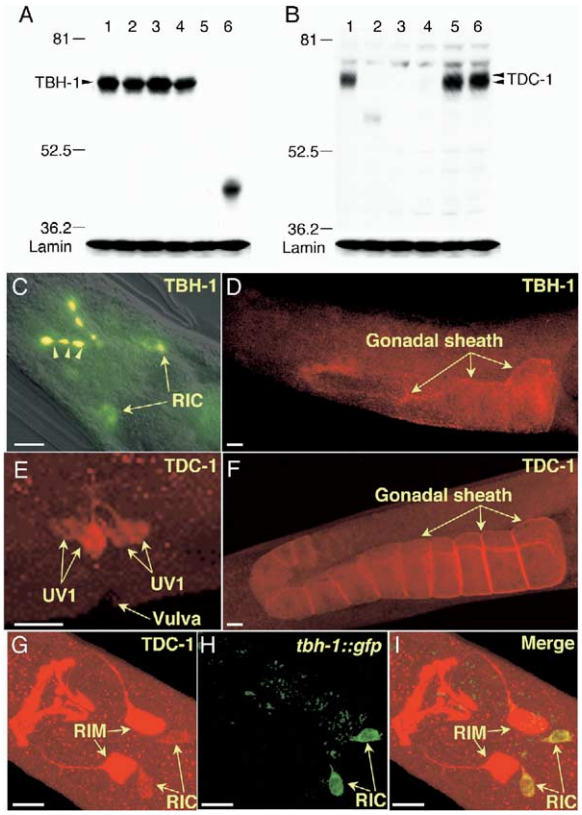


Figure 4. Localization of TBH-1 and TDC-1

(A and B) Western blot analysis of total protein of (1) wild-type, (2) *tdc-1(n3419)*, (3) *tdc-1(n3420)*, (4) *tdc-1(n3421)*, (5) *tbh-1(n3247)*, and (6) *tbh-1(n3722)* animals with (A) TBH-1-antibodies and (B) TDC-1-antibodies. (A) In the *tbh-1(n3722)* mutants, a 45 kDa band is detected, in agreement with the predicted size of the protein that results from the in-frame deletion of the *n3722* allele. (B) The weak 60 kDa band from *tdc-1(n3419)* mutants correlates with the predicted size of the protein of this in-frame deletion allele. (C and D) Whole-mount staining with TBH-1-antibodies. TBH-1 is expressed in the (C) RIC interneurons, where it is mainly localized to synaptic specializations and (D) gonadal sheath cells. (E and F) Whole-mount staining with TDC-1-antisera. TDC-1 is expressed in (E) the UV1 cells in the late L4 larva and the (F) gonadal sheath cell projections between the oocytes in adults. (G–I) Double staining of a *tbh-1*::*gfp* transgenic animal with (G) TDC-1-antisera and (H) mouse monoclonal GFP-antibodies. TDC-1 is expressed in the RIM head neurons and the RIC interneurons. (I) Merged. Anterior is on the left (C–I). Scale bar, 10 μm.

RICs (Figure 4C). No staining was observed in *tbh-1(n3247)* mutants (data not shown). The RIC neurons send processes into the nerve ring, where they make gap junctions with the ASH and AVK neurons and receive synaptic inputs from the OLQ, CEP, URB, and URX sensory neurons; the main synaptic outputs of the RICs are the AVA interneurons and the SMD and SMBD motor neurons (White et al., 1986). Bright staining was observed in RIC synaptic regions, as indicated by a punctate immunofluorescence pattern in the nerve ring, whereas weaker staining was observed in the RIC neuronal processes and cell bodies. By contrast, TBH-1 staining was predominantly localized to the RIC cell bodies of mutants defective in the *unc-104* gene (data not shown), which encodes a neuron-specific kinesin required for the anterograde transport of synaptic vesicles (Hall and Hedgecock, 1991; Otsuka et al., 1991). Axon outgrowth is normal in *unc-104* mutants, but synaptic vesicles remain clustered in cell bodies (Hall and Hedgecock, 1991) and several vesicle-associated proteins, such as synaptotagmin (Nonet et al., 1999), the vesicular acetylcholine transporter UNC-17 (Alfonso et al., 1993), and the vesicular monoamine transporter CAT-1 (Duerr et al., 1999), are mislocalized to neuronal cell bodies in *unc-104* mutants. We suggest that TBH-1, like the closely related dopamine β-hydroxylase (Nelson and Molinoff, 1976), is associated with synaptic vesicles. We also observed punctate TBH-1 staining in the gonadal sheath cells of adult hermaphrodites (Figure 4D). The gonadal sheath is formed by five pairs of cells that envelop most of the gonad arm (Strome, 1986). TBH-1 staining was most prominent in the proximal three pairs of sheath cells, which form a contractile myoepithelium that expels oocytes from the gonad during ovulation. A punctate staining of the gonadal sheath cells is also observed with actin and myosin antibodies (Strome, 1986). Perhaps TBH-1 is associated with actomyosin filaments in the gonadal sheath cells.

TDC-1 was coexpressed with TBH-1 in the RICs and gonadal sheath cells (Figures 4G–4F), suggesting that these cells are octopaminergic. TDC-1 staining was observed in the cell bodies and axonal processes of TDC-1-expressing neurons. The subcellular localizations of TDC-1 and TBH-1 suggest that tyramine is transported from the cytoplasm into synaptic vesicles, where it is converted to octopamine. TDC-1 staining also was present in the gonadal sheath cells and appeared to be localized to the projections of these cells between the oocytes (Hall et al., 1999). The expression of TBH-1 and TDC-1 in the gonadal sheath cells (Figures 4F–4I) may account for the 5-fold increase in octopamine content of adults compared to larvae (Horvitz et al., 1982) and for the dramatic increase in the tyramine content of the *tbh-1* mutants.

