The Role of Tyramine and Octopamine on Short-term Habituation in *Caenorhabditis elegans*

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Abstract

The goal of this thesis is to identify the potential contributions of two largely unstudied monoamines, tyramine and octopamine, in the context of tap habituation; a behavior who's underlying circuitry heavily expresses genes encoding precursors and receptors for both these neurotransmitters. To pursue this goal I compared habituation of the tap withdrawal response in *Caenorhabditis elegans* - a model organism with a fully mapped connectome and proteome, and thoroughly characterized habituation - between wild type worms and worms that were null in tyramine and/or octopamine across different timepoints in young adulthood. I discovered that these gene products contribute to only isolated components of this behavior in an age-dependent manner. Although the impacts of these genes was interesting, the study of worms null in both of these precursors failed to find the striking differences in tap habituation that have been studied in animals expressing mutations in dopamine neurotransmission with much less representation in the neural circuitry responsible for this behavior. With some phenotypes identified by these mutations, their contributions to the biological underpinnings unique to each response-component can now be investigated in more detail.

Lay Summary

Learning to stop paying attention to a repeated stimulus is called habituation. This thesis examined habituation behavior to a startling stimulus using a tiny roundworm. Mutations in specific neurotransmitters altered the way the worms tuned out a repeated mechanical stimulus. Worms were recorded while the Petri plate that contained them was repeatedly tapped, and different aspects of their reversal response (crawling backwards after tap) across time were recorded - i.e., probability, duration, and the speed of each response to tap. Although the proteins studied here didn't demonstrate impacts across all components of this behavior, perhaps more notable was these brain chemicals affected individual aspects of the behavior without impacting others. Additionally, when compared at different ages of adulthood, some of these phenotypes differed. Together, these data provide an enticing hint toward an age-dependent contribution of these proteins on biological mechanisms that combine to produce this form of learning.

Preface

This thesis is based on work conducted in Catharine Rankin's lab at the Djavad Mowafaghian Centre for Brain Health, in the Koerner Pavilion at UBC hospital. While I was responsible for the conception and design of the experiments, as well as the acquisition and analysis of the data, a good portion of the data included were collected by our lab manager, Alvaro Luna, through my guidance and request when access to research facilities became restricted for safety reasons by the pandemic.

Additionally, while this thesis is light due to the profound impacts of COVID-19 in delaying my experiments, I believe that the findings reported here contribute to our current understanding of the function of tyramine and octopamine in behavior and in habituation in *C. elegans*.

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Acknowledgements

I am first and foremost thankful for the opportunity that my supervisor Catharine Rankin has given me by letting me work and learn in her lab and for being patient with me while I had one crisis after another throughout the duration of my degree. She took a chance on me, and for that I will always be grateful! When one project failed, Dr. Rankin would always have another ready for me to try. I am able to present this thesis after the death of several close friends and family members, two unsuccessful projects, countless financial tribulations, visa hardships, and more as a direct result of her continued grace.

Additionally, while every member of the Rankin Lab has helped me and my research in so many ways, I am particularly thankful for the absolute blessing that our lab manager, Alvaro Luna, provided to me. In addition to being unfalteringly kind, compassionate, and educational, Alvaro took the mantle of experimentation from me when my visa to study in Canada unexpectedly terminated and performed many testing paradigms that I couldn't even include in this thesis. I would not have been able to complete this journey without his help.

Finally, my fiancé Gregory Stoddard was my rock during my classes and writing process. Greg was always there as a source of assurance and encouragement and had unwavering faith in my potential. I derived my strength from many people, but my joy originated from my other half.

It was a hard journey, but I hope I can make it worth it. I am not the same person I was when I started this program, and I think most of the changes I went through are for the better. To all the people who helped me, and the list is innumerable, thank you for caring and never giving up on me!

I dedicate this monument of perseverance to my therapist and myself.

Chapter 1: Introduction

Animals can modify their behavior based on past experiences (i.e., learning) and maintain this behavioral change as a result of memory. These conserved biological processes are critical for surviving the constantly changing environments that all animals live in. Learning and the resultant behavioral changes originate in alterations in the organisms' nervous system. The simplest form of learning, habituation, is defined as the decrement in response to repeated stimuli (Harris, 1943). Although simple in presentation, habituation can be considered as a critical type of learning based on 1) it's conservation across phylogeny in every organism tested (Harris, 1943), 2) it's emergence as the earliest form of learning in development (Rankin & Carew, 1987), and 3) it allows the organism to selectively focus its finite attentional to aspects of the environment that are relevant to its survival and reproduction. The molecular changes affecting this essential form of learning have not been extensively studied, and there are still critical gaps in our understanding. The goal of this dissertation is to elucidate the contributions of several poorly researched molecules toward understanding habituation.

1.1 *Caenorhabditis elegans* as a Model of Nervous System and Behavior.

An excellent model organism in which to dissect this simple form of learning is the most systematically studied genetic model organism - *Caenorhabditis elegans*. Since its proposed utility in understanding genetics and the development of nervous systems by Sydney Brenner in 1974, the prolific and collaborative research community that has embraced the worm has wielded the many benefits of *C. elegans* in advancing our understanding of behavior, nervous systems, single neurons, and gene products. At 1mm long and with a life cycle of <3 days, this roundworm offers a high throughput method of understanding habituation from both a holistic and reductionist approach.

The transparency of its cuticle has allowed for the tracing of *C. elegans* cell lineage from a single-celled oocyte to the predetermined 959 somatic cells of the hermaphroditic adult (Sulston, Schierenberg, White, & Thomson, 1983). Among these cells are the 302 neurons comprising the first nervous system to have its connectome mapped (White et al., 1986). In the decades since it's published description the *C. elegans* connectome has been a critical tool in understanding its nervous system and behavior. Genetic analysis and neurophysiological tools (Schafer, 2004) were utilized with the connectome's map to explain the ways in which *C. elegans* can sense and respond to temperature, chemical odorants, and mechanical stimulation. By combining this wiring connectivity knowledge with laser ablation techniques, Chalfie et al. (1985) were able to discover the neural circuits responsible for the worm's tendency to reverse when touched on the head and to move forward when touched on the tail.

Although critical for building our understanding, the *C. elegans'* connectome was not the final tool that scientists were hoping would explain how the nervous system interacts with the environment to result in behavior. Indeed, the investigations of this ostensibly simple nervous system have revealed astonishing intricacy.

1.2 Background

Discoveries within the last 30 years have shown that the nervous system of *C. elegans* is dynamic, rather than static. This was unexpected, given the predetermined cell-lineage of each neuron. This is because, by itself, the connectome can't produce a comprehensive model of how a nervous system produces behavior (Movson, 2012). Connectomes are simply a map of the physical contacts (synapses) through which information travels - such a static frame can't convey how neurons regulate one another in real time. While these maps are critical for building knowledge of circuitry, as seen with the head and tail touch circuits in *C. elegans* (Chalfie et al.,

1985), the basis through which these circuits modulate behavior is a many-layered equation with poorly understood variables. Many neuromodulators such as catecholamines and neuropeptides are known to act on neurons they are not synaptically connected to, rather they are systemically released and act through distant receptors. Thus, the maps of their interactions, the: "catecholominome" and the "neuropeptidome" also reflect important controllers of *C. elegans* behavior that cannot be determined by understanding the physical connectome.

1.2.1 Tap Habituation Neural Circuitry

Shortly after the connectome of *C. elegans* was published (White et al., 1986), Rankin et al. (1990) discovered that *C. elegans* could learn and change behavior in response to repeated tap stimuli. They studied what they termed to be the tap-withdrawal response (TWR), wherein adult worms would crawl backwards in response to mechanical stimuli when their petri plate was tapped. This length of the initial reversal was about 1 mm, the length of the worm, but would become shorter, or even stop altogether, after repeated tap stimuli. In this first study a single measurement of reversal magnitude was used, magnitude was made up of a combination of reversal distance and the probability of reversal (as measured by the percent of worms who reversed at any given stimuli) as a lack of response was measured as "0" distance. In order to ensure that the response magnitude decrement observed was not the result of motor fatigue or sensory adaptation, Rankin et al. (1990) administered a brief electric shock to the petri dish housing the habituated worm as a dishabituating stimulus. Because the electric shock was able to increase the reversal magnitude to tap significantly above habituation levels, the decrement in the TWR was determined to be habituation, a form of non-associative learning.

Rankin and Broster (1992) delivered plate taps to the worms at different Inter-Stimulus Intervals (ISI) to see whether, like other organisms, the rate at which habituation and

spontaneous recovery from habituation occurred were affected by the frequency with which stimuli were delivered. Indeed, worms that received stimuli at shorter ISIs (i.e., more rapid plate taps) showed faster response decrements and faster recovery from a habituated state than worms who had received the same number of stimuli, but with longer ISIs (Rankin & Broster, 1992). This conclusion was consistent with what was known about the cellular equivalent of habituation and spontaneous recovery in other animal models, where-in the synaptic depression and recovery from synaptic depression is similarly dependent on the length of time between stimulation to relevant neurons (Byrne, 1982). The revealed relationship between ISI length and habituation/spontaneous recovery suggested that habituation in *C. elegans* was mediated by more than one cellular process, and that the ISI of the stimuli determined the mechanism activated. Thus, the conclusion was that habituation depended on multiple ISI-dependent molecular mechanisms to result in experience dependent changes (Rankin & Broster, 1992). With a well-characterized tap habituation behavioral paradigm in hand, these mechanisms could now be researched in an organism that uniquely encouraged simultaneous molecular and behavioral understanding.

