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The Role of Serotonin in the Avoidance Attenuation Response of C. elegans

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The Role of Serotonin in the Avoidance Attenuation Response of *C. elegans*

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1. Abstract

Through study of the model organism *Caenorhabditis elegans* and its complex neuronal pathways, research into the underlying mechanisms behind human behaviors is possible. Many of these observable neuronal pathways involve chemosensory interaction between the animal and small molecules in its environment. Through chemosensation, *C. elegans* is capable of detecting and responding to a variety of odors and pheromones, including several ascarosides- small molecules produced by *C. elegans* which enable communication and induce attractant or repulsive responses in other individuals. One ascaroside, Octapamine Succinyl Ascaroside #9 (osas#9), is released by starved L1 *C. elegans* larvae and is known to induce an olfactory avoidance response in individuals at all life stages. However, due to multisensory integration in response to osas#9 and an unknown metabolite in OP50 *E. coli* (a typical food source) the avoidance behavior is attenuated in young adults. This attenuation reaction is hypothesized to be dependent on the ASK and ADF neurons and involve the neurotransmitter serotonin. Avoidance behavior assays and attenuation assays were performed and determined the need for the MOD-1 serotonin-gated chloride channel in attenuation of the osas#9 avoidance pathway in response to an unknown *E. coli* metabolite. Additionally, these assays also pointed to the possible partial involvement of the serotonin uptake transporter MOD-5 and the neuropeptide receptor NPR-1.

2. Background

2.1 *Caenorhabditis elegans* as a Model Organism

Caenorhabditis elegans are small nematode worms, approximately 1 millimeter in length as adults, and are typically found feeding on bacteria-rich regions in soil and fruits (Félix and Braendle, 2010). Fully grown *C. elegans* are simple multicellular organisms and contain about 1000 somatic cells, 302 of which are neurons (Corsi, 2006). Since its first organized use in genetic research in 1963, *C. elegans* has been a commonly used model organism for the study of neural pathways and mechanosensory, chemosensory, and thermosensory behavioral responses of larger and more complex organisms, including humans (Brenner, 1974).

The advantages of *C. elegans* as a model organism are many. Firstly, *C. elegans* are physiologically ideal for studying the correlations between behavioral, genetic, biochemical, and anatomical abnormalities (Ward, 1973). These studies are facilitated by the fully-sequenced genome, extensively studied neuronal structure, and transparent features of the worm at all life stages (Corsi, 2006). In addition to the morphological advantages, *C. elegans* are also self-fertilizing, develop between 300 to 1,000 progeny per single adult, and have a generation time of only 3.5 days at 22 °C , allowing for the quick and effective development of genetic variants for experimental use (Corsi, 2006).

The life cycle of *C. elegans* is short and temperature-dependent. At 22 °C, *C. elegans* reach adulthood in approximately 2.5 days, progressing through the life stages of *in utero* embryogenesis, egg development and hatching, four larval stages (L1-L4), young adulthood, and adulthood. Colder temperatures (between 16 and 20 °C) slow *C. elegans* growth and development. Under conditions of starvation, overcrowding, or freezing temperatures, *C. elegans* follow an alternative life cycle in which the larval L2 stage develops into the pre-dauer and dauer stages. Pre-dauer and dauer specimens exhibit a decreased stress response and may exist in this state for as many as 4 months until conditions improve, followed by entry into the L4 larval state and normal young adult and adult development (Corsi, 2006). The brevity of the generation time as well as the increased species hardiness due to the dauer stage makes *C. elegans* a simple and effective model organism for laboratory use.

2.2 Maintenance of Laboratory-Grown *C. elegans*

C. elegans is an effective model organism for even small laboratories, as very few materials are required for their use. A wide variety of strains are easily ordered from the Caenorhabditis Genetics Center (CGC) and can be easily maintained in a laboratory environment. Worms are typically kept in Nematode Growth Medium (NGM) petri dishes seeded with a live food source of the OP50 strain of *E. coli* (Félix and Braendle, 2010).Worms may be frozen indefinitely in -196 °C liquid nitrogen for long term storage, or may be actively maintained between 16 and 20 °C (Stiernagle, 2006).

2.3 Avoidance Behavior

When organisms are exposed to a threatening external environmental stimulus, they undergo species-specific reactions in which the organism attempts to prevent a negative effect from occurring. These responses, from the fight-or-flight response to the startle reflex, comprise the innate self-preservatory activities known as avoidance behavior. External stimuli observed to induce an avoidance response in most organisms include harmful chemicals, extreme temperature change, physical pain, and alarm pheromones (Andrew, 2012).

Because *C.* elegans are incapable of sight, they must navigate their environments strictly thorugh the senses of taste, smell, and touch. The detection of dangerous external stimuli, including dangerous or communicatory chemical signals, is vital to their survival and social interaction. Due to their relatively advanced neuronal system, *C. elegans* are capable of performing multiple behavioral functions dependent on chemosensation, from food detection to mating to avoidance of dangerous substances (Chute and Srinivasan, 2014), including quinine, glycerol, and other toxic chemicals and plant alkaloids (Hilliard et al., 2002).

