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TYRA-3, A TYRAMINE-ACTIVATED G-PROTEIN COUPLED RECEPTOR, MODULATES LOCOMOTION THROUGH INHIBITION OF DOPAMINERGIC SIGNALING IN *CAENORHABDITIS ELEGANS*

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ABSTRACT

TYRA-3 is a tyramine-activated G-protein coupled receptor in *C. elegans*. TYRA-3 is expressed in dopaminergic ADE and CEP neurons, suggesting it may play a role in modulating dopaminergic signaling. Upon exogenous tyramine exposure, deletion mutants of *tyra-3* are more susceptible to paralysis than wild type animals. *dat-1* deletion mutants, which contain abnormally high concentrations of synaptic dopamine, were also sensitive to paralysis with tyramine exposure. *cat-2* deletion mutants, which contain no detectable dopamine, were partially resistant to tyramine exposure. Double mutants of *cat-2;tyra-3* and *dat-1;tyra*-3 showed moderate sensitivity. These results suggest that TYRA-3 inhibits dopamine release, but additional experiments are necessary to explain the phenotypes of the double mutants.

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BACKGROUND

G Protein-Coupled Receptors

G protein-coupled receptors (GPCRs) are a very diverse and functionally significant family of proteins that serve to convey extracellular signals to intracellular pathways. These receptors are coupled with heterotrimeric G-proteins, a family of proteins that uses GTP to generate the free energy necessary to propagate the intracellular signal. Research on prototypical GPCRs suggests that they contain 7 transmembrane helices (**Figure-1**). Upon binding the signaling ligand, which could be a hormone, lipid, chemokine, biogenic amine, neurotransmitter, etc., ionic interactions between the third and sixth helices are disrupted through conformational changes. This process is thought to activate the G-protein. GTP binds the active G-protein while GDP is released (Kroeze et al., 2003).

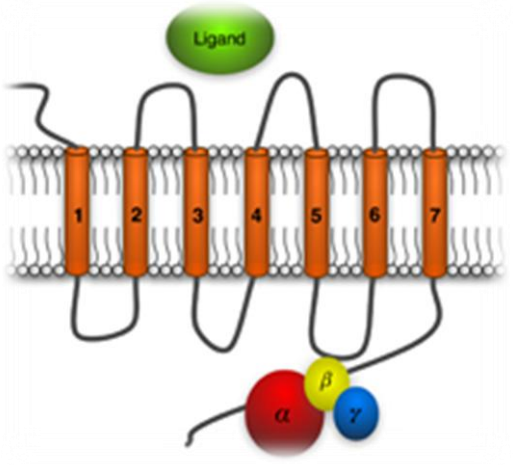

Figure-1: Diagram of Prototypical GPCR. Note the seven transmembrane domains (orange), and the α, β, and γ subunits of the cytoplasmic G-protein complex (Krishnamurthy et al., 2006).

The propagation of the GPCR-mediated intracellular signal proceeds in a widely varying fashion, depending on the specific GPCR. Each G-protein consists of α, β, and γ subunits (red, yellow, and blue in the Figure). The α subunit dissociates upon G-protein activation, and may participate in the cAMP or phosphatidylinositol signaling pathways. Different classes of G_α proteins have been identified. G_s and G_i serve to activate or repress enzymatic or metabolic activity, respectively, through the cAMP pathway. G_q protein, another common G-protein, activates the phosphatidylinositol pathway (Gilman, 1987).

Desensitization of the GPCR typically involves phosphorylation of the protein itself and/or interactions with a cytoplasmic protein known as β-arrestin. β-arrestin binds the receptor-G-protein complex and signals endocytosis of the GPCR. Once inside the cell, the ligand is degraded, β-arrestin is released from the complex, and the GPCR re-associates with the membrane (Filmore, 2004). Phosphroylation of the GPCR by a G-protein coupled receptor kinase results in similar translocation followed by degradation or internalization (Tan et al., 2008).

Approximately one thousand identified GPCRs in humans are particularly consequential in medicinal and behavioral research, as evidenced by the fact that nearly half of all pharmaceuticals on the market target this class of receptors (Voet et al., 2006). The function of most GPCRs is at least partially elucidated. Many serve as receptors for sensory functions like sight and smell. For the 300 or so non-sensory human GPCRs, over half have characterized natural ligands. This information is frequently exploited in drug design, as pharmaceuticals are often targeted to mimic natural ligands to either suppress or activate GPCR signaling (Filmore, 2004) Approximately half of non-sensory

GPCRs remain uncharacterized; with no endogenous ligands identified, these receptors are referred to as orphan GPCRs. Recently, investigations into trace monoamines in vertebrates and invertebrates have begun to elucidate the role of some of these orphan GPCRs (Borowsky et al., 2001).

GPCR Ligands

Classical biogenic amines such as serotonin, norepinephrine, and dopamine act as neurotransmitters that activate GPCRs. These classical amine neurotransmitters represent the primary mechanism by which neurons communicate. A neurotransmitter binding to a neuronal GPCR may trigger relay, amplification, or modulation of neuronal signaling (Lauder, 1993). These signaling pathways, though influenced by very small ligand concentrations, can have broad consequences in motor, cognitive, affective, and arousal functions (Xie and Miller, 2009). This is evident from the dramatic effects of many psychotropic drugs. For example, medications to treat Parkinson's disease target dopamine-specific GPCRs in an effort to improve coordination and decrease tremors; however, the side effects from these dopamine replacement therapies include tics, hallucinations and mood disturbances (Snyder, 2005). Beta-blockers also have diverse consequences; often used to treat heart conditions and anxiety, they compete with norepinephrine for GPCR binding. Beta-blockers are designed to target a limited class of norepinephrine-associated GPCRs and affect melatonin release, intraocular pressure, circulation, and numerous other diverse physical processes (Elliot, 2008). The classical neurotransmitters and their associated GPCRs are extraordinarily consequential in nervous system function.

Trace Amine Neurotransmitters

Recently, a class of trace biogenic amines have been identified as possible neurotransmitters and neuromodulators. These trace monoamines, including β-phenylethylamine, tryptamine, octopamine, and tyramine, are present in minute concentrations in mammalian tissues, and constitute less than 1% of the biogenic amines in most heterologously expressed brain regions (Borowsky et al., 2001; Bunzow et al., 2001). However, judging by the remarkably significant consequences from miniscule variations in classical neurotransmitter activity, it is likely that even such small concentrations of these trace amines can play important physiological roles. A better understanding of the functions of trace amines and their GPCRs may provide important explanations for the pathology of under characterized psychiatric disorders.

Tyramine in particular has been implicated as a possible contributing factor in depression, migraine headaches, schizophrenia, and attention deficit hyperactivity disorder (Wragg et al., 2007). Such hypotheses are relatively recent; tyramine was traditionally thought to be solely a metabolic byproduct or biosynthetic precursor of octopamine (Pirri et al., 2009). TAAR1, a G-protein coupled receptor widely expressed in the mammalian brain, was identified in 2001 as having a high affinity for tyramine (Borowsky et al., 2001). TAAR1 protein is expressed in the dorsal and ventral caudate nucleus, putamen, substantia nigra, nucleus accumbens, ventral tegmental area, locus coeruleus, amygdale, and raphe nucleus; many of these are monoaminergic regions. In the substantia nigra, TAAR1 is co-localized with the dopamine transporter in some

dopaminergic neurons, suggesting that tyramine binding to TAAR1 may indirectly regulate dopamine transporter activity (Xie and Miller, 2009).