The first step in understanding the mechanisms of habituation was to determine the neuronal circuitry in which these mechanisms occurred. Through methodical ablation of the neurons in the touch circuit and observing the effect on tap habituation, Wicks and Rankin (1995) determined that seven mechanosensory neurons (i.e., three anterior, two posterior, and two full body), and 10 interneurons were required for *C. elegans* to reverse when receiving full body mechanical stimulation through its Petri plate being tapped. This tap withdrawal response results from simultaneous activation of the anterior touch circuit (3 sensory neurons) and the posterior touch circuit (two sensory neurons), which drive backward and forward locomotion

respectively; the greater number of anterior neurons tips the response tendency towards backwards locomotion when both circuits are stimulated at the same time by a tap stimulus.

So, if two circuits are involved in habituation of the TWR, the next logical question is which circuit is the site of the mechanisms responsible for habituation? This question was answered by studying each circuit individually and observing which neurons' functioning are required for habituation to occur. Although the unhabituated response of worms to tap is a reversal, very occasionally the stimulated worm will accelerate (i.e., crawl more rapidly) forward. As the tap stimuli are repeated, the proportion of worms that accelerate grows larger (Wicks & Rankin, 1995). However, ablation of the neurons responsible for acceleration or reversal responses reveals that both types of responses, reversals and forward accelerations, will habituate in the absence of the antagonistic circuit. Furthermore, the integration of the habituation curves of forward and reverse responses of sensory neuron ablated animals matches the habituation curve of intact animals. These results suggest that both the forward and reversal touch sensitivity circuits individually habituate to tap stimuli and that the responses we see in the intact circuit are an integration of the two opposing responses (Wicks & Rankin, 1996).

Now that the neural-circuit underlying habituation of the TWR had been well defined, the next question to be addressed was the locus of plasticity. Answering this question was complicated because of the multi-functional aspect of the neurons within the TWR circuitry. In the absence of mechanical stimulation, the interneurons and motor neurons in the TWR circuit also are involved in other behaviors involving reversals, such as spontaneous reversals during roaming and reversals in response to a heat stimulus (Gray et al., 2005). Wicks and Rankin (1997) hypothesized that if the site of plasticity for the habituation of the TWR was among the neurons relevant to these other behaviors, then repeated tap stimuli would also result in a

decrease in spontaneous reversals and reversals in response to thermal cues. However, if the site of TWR habituation was in the sensory neurons or the synapses from the sensory neurons onto the interneurons, then repeated tap stimulation should have no significant impact on the rate of spontaneous reversals or reversals in response to heat. This hypothesis was supported; worms that had been trained with repeated tap stimulation showed no significant differences in the number of spontaneous reversals and reversals to thermal stimuli than worms that had not received tap habituation training (Wicks & Rankin, 1997).

Suzuki et al. (2003) provided evidence that mechanosensory neurons in *C. elegans* changed the way in which they responded over the course of repeated tap stimulation. They used calcium imaging to examine the cell activity of ALM neurons. Because an influx of calcium is a real-time indicator of neurotransmitter release from the neuron, a reduction of calcium within the ALM neuron in response to repeated anterior touch indicates that habituation to mechanical stimulation involves the attenuation of relevant neuron excitability (Suzuki et al., 2003). A similar reduction in calcium influx has been documented for repeated stimulation of the posterior touch receptor neuron, PLM (Kindt et al., 2007), which suggests that both of the antagonistic touch circuits attenuate their ability to respond following repeated stimulation of their unique mechanosensory neurons. To determine whether habituation resulted from neuronal desensitization, O'Hagan et al. (2005) used electrophysiology to measure cell currents of the PLM neuron while repeatedly poking the posterior end of a worm with a glass rod. They found no significant change in signal transduction in the PLM with repeated stimulation, which suggests that the cellular equivalents of habituation are not the result of changes in transduction in the mechanosensory channels themselves (O'Hagan et al., 2005). In support of this hypothesis, direct depolarization of touch cells through optogenetic stimulation of transgenic

worms carrying a light-gated ion channel (i.e., channelrhodopsin) driven in the touch neurons by the *mec-4* promoter resulted in normal habituation of *C. elegans* (Nagel et al., 2005; Leifer et al., 2011;Timbers et al., 2013). This suggests that the mechanism(s) for habituation to tap happen(s) after mechanotransduction. The data from Wicks and Rankin (1997) suggested that habituation likely occurred at the level of the sensory neurons, therefore taken together these data suggest that habituation occurs after transduction within the tap sensory neurons. What could be happening within the body of a mechanoreceptor cell that would so drastically alter behavior? Probably changes in ion channel function, since previously described work from Suzuki et al. (2003) suggests that the process of habituation doesn't result in physiological changes to the neuron. Knowledge of the neural circuit underlying the tap response allowed researchers to identify candidate genes expressed in that neural circuit to investigate the genetic mechanisms underlying habituation. Rankin and Wicks (2000) were the first to report a learning mutant for tap habituation. They discovered that worms with a mutation in *eat-4* had an initial response to tap that was similar to that of wild type but habituated more rapidly at both 10- and 60-second ISIs and failed to dishabituated following an electric shock to the petri plate. EAT-4 is the *C. elegans* ortholog of mammalian vesicular glutamate transporter (VGLUT1) and is expressed in many neurons including touch cells of the TWR circuit (Serrano-Saiz et al., 2013; Lee et al., 1999). Thus, normal glutamate release plays an important role in the habituation and dishabituation of the TWR.

Upstream of glutamate release, Cai et al. (2009) found a potential mechanistic link between repeated touch cell activation and reduced calcium entry into the cell through the potassium channel (*shw-3*) and an accessory subunit (*mps-1*); *mps-1* is expressed in both ALM and PLM neurons (Bianchi et al., 2003). *mps-1* is a gene phylogenetically belonging to the

vertebrate KCNE family, which encodes a single-pass transmembrane protein that changes neuron excitability by assembling with voltage-gated K^+ channels dubbed KHT-1 to control the flow of K^+ ions into the cellular environment (Bianchi et al., 2003). Although worms with an inactive MPS-1 variant have an initial TWR similar to wild type, they habituate much more slowly to tap at both long and short ISIs. Cai et al. (2009) use this data to suggest that the autophosphorylation of the KHT-1/MPS-1 complex is required for the decrease in responsiveness of touch receptor cells in response to repeated mechanical stimulation via reduced K^+ flow. The resultant elongated potentials of mechanoreceptors are proposed to explain the slow recovery of calcium channels within these cells as described by Suzuki et al. (2003). The mechanism through which repeated mechanical stimulation activates the kinase of MPS in wild type worms remains unknown.

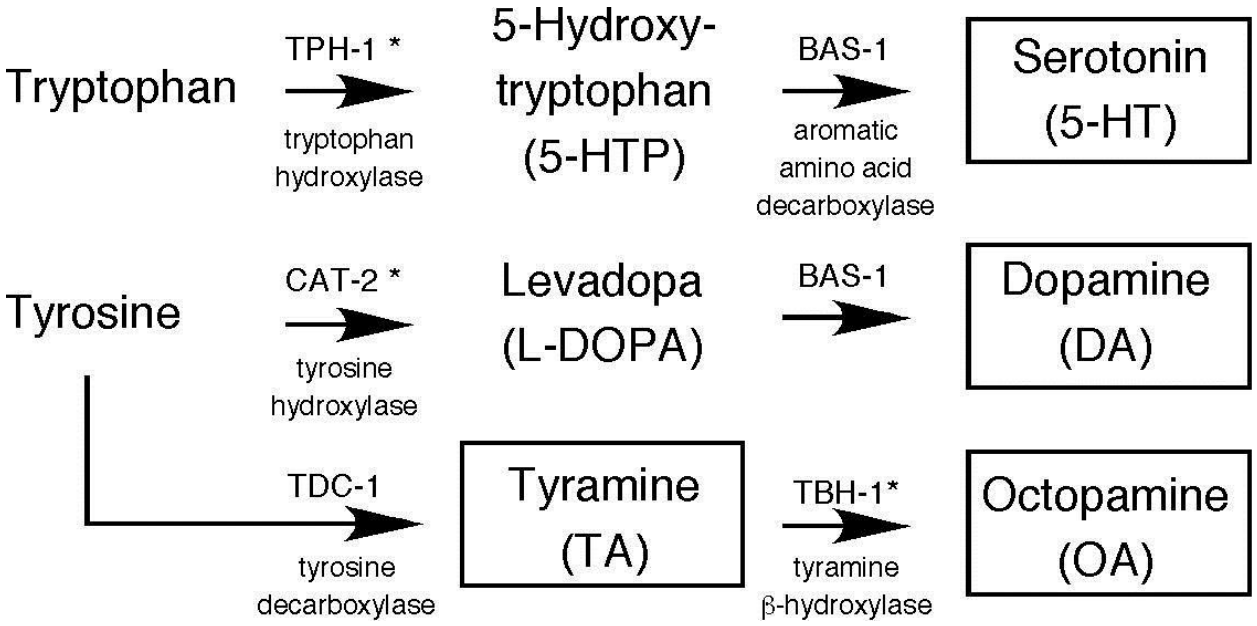
1.3 Biogenic Amines in *C. elegans*

In addition to the expression of genes involved in glutamate neurotransmission in the TWR circuit, several genes related to biogenic amine neurotransmission have been found in the worm. Four biogenic amines have been biochemically identified within the nervous system of *C. elegans* - dopamine, serotonin, tyramine, and octopamine - using methods such as liquid chromatography (LC), thin-layer chromatography (TLC), formaldehyde-induced fluorescence (FIF), and radioenzymatic assays (Sulston et al., 1975; Sanyal et al., 2004; Horvitz et al., 1982; Alkema et al., 2005). These neurotransmitters also have significant roles in non-neuronal cells, but I will focus on their function within the nervous system here. When synthesized and/or catabolized in the nervous system of the hermaphroditic *C. elegans*, biogenic amines regulate a wide variety of behaviors including locomotion, egg laying, pharyngeal pumping, roaming, and aversive responses (Sanyal et al., 2004; Carmel et al., 2005; Hobson et al., 2006; Wragg et al.,

2007; Ben Arous et al., 2009; Bendesky et al., 2011; Mills et al., 2012). Most of what is known about the impact of specific biogenic amines on *C. elegans* behavior has been elucidated through studies that eliminate or reduce the synthesis and/or reception of the amine of interest through loss-of-function mutations in genes encoding the rate limiting step in their respective biosynthesis or receptors. If the changes in behavior noted in these mutants were similar in strains with different mutations in the same gene, or was rescued by the replacement of the gene or by the exogenous application of the neurotransmitter, then the behavior was hypothesized to be mediated by that amine.