2.3.1 Chemosensory neurons

Such avoidance responses depend on an the largest chemosensory organ in nematodes, the amphid. In *C. elegans*, the right and left amphids consist of a set of 12 sensory neurons clustered near the pharyngeal bulb in the head and extend sensory cilia out toward the front tip of the animal. These cilia penetrate the cuticle of the worm and allow exposure to the environment

(Bargmann, 2006). An image depicting the 12 amphid sensory neurons (plus one male-specific sensory neuron) can be seen below in **Figure 1.**

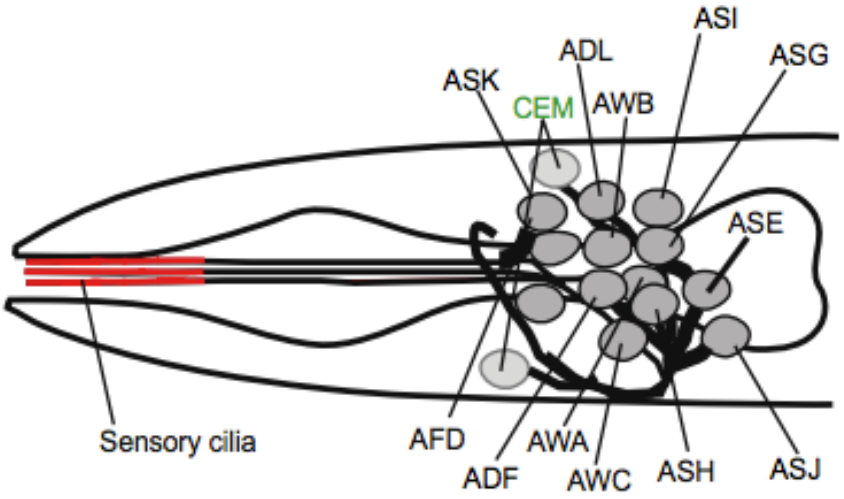

Figure 1: The 12 amphid sensory neurons plus the male-specific sensory neuron CEM (green). Adapted from Chute and Srinivasan, 2014.

11 of these amphid neurons (ADF, ADL, ASE, ASG, ASH, ASI, ASJ, ASK, AWA, AWB, and AWC) directly influence chemosensory behavioral responses, while the twelfth, ADF, mediates thermosensory responses (Inglis et al., 2007). These neurons function through very specific transduction pathways, involving sets of known and unknown components which interact with relevant molecules. An example of a partially known chemosensory neuron pathway is seen in **Figure 2** below and describes the signal transduction of the ASH neuron in response to a chemical repellent (octanol).

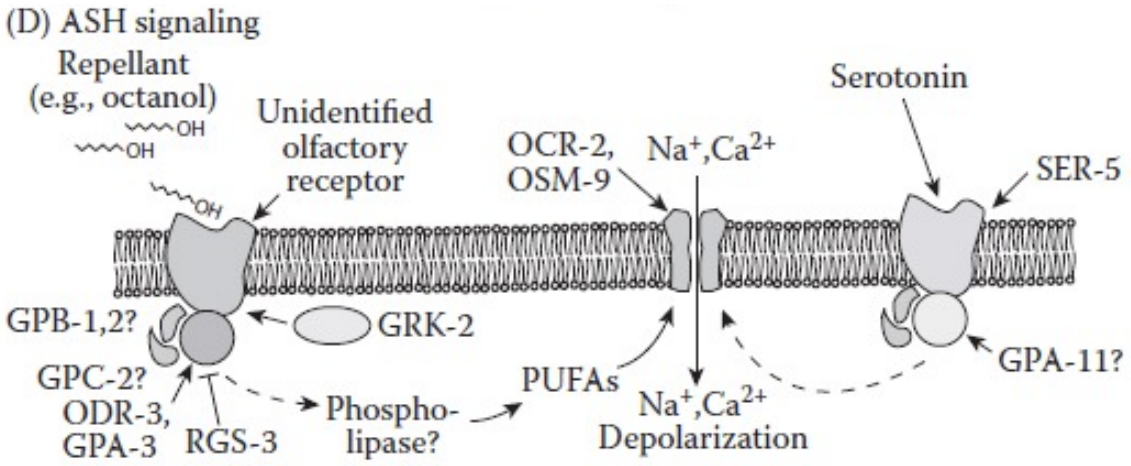

Figure 2: Signal transduction pathway of ASH in response to a chemical repellent (octanol). Dotted lines represent an unknown number of unidentified molecules between known components. Components followed by a question mark represent an unknown placement of that component in the transduction pathway. Adapted from Hart and Chao, 2010.

The role of each known component to a signal transduction pathway is important, as phenotype-altering mutations in those specific components can help to implicate specific neurons in larger pathways. Then, selective ablation of those specific neurons could confirm their involvement in the observed behavior.

2.3.2 G-Protein Coupled Receptors

Additional components that are important to the signal transduction pathways of chemosensory neurons are G-Protein Coupled Receptors (GPCRs). GPCRs are large transmembrane receptors which activate G proteins by binding to external signaling molecules, causing a conformational change. This conformational change activates the G protein's alpha subunit through replacement of bound GDP with GTP and diffusion of its beta - gamma dimer, allowing the G protein to function in signaling (O'Connor and Adams, 2010).

At least 1000 GPCRs are speculated to be encoded in the *C. elegans* genome, most of which are predominantly expressed in chemosensory neurons (Bargmann, 2006). Most of these GPCRs, including the STR-xx, SRA-xx, SRG-xx, SRW-xx superfamilies and ODR-10 are assumed to be olfactory receptors, coupling to heterotrimeric G-proteins. There are multiple receptors expressed in each sensory neuron, any of which possibly having major involvement in the transduction pathways of their respective neurons (Hart and Chao, 2010).

2.3.3 Ascarosides

Interspecies communication is also capable of inducing an avoidance response in *C. elegans* individuals. Various stages of *C. elegans* produce a class of small molecules known as "ascarosides," or glycosides of ascarylose. These ascarosides function as *C. elegans* pheromones, providing methods of communication between individuals by playing a role in several neuronal signaling pathways, depending on their specific molecular structures. The first known ascarosides, including ascr#1, ascr#2, ascr#5, and ascr#8, were identified to regulate dauer formation. Ascarosides can also be found in the pheromones of male and hermaphroditic individuals, enabling chemosensory detection and attraction of a potential mate (Chute and Srinivasan, 2014).

Ascaroside biosynthesis is dependent on side-chain formation via peroxisomal β-oxidation, which shortens long-chain fatty acids and produces acetyl-CoA *in vivo*. (Ludewig and Schroeder, 2013). Biosynthesis of the different ascarosides is known to be dependent on various factors, including the nutritional state of the worm (Chute and Srinivasan, 2014). Studies of loss-of-function mutants including *gpa*-2, *gpa*-3, *srbc*-64, and *srbc*-65 have implicated the involvement of GPCRs in chemosensory detection of ascarosides. A 2012 study conducted by Park et al. found that ascr#2 directly binds to the DAF-37 GPCR, demonstrating the ability of GPCRs to act directly as ascaroside receptors. This binding is extremely structure-specific for both the GPCR and side chain of the ascaroside of interest (Park et al., 2012).