The TAAR1 GPCR signals via the cAMP pathway. Tyramine binding results in activation of cAMP production, therefore it is likely coupled to the G_s heterotrimeric G protein (Tan et al., 2008). Genomic screening has identified six other trace amine associated receptors in humans, though their functions are not well characterized (Miller et al., 2005). Despite evidence of tyramine binding to the mammalian GPCRs, results do not conclusively prove that tyramine is the physiological endogenous ligand for these receptors. Nonetheless, experimental data strongly suggest that tyramine does function as a neurotransmitter in vertebrates.

Tyramine as an Invertebrate Neurotransmitter

The role of tyramine as a neurotransmitter in invertebrates has been convincingly demonstrated (Alkema et al., 2005; Pirri et al., 2009). Tyramine-responsive GPCRs have been identified in fruit flies, locusts, honeybees, silk moths, and nematodes (Saudou et al., 1990; Blenau et al., 2000; Rex and Komuniecki, 2002; Ohta et al., 2003; Rex et al., 2005). As in vertebrates, dopamine and serotonin serve as classical neurotransmitters in invertebrates. Invertebrates utilize octopamine as a major neurotransmitter in lieu of norepinephrine (Roeder et al., 2003). In *C. elegans*, tyramine is a precursor in octopamine synthesis through the following pathway: tyrosine decarboxylase (*tdc-1*) converts tyrosine into tyramine, followed by the conversion of tyramine to octopamine by tyramine β hydroxylase (*tbh-1*). *tdc-1* mutant animals, which lack both tyramine and octopamine, display distinct behavioral phenotypes from *tbh-1* mutants, which lack only

octopamine. This suggests that tyramine independently influences behavior in *C. elegans* (Alkema et al., 2005).

Wild type worms can exhibit behavioral changes in locomotion, feeding, egg-laying, and foraging when abnormal tyramine signaling is induced. *C. elegans* has three tyramine activated G protein coupled receptors—SER-2, TYRA-2, and TYRA-3—and a tyramine-gated chloride channel LGC-55 (**Figure-2)**. *lgc-55* is involved in reversals and head movements; tyramine has been conclusively shown to be the natural ligand for this channel, confirming its role as an inhibitory neurotransmitter in *C. elegans* (Pirri et al., 2009). Upon anterior touch, tyramine is released and through the actions of LGC-55, head oscillations are repressed and the animal reverses. *lgc-55* is thought to play an important role in the *C. elegans* escape response from nematophagous fungi traps (Barron, 1977). It is hypothesized that contact with the constricting hyphal rings, analogous to anterior touch, induces tyramine release through activation of tyraminergic RIM motor neurons. Tyramine binds LGC-55 which is postsynaptic to RIM neurons. Consequently, head movements are suppressed and the worm moves backward to escape the death trap (Pirri et al., 2009).

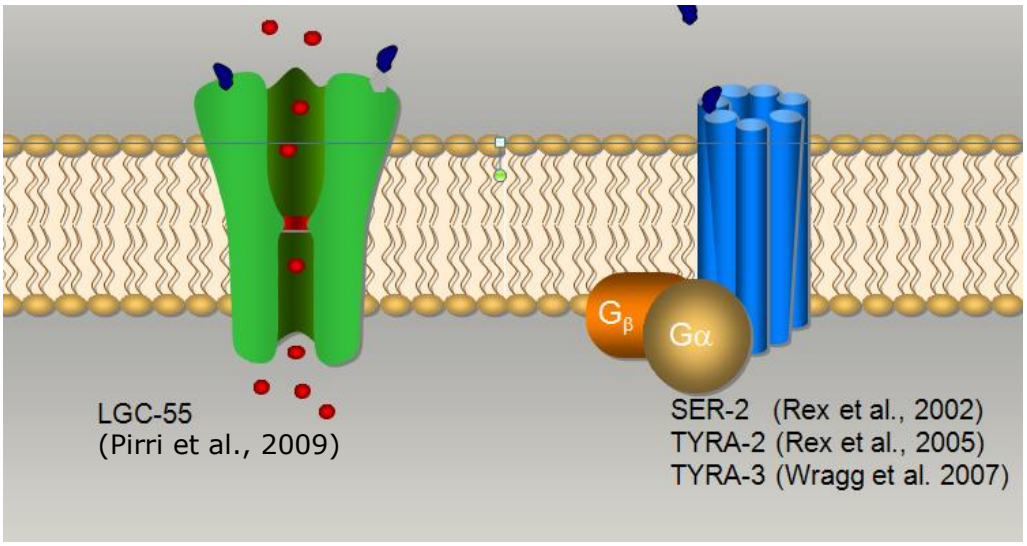

Figure-2: Tyramine Activates LGC-55, SER-2, TYRA-2, and TYRA-3. LGC-55 is a tyramine-gated chloride channel, while SER-2, TYRA-2, and TYRA-3 are tyramine-activated G protein coupled receptors (Donnelly, personal communication)

SER-2 is a tyraminergic GPCR also implicated in the escape response. When expressed in HEK293 cells, intracellular cAMP levels decrease upon exposure to tyramine, suggesting that SER-2 is $G_{i/o}$ (GOA-1) coupled (Rex and Komuniecki, 2002). Tyramine has also been shown to increase Ca^{2+} levels in cells that express *ser-2* (Rex et al., 2004). *ser-2* is expressed in neurons in the head and tail, pharyngeal cells, head muscles, diagonal muscles in males, and some posterior neurons. Generally, the receptor is expressed in sensory, inter- and motor neurons (Tsalik et al., 2003). Thus, mutations in *ser-2* result in observable behavioral modifications. In contrast to the wild type, SER-2 defective worms do not display increased pharyngeal pumping in the presence of endogenous tyramine (Rex et al., 2004). This behavioral phenotype suggests that SER-2 could affect feeding and foraging behavior through tyraminergic neuromodulation. Additionally, *ser-2* mutant worms appear to be defective in making deep ventral (omega) turns. SER-2 is thought to enable completion of tight, closed omega turns through

inhibition of GABAergic signaling (Donnelly, personal communication). Backward locomotion, suppression of head oscillations, and omega turns are observed as part of the *C.* elegans escape response from nematophagous fungi, thus both *ser-2* and *lgc-55* are thought to play a role in the tyramine-modulated escape (Donnelly and Alkema, personal communication).

TYRA-2 is another tyramine responsive GPCR in *C. elegans*. Sequence homology suggests that it is likely G_i coupled. Like SER-2, TYRA-2 protein expressed in COS-7 cells binds tyramine with a higher affinity than other physiologically relevant biogenic amines. TYRA-2 has a much more restricted expression than SER-2, as it has been identified only in specific pharyngeal neurons, amphid neurons, and neurons in the nerve ring, body and tail. TYRA-2 is not expressed in muscle. Very little behavioral data is available, but it is predicted that TYRA-2 may play a similar role as SER-2 in the stimulation of pharyngeal pumping (Rex et al, 2005).