The biosynthesis of biogenic amine neurotransmitters is a multistep process, apart from tyramine (Figure 1). Two amino acids, tyrosine and tryptophan, can be catalyzed by various enzymes to eventually synthesize dopamine, tyramine, or serotonin (5-HT). Tyramine can then be converted into octopamine through tyramine β-hydroxylase (TBH-1) (Alkema et al., 2005). For dopamine, tyrosine is first hydroxylated into tyrosine hydroxylase (TH) in a rate limiting step encoded by the *cat-2* gene, which produces L-dopa (Sulston et al., 1975). 5-HT is similarly resultant from the hydroxylation of tryptophan into 5-hydroxytryptophan (5-HTP) through its rate-limiting enzyme encoded by the *tph-1* gene (Loer & Kenyon, 1993). Both L-dopa and 5-HTP are then decarboxylated by the same amino acid decarboxylase (AAAD), encoded by the gene *bas-1* (Loer & Kenyon, 1993) to correspondingly produce dopamine or serotonin. Tyramine is the decarboxylated product of tyrosine through another AAAD, *tdc-1* (Hare & Loer, 2004).

Figure 1. Biosynthetic Pathways of Biogenic Amines. This figure shows the pathways through which *C. elegans* produces its biogenic amines. Asterisks indicate that a cofactor encoded by *cat-4* is required in that step. From "Biogenic Amine Neurotransmitters in *C. elegans*", by D. Chase and M. Koelle, 2007, *WormBook*. Copyright 2007 by Daniel L. Chase and Michael R. Koelle.



1.3.1 The Role of Dopamine in *C. elegans* Behavior

The most extensively studied biogenic amine in *C. elegans* is dopamine (DA). Within and across phylogeny, including *C. elegans,* DA is essential for many forms of learning. The eight dopaminergic neurons in the hermaphroditic *C. elegans* connectome, as indicated by expression of the genes required for DA biosynthesis and release, are associated with feeding and reduction of locomotion in response to environmental cues. DA is delivered to the rest of the organism through 429 dopaminergic synapses as well as extrasynaptically after being released into the body cavity of the worm by the DA neurons (Sulston et al., 1975; Chase et al., 2004). DA neurons are also hypothesized to be mechanoreceptive in nature, due to the expression of the mechanotransduction channel TRP-4 in all eight dopaminergic neurons (Sawin et al., 2000; Li et al., 2006; Stephens et al., 2006; Li et al., 2011). The *C. elegans* genome encodes four different dopamine receptors including the D1-like receptors (*dop-1* and *dop-4*), which are expressed both in the nervous system and in non-neuronal cells, as well as D2-like receptors (*dop-2* and *dop-3*), which are proposed to have a strictly neuromodulatory role as a result of their exclusive expression in neuronal cells (Suo et al., 2003; Tsalik et al., 2003; Sugiura et al., 2005).

C. elegans finds and remains on food patches through DA mediated mechanosensation (Sawin et al 2000). The mechanoreceptive nature of dopaminergic neurons is demonstrated through locomotor slowing response, in which the worm detects patches of food through contact with the unique texture of bacterial lawns on which they feed. Well-fed wild type worms slow down when they encounter a patch of food in a well-characterized behavior termed the ‘basal-slowing response’ to facilitate consumption. This behavior is mediated through DA; well-fed *cat-2* mutants, that have reduced levels of dopamine, fail to reduce their velocity once they have encountered a patch of food and move at a rate similar to well-fed wild type worms that are not

on bacteria (Sawin et al., 2000). DA is thought to mediate the basal-slowing response through mechanosensation rather than nutrition detection, because well-fed wild type worms (but not *cat-2* mutants) still reduce locomotion when encountering texturally similar but bacteria-free media (Sawin et al., 2000). The basal slowing response is hypothesized to be mediated through DOP-1 and DOP-3 dopamine receptors acting antagonistically to each other in response to changing mechanosensory information. Evidence supporting this hypothesis is that *dop-3* mutants fail to slow down when encountering a patch of food; this slowing defect is rescued by mutations in *dop-1* (Chase et al., 2004). Put another way, *dop-3* and *dop-1* double mutants act like wildtype worms, because worms examined on food patches move more slowly than worms off-of food (Chase et al., 2004). This implies that these receptors work antagonistically to mediate the wildtype basal slowing response.

Once a food patch has been consumed, DA mediated food searching takes place in the immediate vicinity through 'area-restrictive search' behavior. Well-fed wild type worms (but not *cat-2* mutants) will sweep their heads around more frequently immediately after being removed from food compared to 30-minutes after removal from food (Hills et al., 2004). If no food is found within 30-minutes, wild type worms will decrease the frequency of sharply angled head turns to promote the search of bacteria in distant areas. *cat-2* worms do not increase the amount of high-angle head turns immediately after being removed from food and spend less time searching the immediate area as a result. This effect was rescued with exogenous application of DA.

These two distinct behavioral modifications of DA neurotransmission support *C. elegans* quickly locating and remaining on patches of food. To make sense of how these behaviors would be adaptive, one must only be reminded that *C. elegans* needs to constantly eat food to survive. If

a well-fed worm wanders off a patch of food, the area-restrictive search behavior increases the high-degree turns to help them search for food more efficiently. If food is found, the basal-slowing response encourages worms to stay in the vicinity. If the immediate area is devoid of food, the area-restrictive searching is silenced after a short time so that the worm can move to distant areas for new food patches.

DA synthesis also modulates the transition between swimming and crawling behavior. These mutually exclusive forms of locomotory gaits are normally transitioned between liquid to solid land through mechanoreception of ADE and PDE neurons acting upon DOP-1 and DOP-4 receptors (Vidal-Gade et al., 2011).

Interestingly, well-fed wild type worms habituate to tap more rapidly while off food than on food, likely because the frequent pausing and reversals are deleterious to searching for food. This altered rate of habituation was found to be mediated by DA. Kindt et al. (2007) and Sanyal et al. (2004) demonstrated that worms who have had their D1-like dopaminergic receptors, *dop-1*, or a dopamine biosynthesis precursor encoded by *cat-2*, genetically removed habituate much more quickly than wild type worms while on food, and at a similar rate to wild type worms if both are off of food. The normal rate of habituation is successfully restored in these mutants when exogenous dopamine is supplied through the agar on the Petri plate (Figure 2). Although dopamine isn't synthesized in any of the neurons in the tap habituation circuit, *dop-1* is expressed in the anterior (ALM) and posterior tap sensory neurons (PLM), which are suggested to be the loci of plasticity for habituation to tap. The loss of this on-off food phenotype observed for *dop-1* and *cat-2* worms was demonstrated to be a result of dopaminergic signaling in the ALM neurons. Calcium imaging revealed that while initial responses to anterior touch were the same between mutants and controls, transient calcium in the ALM neurons decreased at a much

faster rate in *dop-1* mutants than wild type worms in response to repeated touch (Kindt et al., 2007). Calcium imaging studies of the ALM neuron in *dop-1* mutants both on and off of food showed that calcium influx was not different in the two conditions and was reduced at a rate similar to that of wild type worms off of food in response to repeated tap stimulation (Kindt et al., 2007) Additionally, the rapid rate of *dop-1* loss-of-function mutants' habituation to tap was rescued to wild type levels when a functional *dop-1* receptor transgene was expressed in ALM, suggesting that the DOP-1 receptor functions within ALM (and possibly PLM, but this has not been tested) to negatively regulate touch sensitivity (Sanyal et al., 2004). Therefore, the rapid habituation of wild type worms in the absence of food is correlated with a similarly rapid attenuation of ALM excitability.

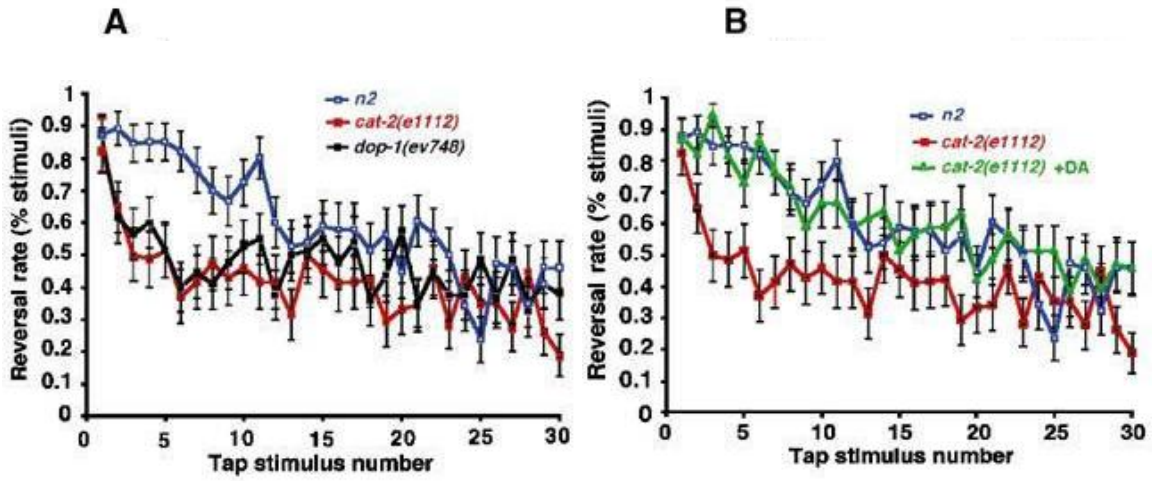
DA is also involved in another form of learning; state-dependent olfactory adaptation. Colbert and Bargmann (1995) found that when exposed to the same attractive odorant normally released by nutritious bacteria (i.e., benzaldehyde) over a prolonged period, *C. elegans* would no longer orient itself toward that specific volatile chemical. Although all chemicals tested were sensed by a single pair of sensory neurons - AWC, specifically - several mechanisms were implicated in the adaptation. Depending on which mechanism was activated, that specific chemical odorant, or multiple odorants, could be adapted to (Colbert & Bargmann, 1995). In Bettinger and McIntire (2004), adaptation is suggested to show state dependence because wild type worms who initially adapt to benzaldehyde while also exposed to intoxicating levels of ethanol will no longer have a decremented response if the they are re-exposed to benzaldehyde while not under the intoxicating influence of ethanol. State-dependent olfactory adaptation is dependent on DA biosynthesis because *cat-2* mutants retain their decremented olfactory response upon re-exposure to benzaldehyde even if their level of inebriation is different from their level of

intoxication at the time of primary exposure (Bettinger & McIntire, 2004). The role of DA biosynthesis in state-dependent olfactory adaptation, however, remains largely unknown.