One ascaroside of interest is generated from the succinylation of the *C. elegans* neurotransmitter octapamine, which forms a side chain on the ascarylose sugar base. This ascaroside is most structurally similar to ascr#9, connected to the nitrogen of octapamine. This ascaroside was named Octapamine Succinylated Ascaroside #9 (osas#9) according to WormBase's Small Molecule Identifier standard naming conventions (Ludewig, 2013). The chemical structure of osas#9 and related ascarosides, adapted from Artyukhin et al (2013), can be seen in **Figure 3** below.

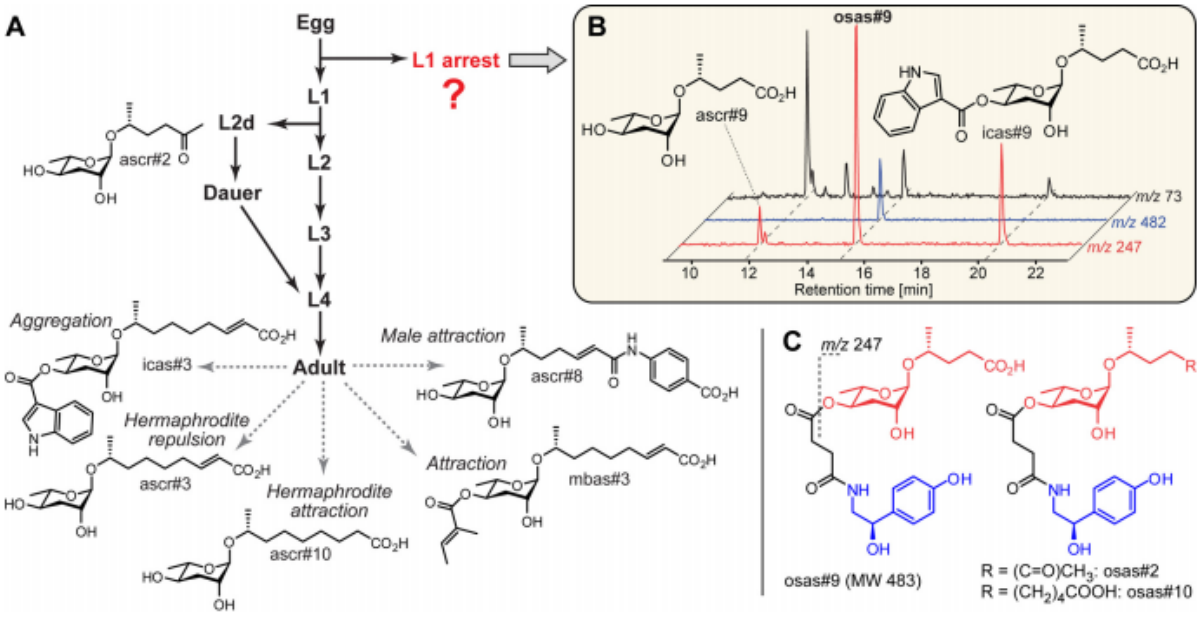

Figure 3: Ascaroside Chemical Structures
The proposed chemical structures of various ascr#9 derivatives, including osas#2, osas#9, and osas#10. Adapted from Adapted from Artyukhin et al.

The structure of osas#9 was confirmed via NMR comparison of synthetic osas#9 and osas#9 produced *in vivo*. Through HPLC and NMR analysis, osas#9, has been identified as a component of the chemical signals released by starved L1 larvae. Osas#9 may be isolated via preparative HPLC for use in avoidance behavioral assays. When exposed to concentrated osas#9, all life stages of *C. elegans* have been known to respond with immediate avoidance of the treated area through locomotory reversal. As osas#9 is associated with starved L1 larvae, this finding draws the conclusion that osas#9 may be used as a component of an intraspecies signal to communicate the absence of available food. The study of osas#9's role in avoidance behavior has higher implications for the purpose of biogenic amine succinylation as a method of ascaroside-based communication in *C. elegans* (Artyukhin et al., 2013).

2.3.4 Multisensory integration

Further evidence of the biological importance of osas#9 in *C. elegans* is the ability of the avoidance response to be attenuated in the presence of a food source. The ability of *C. elegans* to gather information obtained from multiple senses, in this case olfaction of both osas#9 and the

food source simultaneously, is known as multisensory integration. Artyukhin et al. discovered the attenuation of this characteristic avoidance response in the presence of both osas#9 and an *E. coli* foodsource, signifying that a metabolite in *E. coli* may be responsible for this behavior. (Artyukhin et al., 2013). The attenuation of the avoidance response is an example of the adaptation of neuronal signaling pathways under multisensory integration.

In 2015, Turland confirmed the dependency of *E. coli* concentration on the attenuation of the avoidance response to osas#9 as well as established the minimum ideal concentration of the master stock of *E. coli* extract to be 1/1000. To date, no attenuating *E. coli* metabolite has been identified and the neuronal pathway remains largely unknown, however two neurons, including ASK and ADF, have been identified to be at least partially involved in the attenuation pathway, and one neuron, ASH, has been confirmed to be necessary in the detection of osas#9 (Turland, 2015; Yabut, 2017).

2.4 Serotonin

In 2017, Yabut examined the attenuation of the osas#9 avoidance response in a variety of genetically mutated strains of *C. elegans* and found that attenuation is not observed in *tph*-1 mutated strains. As *tph*-1 encodes tryptophan hydroxylase, an enzyme that catalyzes serotonin biosynthesis, it is likely that serotonin is in part required for attenuation (Yabut, 2017).