Surprisingly, we found that TDC-1 was highly expressed in a few cells that did not express TBH-1. We observed bright staining of a pair of neurons in the lateral ganglion. We identified these cells as the RIM motor neurons (Figure 4G). Four uterine cells, which we identified as the UV1 cells (Figure 4E), also expressed TDC-1. Expression in the uterine cells was not observed until the late L4 stage, the time at which the UV1 cells are generated (Newman et al., 1996). The expression of TDC-1 but not TBH-1 in the RIMs and UV1 cells suggests that these cells use tyramine in signaling, although we cannot exclude the possibility that tyramine serves as an intermediate for the biosynthesis of another molecule in these cells. The octopaminergic RIC neurons expressed TDC-1 at much lower levels than do the tyraminergic RIM neurons (Figure 4G). Perhaps this difference results in a complete conversion of tyramine into octopamine in the synaptic vesicles of the RIC neurons.

tdc-1 Mutants Are Hyperactive in Egg Laying

tbh-1 and *tdc-1* deletion mutants were viable and healthy and had normal brood sizes (data not shown). As will be reported elsewhere, *tbh-1* and *tdc-1* mutants both had a slightly reduced locomotion rate and de-

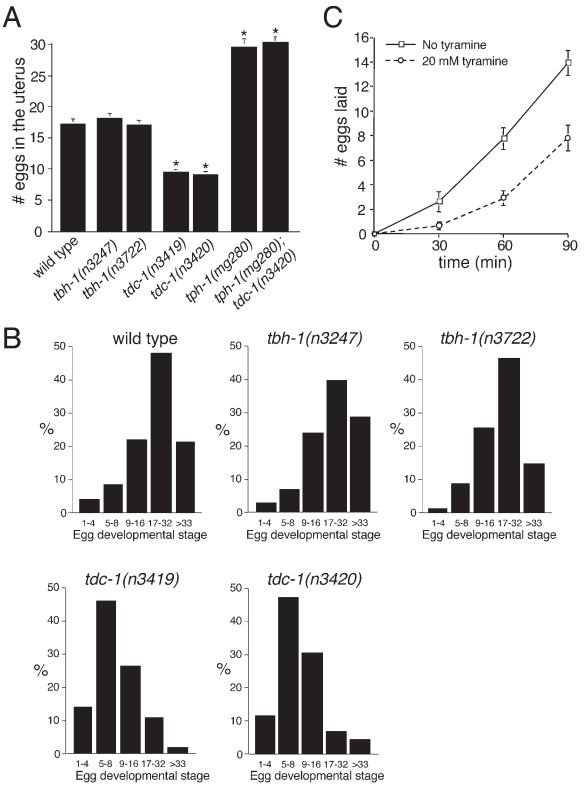


Figure 5. *tdc-1* Mutants Are Hyperactive in Egg Laying

(A) Number of unlaid eggs in the uterus of wild-type, *tbh-1*, and *tdc-1* animals. *tdc-1* mutants had fewer eggs in the uterus. *Statistical difference from wild-type. $p < 0.0001$, Student's t test. (B) Distributions of the stages of freshly laid eggs in wild-type (n = 135), *tbh-1(n3247)* (n = 123), *tbh-1(n3722)* (n = 95), *tdc-1(n3419)* (n = 140), and *tdc-1(n3420)* (n = 154) animals. *tdc-1* mutants laid eggs at an earlier stage (<8 cells) than did wild-type and *tbh-1* animals. The distribution of the stage of the laid eggs of the *tdc-1* mutants is statistically significantly different (*) from those of the wild-type and the *tbh-1* mutants; χ^2 test, $p < 0.001$. (C) Exogenous tyramine inhibits egg laying. Single wild-type animals were transferred to petri dishes with bacteria containing either no tyramine (n = 13) or 20 mM tyramine (n = 12). At the indicated times after transfer, the number of eggs laid was counted. Error bars indicate standard errors of the means.

fects in the inhibition of pharyngeal pumping and egg laying in the absence of food (M.J.A. and H.R.H., unpublished data). Since *tdc-1* and *tbh-1* mutants both lack octopamine, the behavioral defects that *tbh-1* and *tdc-1* mutants have in common suggest a role for octopamine in the modulation of locomotion, pharyngeal pumping, and egg laying. *tdc-1* mutants also had defects not shared with *tbh-1* mutants: *tdc-1* mutants were hyperactive in egg laying in the presence of food, failed to suppress head oscillations in response to touch, and had defects in reversal behavior (see below).

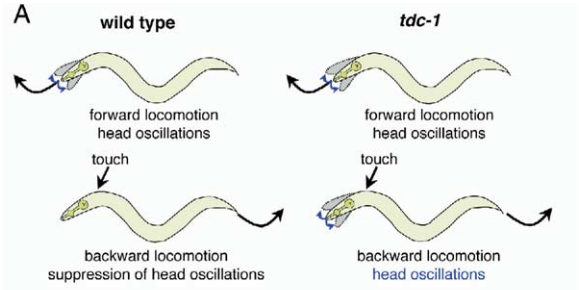
tdc-1 mutants had a reduced number of eggs in the uterus compared to wild-type animals and *tbh-1* mutants (Figure 5A). The egg-laying rate of *tdc-1* mutants was comparable to those of wild-type and *tbh-1* mutant animals [wild-type, 9.6 ± 0.5; *tbh-1(n3247)*, 9.5 ± 0.5; *tbh-1(n3722)*, 10.0 ± 0.5; *tdc-1(n3419)*, 9.2 ± 0.5; and *tdc-1(n3420)*, 10.0 ± 0.5 eggs/hr]. However, *tdc-1* mutants laid their eggs at an earlier developmental stage than did wild-type animals and *tbh-1* mutants. Specifically, the wild-type and *tbh-1* mutants laid most eggs at the nine-cell to comma stage. *tdc-1* mutants laid most of their eggs at the 1–8 cell stage (Figure 5B), suggesting that the time between fertilization and egg laying was reduced in *tdc-1* mutants.