DA also regulates decision making in *C. elegans*. D1-like receptor *dop-1* works antagonistically with D2-like receptors *dop-2* and *dop-3* in wild type animals to regulate the decision of whether to cross an osmotically aversive barrier to reach a chemoattractant on the other side. Compared to wild type worms, *dop-1* mutants are more likely to cross the osmotic barrier. *dop-2* as well as *dop-3* mutants will cross that barrier even less than wild type worms in the same assay (Wang et al., 2014). Regarding aversive chemical odorants, dopaminergic signaling through DOP-3 receptors in ASH to reduce avoidance to 1-octanol (Ezak & Ferkey, 2010).

Dopamine antagonistically mediates many behaviors that are promoted by 5-HT in *C. elegans*. These inhibitory effects extend to 5-HT promoted behaviors including egg laying, pharyngeal pumping, and swimming behavior (Osuna-Luque et al., 2018; Vidal-Gade et al., 2011; Weinshenker et al., 1995).

Figure 2. Normal Tap Habituation Requires DA Signaling. This figure shows that tap habituation curves steepen if worms have loss-of-function mutations in *cat-2* or *dop-1* (A), but this phenotype can be rescued in *cat-2* mutants with the exogenous application of dopamine (B). Figure adapted with permission from "Dopamine Modulates the Plasticity of Mechanosensory Responses in *Caenorhabditis elegans*", by Sanyal, S et al., 2004, *The EMBO Journal*, *23*(2), p. 478. Copyright 2004 by European Molecular Biology Association.



1.3.2 The Role of Serotonin in *C. elegans* Behavior

Serotonin (5-HT) is made in eight other neurons in the hermaphroditic *C. elegans*. Similar to DA, the implications of 5-HT on behavior have largely been elucidated through examination of worms with mutations that don't biosynthesize 5-HT or through the exogenous application of 5-HT to the environments of wild type worms to alter the effects of this biogenic amine after production. Exogenous application of 5-HT to wild type worms increases the effects that 5-HT has on behavior; this method has resulted in dramatic inhibition of locomotion and defecation, while increasing egg laying and pharyngeal pumping (Carnell et al., 2005; Horvitz et al., 1982; Mendel et al., 1995; Naicaris & Avery, 2003; Rogers et al., 2001; Sawin et al., 2000; Ségalat et al., 1995; Waggoner et al., 1998; Weinshenker et al., 1995). Worms with mutations in the biosynthetic enzymes that are required for 5-HT production (i.e., TPH-1) show the inverse of some of behavioral alterations made by over exposure to 5-HT (Sze et al., 2000; Yemini et al., 2013).

While DA biosynthesis is responsible for the behavior of well-fed wild type worms slowing down upon mechanostimulation from a food patch, 5-HT biosynthesis is required for the enhanced slowing response observed in food-deprived wild type worms entering a patch of food (Sawin et al., 2000). These two behavioral modifications are suggested to be adaptive dependent on context; DA encourages well-fed worms to stay on food, but 5-HT signaling kicks in when animals have been food deprived and forces worms to stay on any food patches they find. Worms that don't have the cofactor required for both DA and 5-HT biosynthesis, *bas-1*; or another cofactor required for DA, 5-HT, and OA biosynthesis, encoded by *cat-4* mutants; but not *cat-2* mutants, fail to demonstrate an enhanced slowing response upon reentering food after being food deprived for at least 30 minutes. Because BAS-1 and CAT-4 are enzymes required

for biosynthesis of both DA and 5-HT, the inability of *cat-2* mutants, which are deficient in only DA, to elicit the enhanced slowing response suggests that the enhanced slowing response is a result of 5-HT (Figure 1). Furthermore, the enhanced slowing response can be restored in *bas-1; cat-4* double mutants with exogenous application of 5-HT, but not DA (Sawin et al., 2000). Although Sawin et al. (2000) didn't test for the enhanced slowing response in *tph-1* null mutants, which lack 5-HT but no other biogenic amines, later studies did test with *tph-1* null mutants and were able to identify an enhanced slowing response (Gürel et al., 2012; Zhang et al., 2005). 5-HT is proposed to induce the enhanced slowing response through at least two parallel pathways involving 5-HT receptor MOD-1 in sensory neurons and reuptake transporter MOD-5 within the NSM neuron (Gürel et al., 2012; Ranganathan et al., 2000; Ranganathan et al., 2001; Sawin et al., 2000).

The role of 5-HT in locomotion in the presence of food is more controversial. Studies by Ben Arous et al. (2009) and Churgin et al. (2017) found very different results. While both groups suggested that 5-HT is required for appropriately adapted behavior from feeding to fasting, the exact contribution of this monoamine becomes a topic of conflicting results depending on which study you are looking at. Churgin at el. (2017) found that *tph-1* worms roam *more* than wild type in the presence of food, a finding that is also supported by other research by Flavell et al. (2013), but that the proportion of time spent roaming while food deprived is similar to that of N2. Conversely, Ben Arous et al. (2009) found that *tph-1* worms are roaming deficient, with *tph-1* mutants spending *less* time roaming than N2 regardless of the presence of a bacterial lawn. To summarize, both papers found that the adaptation of fraction of time spent roaming from well-fed to starved conditions requires the presence of 5-HT, with *tph-1* mutants showing a smaller difference than wild type worms in roaming fraction between these two states. The discrepant

findings are that Ben Arous et al. (2009) found that *tph-1* null worms are roaming deficient overall, while Churgin et al. (2017) suggested that *tph-1* null worms have enhanced roaming ability. This discrepancy may be the result of testing methods, because Churgin et al. (2017) observed the locomotory states of their worms in a liquid medium while Ben Arous et al. (2009) tracked locomotory states on solid agar. Perhaps the effects of 5-HT on locomotory states is dependent on the housing medium as well as the presence of food.

In addition to food-related locomotor behaviors, 5-HT is also involved in olfactory adaptation. Nuttley et al. (2002) suggested that, unless the chemoattractant benzaldehyde accurately predicted the presence of food, wild type *C. elegans* no longer oriented towards the smell. In the absence of *Escherichia coli*, benzaldehyde was proposed to become an aversive stimulus that was associated with nutrient-poor environments. Interestingly, exogenous application of 5-HT stopped this adaptation from occurring in the absence of *E. coli*. The presence of food also had no effect on the level of olfactory adaptation to benzaldehyde in 5-HT biosynthesis null worms (Nuttley et al., 2002). Hence, 5-HT signaling is required for food-odor associative learning. As worms age they become more attracted to benzaldehyde, as demonstrated by stronger chemotaxis and delayed olfactory adaptation - an aging characteristic that is proposed to be mediated by serotonergic signaling in a mechanism of associating this odorant with nutrition (Tsui & van der Kooy, 2000).

Serotonergic signaling has also been implicated in *aversive* conditioning through taste and smell. Wild type worms can learn to avoid specific toxic bacterial metabolites after ingestion leads to visceral malaise as part of an adaptive strategy to predict viable food sources. *tph-1* null mutants have reduced associative learning capabilities for these metabolites, requiring more exposure to the toxin to stop chemotaxis (Ballestriero et al., 2016). Similarly, 5-HT released

through ADF and received through the 5-HT-gated ion channel MOD-1 promotes aversive learning to pathogenic bacteria (Zhang et al., 2005). *C. elegans* can also negatively associate a normally attractive ion concentration of NaCl in a type of associative learning called gustatory plasticity, in which serotonergic (along with dopaminergic and glutaminergic) signaling has been implicated (Hukema et al., 2008).

1.3.3 The Role of Octopamine and Tyramine on *C. elegans* Behavior

Octopamine and its precursor tyramine are synthesized in *C. elegans* neurons (Horvitz et al., 1982; Alkema et al., 2005). The gene coding for the catalysis of tyramine into octopamine, *tbh-1*, is expressed in only a subset of tyraminergic cells, suggesting there may be independent functions for the two biogenic amines rather than tyramine being one step of a biosynthetic process to the more well-known octopamine (Alkema et al., 2005). Octopamine, which is found in amounts that are radio-enzymatically detectable in wild type worm extracts, was originally proposed to be the antagonist of serotonergic signaling. Exogenous application of either tyramine or octopamine leads to hyperactivity in the presence of food and reduces 5-HT mediated pharyngeal pumping and egg laying, while application of pharmacological antagonists to tyramine and octopamine had opposite effects (Horvitz et al., 1982; Naicaris & Avery, 2003; Packham et al., 2010; Ségalat et al., 1995). All these behaviors are normally stimulated by the presence of food, so it was proposed that octopamine acted antagonistically to the gene products released from the presence of food (at the time, this was proposed to be 5-HT). Octopamine concentrations in *C. elegans* extracts increased with the age of the animal, so Horvitz et al. (1982) also proposed that octopamine's main role was in adult behaviors.