5-hydroxytryptamine (5-HT), also called serotonin, acts as a monoamine neurotransmitter and neuromodulator in many animal species, including *C. elegans* (Sawin et al., 2000). In humans and other vertebrates, serotonin plays a role in a wide variety behavioral responses, including sleep modulation and sexual response. It functions in the nervous system by acting as a transmitter and producing various paracrine and hormonal effects. Deficiencies in serotonin have been linked to such disorders as depression, posttraumatic stress, and epilepsy (National Center for Biotechnology Information, 2017).

In vivo serotonin release in *C. elegans* is known to modulate locomotion and also has implications in the stimulation of egg laying and pharyngeal pumping (Chase and Koelle, 2007). Serotonin signaling is a major part of the stress response in both mammals and *C. elegans* (Tatum et al., 2014). When exposed to a bacterial lawn of OP50 as a food source, starved animals are more likely to slow their locomotory rate than well-fed animals. This behavior is known as the “enhanced slowing response,” which has been observed to require serotonin. Sawin

et al. conducted a 2000 study in which the enhanced slowing response was rescued in strains mutant for serotonin biosynthetic enzymes with the introduction of 2 mM exogenous serotonin (Sawin, et al., 2000).

2.4.1 *C. elegans* Serotonergic Elements

Serotonin is utilized and synthesized in several elements in wildtype *C. elegans* models. Serotonin is biosynthesized in 8 types of *C. elegans* neurons, including CP1-6, R1/R3/R9, NSM, HSN, VC4-5, ADF, RIH, and AIM. Of these 8 neurons, ADF, a sensory neuron, has been identified to be a part of the attenuation pathway (Chase and Koelle, 2007).

In *C. elegans,* several receptors are known to bind serotonin that may have a role in the attenuation pathway. Of these, three are GPCRs (SER-1, SER-4, and SER-7), and one is a serotonin-gated channel (MOD-1). Also potentially relevant to the attenuation pathway are the serotonin neurotransmitter uptake transporter (MOD-5) and the neuropeptide receptor (NPR-1), which modulates aerotaxis and feeding behavior (Gurel et al., 2012).

Of particular interest to this project is the serotonin-gated chloride channel, MOD-1. MOD-1 is dependent on the internal concentration of chloride ions rather than cations as is common in most *C. elegans* channels. According to a 2012 study, MOD-1 is one of the major receptors (together with SER-4) which allow *C. elegans* to slow locomotion via exogenous and endogenous serotonin, also known as serotonin paralysis (Gurel et al., 2012). While *mod*-1 mutants have previously been studied to be defective in the enhanced slowing response (Ranganathan et al., 2000), further study has proven that *mod*-1 requires a background mutation, n4954, to be defective (Gurel et al, 2012). *Mod-1* is expressed in 11 cells in *C. elegans*, including 6 interneurons (AIA, AIB, AIY, AIZ, RIB, RIC), 4 motor neuron (DD, RIM, RME, and VD), and 1 neuron that acts as both (RID) (Bhatla, 2009).

3. Methods and Materials

3.1 Strains

The Bristol N2 wildtype strain was used and obtained from the Caenorhabditis Genetics Center (CGC). The N2 strain was utilized in all experiments as a baseline control. A mutant *tph*-1 strain was obtained from the CGC and used to confirm previous findings. Strains with point mutations of genes coding for various serotonin receptors and serotonin-gated channels, including MT9668, MT9771, DA1814, AQ866, RB1584, and CX4148, were also received from the CGC.

3.2 Maintenance of *C. elegans*

All strains of *C. elegans* were maintained on NGM agar plates seeded with 3 drops of *E. coli* OP50 as a food source. Petri dishes were kept in plastic containers at 20 °C. Worms were transferred between plates using a flame-sanitized platinum wire periodically to prevent overcrowding. Contaminated plates were treated with a diluted bleach solution and the washed eggs were transferred to a clean seeded plate.

3.3 Avoidance Behavior Assays

Drop assays were performed to test the avoidance behaviors of the wildtype strain when exposed to different solutions of interest. The young adult worms from each plate were transferred onto a clean unseeded plate with a pipette, washing with M9 buffer twice. After being transferred, the young adults were allowed to freely move around the plate for 3 hours. A drop of solvent control (DI H_2O) was pipetted by mouth on the tails of individual worms, allowing the solvent to envelop the worm. Within 4 seconds of the drop being placed, behavior was quantified as either “avoidance” or “no avoidance” depending on the worm’s locomotion; “Avoidance” is defined as either the backward motion of at least one body length or a head turn of greater than 90 degrees. 20 young adult worms per plate were assayed. The process was repeated for a solution of interest, 2M glycerol, which is a known repellent. A separate assay was completed using 1 μM osas#9 as the solution of interest. Both assays were performed at least 3 times per strain on 3 separate days.

3.4 Avoidance Attenuation Assays

Similar to the avoidance behavior assay (drop assay), an avoidance attenuation drop assay was performed on several different strains. This assay tests the avoidance reaction of any given strain in comparison to solutions of interest combining a constant concentration of osas#9 with varying concentrations of *E. coli* extract. Mixtures prepared for this set of assays included 1 µM osas#9 and DI H2O as positive and negative solvent controls, and 1 µM osas#9 + 1/1000 extract, 1 µM osas#9 + 1/2000 extract, and 1 µM osas#9 + 1/10000 extract.

These dilutions were produced by diluting the master stock of *E. coli* extract in 1 mL 100% EtOH to 10% using 10 µL of master stock and 90 µL of 100% EtOH. 5%, and 1% extract stocks were produced by mixing 40 µL and 10 µL of the 10% stock with 40 µL and 90 µL of 100% EtOH, respectively. 98 µL of DI H_2O, 11 µL of 10 µL osas#9 in EtOH, and 1 µL of 10% extract stock were combined to produce 1 µM osas#9 + 1/1000 extract dilutions. This was repeated using 1 µL of 5% extract stock and 1% extract stock to produce 1 µM osas#9 + 1/2000 and 1 µM osas#9 + 1/10000 extract dilutions respectively.