TYRA-3 is the most recently described tyramine activated GPCR in *C. elegans*. TYRA-3 was suspected as a possible tyramine receptor because of its sequence similarity to a newly characterized tyramine-specific GPCR in insects (Wragg et al., 2007). Preliminary data confirms a high affinity for tyramine. Extrapolation from sequence similarities suggests that TYRA-3 is G_q coupled (**Figure-3**) (diagram left side) and thus affects signaling in the phosphatidylinositol pathway. TYRA-3 is essential for inhibition of aversive responses to dilute octanol (Wragg et al., 2007). Aside from this, the role of TYRA-3 in *C. elegans* behavior is largely unexplored. It is hypothesized that *tyra*-3 may play a regulatory role in dopaminergic signaling because it is expressed in dopaminergic ADE/CEP neurons (Wragg et al., 2007).

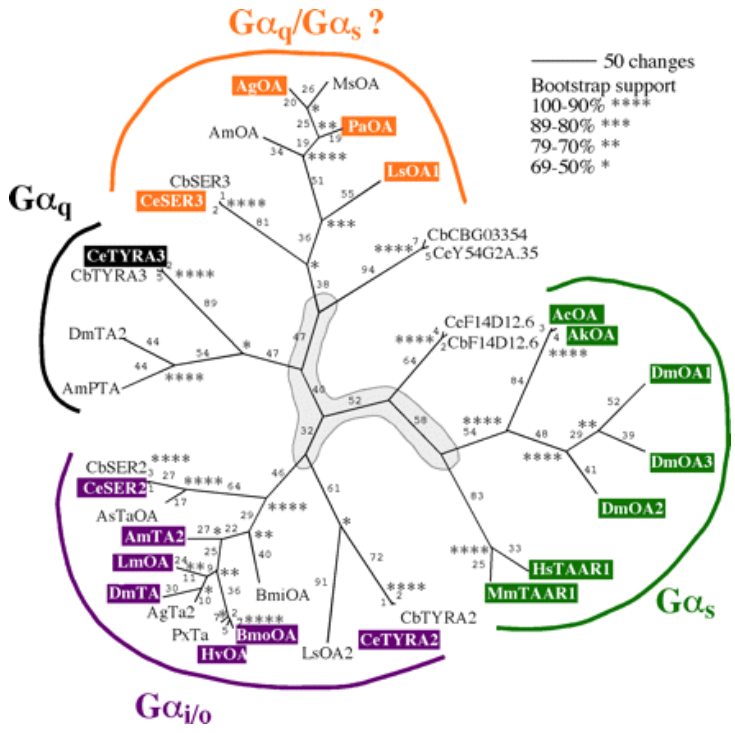

Figure-3: Phylogenetic Relationships Among Biogenic Amine G-Protein-Coupled Receptors from Other Bilaterian Species. Note that *C. elegans* TYRA-3 (black box, left side) is G_q coupled (Wragg et al., 2007).

Dopamine Signaling in *C. elegans*

Dopamine has been shown to play a role in numerous nematode behaviors, including learning and locomotion (McDonald et al., 2006). Dopamine is synthesized in 8 neurons in hermaphrodite *C. elegans:* ADE(R and L), PDE(R and L), CEPD(R and L) and CEPV(R and L) (**Figure-4**) (Chase and Koelle, 2007). The ADE and PDE neurons, also known as the anterior and posterior deirid sensilla, are involved in mechanical texture sensation. ADE neurons are located posteriorly and ventrally to the terminal bulb. The dorsal ADE process sends off a short branch on the side, which extends to the lateral wall and terminates as a cilium (Altun and Hall, 2008). PDE sensory neurons are located

next to dorsal body wall muscle along the lateral side of the posterior body. Neurons of the cephalic sensillia, the CEP neurons have cell bodies and dendrites that extend to the tip of the nose. Like ADE and PDE neurons, CEP neurons are involved in mechanosensory functions (Altun and Hall, 2008).

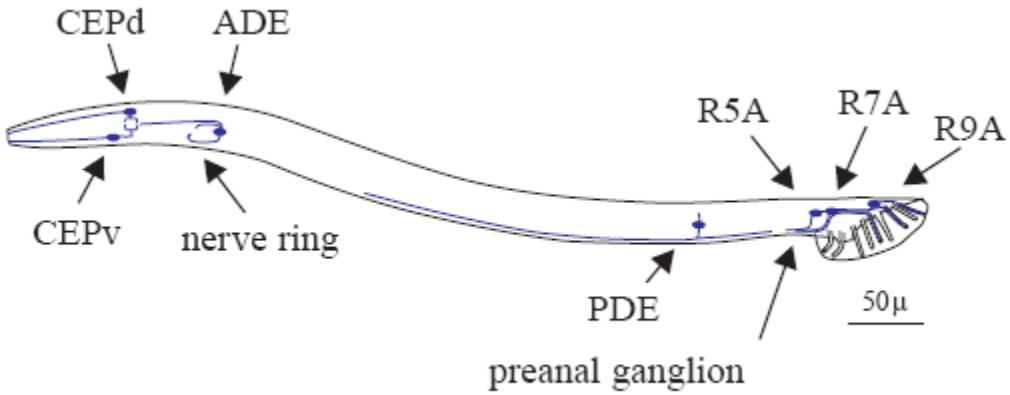

Figure 4: Dopaminergic Neurons in Male *C. elegans*. ADE, CEPd, CEPv, and PDE neurons are dopaminergic neurons in both male and hermaphrodite *C. elegans*. Note that ray neurons R5A, R7A and R9A are only present in males (Lints and Emmons, 1999).

Two genes vital in regulating dopamine levels are *cat-2* and *dat*-1 (**Figure-5).** Mutants in *cat-2* and *dat-1* genes display unique phenotypes that demonstrate the significance of dopaminergic signaling in the nematodes. The *cat-2* gene encodes tyrosine hydroxylase which catalyses the rate-limiting step in dopamine synthesis. Consequently, no dopamine is detectable in *cat-2* mutant animals (Nass et al., 2002). These mutants fail to exhibit area-restricted search behavior. Area-restricted search in *C. elegans* refers to the changes in turning frequency in response to food availability (Hills et al., 2004). Wild type animals exhibit a high turning frequency in areas with food, and a low turning frequency in areas with no food. *Cat-2* mutants have a low turning frequency under both conditions, suggesting that dopamine is required to stimulate

turning in response to food (Hills et al., 2004). Additionally, wild type animals exhibit a basal slowing response when they encounter bacteria or a substance of comparable texture, whereas *cat-2* mutants fail to slow locomotion in response to food (Sawin et al., 2000). As evidenced by these phenotypic changes, dopaminergic signaling is crucial for maintaining efficient foraging behaviors through modulation of turning and locomotory speed in *C. elegans.*