Octopamine has indeed been found to antagonistically regulate other behaviors that are serotonin dependent. Churgin et al. (2017) proposed that while serotonergic signaling mediated

food-related locomotion, octopamine regulated starvation related locomotion. In wild type worms, octopamine is released from the RIC interneurons and activates SER-3 and SER-6 receptors in the SIA neurons to promote roaming during fasting. Fasting *tdc-1* mutants, that lack both tyramine and octopamine, spend a comparable ratio of time dwelling or roaming to well-fed wild type animals, which suggests that these biogenic amines are required to produce fasting-appropriate locomotion. Both fasting *tdc-1* and fasting *tbh-1* mutants show increased quiescence (a sleep-like state) and dwelling along with decreased roaming compared to fasting wild type worms. Exogenous application of octopamine to fasting *tbh-1* mutants restored the proportion of time spent quiescent vs dwelling to that of fasting wildtype worms, which suggests that octopamine signaling is responsible for the hyperactivity seen in fasting wild type worms (Churgin et al., 2017). Tyramine is proposed to have antagonistic effects to octopamine on fasting appropriate locomotion, because exogenous application of tyramine to fasting wild type and *tdc-1* null worms promoted reduced locomotion activities typical of feeding worms, but the pathway through which these behaviors are regulated has not been elucidated (Churgin et al., 2017). It is suggested, however, that octopamine works within this pathway to signal nutrient deprivation. The DAF-12 nuclear hormone receptor forms different complexes in the presence or the absence of food. In the absence of food, *tbh-1* promoters can bind to the DAF-12/DIN-1 complex. In the presence of food TBH-1 does not bind to the DAF-12, because DAF-12 does not form the complex with DIN-1. When TBH-1 binds to the DAF-12/DIN-1 complex it upregulates biosynthesis of TBH-1 in the RIC neurons (Tao et al., 2016).

Octopamine is proposed to play a role in developmentally timed quiescence as well. Quiescence in *C. elegans* is temporally aligned with the molting of their cuticles as they transition from previous larval stages of development. During this molting period, they enter a

period known as lethargus, which is interspersed with quiescence and random bouts of activity (Raizen et al., 2008). Both *tdc-1* and *tbh-1* loss of function mutants exhibited an increased amount of quiescence during lethargus while transitioning from L4 to adulthood compared to wild type worms, suggesting that octopamine suppresses quiescence during developmentally timed lethargus (Singh et al., 2014). Developmentally timed quiescence is enhanced by 5-HT, as *ser-4* loss of function mutants have decreased total rest during molting (Singh et al., 2014). The mechanisms of how serotonergic and octopaminergic signaling alter developmentally timed quiescence have not been examined, so it is unclear if they are antagonistic within the same circuits.

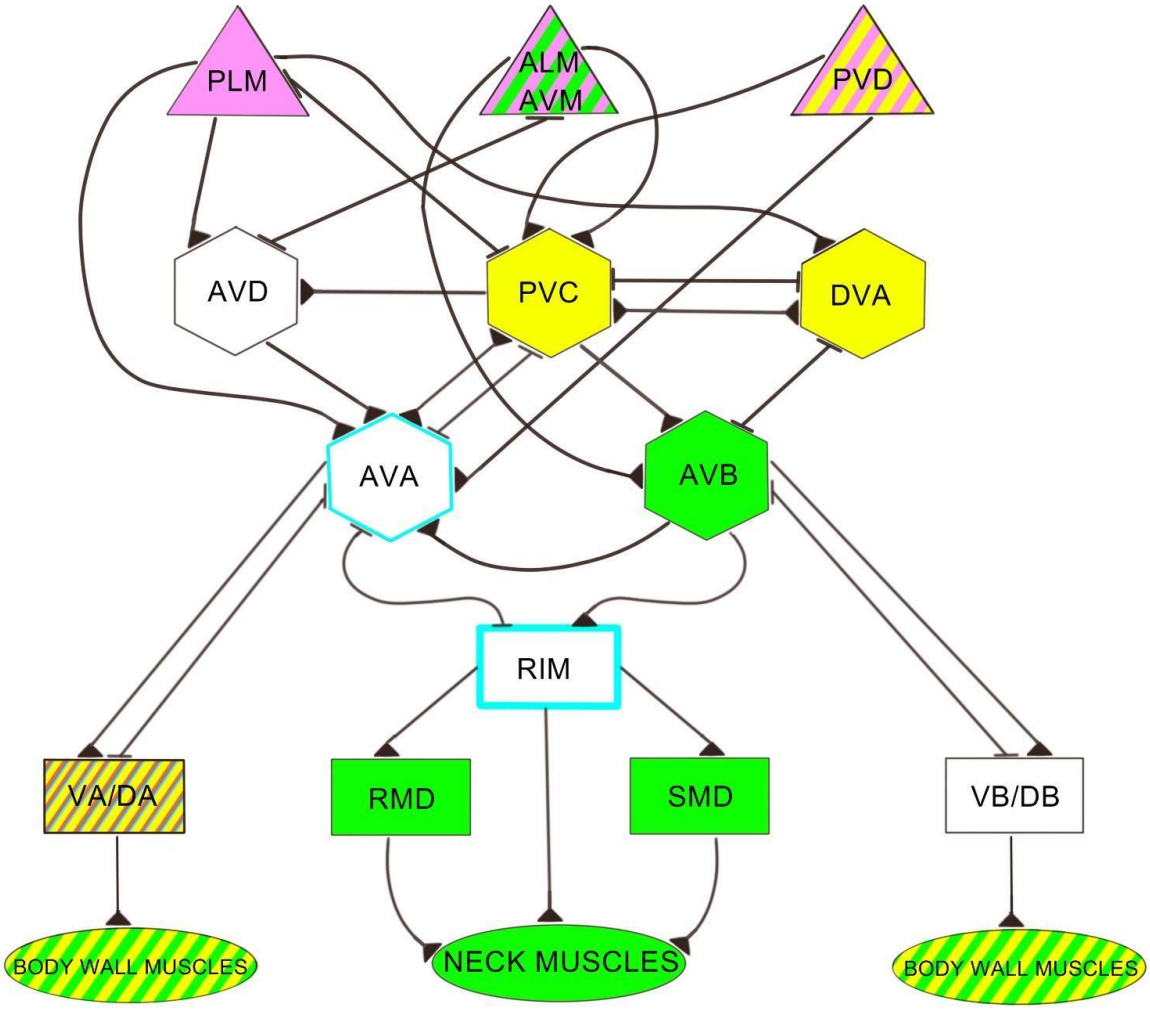
Additional evidence for tyramine and octopamine antagonizing 5-HT are the involvement of these biogenic amines in responses to aversive olfactory stimuli. As explained previously, 5-HT can both increase and maintain sensitivity to aversive olfactory stimuli. One such example is the increased responsiveness of worms to dilute octanol in the presence of either food or serotonergic signaling through modulation of ASH signaling (Chao et al., 2004). Treatment of wild type worms with exogenous tyramine or octopamine abolished the food and 5-HT mediated increased sensitivity to dilute octanol, while *tdc-1* and *tbh-1* mutants had increased responsiveness to octanol, similar to exogenous 5-HT treatment on wild type worms (Wragg et al., 2007). Although tyraminergic and octopaminergic signaling exhibit similar effects on 5-HT mediated sensitivity to octanol aversion, both biogenic amines have independent circuits and receptors through which they inhibit aversive olfactory behaviors (Wragg et al., 2007). Although all three octopamine receptors have implications in postponing this aversion, OCTR-1 is the most heavily implicated because *octr-1* is activated specifically in response to food or 5-HT signaling (Harris et al., 2010). This signaling cascade has been used to liken octopaminergic

signaling to noradrenergic signaling in mammals in the inhibition of olfactory nociception, which is processed through norepinephrine receptors that have a configuration that is most similar to that of OCTR-1 in invertebrates (Mills et al., 2010; Nai et al., 2010).

Tyramine has been proposed to suppress head oscillations during spontaneous reversal but has not been studied in the context of tap withdrawal response and habituation (Alkema, Hunter-Ensor, Ringstad, & Horvitz, 2005). However, based on the location of tyramine receptors within the tap circuit I had a strong hypothesis that it would be involved in tap habituation (Figure 3). The tap withdrawal response circuit expresses all four known tyramine receptors: *tyra*-2, *tyra-3*, *ser-2*, and *lgc-55* (Kratsois et al, 2017; Pirri & Alkema, 2012; Rex et al., 2005; Spencer et al., 2011; Tsalik et al., 2003; Zheng et al., 2015). Additionally, *tdc-1* is likely expressed in two neuron classes in this circuit (Alkema et al., 2005; Lemieux et al., 2015; Spencer at al., 2011). While most receptor and biosynthetic gene expression reported in literature is largely agreed upon, some reporter constructs have revealed additional neurons within the tap-circuit that express *tdc-1* and *tyra-3*. These unique findings come from a study that examined genome wide cell specific expression profiles across development of the hermaphroditic worm using mRNA tagging (Spencer et al., 2011), whereas the other studies mentioned generally used GFP-fusion promoter constructs strictly in adult worms. Spencer et al. (2011) also reported the expression of *octr-1*, an octopamine receptor, within the motor neurons driving reversal in response to tap. The unique expression profiles reported by Spencer et al. (2011) may result from their utilization of a promoter construct that encompassed potential post-transcriptional gene regulation via mRNA, as well as the inclusion of embryonic and larval expression patterns - techniques that no other paper used in unison. The weight of empirical support for the expression of relevant genes within a specific cell is indicated in Figure 4 through the thickness of the color

used to represent that gene. For example, the expression *tyra-3* and *octr-1* in the VA motor neurons is only reported by Spencer et al. (2011) so the stripe of color representing these genes is thinner. Furthermore, the expression of tyramine receptors on the mechanosensory neurons, which receive no direct synaptic input, strongly suggests that tyraminergic signaling can act extrasynaptically in a fashion similar to dopaminergic signaling within the same circuit (Branicky & Schafer, 2009; Donnelly et al., 2013). Indeed, some components of the touch escape response have been found to be mediated by the slow acting metabotropic tyramine receptor, SER-2 in a way that is necessarily extra-synaptic due to the lack of direct connection to any tyraminergic cells (Clark, 2014; Rex et al., 2004; Tsalik et al., 2003). Outside of the neural circuit of the tap withdrawal response, the body wall musculature expresses tyramine receptors that may affect the ability of the worms to respond to tap (Kudlow et al., 2012; Tsalik et al., 2003). Because of the high numbers of genes related to octopamine and tyramine signaling in the tap circuit I examined the roles of tyramine and its biosynthetic product, octopamine, in tap habituation in *C. elegans*.