In order to confirm the need for serotonin in the attenuation pathway, an attenuation assay was performed on *tph*-1, a mutation of *C. elegans* incapable of biosynthesizing serotonin via tryptophan hydroxylase. 20 worms per plate were tested with a drop of each of the two controls and three solutions of interest. Their avoidance reactions were quantified in the same manner as the drop test from **Section 3.3.** This attenuation assay was completed in triplicate with one trial each day over a course of 3 days.

In order to identify the exact role that serotonin plays in attenuation, several strains with mutations in genes involving serotonin receptor GPCRs, a neuropeptide receptor GPCR, and serotonin-related channels were assayed at least 3 times on 3 different days in the same manner. The results were compared to three trials of the wildtype, N2. A table of assayed mutant strains and their modifications can be seen below.

Table 1: Assayed mutant strains and their modifications

Strain	Modified Gene	Modified Allele	Phenotype
DA 1814	*ser*-1	ok345	5-HT2 receptor, GPCR
AQ 866	*ser*-4	ok512	5-HT1 receptor, GPCR
RB 1585	*ser*-7	ok1944	5-HT6 receptor, GPCR
CX 4148	*npr*-1	ky13	Neuropeptide receptor, GPCR
MT 9668	*mod*-1	ok103	Serotonin-gated chloride channel
MT 9772	*mod*-5	n3314	Serotonin uptake transporter

3.5 Well-fed Avoidance Behavior Assay

The avoidance behavior assay from **Section 3.3** was repeated on well-fed MT9668 (*mod*-1) mutants to confirm the lack of attenuation in the presence of food. The assay was performed on the plates on which the worms were raised and used DI H_2O as a solvent control and osas#9 as a solution of interest. This assay was repeated three times on three different days.

3.6 Exogenous Rescue Attenuation Assay

A rescue assay using exogenous serotonin was performed to attempt to restore the ability of the mutant worms to attenuate their avoidance response to osas#9 when exposed to a food source. 1.5g NaCl, 1.75g bacto peptone, and 8.5g agar were dissolved in 500 mL of DI H_2O and

autoclaved for 30 minutes. The solution was allowed to cool to approximately 50 °C. 25 mL of 1M KH_2PO_4 buffer, 1 ml of 1M $MgSO_4$, 1 ml of 1M $CaCl_2$, 1 mL of cholesterol stock (5 mg/mL in ethanol), and 425.36 mg serotonin hydrochloride powder (MW= 212.68 Da) were added to the cooled solution, bringing the final concentration of serotonin to 4 mM. The solution was poured into 50 5 cm plates under a fume hood and allowed to cool overnight. The 4mM serotonin NGM plates were stored at room temperature until use.

The avoidance attenuation assays from **Section 3.4** were repeated, transferring the starved worms to these 4mM serotonin NGM plates instead of standard NGM plates and allowing the worms to move freely about the plate for 3 hours. MT 9668 (*mod*-1) and *tph*-1 mutant strains were assayed using DI H_2O and 1 μM osas#9 as solvent controls and 1 μM osas#9 + 1/1000 extract, 1 μM osas#9 + 1/2000 extract, and 1 μM osas#9 + 1/10000 extract as solutions of interest. The assay was performed three times per strain on three different days.

3.7 Statistical Analysis

Statistical analysis was performed using the Data Analysis Tools contained in the FUNCRES.XLAM add-on to Microsoft Excel. Mann-Whitney U tests and parametric one-way ANOVAS were used to determine statistical significance.

4. Results

Drop assays were performed to confirm the tendency of starved wildtype *C. elegans* to avoid osas#9 and develop a baseline for further study. **Figure 4** below shows the results of the assay of N2 avoidance of 2M glycerol (3A) and 1µM osas#9. (3B).

(A) **(B)**

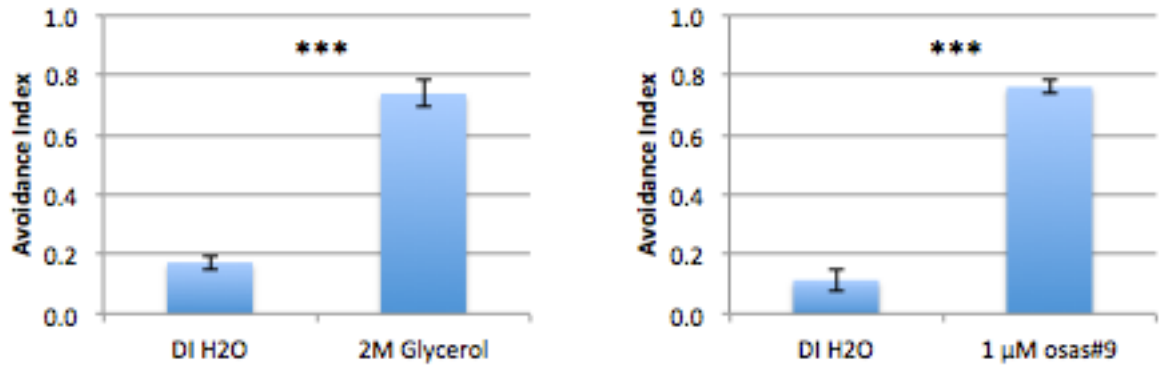

Figure 4: Wildtype avoidance assays
(A) Avoidance of wildtype (N2) samples to solvent control (DI H_2O) and solution of interest (2M glycerol). P =0.0009, N=11. (B) Avoidance of N2 samples to solvent control (DI H_2O) and solution of interest (1µM osas#9). P=0.0275, N=4. P-values were obtained via Mann-Whitney tests. Error bars represent SEM.

Several starved strains were then assayed to determine their ability to attenuate the avoidance response to osas#9 when exposed to varying levels of *E. coli* extract. A graph of the results of each assay is below in **Figure 5**.