Thus, in the presence of food *tdc-1* mutants, unlike *tbh-1* mutants, are hyperactive in egg-laying behavior, suggesting that tyramine plays a role independent of octopamine in the inhibition of egg laying in vivo. We therefore tested the effect of exogenous tyramine on egg laying. Egg laying was inhibited on Petri plates containing 20 mM tyramine (Figure 5C). The inhibitory effect of exogenous tyramine on egg laying was similar to the inhibitory effect of exogenous octopamine (Horvitz et al., 1982). Although our data indicate that tyramine inhibits egg laying in vivo, we cannot exclude that the egg-laying constitutive phenotype observed in the *tdc-1* mutants is caused by defects in both octopamine and tyramine signaling. Egg laying is regulated in part by the serotonergic HSN neurons, which induce contraction of the vulva muscles (Trent et al., 1983). Serotonin-deficient *tph-1* mutants are egg-laying defective (Egl-D): they retain more eggs in the uterus, and eggs are laid at a later stage (Sze et al., 2000). *tph-1; tdc-1* double mutants were Egl-D, similar to the *tph-1* single mutant (Figure 5A), indicating that *tdc-1* acts upstream of and/or parallel to *tph-1* in egg laying.

Anterior Touch Sensory Neurons Mediate the Suppression of Head Oscillations

We found that *tdc-1* mutants fail to suppress head oscillations in response to anterior touch (see below). *C. elegans* locomotion is accompanied by oscillatory head movements during which the tip of the nose moves rapidly from side to side (Figure 6A) (Croll and Smith, 1978). The tip of the nose contains the endings of several sensory neurons, and head oscillations may allow the animal to explore its immediate environment and may contribute to chemotactic and thermotactic behaviors. Locomotion and head movements are controlled by different muscle groups: locomotion is controlled by the body wall muscles and is restricted to dorsal/ventral flexures, while head movements are controlled by eight radially symmetric muscle groups that allow *C. elegans* to move its head through 360° (White et al., 1986). Head movements are regulated independently from locomotion, since animals that were feeding but not moving still displayed head oscillations (our unpublished data).

Light touch of an eyelash to the anterior half of the body induces a backing response (Chalfie et al., 1985). We found that, during this backing response, head oscillations were suppressed in wild-type animals (Figure 6A; see Movie S1 in the Supplemental Data available with this article online); head oscillations resumed as soon as forward locomotion was reinitiated. The analysis of the *C. elegans* touch response has led to the identification of the responsible mechanosensory neurons and the characterization of the neural circuit that controls forward and backward locomotion by *C. elegans* (Chalfie et al., 1985). Light anterior touch, sensed by the ALM/AVM mechanosensory neurons, in-



B

Genotype	Suppression of head oscillations
wild type	96% (n =125)
tbh-1(n3247)	92% (n =112)
tbh-1(n3722)	94% (n =98)
tdc-1(n3419)	0% (n > 200)
tdc-1(n3420)	0% (n > 200)
tdc-1(n3420), nEx1180	83% (n =132)
tdc-1(n3420), nEx1181	0% (n =87)
cat-1(e1111)	0% (n =97)
tph-1(mg280)	81% (n =133)
cat-2(e1112)	93% (n =103)
unc-25(e156)	85% (n =99)
unc-25(e156); tdc-1(n3420)	0% (n = 88)
unc-3(e151)	89% (n =98)
unc-3(e151); tdc-1(n3420)	0% (n =78)

C

Stimulus	Suppression of head oscillations	Sensory neurons involved
Spontaneous	20% (n =104)	-
Anterior touch (adult)	96% (n =125)	AVM/ALM*
Anterior touch (L1)	87% (n =96)	ALM*
Nose touch	29% (n =90)	ASH/FLP
Octanol	28% (n =85)	ASH
4M Fructose	22% (n = 83)	ASH
Harsh touch	30% (n =90)	PVD
Posterior touch	0% (n =140)	PLM

D

Ablated	Suppression of head oscillations
Mock	100% (12/12)
RIM	0% (0/12)
RIC	100% (7/7)
AVA	0% (0/7)
AVD	28% (2/7)
AVE	100% (6/6)

Figure 6. *tdc-1* Mutants Fail to Suppress Head Oscillations in Response to Anterior Touch

(A) Forward locomotion of wild-type animals is accompanied by oscillatory head movements. Anterior touch of wild-type animals with an eyelash induces backing during which head oscillations are suppressed. *tdc-1* mutants fail to suppress head oscillations during backing.

(B) Suppression of head oscillations in response to anterior touch was scored during backing.

(C) The AVM and ALM mechanosensory neurons mediate suppression of head oscillations. Animals were scored for the suppression of head oscillations during spontaneous reversals, in response to gentle anterior or posterior touch, nose touch, harsh touch, octanol, and osmotic (4 M fructose) avoidance. Animals were scored only if they made at least one backward body bend during a spontaneous reversal or in response to the stimulus. Animals that did not display head oscillations during backward locomotion were scored as positive. Sensory neurons that mediate the responses to the various stimuli are indicated. The suppression of head oscillations in response to anterior touch is significantly different (*) from the response to other stimuli; χ^2 test, $p < 0.001$.

(D) Animals in which RIM motor neurons or the AVA or AVD backward command neurons were ablated failed to suppress head oscillations in response to anterior touch.

duces a backing response, whereas light posterior touch, sensed by the PLM mechanosensory neurons, accelerates forward locomotion. *mec* mutants, which lack functional touch cells, do not back in response to anterior touch. We found that adult *mec-4* and *mec-7* mutants did not suppress head oscillations in response to anterior touch, indicating that the touch cells mediate the suppression of head oscillations (data not shown). In young larvae (L1), the anterior touch response depends solely on the ALM touch sensory neurons, because the AVMs develop in the L4 larval stage (Chalfie et al., 1985). Young larvae also suppressed head oscillations in response to anterior touch, indicating that the ALM touch sensory neurons are sufficient to mediate the touch response to the suppression of head oscillations. Gentle posterior touch, sensed by the PLM neurons, never induced the suppression of head oscillations. Forward locomotion was always associated with head oscillations.