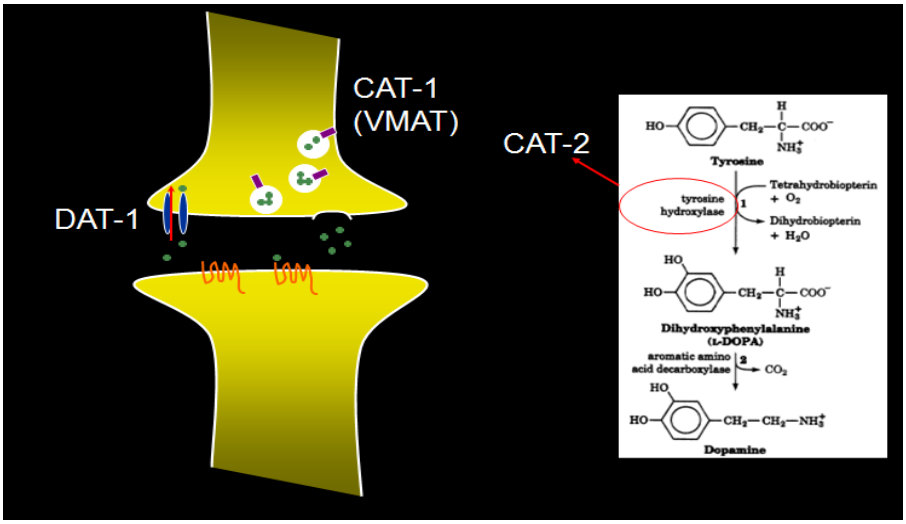

Figure-5: Diagram of DAT-1 and a Dopaminergic Neuron. DAT-1 is a dopamine reuptake transporter. *cat-2* encodes tyrosine hydroxylase, the rate-limiting step in dopamine synthesis (McDonald, personal communication).

In contrast to *cat-2* mutants, *dat-1* mutant animals are thought to have potentiated dopamine activity. *dat-1* encodes a dopamine transporter necessary for the re-uptake of dopamine into presynaptic neurons (Jayanthi et al., 1998). Thus, loss-of-function mutations in *dat-1* result in increased synaptic dopamine concentrations. Intriguingly, *dat-1* mutants paralyze rapidly when swimming in water while wild type worms continue to thrash. This suggests that dopamine spillover into extrasynaptic sites negatively regulates locomotion (McDonald et al., 2007). Not all dopamine GPCRs are post

synaptic to dopaminergic neurons; spillover of dopamine into extrasynaptic sites could activate these GPCRs, leading to this unique phenotype (McDonald et al., 2007).

PROJECT PURPOSE

The purpose of this project was to determine the role of TYRA-3 in modulating dopaminergic signaling in *C. elegans*. Using confocal microscopy, the expression pattern of TYRA-3::GFP was analyzed to confirm expression in dopaminergic cells. Animals mutant in *tyra-3*, *cat-2* (loss of dopamine synthesis), and *dat-1* (loss of dopamine re-uptake transporter), as well as double mutants, were studied using exogenous tyramine drug assays. The goal of these experiments was to define neural circuits that may involve the interaction of TYRA-3 and dopaminergic signaling.

METHODS

C. elegans Strains

All strains were obtained from the *C. elegans* Genetics Center (CGC) unless otherwise noted. Worms were cultured at room temperature (22°-24°C) on nematode growth media (NGM) agar plates. Plates were seeded with OP50 *E. coli* as a food source (Brenner, 1974). The wild-type strain used was Bristol N2. Mutant strains used in this study were *tyra-3(ok325), lgc-55(tm2913), tdc-1(ok914), cat-2(tm2261, e1112),* and *dat-1(ok157).* A *ptyra-3*::TYRA-3::GFP rescue line from R. Komuniecki was also used.

Cat-2(tm2261) worms were backcrossed with N2 worms prior to experiments to eliminate background mutations. *cat-2;tyra-3* double mutants were made using this backcrossed *tm2261* allele. The *cat2;tyra-3* cross was initiated by plating 8 *cat-2* males with 2 *tyra-3* hermaphrodites. In F_1 offspring, 8 males were picked and again plated with 2 *tyra*-3 hermaphrodites. In F_2 offspring, approximately 12 L4 animals were isolated to individual plates and allowed to reproduce. Homozygosity of *cat-2;tyra-3* double mutants was confirmed using PCR from these progeny. The same procedure was used to obtain *dat-1;tyra-3* double mutants.

Worm Lysis and PCR

Approximately 3-5 worms were picked and placed in 5 µL of 1X Prot K lysis buffer. The worms were lysed at 65°C for 1 hour and 95°C for 15 minutes in an Eppendorf Thermocycler. For each PCR reaction, a 25µL mix was used containing 2.5 µL 10X PCR Buffer, 2.5 µL of 2 mM DNTPs, 0.1 µL Taq polymerase, 1.5 µL worm

lysate, 0.25 µL of each primer, and 17.9 µL dH_2O. Primers used for confirming *cat-2* deletion were tm2261 external forward (5'-GATTCTCCAACAACTGAACGACG-3') and tm2261 external reverse (5'-GTGCTCTTCCTCCGTGTAGTC-3'). Primers used for confirming *dat*-1 deletion were ok157 internal forward (5'-GGGCTTATTGATTGCAGTGTTC-3') and ok157 external reverse (5'-CCCTCGCATTGACGAATTTG-3'). The samples were heated at 94°C for 2 minutes in an Eppendorf Thermocycler, followed by 35 cycles consisting of 94°C for 15 seconds, 60°C for 45 seconds, and 72°C for 1 minute. Finally, the samples were heated at 72°C for 5 minutes. Agarose gel electrophoresis was used to visualize PCR products.

Tyramine Behavioral Assays

Exogenous tyramine assays were conducted at room temperature (22°-24°C) on 1.7% agar plates containing tyramine hydrochloride (Sigma) and 2 mM acetic acid. Young adult animals used were selected at the L4 stage 24 hours prior to assay. For the assay, worms were transferred to drug plates for a 20 minute period. The number of immobilized worms was scored every minute. Only worms that had no significant movement for 5 seconds were counted as immobilized. At least 9 trials of an average of 12 worms each were performed for each drug and strain combination in this study.

TYRA-3 Expression Analysis

ptyra-3::TYRA-3::GFP;*lgc-55*::mCherry and *ptyra-3*::TYRA-3::GFP;*tdc-1*::mCherry were visually analyzed using confocal microscopy (Zeiss). The images were

formatted using ImageJ software. Cell identification was done by comparing cell morphology to the known *C. elegans* neuronal diagram.

RESULTS

TYRA-3 is a G-protein coupled receptor that has been shown to bind tyramine with high affinity (Wragg et al., 2007). Its role in *C. elegans* behavior is largely unexplored. To investigate the function of TYRA-3, worms with loss of function deletion mutations in the receptor were exposed to drug plates with various concentrations of tyramine. These assays were performed in parallel with wild type and *tyra-3* rescue mutants containing a *ptyra3*::TYRA::GFP extra-chromosomal array in the *tyra-3* deletion background. At a 10 mM tyramine concentration, a clear difference in immobilization between *tyra-3* mutants and wild type worms was observed (**Figure-6**). *tyra-3* mutants (red curve) were hyper-sensitive to paralysis in response to exogenous tyramine exposure. The wild type phenotype (blue curve) was completely restored with the extra-chromosomal rescue (green curve).