Figure 3. Distribution of Tyramine in the Tap Habituation Circuit. This figure shows the distribution of tyramine biogenic precursors and receptors within the tap habituation circuit. Each neuron is colored-coded or outlined in the color representing which tyramine receptors or biogenic precursors are represented within that cell, respectively. Sensory neurons are represented by triangles, command interneurons are represented by hexagons, and motor neurons are represented by rectangles. All neurons shown represent bilateral classes of neurons aside from AVM and motor neurons. Two interneurons, AVA and AVB, are proposed to be the neurons responsible for forward locomotion or reversal, respectively. Synaptic connections to a cell are represented by triangles, and electrical gap junctions are represented by flat lines on each connecting cell. Neurons containing the biosynthetic precursor of tyramine are highlighted in cyan, with the relative thickness of the line denoting the amount of support within literature of this gene being expressed in these neurons. All four known tyramine receptors, and one octopamine receptor are present in the tap habituation circuit, and their expression is denoted using pink for *tyra-2*, yellow for *ser-2*, green for *lgc-55*, *orange* for *tyra-3*, and slate for *octr-1*. *tdc-1*, represented by cyan, is present in AVA and RIM. The support for the expression of these receptors within each cell according to the literature is indicated by the width of the line representing each receptor filling in the cell.


PLM
ALM
AVM
PVD
AVD
PVC
DVA
AVA
AVB
RIM
VA/DA
RMD
SMD
VB/DB
BODY WALL MUSCLES
NECK MUSCLES
BODY WALL MUSCLES

1.4 Goals

Based on the gaps in the literature, and the expression of tyramine precursor enzymes and receptors on the neurons of that tap circuit, this project was designed to investigate the roles of tyramine and/or octopamine in the ability of *C. elegans* to habituate to tap stimuli. By comparing worms with null mutations for genes critical for tyramine and/or octopamine neurotransmission at early and late adulthood, our understanding of the role of these biogenic amines in habituation will be better elucidated. The objectives of my research are:

1) To elucidate the impacts that tyramine and octopamine have on tap habituation through the examination of their absence of biosynthesis with well-fed 96-hour post-synchronization adult worms (Experiment 1).

2) Testing whether any phenotypes discovered in Goal 1 are present earlier in worm development by testing tyramine and/or octopamine null worms vs wild type worms at 72-hours post-synchronization (Experiment 2).

3) Identifying potential age-mediated phenotypes by comparing each strain against itself at 72- and 96-hours post-synchronization worms (Experiment 3).

Chapter 2: Methods

2.1 General Methods

2.1.1 Worm Maintenance

Animals were maintained on nematode growth medium (NGM) seeded with 50 µl of *E. Coli* (OP50) as described by Brenner (1974). The Bristol N2 was used as a wild type. Strains obtained from the *Caenorhabditis* Genetics Center (University of Minnesota, Minneapolis, MN) include: RB993 (*tdc-1[ok914]*), and RB1161 (*tbh-1[ok1196]*). Strains obtained from the Mitani lab (Tokyo Women's Medical University, Japan) include: MT10661 (*tdc-1[n3420]*), MT13113 (*tdc-1[n3419]*), and MT9455 *(tbh-1[n3247]*). All strains were maintained for no more than 30 generations (with new colonies made daily) to avoid accumulation of spontaneous mutations. If plates became visibly contaminated by mold or foreign bacteria, the strain was cleaned by placing 20 gravid adult hermaphrodites into a 5 µL drop of 5%-hypochlorite: 1-M-NaOH (1:1) (bleaching) solution that had been positioned on a new plate, away from the bacterial lawn, in order to kill the contamination in the next generation. Adults were dissolved and by the time eggs hatched the bleach had soaked into the agar. Contamination-free newly hatched worms crawled to the bacterial lawn.

2.1.2 Strains Tested

The genetic toolbox available for *C. elegans* research allows for the creation of many mutations that may render a gene null in function. Mutations can either be created with either forward genetic approaches, wherein mutagens are applied until a specific phenotype is affected and the whole genome of the animal is mapped to see what has changed, or reverse genetics techniques, in which the function of a specific gene is characterized by inactivating the expression of that gene/isolating a loss-of-function allele (Boulin & Hobert, 2012). Whether

experimentally generated from forward or reverse genetic screening techniques, worm strains always carry a risk of having an off-target mutation. These potential off-target mutations may phenotypically alter the behavior of the strain being examined in a way that is not the result of the gene of interest. Separate alleles that have been experimentally confirmed to have a complete loss of function of that gene of interest (e.g., no production of that protein) may still behave differently from each other, which suggests that an off-target mutation that is idiosyncratic to a specific strain may be altering the behavior. It is entirely possible to have a behavioral phenotype that is only observed in one loss-of-function allele, while another allele that doesn't contain the off-target mutation does not show the same behavioral phenotype.

To control for this possibility, at least two different curated alleles were tested for each of the biogenic amines tested. Thus, for the enzyme responsible for the synthesis of octopamine, two alleles (*tbh-1[n3247]* and *tbh-1[ok1196]*) were tested and for the enzyme responsible for the synthesizing tyramine, three alleles (*tdc-1[n3420]*, *tdc-1[n3419]*, and *tdc-1[ok914]*) were used. Only the behavioral differences that were consistently observed between all loss-of-function alleles for a given gene and wild type worms were likely to be the result of the gene product of interest; thus only those differences will be the focus of the results section.

2.2 Behavioral Testing

Worms were age-synchronized by using a platinum wire pick to transfer 5-10 young gravid adult worms onto NGM plates that had been poured 48-hours prior and seeded with 50 μL of E. coli (OP50) 24-hours earlier and left to dry overnight in an environmentally regulated room at approximately 40% RH and 20 °C until ready to use. Gravid worms were removed from these plates and discarded after two- to four-hours, leaving behind eggs that would hatch into ~ 80-120 worms on each plate. In order to minimize mechanical stimulation outside of the testing

assay, these plates were wrapped in Parafilm and left on a foam-padded shelf suspended by vibration isolating cords within an environmentally controlled room (~40+/-5 % RH and 20+/-1 °C) until the worms reached the appropriate age, at which point the plates were always handled on a foam layer until they were placed directly on the Multi-Worm Tracker.

2.2.1 Multi-Worm Tracker Recording

Tracking was recorded using the Multi-Worm Tracker (MWT) apparatus with program version 1.2.0.2 (Swierczek et al., 2011). Plates containing age-synchronized worms were secured onto the MWT platform and were visualized using a Falcon 4M30 camera (Dalsa) with a 60mm f-number 4.0 Rodagon (Rodenstock) lens set 40cm above the tracking platform. Images were captured from a round region of interest within the perimeter of the secured plate as denoted on the MWT program interface using a capture card PCOe-1427 CameraLink (National Instruments).

2.2.2 Tap-Stimulation

Age-synchronized worm colonies were grown at 20 °C in Parafilm wrapped plates on vibration isolating shelving until either 72- (i.e., young adult) or 96-hours (i.e., egg-laying adults) after the removal of un-mated progenitor hermaphrodites. Once the desired age was reached, these colonies were either secured to the MWT platform within the unwrapped plates they had been reared on. Immediately before the beginning of tracking, the lids of plates in all experimental conditions were lifted, wiped with a Kimwipe to remove condensation, and placed back over the plate to stimulate the brief air-puff and get the worms active enough to be recorded by the MWT software. Following the return of the lid, the tracking software was started, and it began recording the worms as they acclimated to their new environment over the course of a 10-minute pre-plate period. Starting from 600 seconds, taps were delivered through an

electromagnetic tubular push solenoid that drove a plunger onto the side of the plate at a 10-second ISI. Tracking continued until 10-seconds after the final of 30 taps.

2.2.3 Operational Criteria for Including Tap-Elicited Reversal-Responses

Tap-elicited reversals were operationally defined as in previous research as backwards crawling that occurred within 1 second of the solenoid tap being delivered. Choreography software screened and included responses for later analysis only if objects matching the specified pixels size (i.e., worms) persisted for at least 20 seconds and had moved at least two body lengths at some point during the tracking in order to exclude shadows from worm tracks or other non-worm artifacts from contributing to the data. The following commands were passed to the Choreography software (version 1.3.0_r1035; Swierczek et al., 2011) to implement these criteria: -p 0.027 -s 0.1 -t 20 -M 2 --shadowless -S -o nNss*b12xyMmeSakcr --plugin Reoutline::exp --plugin Respine --plugin MeasureReversal::tap::dt=1::collect=0.5::postfix=trv --plugin MeasureReversal::puff::dt=3::collect=0.5::postfix=prv --plugin MeasureReversal::postfix=txt.

2.2.3.1 Speed Calculation

The speed data was calculated over a 0.1 second time window. The Choreography output calculates the mean speed of all worms that meet the operational criteria within a frame (i.e., mean speed per frame). The mean speed of worms on a plate was calculated as the sum of mean group speed per frame/ number of frames during the specified time period, which in this case was the 1 second following tap stimulation. These plate speed averages were then combined and averaged with the mean speeds of other plates within each experimental condition to create the mean speed to each tap.