We noticed that head oscillations were usually not suppressed during spontaneous reversals. We asked whether other stimuli that, like gentle touch, induce backward locomotion led to the suppression of head oscillations. Nose touch, volatile repellents, and high osmolarity induce an avoidance response and are mainly sensed by the ASH sensory neurons (Kaplan and Horvitz, 1993). Backward locomotion induced by those stimuli usually did not suppress head oscillations (Figure 6B). The *C. elegans* response to harsh touch with a platinum wire is mediated by the PVD sensory neurons and results in either the acceleration of forward locomotion or a backing response (Way and Chalfie, 1989). Head oscillations were not suppressed in most animals in which backward locomotion was induced by harsh touch. These observations indicate that backward locomotion is not sufficient for this suppression. Is backward locomotion necessary for the suppression of head oscillations in response to anterior touch? To address this question, we examined the response of *unc-3* mutants to anterior touch. The UNC-3 protein is a member of the Olf-1/EBF family of transcription factors and is required for the axonal outgrowth of the motor neurons of the ventral cord (Prasad et al., 1998). *unc-3* mutants are largely immobilized. We found that *unc-3* mutants still displayed normal head oscillations, presumably because the ventral cord motor neurons do not innervate the head muscles. Anterior touch of *unc-3* mutants did not induce backward locomotion but did suppress head oscillations (Figure 6C), indicating that backward locomotion is not required for the suppression of head oscillations.

tdc-1 Is Required to Suppress Head Oscillations in Response to Anterior Touch

tdc-1 mutants normally induced backing in response to light touch but failed to suppress head oscillations during backward locomotion (Figures 6A and 6B; Movie S2). This defect in the suppression of head oscillations was rescued by a *tdc-1* genomic clone [*tdc-1(n3420); nEx1180*] but not by a mutant of this clone carrying a frameshift mutation in *tdc-1* [*tdc-1(n3420); nEx1181*]. Octopamine-deficient *tbh-1* mutants, dopamine-deficient *cat-2* mutants, and serotonin-deficient *tph-1* mutants did suppress head oscillations (Figure 6C). However, *cat-1* mutants failed to suppress head oscillations in response to anterior touch. *cat-1* encodes a vesicular monoamine transporter that can transport biogenic amines, including tyramine, when expressed in mammalian cells (Duerr et al., 1999). We suggest that tyramine and *cat-1*-mediated tyramine transport into synaptic vesicles are essential for the suppression of head oscillations in response to anterior touch.

Tyramine May Suppress Head Oscillations by Inhibiting Head Muscle Contractions

How are head oscillations regulated? The *C. elegans* head muscles are divided in eight radially symmetric sectors that are independently innervated by different classes of motor neurons (White et al., 1986), including the cholinergic RMD and SMD motor neurons (J. Duerr,

personal communication), the GABAergic RME motor neurons (McIntire et al., 1993), and the tyraminergic RIM motor neurons (this manuscript; see Figure 8). Acetylcholine acts as an excitatory neurotransmitter at the neuromuscular junction (Lewis et al., 1980), whereas GABA is the main inhibitory neuromuscular transmitter in *C. elegans* (McIntire et al., 1993). *cha-1(p1152)* mutants, which have greatly reduced choline acetyltransferase activity, display uncoordinated locomotion (Rand and Russell, 1984) and uncoordinated head movements with few head oscillations (data not shown). Ablation of the cholinergic RMD neurons results in the loss of head oscillations (Hart et al., 1995; J. Kaplan and H.R.H., unpublished data). GABA-deficient *unc-25* mutants and RME-ablated animals display loopy head oscillations (McIntire et al., 1993). These observations indicate that acetylcholine and GABA are required for normal head oscillations.

The cholinergic RMD and SMD neurons are coupled by gap junctions and independently innervate the different muscle sectors (White et al., 1986). In addition, the SMD neurons make reciprocal synaptic connections with the GABAergic RME neurons. The RME neurons may coordinate head muscle contractions induced by the cholinergic RMD and SMD neurons by preventing the simultaneous contraction of opposing muscle sectors. The coordinated rhythmic contraction of opposing muscle sectors may lead to head oscillations. *tdc-1* mutants display normal head oscillations during forward locomotion, suggesting that tyramine is not required for head oscillations but rather is required only for the suppression of head oscillations in response to anterior touch.

To determine whether the suppression of head oscillations in response to anterior touch is GABA dependent, we examined the anterior touch response in *unc-25* mutants. *unc-25* mutants had a reduced backing response but nonetheless suppressed head oscillations (Figure 6C), indicating that GABA is not required for the suppression of head oscillations.

In response to harsh touch, *unc-25* mutants simultaneously contract their ventral and dorsal body muscles, resulting in a shrinkage in body length rather than in backward movement (McIntire et al., 1993). *unc-25* mutants did not shrink in response to gentle anterior touch (our unpublished data). However, *unc-25; tdc-1* double mutants showed a localized shrinking of head muscles in response to gentle anterior touch. These observations indicate that tyramine acts to inhibit head muscle contraction in response to anterior touch. We suggest that tyramine release from the RIMs relaxes the head muscles, thereby suppressing head oscillations. By contrast, GABA-induced head muscle relaxation may not be triggered by anterior touch.

tdc-1 Mutants Have Defects in Reversal Behavior

We noticed that in response to anterior touch *tdc-1* mutants backed less than did wild-type animals and often displayed slightly jerky backward locomotion. Wild-type animals and *tbh-1* mutants reversed on average 3.6 to 3.5 body bends, respectively, in response to anterior touch (Figure 7A). *tdc-1* mutants initiated backward locomotion normally in response to anterior touch but backed on average only 2.2 body bends.

C. elegans reversal frequency can be modulated by chemosensory cues (Pierce-Shimomura et al., 1999), humidity (Zhao et al., 2003), temperature, and food (Tsalik and Hobert, 2003) and allows the animal to explore its environment in search of favorable conditions. On plates without food, wild-type animals made approximately 15 spontaneous reversals in 5 min (Figure 7B). Similarly, *tbh-1* mutants made about 16 reversals. By contrast, *tdc-1(n3420)* and *tdc-1(n3419)* mutants made 30 reversals in 5 min. However, as with the reduced backing response induced by touch, *tdc-1* mutants backed less far during spontaneous reversals than did wild-type or *tbh-1* mutant animals (data not shown). These data suggest that *tdc-1* mutants fail to sustain backward locomotion once it is initiated and suggest a role for tyramine in reversal behavior.