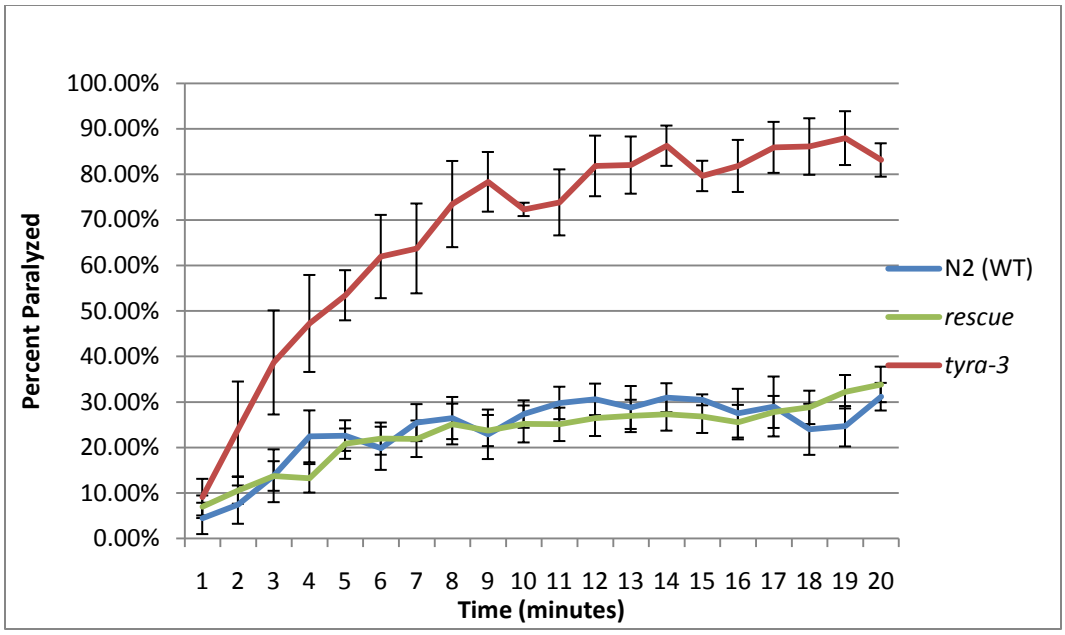

Figure-6: *tyra-3* Loss of Function Mutants are Sensitive to Paralysis on 10 mM Tyramine. Exogeneous tyramine behavioral assays were performed as described in Methods. Each point represents the mean of at least nine trials. Error bars denote standard error.

Confocal microscopy of the translational GFP fusion *ptyra3::*TYRA-3::GFP strain was used to analyze the TYRA-3 expression pattern. The strain was crossed with *lgc*-55::mCherry and *tdc-1::*mCherry to visualize the relative positioning of cells. TYRA-3::GFP was identified in dopaminergic CEP neurons (**Figure-7)** and in dopaminergic ADE neurons (**Figure-8**). Expression was observed in additional neurons that were not readily identifiable given lack of co-expression with the known *lgc-55::*mCherry and *tdc-1*::mCherry reporters.

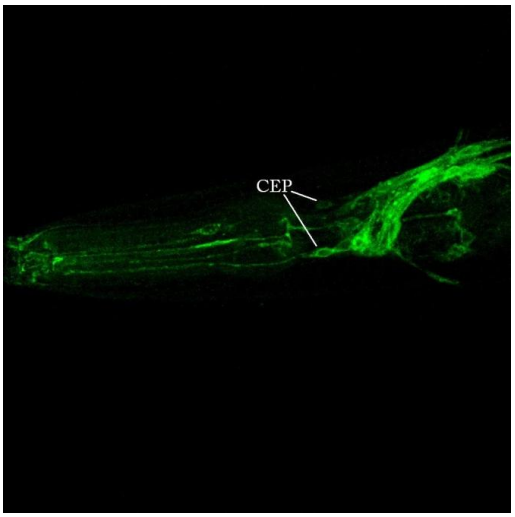

Figure-7: **TYRA-3 Expression in Dopaminergic CEP Neurons.** The TYRA-3::GFP fusion protein fluoresces green.

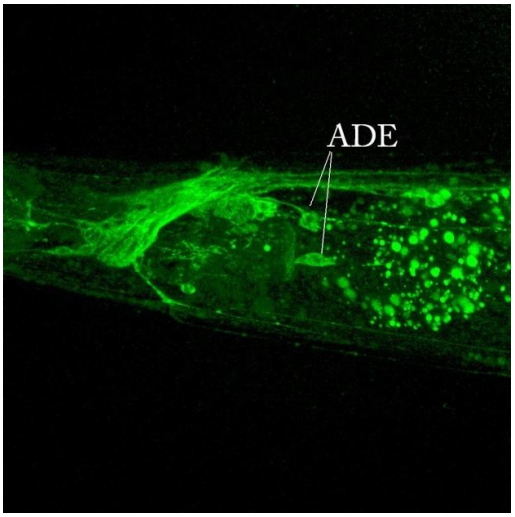

Figure-8: TYRA-3 Expression in Dopaminergic ADE Neurons. The TYRA-3::GFP fusion protein fluoresceses green.

Since TYRA-3 is expressed in dopaminergic neurons, additional drug assays were performed to investigate the influence of TYRA-3 on dopamine signaling. *dat-1* loss of function mutants lacking the dopamine reuptake transporter, as well as *dat-1;tyra-3* double mutants, were assayed on plates containing various concentrations of tyramine (**Figure-9**). At 10 mM tyramine, both *dat-1* (purple curve) and *dat-1;tyra-3* mutants (turquoise curve) were more sensitive to paralysis than wild type (blue curve) and less sensitive to paralysis than *tyra-3* mutants (red curve).