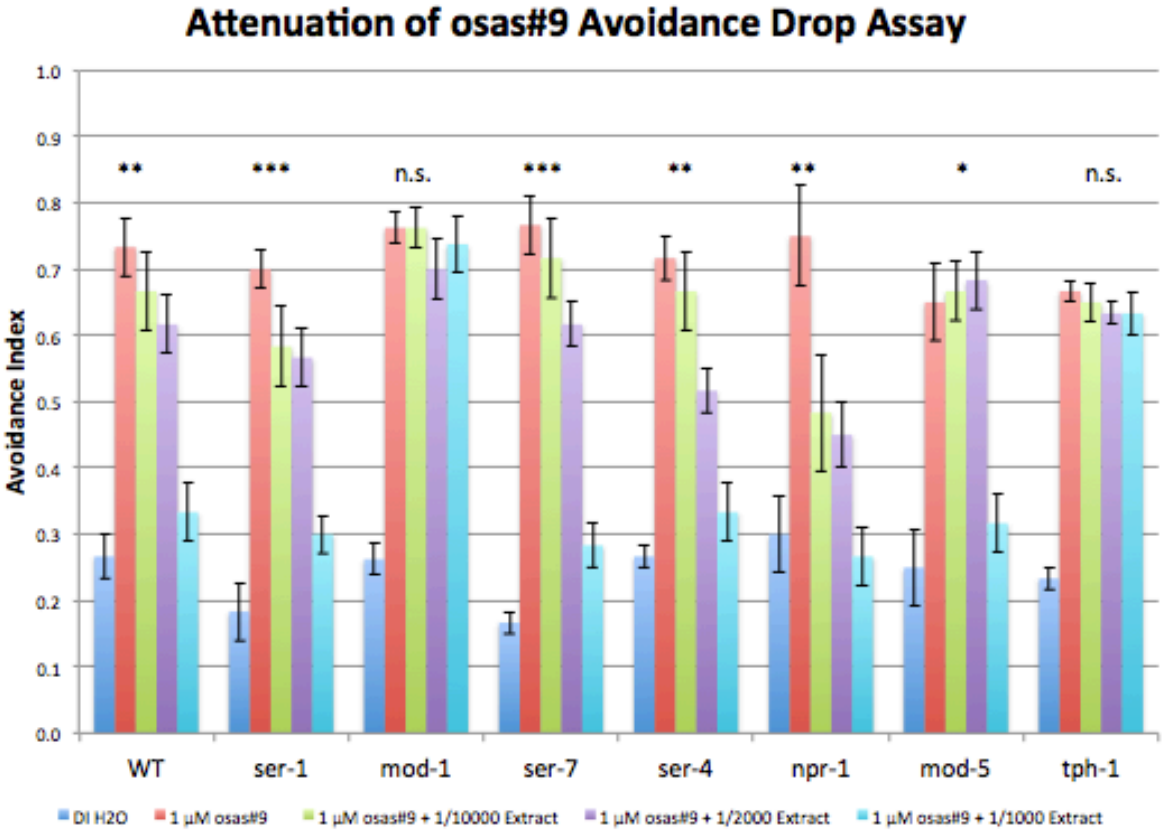

Figure 5: Avoidance attenuation assays of wildtype strain and 7 mutant strains
All P values calculated comparing positive control (1 µM osas#9) and 1 µM osas#9 + 1/1000 extract using one-way ANOVA. Non-significant strains include *mod*-1 (p=0.6278, N=4) and *tph*-1 (p=0.4217, N=3).

The wildtype strain accurately shows the expected response of a negative correlation between extract concentrations and avoidance index. Of the 7 tested mutant strains, 5 showed a statistically significant difference between the positive control (1 µM osas#9) and the 1 µM osas#9 + 1/1000 extract, verifying the strain's ability to attenuate the osas#9 avoidance response in the presence of *E. coli*. The remaining two strains, mutant for *mod*-1 and *tph*-1, did not display statistically significant differences (P=0.6278 and P=0.4217, respectively), drawing the conclusion that both the MOD-1 serotonin-gated channel and TPH-1 are required for attenuation.

These results confirm the previous finding that TPH-1 is required for the attenuation of the avoidance response to osas#9 and implies the direct or indirect involvement of MOD-1. More specifically, individuals that are incapable of chloride uptake via the MOD-1 protein are not able to detect or respond to the metabolite in *E. coli* responsible for attenuation. This MOD-1 protein

requires serotonin to act as a proper chloride channel, the major source of which could be from TPH-1 in wildtype strains.

While they do show a statistically significant difference when comparing the positive control to the 1/1000 extract, there is a slight visual deviation from the wildtype for the *mod*-5 and *npr*-1 mutants in the 1/10000 and 1/2000 extracts in **Figure 5**. At smaller concentrations (<1/1000), *mod*-5 appears to not respond to the extract and *npr*-1 appears to respond more than the wildtype. While this finding is not entirely significant, it does imply that NPR-1 and MOD-5 may be partially involved in the attenuation response and suggests further research.

In order to provide more evidence of the need for MOD-1 in the attenuation response, an avoidance attenuation assay was performed on well-fed *mod*-1 mutant samples. The results of this assay can be seen below in **Figure 6**.