The RIM Motor Neurons Modulate Reversal Frequency and Are Required for the Suppression of Head Oscillations

The defects of *tdc-1* mutants in egg laying, suppression of head oscillations, and reversal behavior were not shared by the *tbh-1* mutants, suggesting a distinct role for tyramine in these behaviors. However, since *tdc-1* mutants also lack octopamine, we could not exclude a role for octopamine in these behaviors. We therefore analyzed the behavior of animals in which either the tyraminergic or octopaminergic neurons were killed by laser ablation. We found that animals in which the tyraminergic RIMs were ablated had an impaired backing response to anterior touch and failed to suppress head oscillations (Figure 6C). The RIM-ablated animals also showed a dramatic increase in the number of spontaneous reversals, similar to *tdc-1* mutants (Figure 7C) and consistent with findings by Zheng et al. (1999), who reported that RIM ablations lead to a decrease in forward run duration. By contrast, mock-ablated and RIC-ablated animals showed a normal backing response and had no defects in the suppression of head oscillations in response to anterior touch (Figure 6C). In addition, the reversal frequency of RIC-ablated animals was similar to that of mock-ablated animals (Figure 7C). Thus, RIM-ablated animals have defects in reversal behavior and the suppression of head oscillations that are shared by *tdc-1* mutants, indicating that the tyraminergic RIM motor neurons modulate reversal behavior are required for the suppression of head oscillations.

The AVA and AVD Backward Locomotion Command Neurons Are Required for the Suppression of Head Oscillations in Response to Anterior Touch

The ALM/AVM and PLM mechanosensory neurons provide inputs to four pairs of locomotion command interneurons: the PVCs and AVBs, which are mainly required for forward locomotion, and the AVAs, AVDs, and AVEs, which generally drive backward locomotion (Chalfie et al., 1985). Since the tyraminergic RIM motor neurons are connected by gap junctions to the AVA and AVE

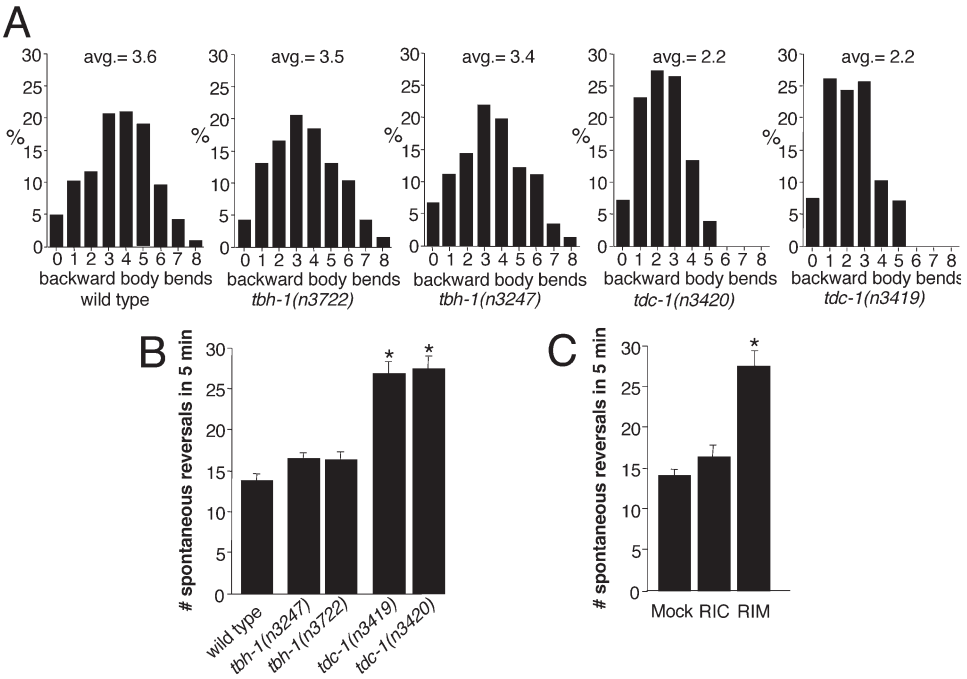


Figure 7. *tdc-1* Mutants and RIM-Ablated Animals Have a Reduced Backing Response and an Increase in Spontaneous Reversals

(A) Distribution of number of backward body bends in response to anterior touch of wild-type animals (n = 279), *tbh-1* mutants [*tbh-1(n3247)*, n = 92; *tbh-1(n3722)*, n = 148], and *tdc-1* mutants [*tdc-1(n3420)*, n = 244; *1 tdc-1(n3419)*, n = 232]. The distribution of the number of backward body bends of the *tdc-1* mutants is significantly different from those of the wild-type and the *tbh-1* mutants; χ^2 test, $p < 0.001$. (B) Number of spontaneous reversals in 5 min of well-fed wild-type (n = 23), *tbh-1(n3247)* (n = 20), *tbh-1(n3722)* (n = 20), *tdc-1(n3420)* (n = 21), and *tdc-1(n3419)* (n = 23) animals on plates devoid of food. (C) Laser ablations of RIM motor neurons led to an increase in the number of spontaneous reversals. Mock (n = 12), RIM (n = 12), RIC (n = 7). Error bars indicate standard errors of the mean. *Statistical difference from wild-type; Student's t test, $p < 0.0001$.

backward command neurons (Figure 8), we tested the role of the AVA and AVE neurons in the suppression of head oscillations in response to anterior touch (Figures 6D and 7C). AVA-ablated animals back in response to anterior touch, but backing is uncoordinated (Chalfie et al., 1985). We found that head oscillations were not suppressed in response to anterior touch in AVA-ablated animals, suggesting that the gap junctions between the RIM and AVA neurons are important in linking the touch response to the suppression of head oscillations. By contrast, AVE-ablated animals showed a normal backing response and normally suppressed head oscillation in response to anterior touch.