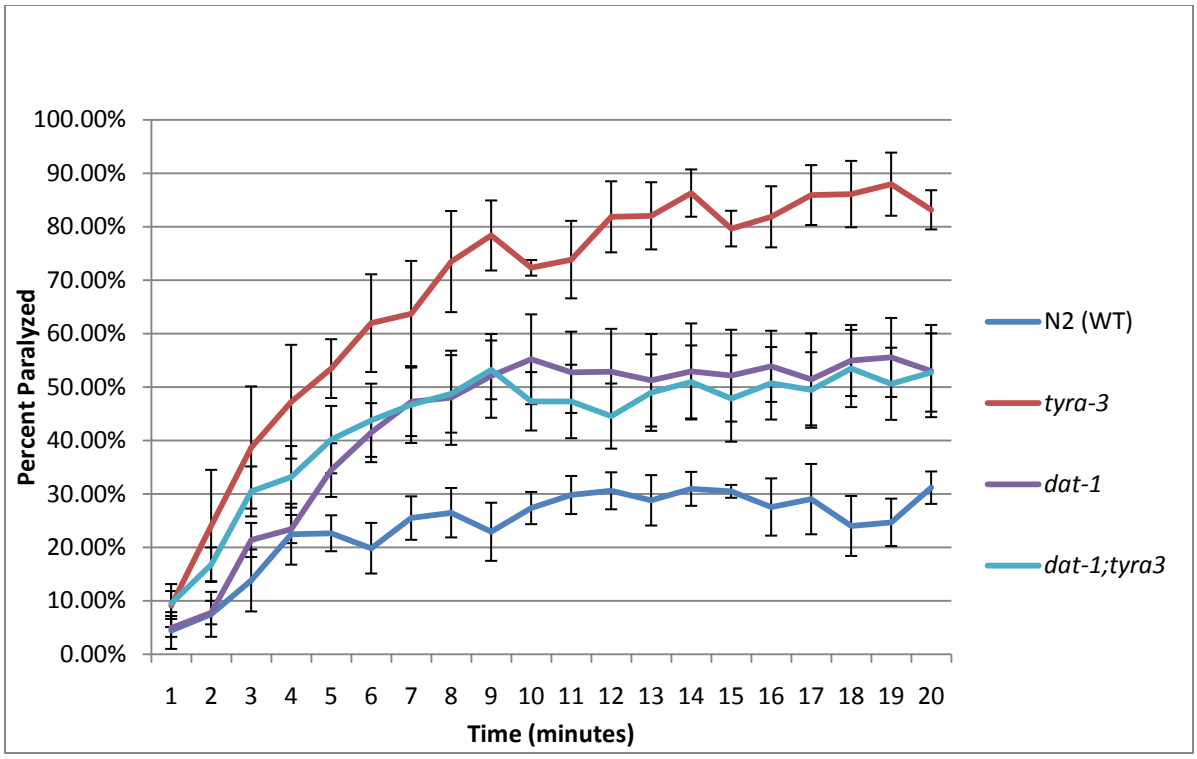

Figure-9: *dat-1* and *dat-1;tyra-3* Loss of Function Mutants are Moderately Sensitive to Paralysis on 10 mM Tyramine Plates. Each point represents the mean of at least nine trials. Error bars denote standard error.

cat-2 and *cat-2;tyra-3* loss of function mutants (producing no dopamine) were also tested on varying concentrations of tyramine (**Figure-10**). At 10 mM tyramine, *cat-2;tyra-3* double mutants (pink curve) showed higher sensitivity than wild type and lower sensitivity than *tyra-3* mutants, similar to *dat-1* and *dat-1;tyra-3* mutants. *cat-2* mutants (orange curve) showed a slight resistance to paralysis at 10 mM tyramine. This phenotype was more pronounced at 20 mM tyramine (**Figure-11**).

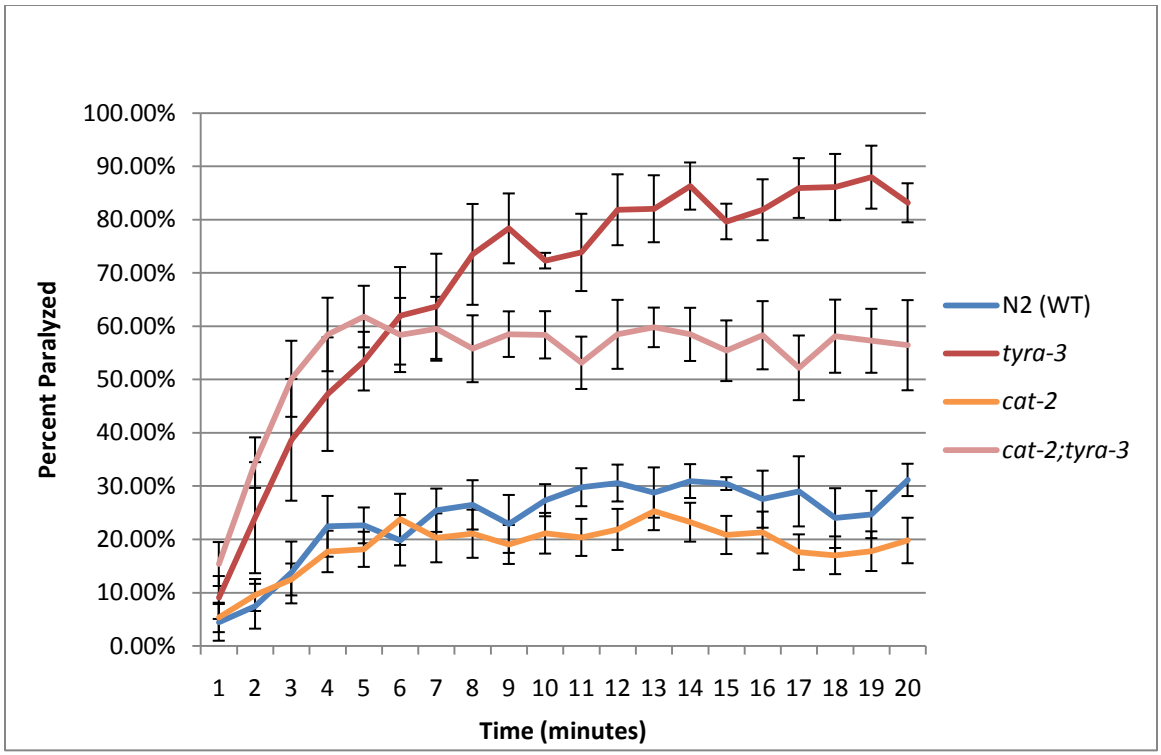

Figure-10: *cat-2;tyra-3* Loss of Function Mutants are Moderately Sensitive to Paralysis at 10 mM Tyramine. Each point represents the mean of at least nine trials. Error bars denote standard error.

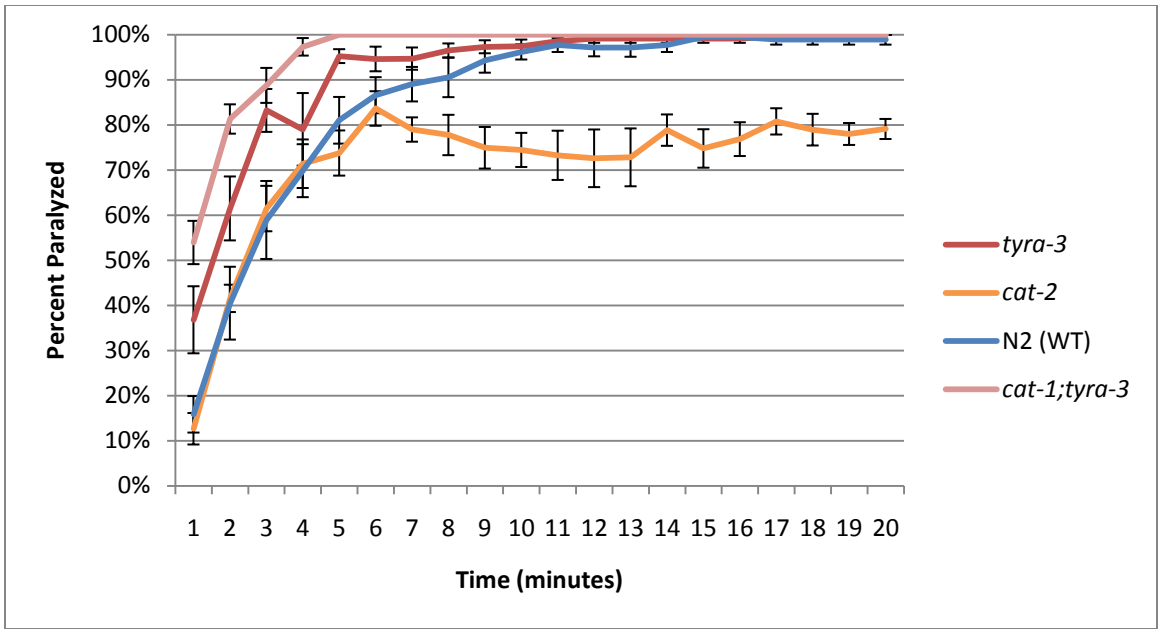

Figure-11: *cat-2* Loss of Function Mutants are Resistant to Paralysis at 20 mM Tyramine. Each point represents the mean of at least nine trials. Error bars denote standard error.