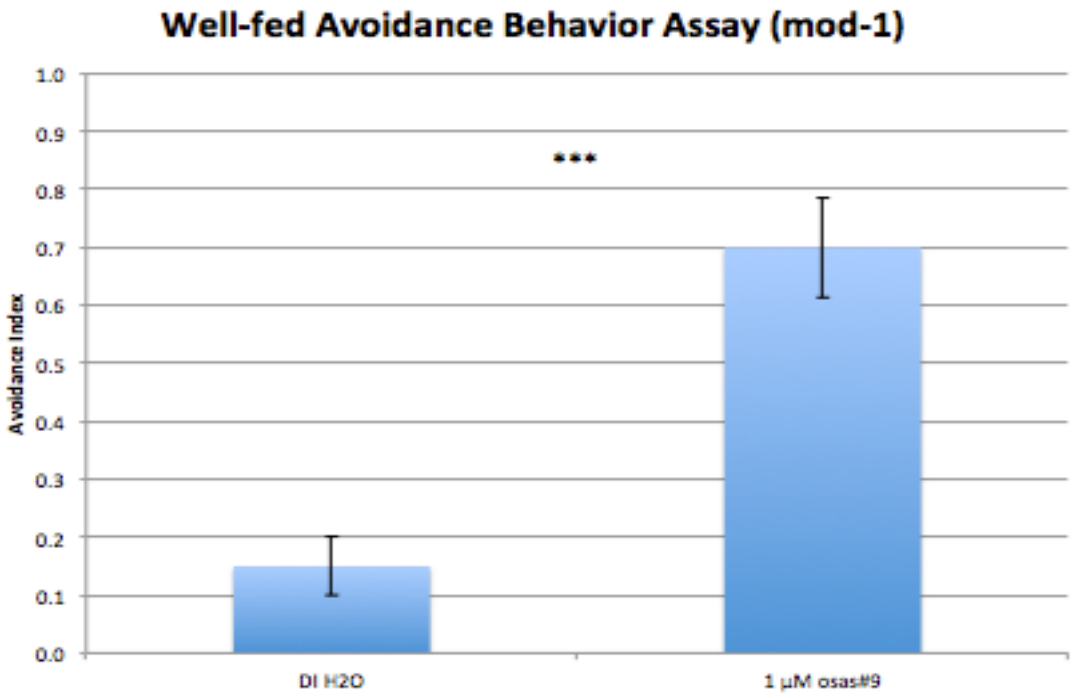

Figure 6: Well-fed avoidance behavior assay (*mod-1)*
Avoidance of mutant *mod*-1 samples to solvent control (DI H_2O) and solution of interest (1 µM osas#9). P =0.0007, N=3. Significance determined via one-way ANOVA.

The difference between the solvent control and the solution of interest is statistically significant, implying the inability of *mod*-1 mutants to attenuate the osas#9 avoidance response

even in the presence of a large amount of food. This provides further evidence into the need for MOD-1 in the attenuation pathway.

Finally, an exogenous rescue via exposure to 4mM of exogenous serotonin was attempted for the *mod*-1 and *tph*-1 mutant samples in order to restore the ability of both strains to attenuate the osas#9 avoidance response. The results of an attenuation behavior assay on these attempted rescues can be seen below in **Figure 7**.

(A) **(B)**

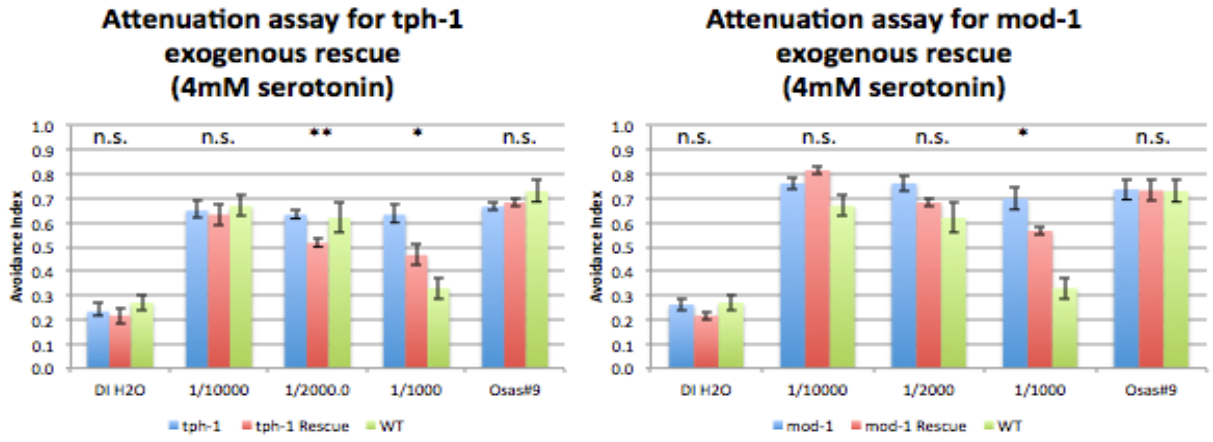

Figure 7: Avoidance attenuation assays for exogenous rescues of *tph-1* and *mod-1*
(A) Avoidance attenuation assays of rescued *tph*-1 strain after treatment with 4mM serotonin. There is a statistically significant difference between *tph*-1 and *tph*-1 rescue for 1 µM osas#9 + 1/2000 extract (P=0.0078, N=3) and 1 µM osas#9 + 1/1000 extract (P=0.0394, N=3). (B) Avoidance attenuation assays of rescued *mod*-1 strain after treatment with 4mM serotonin. There is a statistically significant difference between *mod*-1 and *mod*-1 rescue for 1 µM osas#9 + 1/1000 extract (P=0.0401, N=4). WT provided for visual comparison. All p-values determined via one way ANOVA. Error bars represent SEM.

While a statistically significant difference was achieved for the 1 µM osas#9 + 1/1000 extracts in both *tph*-1 to *tph*-1 rescues (P=0.0394) and *mod*-1 to *mod*-1 rescues (P=0.0401), neither rescue was able to completely restore the attenuation response. The *tph*-1 rescue was able to increase attenuation over the *tph*-1 mutant by approximately 16%, while the *mod*-1 rescue was only able to increase attenuation by approximately 13%.

5. Discussion

This project aimed to investigate the possible role(s) of serotonin in the neuronal pathway responsible for attenuation of the avoidance response to osas#9 in the presence of an *E. coli* food source. Confirmation of these possible roles is capable of implicating several neurons in this neuronal pathway and directing future studies.