Cell ablation studies using laser microsurgery support a model in which the ALM and AVM touch neurons activate the AVD backward command interneuron via gap junctions and inhibit forward locomotion command neurons through synaptic connections with the PVC and AVB forward command neurons (Chalfie et al., 1985; Zheng et al., 1999). To test the role of the AVD neurons in coupling the touch response to the suppression of head oscillations, we ablated the AVD neurons. Five of seven AVD-ablated animals failed to suppress head oscillations in response to anterior touch, consistent with the hypothesis that the AVD neurons transduce the touch response to the RIM neurons. These observations suggest that ALM/AVM sensory neurons stimulate the release of tyramine from the RIM neurons through the activation of the AVA and AVD backward locomotion command neurons (Figure 8).

Discussion

tdc-1 and *tbh-1* Are Required for Octopamine Biosynthesis

We identified two *C. elegans* genes that are required for the biosynthesis of octopamine, a major invertebrate neurotransmitter/neuromodulator. *tbh-1* encodes tyramine β-hydroxylase, which converts tyramine to octopamine. *tdc-1* encodes tyrosine decarboxylase, which mediates the first step in octopamine biosynthesis, the conversion of tyrosine into tyramine. TDC-1 is closely related to PLP-dependent decarboxylases required for catecholamine, indolamine, and histamine biosynthesis in the animal nervous systems. Tyrosine decarboxylases have been identified in organisms that do not contain octopamine such as plants (Facchini et al., 2000) and bacteria (Lucas et al., 2003). However, TDC-1 is less similar to these enzymes than it is to the animal AADCs, suggesting that tyrosine decarboxylases evolved independently in animals.

Tyraminergic Cells Are Distinct from Octopaminergic Cells

TBH-1 and TDC-1 are coexpressed in the RIC interneurons and gonadal sheath cells, indicating that these

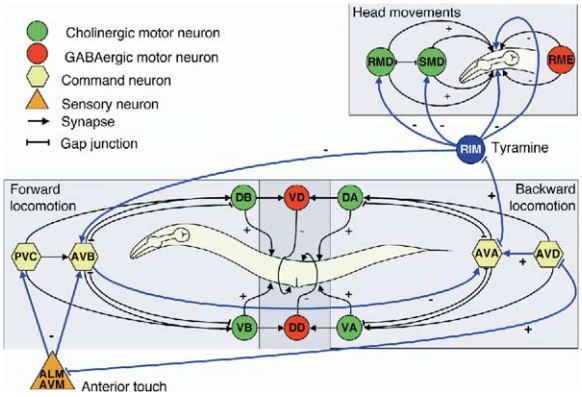


Figure 8. Model: The Tyraminergic RIM Motor Neurons Modulate Reversal Frequency and Are Required for the Suppression of Head Oscillations

Schematic representation of the neural circuit that controls locomotion and head movements. Synaptic connections (arrows) and gap junctions (bars) are as described by White et al. (1986). Excitatory cholinergic motor neurons are represented by green circles; inhibitory GABAergic are represented by red circles. Locomotion command neurons required for the control of forward (AVB, PVC) and backward (AVA, AVD) locomotion are depicted as yellow hexagons. Sensory neurons that detect anterior touch (AVM, ALM) are shown as an orange triangle. Hypothesized excitatory (+) and inhibitory (–) connections of neurons in this circuit are based primarily on the identification of neurotransmitters and laser ablation and genetic studies (J. Duerr, personal communication; Chalfie et al., 1985; McIntire et al., 1993; Zheng et al., 1999). Connections that are hypothesized to be important for the suppression of head oscillations in response to anterior touch are shown in blue. See text for details.

cells are octopaminergic. The RIM motor neurons and the UV1 cells express TDC-1 but not TBH-1, indicating that these cells are tyraminergic. Thus, *C. elegans* has tyraminergic cells that are distinct from octopaminergic cells. This observation indicates that tyramine is not simply a biosynthetic precursor for octopamine. In addition, the differential expression of TDC-1 and TBH-1 in gonadal cells indicates that tyramine and octopamine both likely act in nonneural tissues. A precedent for the synthesis of independent neurotransmitters through the differential expression of a decarboxylase and a β-hydroxylase exists in vertebrates: noradrenergic cells express DDC and DBH, while dopaminergic cells express DDC but not DBH (Muller et al., 1984; Mercer et al., 1991; Chatelin et al., 2001). Tyramine-like immunoreactivity has been detected in presumptive nonoctopaminergic regions (Monastirioti et al., 1995; Stevenson and Sporhase-Eichmann, 1995) of the *Drosophila* (Nagaya et al., 2002) and locust (Donini and Lange, 2004) nervous systems. Our findings suggest that insects have neurons that contain tyramine independently of octopamine as a result of the differential expression of the two octopamine biosynthetic enzymes.

The molecular characterization of the gene that encodes the *C. elegans* tyrosine decarboxylase has allowed us to identify putative orthologs required for tyramine biosynthesis in *Drosophila*, the mosquito, and the honeybee. Analysis of the expression patterns of such tyrosine decarboxylases and tyramine β-hydroxylases should allow the identification of tyraminergic and octopaminergic cells in other invertebrates. Furthermore, since octopamine-deficient *Drosophila* are female sterile (Monastirioti et al., 1996), the invertebrate tyrosine decarboxylases we have identified could define a target class for the management of invertebrate pests.