DISCUSSION

tyra-3 mutants, lacking a functional TYRA-3 G protein-coupled tyramine receptor, are more sensitive to paralysis than wild type worms upon exposure to exogenous tyramine. *tyra-3* mutants expressing a *ptyra-3::*TYRA-3::GFP extrachromosomal array display a wild type phenotype, confirming that the apparent tyramine sensitivity of *tyra-3* is due to loss of function in the TYRA-3 receptor (**Figure-6)**. These results at first seem counter-intuitive, as one would predict that the loss of a tyramine receptor would make the worms more *resistant* to tyramine paralysis, but this may be partially explained by interactions between tyraminergic and dopaminergic signaling. The strong expression of GFP tagged TYRA-3 in dopaminergic ADE and CEP neurons (**Figure-7, Figure-8**) suggests that the binding of tyramine to TYRA-3 could have some effect on dopaminergic signaling in these neurons. The time response curves for *cat-2* and *dat-1* mutant worms, which contain no dopamine and high synaptic dopamine, respectively, support a model involving suppression of dopamine signaling by the TYRA-3 receptor.

Contrary to sequence homology analysis that suggests TYRA-3 is G_q coupled (Wragg et al., 2007), the time response to tyramine exposure for *tyra-3, cat-2,* and *dat-1* mutants suggests that TYRA-3 is G_i coupled. In principal, tyramine binding to TYRA-3 leads to dissociation of the G_i protein. The dissociated G_i protein suppresses enzymatic activity by inhibiting the production of cAMP from ATP (Gilman, 1987). This change in enzymatic activity could ultimately down regulate dopaminergic signaling through a number of mechanisms. Following this model, hypersensitivity of *tyra-3* loss of function

mutants to tyramine may be due, in part, to lack of repression of dopamine release. Excessive dopamine signaling, as in *dat-1* mutants, has been shown to cause paralysis in swimming assays (McDonald et al., 2007). Without inhibition of dopaminergic signaling by TYRA-3, spillover of dopamine into extrasynaptic sites negatively regulates locomotion.

The phenotypes observed for *dat-1* and *cat-2* are consistent with previously established behaviors; *dat-1* mutants paralyze when swimming, while *cat-2* mutants display a lack of basal slowing when encountering food (Sawin et al., 2000). Hypersensitivity to paralysis of *dat-1* loss of function mutants exposed to 10 mM tyramine (**Figure-9)** may be the result of excessive dopamine partially outweighing repressive effects of TYRA-3. The partial resistance of *cat-2* mutants to exogenous tyramine exposure (**Figure-11**) also supports the theory that dopamine release leads to the paralysis observed in *dat-1* and *tyra-3* mutants. Sufficient tyramine exposure leads to paralysis in wild type worms, but without dopamine to repress locomotion, *cat-2* mutants are resistant to this effect. The effects of tyramine exposure on these mutants demonstrate a significant interaction between dopaminergic and tyraminergic nervous systems. Dopamine contributes to the paralysis observed in *dat-1, tyra-3* and wild type worms when exposed to exogenous tyramine. *tyra-3* mutants are the most sensitive to paralysis, supporting the hypothesis that tyramine binding to TYRA-3 inhibits dopamine release through suppression of the cAMP pathway

According to this model, it was predicted that *dat-1;tyra-3* mutants would be more sensitive to paralysis than *dat-1* mutants, and *cat-2;tyra-3* mutants would show the same resistance as *cat-2* mutants. However, this was not the case. Both *cat-2;tyra-3* and

dat-1;tyra-3 double mutants were similarly sensitive to paralysis on 10 mM tyramine plates (**Figure-9, Figure-10)**. This suggests that TYRA-3 is likely influencing locomotion through actions in other neurons in addition to ADE and CEP. This hypothesis could be tested by rescuing the *tyra-3* deletion through expression of a TYRA-3 extra-chromosomal array with a dopamine-specific promoter (ie, *pdat-1*). This would express the functional TYRA-3 receptor only in dopaminergic cells. By comparing the time response of these mutants on tyramine plates to results for *tyra-3* and wild type worms, it may be possible to gain insight on the effects of TYRA-3 outside of dopaminergic signaling.

It would also be useful to cross the *ptyra-3*::TYRA-3::GFP worms with additional mCherry tagged mutants. This could help with identification of neurons where TYRA-3 is expressed. Since dopamine is often associated with attention (Chase and Koelle, 2007), an assay measuring attention behaviors in the worm could also be useful in establishing the actions of TYRA-3. It is possible that tyramine binding to TYRA-3 in the escape response could serve to heighten attention in the worm. Additionally, drug assays on aldicarb, an acetylcholine esterase inhibitor, could provide come clues about the interaction of TYRA-3 with other neurotransmitters. Excess acetylcholine concentration leads to paralysis by contracting body wall muscles, while a lack of acetylcholine causes resistance to paralysis (Nguyen et al., 2005). If TYRA-3 is expressed in cholinergic or GABAergic neurons, it is expected that exposure to aldicarb and tyramine simultaneously will paralyze *tyra-3* mutant animals at a different rate than wild type.

. In context of the tyramine-modulated escape response from nematophagous fungi, tyramine binding to TYRA-3 could positively regulate locomotion. Activation of LGC-55 leads suppression of head movements and initiates a reversal to escape the death trap (Pirri et al., 2009), then SER-2 modulates the omega turn so the worm can move continue in the same direction moving forward (Donnelly, personal communication). TYRA-3 may inhibit dopamine, leading to an increase in velocity after the omega turn. TYRA-3 inhibition of dopamine may also prevent basal slowing and thus encourage escape. The reaction of *tyra-3* loss of function mutants to anterior touch could elucidate the role of *tyra-3* in the escape response. Various parameters of the escape response, including turning, reversals, and locomotory speed of *tyra-3* mutants could also be quantified and analyzed.

BIBLIOGRAPHY

Alkema MJ, Hunter-Ensor M, Ringstad N, Horvitz HR (2005) Tyramine Functions Independently of Octopamine in the *Caenorhabditis elegans* Nervous System. *Neuron* 46: 247-260.

Altun ZF, Hall DH (2008) Handbook of *C. elegans* Anatomy. In *WormAtlas*. http://www.wormatlas.org/hermaphrodite/hermaphroditehomepage.htm

Barron, GL (1977) The Nematode-Destroying Fungi. *Topics in Mycobiology* 1.

Blenau, W., Balfanz, S., and Baumann, A (2000) Amtyr1: characterization of a gene from honeybee (Apis mellifera) brain encoding a functional tyramine receptor. *Journal of Neurochemistry* 74: 900–908.