2.2.3.2 Response Probability

Response probability indicates the proportion of worms that responded to each of the 30 stimuli. The small number of worms that were reversing at the time of stimulation were removed from analysis. For computational purposes, objects that reversed less than 30 μm were considered to have not reversed. Response probability was calculated by dividing the number of worms that reverse after a stimulus is delivered by the total number of worms being currently tracked on the dish.

2.2.3.3 Response Duration

Response duration indicates length of time, in seconds, that objects meeting the operational criteria were reversing following the tap stimulus. Tap reversal can't effectively be differentiated from forward movement using morphology analysis because the resolution being captured in these experiments leaves the head and tail tips of worms largely indistinguishable, however the tap withdrawal response can be indirectly assessed (Swierczek et al., 2011). When worms respond to tap by reversing, their backwards speed is much faster than their previous forward speed, therefore worms that change their direction of movement and increase their rate of speed are presumed to have reversed (Guiles, 2012; Swierczek et al., 2011).

2.2.4 Tap Habituation

Tap habituation data was calculated using output from a Python program written by Joseph Liang to collect mean reversal speed duration, and probability responses to taps. Response level, when talking about response probability, refers to the proportion of all recognized objects that reversed in response to tap. When discussing response duration or speed, the response level is the average response measurement of all worms on the plate that met the operational criteria for having responded to tap. Initial response was defined as response level to

the first tap. Final response was defined as the response level to the 30th tap. The habituation curve was constructed using mean responses to all taps to create a line plot.

2.3 Statistical Analyses

2.3.1 Assessment Across all Response-Components

The "N" for all analyses was the number of plates rather than the number of worms or the average of each of the 30 total responses to tap in order to make a more conservative estimate of sample size. Analyses were performed on two different kinds of data summaries. In the first type of calculation we were investigating the differences between alleles in their average response to tap across all three response measures (i.e., probability, duration, and speed), so the plate's response to all 30 taps were averaged to produce one number per plate for each response-component (e.g., Figure 4G-I). The amount of habituation for each plate of worms was quantified as the arithmetic difference in response between the initial and final response to tap for each of the alleles (e.g., Figure 4J-L). This quantification of habituation was sensitive enough to enable wild type worms to be compared to mutants even if there were differences in initial tap response-levels, while comparison of average responses to all 30 taps granted the ability to screen for potential hyper/hypo-responsive mutants.

2.3.2 Statistical Analyses of a Given Response-Component

Data were analyzed using a one-way Analysis of Variance (i.e., ANOVA) between the six strains tested (i.e., one wild type, two alleles of *tbh-1*, and three alleles of *tdc-1*). If more than one dependent variable (i.e., strain and age) were evaluated, as was the case in experiment 3, then the main effects and the interactions between variables were evaluated in a two (72/96-hours) x six (Strains) ANOVA (i.e., two-way ANOVA). The analyses relevant to each experimental design were repeated for both average responses and depth of habituation across all

three response-components with the understanding that multiple ANOVAs are appropriate when the outcome variables are conceptually independent (Huberty & Morris, 1989). Previous research from the Rankin lab has indicated that tap-elicited response probability, duration, and speed have different biological underpinnings at both the level of habituation and average responses to tap (Giles, 2012; McDiarmid et al., 2017), thus data for these measures is considered independently. All statistical tests were considered to have significantly different average responses or response-decrements in any given response-component if the probability that the responses were the same was less than 5% (i.e., $p < 0.05$), however the only results considered in detail were those in which all alleles for a gene were significantly different from wild-type worms.

Multiple biosynthesis-null alleles of *tbh-1* and *tdc-1* were utilized for every experiment in this thesis. While the lack of tyramine and octopamine biosynthesis or lack of octopamine biosynthesis and tyramine overexpression had previously been experimentally confirmed for *tbh-1* and *tdc-1* mutants, respectively, it is always possible that the methods of mutagenesis may result in off-target genetic changes that affect the behavior of interest. Only the phenotypes in which all relevant alleles of a gene significantly differed from wild type are being discussed here, because consistently different behavioral patterns across mutants are more likely to have resulted from the alterations to the gene of interest rather than unknown mutations.

2.3.2.1 Assessment of Total Average Stimulus Responses

The mean plate-responses to each of the 30 stimuli were grouped together to represent each plate's average response to all taps on a given response-component. This gives an average response size across all 30 stimuli. When allele was the only dependent factor being considered, as was the case in Experiments 1 and 2, a one-way ANOVA was used to determine if there was

evidence that there was a significant difference between the strain means. When the one-way ANOVA indicated that the differences of at least one of the allele means were unlikely to be the result of random sampling, a planned Dunnett's post hoc test was computed with multiplicity adjusted p-values in order to conservatively determine which genes consistently differed from N2 across all alleles.

When both strain and age were dependent factors being considered, as was the case in Experiment 3, a two-way ANOVA was performed to determine how these two independent variables in combination affect a relevant response-component. When the two-way ANOVA revealed that differences in an allele's response measurement depended on the age at which the worms were tested, or vice versa, through a significant main effect, then a Šidák's multiple comparison post hoc test was used to determine which of the alleles were significantly different from themselves on a given behavioral measure at different points in adulthood. Šidák's post hoc test was used here because the purpose of Experiment 3 was to determine the ways in which each independent allele differed across the level of the second variable (i.e., age at test); the Šidák method is the most powerful post hoc test available for comparing a selected set of means given that each comparison is independent of the others (Abdi, 2007).

2.3.2.2 Assessment of Degree of Habituation

The overall extent of decrement from the beginning to the end of stimulation for a given response-component, or the degree of habituation, called "response-decrement" was measured by subtracting the initial stimuli response from the final stimuli response. This meant that each plate had one score representing an average response-decrement for all worms on that plate. As with the plate-response average scores, this response-decrement-score was analyzed using a one-way ANOVA and Dunnett's post hoc test when applicable for Experiments 1-2, and a two-way

ANOVA with a Šidák post hoc test for Experiment 3 to determine the ways in which the decrement of response differed between each allele and wildtype or between each allele across adulthood, respectively.

2.3.2.3 Statistical Software

All statistical analyses were performed using GraphPad Prism version 9.0.0 for Windows 64-bit, GraphPad Software, San Diego, California USA, www.graphpad.com.

Chapter 3: Results

3.1 Introduction

In order to further investigate the role of tyramine and octopamine in habituation of the tap withdrawal response in *C. elegans*, we obtained strains of worms with mutations in genes critical for the biosynthesis of each amine and then observed the response of each mutant to repeated tap stimuli at 10-second ISI. The neural circuit that mediates the TWR in *C. elegans* includes several sites of tyramine synthesis and many points of expression of tyramine and octopamine receptors (Figure 3), suggesting that these should have a significant impact on the way that this nematode habituates (Alkema et al., 2005; Kratsois et al., 2017; Lemieux et al., 2015; Pirri & Alkema, 2012; Rex et al., 2005; Spencer et al., 2011; Tsalik et al., 2003; Zheng et al., 2015). Furthermore, the expression of tyramine receptors on the mechanosensory neurons, which receive no direct synaptic input, strongly suggests that tyraminergic signaling can act extrasynaptically in a fashion similar to dopaminergic signaling within the same circuit (Branicky & Schafer, 2009; Donnelly et al., 2013). Outside of the neural circuit of the tap withdrawal response, the body wall musculature expresses tyramine receptors that may affect the ability of the worms to respond to tap (Kudlow et al., 2012; Tsalik et al., 2003).

In addition to the high number of genes related to tyramine and octopamine signaling within the tap circuit itself, tyramine and octopamine are the most extrasynaptically active monoamine neurotransmitters, with 94 and 100% respectively of all receptor expressing cells receiving no synaptic input from cells that synthesize their corresponding neurotransmitter (Bentley et al., 2016). This so-called monoamine connectome is proposed to wirelessly deliver tyramine and octopamine to cells encoding tyramine and octopamine receptors beyond what is demonstrated in Figure 3.

3.2 Effects of Tyramine and Octopamine on Response Duration at 96-Hours Post-Egg-Lay

The neural circuit that controls the TWR in *C. elegans* contains seven sensory neurons, four pairs of command interneurons, and two pools of motor neurons responsible for forward and backward locomotion (Rose & Rankin, 2001; Wicks & Rankin, 1995), as seen in Figure 3. Basically, all the cells represented in this behavioral circuit either produce or receive tyramine or octopamine, including the body wall muscles! In order to further investigate the role that these heavily represented monoamines might play in the neural plasticity that underlies tap habituation in *C. elegans*, I sought to determine the ways in which worms that didn't produce one or both of these molecules differed in their responses to repeated taps. In the first experiment worms were tested 96-hours after synchronization. At this time these adult worms are early middle age and still producing eggs.