Tyramine Acts as a Neurotransmitter/Modulator in *C. elegans*

tdc-1 mutants are deficient in both tyramine and octopamine, whereas *tbh-1* mutants are only octopamine deficient. Since *tdc-1* mutants have behavioral defects not shared by *tbh-1* mutants, tyramine likely has a specific role in these behaviors. Specifically, *tdc-1* mutants but not *tbh-1* mutants are hyperactive in egg laying and display defects in reversal behavior and in the suppression of head oscillations in response to anterior touch. That exogenous tyramine inhibits egg laying supports the hypothesis that tyramine inhibits egg laying in vivo. The tyraminergic UV1 cells are the likely source of the tyramine that controls egg laying. The UV1 cells are part of the somatic gonad, form adherens junctions with the utse and the vulF vulval cells, and help connect the uterus with the vulva. The UV1 cells contain neurosecretory vesicles and express neuropeptides (Schinkmann and Li, 1992) as well as several neurosecretory proteins, including SNT-1 synaptotagmin (Nonet et al., 1993), UNC-64 syntaxin (Saifee et al., 1998), UNC-11 AP180 (Nonet et al., 1999), and IDA-1 phogrin-IA-2 (Zahn et al., 2001). The close proximity of the UV1 cells to the egg-laying neuromusculature suggests a paracrine role for tyramine in the inhibition of egg laying.

Like *tdc-1* mutants, RIM-ablated animals had defects in reversal behavior and failed to suppress head oscillations in response to anterior touch. This observation indicates that the tyraminergic RIMs act to modulate reversal behavior and to suppress head oscillations in response to anterior touch.

Tyramine has long been thought to be simply a biosynthetic precursor of octopamine. However, the identification in *Drosophila* (Saudou et al., 1990), the locust (Vanden Broeck et al., 1995), the honeybee (Blenau et al., 2000), the silk moth (Ohta et al., 2003), and *C. elegans* (Rex and Komuniecki, 2002) of G protein-coupled receptors that respond to tyramine has led to the suggestion that tyramine may itself act as a neurotransmitter. In addition, flies that contain a transposon inserted near a gene that encodes tyramine-responsive receptor have an impaired response to repellents, suggesting a role for this receptor in olfaction (Kutsukake et al., 2000). However, for none of these receptors has tyramine been shown to be the endogenous ligand. Exogenous tyramine increases chloride conductance in the *Drosophila* Malpighian tube (Blumenthal, 2003) and modulates excitatory junction potentials in *Drosophila* body wall muscle (Kutsukake et al., 2000) and locust oviduct muscle (Donini and Lange, 2004); whether endogenous tyramine acts similarly is unknown. In addition, exogenous tyramine can rescue defects in cocaine sensitization in *Drosophila iav*/TRPV channel mutants (McClung and Hirsh, 1999); however, whether endogenous tyramine plays a role in cocaine sensitization is unclear, since the previously reported reduced

octopamine and tyramine levels in *iav* mutants could not be confirmed in a recent study from the same laboratory (Gong et al., 2004).

Tyramine is present in trace amounts in the mammalian brain (Boulton, 1976). Although the physiological roles of trace amines, such as tyramine and octopamine, is unclear, trace amines have been implicated in the etiology of a variety of neurological and neuropsychiatric disorders (reviewed by Branchek and Blackburn, 2003). The recent identification of mammalian receptors that respond to tyramine (Borowsky et al., 2001) suggests that tyramine may function independently of the classical neurotransmitters. Our identification of specific tyraminergic cells in *C. elegans* and our characterization of behaviors regulated by tyramine establish tyramine as an endogenous neurotransmitter/neuromodulator and may help reveal physiological roles for tyramine in both invertebrates and vertebrates.

Tyramine Links the Neural Circuit that Regulates the Touch Response with the Neural Circuit that Regulates Head Movements

Where does tyramine act in the neural circuit that links the touch response to the suppression of head oscillations? Chalfie et al. (1985) proposed that tactile stimulation of the ALM/AVM anterior touch sensory neurons leads to the activation of the AVD command neurons, which in turn activate the AVA command neurons (Figure 8). The AVA neurons make gap junctions with the RIM motor neurons (White et al., 1986). Our genetic and laser ablation studies are consistent with a model in which the RIM neurons are activated through these gap junctions by the AVA neurons, leading to the release of tyramine and the inhibition of the cholinergic RMD and SMD head motor neurons, of head muscle contractions, and consequently of head oscillations. Thus, the tyraminergic RIMs link the locomotory neural circuit with the neural circuit that controls head movements and in this way help to generate a complex coordinated behavioral response.

Head oscillations are suppressed less often when backing is induced by harsh touch, which is sensed by the PVD neurons, or by nose touch and osmotic stimuli, which are sensed by the ASH neurons. We observed that animals back further in response to anterior touch than to nose touch, osmotic avoidance, or harsh touch (data not shown). The ASH neurons provide synaptic inputs to the AVA and AVD backward command neurons, and the PVD neurons provide synaptic inputs to the AVA neurons (White et al., 1986). Perhaps upon stimulation the ALM/AVM mechanosensory neurons provide a greater input to the AVA neurons than do the ASH and PVD neurons, and this greater input is required to trigger sufficient tyramine release from the RIM neurons. Alternatively, other neural connections may also play a role in the suppression of head oscillations in response to anterior touch. For instance, the ALM mechanosensory neurons also have synaptic outputs to the RMD head motor neurons, and the ALM neurons may inhibit the RMD motor neurons in response to anterior touch.

The RIM neurons also provide synaptic inputs to the AVB forward command neurons (White et al., 1986). Tyramine release from the RIMs may link the activation of the AVA backward command neurons with the inhibition of the AVB neurons. Failure to properly inhibit the AVB neurons during backing may lead to premature reinitiation of forward locomotion, as observed in *tdc-1* mutants and RIM-ablated animals.

If tyramine release from the RIMs inhibits the AVB forward command neurons, why might *tdc-1* mutants and RIM-ablated animals have an increase rather than a decrease in their spontaneous reversal frequency? Although the locomotion command neurons were defined as forward and backward command neurons with respect to the touch response (Chalfie et al., 1985), several studies have shown that these neurons cannot be strictly categorized into forward and backward neurons but rather can play a role in both directions of locomotion (Hart et al., 1995; Zheng et al., 1999; Brockie et al., 2001). The locomotion command neurons make multiple reciprocal synaptic connections (White et al., 1986), which may coordinate the activities of the antagonistic forward and backward locomotory circuits and allow the animal to switch its direction of locomotion. The connectivity of the RIM motor neurons with the AVB and AVA locomotion command neurons may facilitate the coordination between the forward and backward locomotion motor programs so that RIM ablation or tyramine deficiency perturbs this coordination and consequently leads to an increase in the spontaneous reversal frequency.