The data confirms the involvement of serotonin in the attenuation pathway. In **Figure 5**, no statistical difference was found between the positive control (1 μM osas#9) and the 1 μM osas#9 + 1/1000 extract trials of *mod*-1 (P=0.6278) and *tph*-1 (P=0.4217), implying the inability of such mutants to attenuate the osas#9 avoidance response through detection of the unknown *E. coli* metabolite. This finding points to the involvement of the serotonin-gated chloride channel MOD-1 and the serotonin biosynthesizer TPH-1 in the response. **Figure 5** also visually opens up several other receptors, including NPR-1 and MOD-5, as possible candidates fornpr further study. Attempts to provide more evidence as to the involvement of MOD-1 resulted in a statistically significant difference between the solvent control and the solution of interest in well-fed *mod*-1 mutants (Figure 6, P=0.0007) and provided a proof of concept that rescue of the *mod*-1 strain may be capable of restoring its ability to attenuate the osas#9 avoidance response (Figure 7B, P=0.0394).

Exposure to 4mM exogenous serotonin was not an effective technique in completely rescuing the attenuation of the avoidance response to osas#9. However, this result is to be expected as exogenous serotonin is known to decrease locomotory movement in wildtype (N2) samples (Gurel et al., 2012). An initial trial of increasing the concentration of exogenous serotonin from 4mM to 8 mM for *mod*-1 rescues resulted in a lack of avoidance response to either 1 μM osas#9 or 2M glycerol. Future experiments may be capable of rescuing the attenuation response through an endogenous rescue or through titration of exogenous serotonin in order to minimize the paralyzing response to serotonin in N2 samples.

Additionally, this research draws attention a connection between the ability of *mod*-1 mutants to attenuate their avoidance response to osas#9 and perform enhanced slowing in response to an apparent *E. coli* metabolite. (Chase and Koelle, 2006). For this reason it is possible that the mechanisms for enhanced slowing in starved worms and attenuation of the osas#9 avoidance response are related, including similar *E. coli* metabolite uptake and detection.

Further continuation of this project would benefit to study into possible correlations between enhanced slowing and attenuation of starved *C. elegans* samples in response to a food source.

This project opens up the opportunity for directed study into the neuronal pathways involved in the attenuation response. Previous research has theorized a working neuronal pathway involving ASH as the primary sensory neuron facilitating osas#9 avoidance, while ADF and ASK may work cooperatively in detecting the unknown *E. coli* metabolite and attenuating the avoidance response. The working model developed by Yabut is below in **Figure 8.**

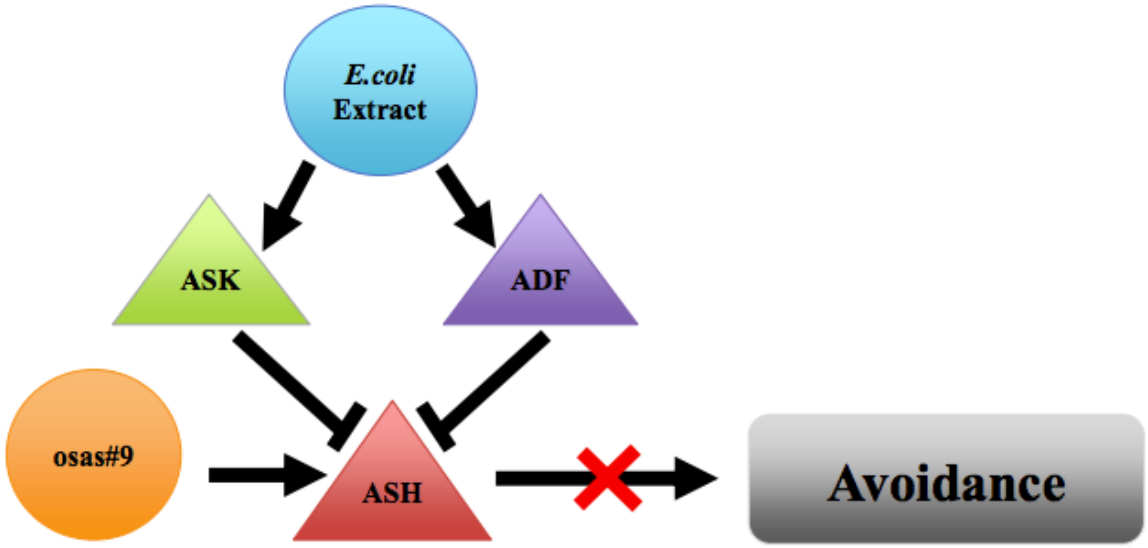

Figure 8: A working model of the neuronal pathway for attenuation of the osas#9 avoidance response. Adapted from Yabut, 2017.

These findings may also implicate several interneurons in the neuronal signaling pathway of attenuation, including AIA, AIB, AIY, and AIZ. For example, the AIA interneuron, which is known to utilize MOD-1, forms a gap junction with ADF and is synapsed onto and synapses onto both ASK and ASH. Further study would provide important insights into the working model of the neuronal pathway of osas#9 avoidance attenuation in young adults. For example, attenuation avoidance assays, as described in **Section 3.4**, conducted on *C. elegans* individuals ablated for ADF, ASK, and/or any of the possible MOD-1 utilizing interneurons would be capable of determining each neuron's involvement in the attenuation pathway.

Once more neurons are identified to be involved in the attenuation response to osas#9 avoidance and the neuronal signaling pathway becomes more complete, proper identification of

the *E. coli* metabolite responsible for this attenuation response may become a reality. Assays of isolated known chemical compounds detected by the involved sensory neurons could confirm the responsible metabolite if they invoke a similar attenuation response as the *E. coli* extract. If not, it may confirm the need for multiple synergistic metabolites or the existence of another unidentified sensory neuron.

6. Conclusions

The study of the neuronal pathways involved in multisensory integration is important for research into many human mental illnesses and disorders, including autism, attention deficit disorder, and developmental dyslexia. The use of the organism *C. elegans* as a model of neuronal activity and behavior is effective in designing straightforward and reproducible assays to better understand similar mechanisms in humans. The results of this study further assert the possibility of major multisensory integration malfunctions due to small genetic mutations in the elements involved in the related neuronal pathways. Understanding the roles and functions of these key elements in *C. elegans* models, including those enabling olfactory detection of environmental signals, can lead to a higher understanding of innate human behavior.

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