Brenner, S (1974) The genetics of *Caenorhabditis elegans*. *Genetics Society of America* 77: 71-94.

Borowsky B, Adham N, Jones KA, Raddatz R, Artymyshyn R, Ogozalek KL, Durkin MM, Lakhlani PP, Bonini JA, Pathirana S, Boyle N, Xiaosui P, Kouoranova E, Lichtblau H, Ochoa FY, Branchek TA, Gerald C (2001) Trace amines: identification of a family of mammalian G protein-coupled receptors. *Proceedings of the National Academy of Sciences* 98**:** 8966-*8967.*

Bunzow JR, Sonders MS, Arttamangkul S, Harrison LM, Zhang G, Quigley DI, Darland T, Suchland KL, Pasumamula S, Kennedy JL, Olson SB, Magenis RE, Amara SG, Grandy DK (2001) Amphetamine, 3,4-methylenedioxymethamphetamine, lysergic acid diethylamide, and metabolites of the catecholamine neurotransmitters are agonists of a rat trace amine receptor. *Molecular Pharmacology* 60: 1181-1188.

Chase DL, Koelle MR (2007) Biogenic amine neurotransmitters in *C. elegans*. *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.132.1.

Elliot C (2008) In Defense of the Beta Blocker. *The Atlantic*.

Filmore D (2004) It's a GPCR World. *Modern Drug Discovery* 11: 24-28.

Gilman AG (1987) G Proteins: Transducers of Receptor-Generated Signals. *Annual Review of Biochemistry* 56: 615-49.

Hills T, Brockie PJ, Maricq AV (2004) Dopamine and Glutamate Control Area-Restricted Search Behavior in *Caenorhabditis elegans*. *The Journal of Neuroscience* 24: 1217-1225.

Jayanthi LD, Apparsundaram S, Malone MD, Ward E, Miller DM, Eppler M, Blakely RD (1998) The *Caenorhabditis elegans* Gene *T23G5.5* Encodes an Antidepressant- and Cocaine-Sensitive Dopamine Transporter. *Molecular Pharmacology* 54: 601-609.

Krishnamurthy N, Brown DP, Kirshner D, Sjolander K (2006) PhyloFacts: an online structural phylogenomic encyclopedia for protein function and structural classification. *Genome Biology* 7: R83.

Kroeze W, Sheffler D, Roth B (2003) G-Protein-Coupled Receptors at a Glance. *Journal of Cell Science* 116: 4867-4869.

Lauder JM (1993) Neurotransmitters as growth regulatory signals: role of receptors and second messengers. *Trends in Neuroscience* 16: 233-240.

Lints R, Emmons SW (1999) Patterning of dopaminergic neurotransmitter identity among Caenorhabditis elegans ray sensory neurons by a TGFb family signaling pathway and a Hox gene. *Development* 126: 5819-5831.

McDonald PW, Jessen T, Field JR, Blakely RD (2006) Dopamine Signaling Architecture in *Caenorhabditis elegans*. *Cellular and Molecular Neurobiology* 26: 591-616.

McDonald PW, Hardie SL, Jessen TN, Carvelli L, Matthies DS, Blakely RD (2007) Vigorous motor activity in *Caenorhabditis elegans* requires efficient clearance of dopamine mediated by synaptic localization of the dopamine transporter DAT-1. *Journal of Neuroscience* 27: 14216-14227.

Miller GM, Verrico CD, Jassen A, Konar M, Yang H, Panas H, Bahn M, Johnson R, Madras BK (2005) Primate trace amine receptor 1 modulation by the dopamine transporter *The Journal of Pharmacology and Experimental Therapeutics* 313: 983-994.

Nass R, Hall DH, Miller DM, Blakely RD (2002). Neurotoxin-induced degeneration of dopamine neurons in Caenorhabditis elegans. *Proceedings of the National Academy of Sciences* 99: 3264-3269.

Ohta, H., Utsumi, T., and Ozoe, Y (2003). B96Bom encodes a *Bombyx mori* tyramine receptor negatively coupled to adenylate cyclase. *Insect Molecular Biology* 12: 217–223.

Pirri JK, McPherson AD, Donnelly JL, Francis MM, Alkema MJ (2009) A Tyramine-Gated Chloride Channel Coordinates Distinct Motor Programs of a *Caenorhabditis elegans* Escape Response. *Neuron* 62: 526-538.

Rex E, Komuniecki RW (2002) Characterization of a tyramine receptor from *Caenorhabditis elegans*. *Journal of Neurochemistry* 82: 1352–1359.

Rex E, Molitor SC, Hapiak V, Xiao H, Henderson M, Komuniecki R (2004) Tyramine receptor (SER-2) isoforms are involved in the regulation of pharyngeal pumping and foraging behavior in *Caenorhabditis elegans*. *Journal of Neurochemistry* 91: 1104-1115.

Rex E, Hapiak V, Hobson R, Smith K, Xiao H, Komuniecki R (2005) TYRA-2 (F01E11.5): a *Caenorhabditis elegans* tyramine receptor expressed in the MC and NSM pharyngeal neurons. *Journal of Neurochemistry* 94: 181–191.

Roeder T, Seifert M, Kahler C, Gewecke M (2003) Tyramine and octopamine: antagonistic modulators of behavior and metabolism. *Archives of Insect Biochemistry and Physiology* 54: 1-13.

Saudou F, Amlaiky N, Plassat JL, Borrelli E, and Hen R (1990) Cloning and characterization of a *Drosophila* tyramine receptor. *The EMBO Journal* 9: 3611–3617.

Sawin ER, Ranganathan R, Horvitz HR (2000) *C. elegans* locomotory rate is modulated by the environment through a dopaminergic pathway and by experience through a serotonergic pathway. *Neuron* 26: 619–631.

Snyder B (2005) Where are the New Drugs: The Push to Improve the Pipeline. *Lens*.
Tan CM, Brady AE, Nickols HH, Wang Q, Limbird LE (2004) Membrane trafficking of G protein-coupled receptors. *Annual Review of Pharmacology and Toxicology.* 44: 559–609.

Tan ES, Groban ES, Jacobson MP, Scanlan TS (2008) Toward Deciphering the Code to Aminergic G Protein-Coupled Receptor Drug Design. *Chemistry and Biology* 15: 343-353.

Tsalik EL, Niacaris T, Wenick AS, Pau K, Avery L, Hobert O (2003) LIM homeobox gene-dependent expression of biogenic amine receptors in restricted regions for the *C. elegans* nervous system. *Developmental Biology* 263: 81-102.

Voet D, Voet J, Pratt C *Fundamentals of Biochemistry: Life at the Molecular Level*. 2nd ed. USA: Wiley, 2006. 757-759.

Wragg RT, Hapiak V, Miller SB, Harris GP, Gray J, Komuniecki PR, Komuniecki RW (2007) Tyramine and octopamine independently inhibit serotonin-stimulated aversive behaviors in *Caenorhabditis elegans* through two novel amine receptors. *Journal of Neuroscience* 27: 13402-13412.

Xie Z, Miller GM (2009) Trace amine-associated receptor 1 as a monoaminergic modulator in brain. *Biochemical Pharmacology* 78: 1025-1104.