Why might *C. elegans* suppress head oscillations in response to anterior touch but not in response to nose touch or posterior touch? The touch response of *C. elegans* could allow the animal to escape from nematophagous fungi, which use trapping devices along their hyphae to catch live nematodes (Barron, 1977). Fungi such as *Arthrobotrys dactyloides* and *Dactylaria brochopaga* use constricting rings to entrap nematodes. When a nematode moves into the ring, the contact triggers the swelling of the ring cells and can lead to the capture of the nematode. There is a lag time between the initial contact and the closure of the ring, allowing some nematodes to withdraw from the ring before being caught (Barron, 1977). The tyramine-mediated suppression of head oscillations in response to anterior touch may allow the nematode to smoothly retreat without activating of the surrounding fungal ring and thereby increase the chances of the nematode's escape from this death trap.

Experimental Procedures

Strains, Molecular Biology, and Germline Transformation

All strains were cultured at 20°C on NGM agar plates with the *E. coli* strain OP50 as a food source (Brenner, 1974). *tbh-1* and *tdc-1* deletion alleles were obtained by screening a chemical deletion library (Dong et al., 2000; Ranganathan et al., 2000). All deletions strains were outcrossed at least six times. Full-length *tbh-1* cDNA sequence was obtained from expressed sequence tag (EST) clone yk722g9. Partial *tdc-1* cDNA sequences were obtained from EST clones yk374c1 (*tdc-1a*) and yk303a5 (*tdc-1b*). The 5′ end sequence of the *tdc-1* cDNA was determined by 5′ RACE. Standard techniques for molecular biology were used (Sambrook et al., 1989). ClustalW alignments (Thompson et al., 1994) were carried out using MacVector software (Accelrys).

A *tbh-1::gfp* transcriptional fusion construct was made by clon-

ing a 4.5 kb *tbh-1* promoter fragment corresponding to nucleotide (nt) –4537 to +17 relative to the translational start site into the vector pPD95.67. A *tdc-1::gfp* reporter construct was obtained by cloning a PstI fragment corresponding to nt –4423 to +443 into the vector pPD95.69. GFP constructs were injected at 80 ng/μl into *lin-15(n765ts)* animals along with the *lin-15* rescuing plasmid pL15EK at 50 ng/μl. A *tdc-1* genomic NsiI fragment, corresponding to nt –917 to +2522, and a fragment corresponding to nt –4423 to +3042 relative to the translation start site were subcloned in pBSK (Stratagene), resulting in pGTDC1 and pGTDC2, respectively. pGTDC2-stop was derived from pGTDC2 by filling in and religating an AvrII site at nt 10541 of cosmid K01C8. pGTDC1 (*nEx1105*) was injected at 50 ng/μl, and pGTDC2 (*nEx1180*) and pGTDC2-stop (*nEx1181*) were injected at 2.5 ng/μl into *tdc-1(n3420); lin-15(n765ts)* animals along with the *lin-15* rescuing plasmid.

HPLC Analysis, TLC, and Decarboxylase Activity Assays

Octopamine levels were quantified using HPLC coupled with electrochemical detection (see Supplemental Data). TLC (Eaton and Mullins, 1988) and TDC activity assays (McClung and Hirsh, 1999) were performed as described with slight modifications (see Supplemental Data).

Immunohistochemistry and Microscopy

TBH-1 antibodies were raised in rabbits against a GST-TBH-1 (aa 244–586) fusion protein. TDC-1 antibodies were raised in rabbits against a GST-TDC-1A (aa 534–650) fusion protein. TBH-1 and TDC-1 antibodies were purified and used for Western blot analysis and immunohistochemistry using standard methods (see Supplemental Data). Identifications of TDC-1- and TBH-1-expressing cells were based on cell body positions and axon morphologies of strains that expressed *tdc-1* and *tbh-1 gfp*-reporter genes and by immunostaining (see Supplemental Data). Cell ablations using laser microsurgery ablations were performed during the second larval stage (L2) as previously described (Avery and Horvitz, 1989). GFP reporters were used to facilitate cell identifications and to confirm cell ablations at the L4 stage (see Supplemental Data). The integrated *tbh-1:: gfp* transgenic line, *nls107*, was used for RIC ablations. An integrated *nmr-1::gfp* transgenic line, *akIs3* (Brockie et al., 2001), was used for RIM, AVA, AVD, and AVE ablations.

Behavioral Assays

Behavioral assays were performed with young adults at room temperature (22°C–24°C); the different genotypes were scored in parallel. Egg-laying assays were performed as described by Koelle and Horvitz (1996). Suppression of head oscillations was tested by touching animals that were outside the bacterial lawn with a fine eyelash behind the posterior bulb of the pharynx. The presence or absence of head oscillations was scored during the backing response. Animals that showed no head oscillations during backward locomotion were scored as positive. More than 60 animals were scored for each genotype. Nose touch and osmotic avoidance touch were tested as described (Bargmann et al., 1990; Kaplan and Horvitz, 1993). Response to harsh touch can be assayed only in animals that do not have functional light touch sensory neurons and were scored in *mec-7(e1527)* mutants (Way and Chalfie, 1989). Animals were tested 24 hr after they were picked as late L4 larvae. Laser-ablated animals were tested at least 20 times; at least six animals were examined for each ablated neuron class. Reversal assays were performed as described (Tsalik and Hobert, 2003). Any backward movement was scored as a reversal.

Supplemental Data

The Supplemental Data include Supplemental Experimental Procedures and two supplemental movies and can be found with this article online at http://www.neuron.org/cgi/content/full/46/2/247/DC1/.

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Note Added in Proof

While this paper was in press, Cole et al. (2005) reported the identification of two *Drosophila* tyrosine decarboxylase genes, *dTdc1* (CG30445) and *dTdc2* (CG30446). *dTdc2* mutants lack neural tyramine and octopamine, and TDC2 (CG30446) was identified as the closest homolog of TDC-1 in this study.