In this experiment we tested plates of 96-hour old wild type worms, two strains with mutations in *tbh-1,* and three strains with mutations in *tdc-1*. 30 taps were delivered with a 10-second ISI to plates of worms that had previously been sitting undisturbed for 10 minutes on the MWT platform. Although the MWT software tracks 18 standard behavioral measures, only the probability of response, response duration, and response speed were considered here. The data in Figure 4 Panels A, B, and C shows the raw habituation data for wild-type worms and the two *tbh-1* alleles. Visual examination of the graphs for probability, duration and speed shows that for each measure the data from the two alleles brackets the wild type data, indicating there were no consistent differences between the *tbh-1* worms and the wild-type worms. In Figure 4 the panels D, E, and F shows the raw habituation data for probability, duration and speed for wild type worms and the three alleles of *tdc-1* when all worms are 96-hours of age. In panels D and F, the *tdc-1* alleles again bracket the wild-type worms, indicating no consistent differences in response

probability and speed, respectively. In contrast, for duration the *tdc-1* worms showed consistently smaller responses than wild-type worms (Figure 4E), suggesting a role for *tdc-1* in response duration at 96-hours. These observations were confirmed by statistical analyses. The one-way ANOVAs were performed on all strains tested (i.e., N2, *tbh-1[n3247]*, *tbh-1[ok1196]*, *tdc-1[n3420]*, *tdc-1[n3419]*, and *tdc-1[ok914]*)for the average plate response probability, duration and speed with strains compared to each other at 96h were significantly different for all three response measures; probability: F (5, 89) = 6.283, $p < 0.0001$, duration: F (5, 89) = 22.81, $p < 0.0001$, speed: F (5, 89) = 8.201, $p < 0.0001$. Post hoc analysis (Table 1; Figure 4G) conducted using a Dunnett's correction on Response Probability at 96-hours revealed no consistently significant differences across alleles compared to N2: *tdc-1(ok914)* was the only significantly different allele from N2, while *tdc-1(n3420)*, *tdc-1(n3419)*, and both the *tbh-1(n3427)* and *tbh-1(ok1196)* alleles were non-significant (Table 1, Figure 4G). Similarly, a Dunnett's post hoc analysis of Response Speed at 96-hours only found one statistically significant difference when comparing N2 and *tbh-1(n3247)*, with no consistent differences within alleles (Table 1, Figure 4I). Interestingly, the Dunnett's post hoc test of response duration at this time point identified consistently significant differences between N2 and all three alleles of *tdc-1*, although only one strain of *tbh-1* was significant (i.e., *tbh-1[n3247]*) while the other (i.e., *tbh-1[ok1196]*) was non-significant (Table 1; Figure 4H). To summarize, the only difference consistent for all alleles of a given gene for average response magnitude was that all three alleles of *tdc-1* responded for significantly less time on average than N2 at 96-hours of age.

In order to assess significant differences in degree or amount of habituation between N2, both alleles of *tdc-1*, and all three alleles of *tbh-1*, an additional one-way ANOVA was performed on each of the above data-sets, this time looking at the amount of response-decrement

between the initial and final response to tap for each strain instead of the total average tap response to all 30 taps. Although these one-way ANOVAs revealed significant differences in response-decrement between the strains at 96-hours on probability (F [5, 85] = 10.84, $p <$ 0.0001), duration (F [5, 85] = 5.066, p = 0.0004), and speed (F [5, 85] = 4.836, p = 0.0006), there was no measure on which all alleles of a gene had consistently significant different degree of response-decrement compared N2 as assessed by a Dunnett's post hoc test. Some individual strains had significantly larger response-decrements compared to N2 for probability of responding, including *tbh-1(ok1196)*, *tdc-1(n3420)*, and *tdc-1(ok914)*, while at least one allele of each gene were non-significant (i.e., *tbh-1[n3427]* and *tdc-1[n3419]*) (Table 2; Figure 4J). In response-decrement of duration, the Dunnett's post hoc comparisons for all strains were non-significant against N2 (Table 2; Figure 4K). Similarly, none of the strains' response-decrements in speed were significantly different from that of N2 (Table 2; Figure 4L).

Figure 4. Genetic Dissection of Response-Components Among 4-Day-Old Worms. The strains indicated below show significant effects for all three response measures of reversal-response to tap (ordinary one-way ANOVA across strains for both the average response to all response measures and response decline from the 1st to 30th taps). '*' indicates $p < 0.05$, '***' indicates $p \leq 0.001$, '****' indicates $p \leq 0.0001$, and 'n.s.' indicates $p < 0.05$ on a Dunnett's post hoc analysis. A-C) The three response-components' tap-habituation curves of both alleles of *tbh-1* vs N2 at 96-hours. D-F) The three response-components' tap-habituation curves of all three alleles of *tdc-1* vs N2 at 96-hours. G-I) All three alleles of *tdc-1* respond for a significantly shorter duration than N2 on average H-J) No consistent significant differences in the response-decrement between each genotype and N2 on any response measure.

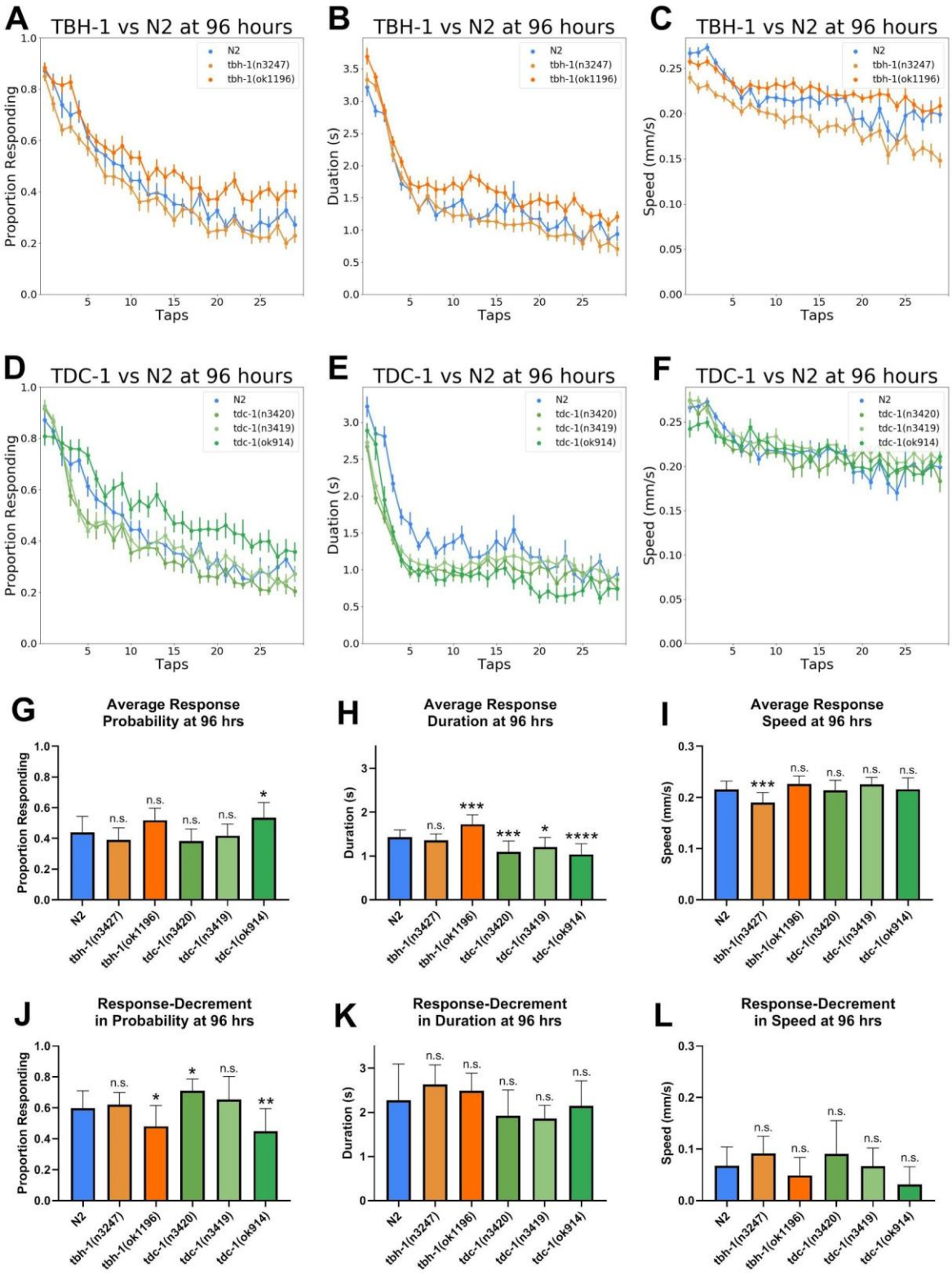

A
TBH-1 vs N2 at 96 hours
N2
tbh-1(n3247)
tbh-1(ok1196)
Proportion Responding
Taps
B
TBH-1 vs N2 at 96 hours
Duation (s)
C
TBH-1 vs N2 at 96 hours
Speed (mm/s)
D
TDC-1 vs N2 at 96 hours
tdc-1(n3420)
tdc-1(n3419)
tdc-1(ok914)
E
F
G
Average Response Probability at 96 hrs
H
Average Response Duration at 96 hrs
I
Average Response Speed at 96 hrs
J
Response-Decrement in Probability at 96 hrs
K
Response-Decrement in Duration at 96 hrs
L
Response-Decrement in Speed at 96 hrs

Table 1. The Means and Standard Deviations of Average Response-Component Measures for Each Strain at 96-Hours Post-Egg-Lay. The means and standard deviations of each strain's probability, duration, and speed across all 30 taps when worms are 96-hours old. Green cells indicate a significant difference (i.e., $p < 0.05$) whereas empty (white) cells indicate no significant difference (i.e., $p > 0.05$, n.s.) on a Dunnett's post-hoc corrected analysis between the response-component of that mutant strain and wild type control (i.e., N2). Of the green cells, only those whose text is red is considered to have consistent significant differences from N2. N = minimum 14 plates per strain across three days of testing. To see the complete response curves for each of these strains, consult Figure 4.

Strain	Probability		Duration		Speed	
	M	*SD*	*M*	*SD*	*M*	*SD*
N2	0.440	0.104	1.432	0.163	0.216	0.0163
tbh-1(n3247)	0.390	0.0784	1.359	0.149	0.190***	0.0194***
tbh-1(ok1196)	0.518	0.0786	1.727**	0.216**	0.226	0.0162
tdc-1(n3420)	0.384	0.0785	1.099***	0.242***	0.214	0.0197
tdc-1(n3419)	0.418	0.0754	1.204*	0.222*	0.226	0.0133
tdc-1(ok941)	0.535*	0.0986*	1.038****	0.241****	0.316	0.022

* $p < .05$. ** $p < 0.01$. *** $p < 0.001$. **** $p < 0.0001$.

Table 2. The Means and Standard Deviations of Average Response-Decrements Between 1st and 30th Tap for all Response-Components of Each Strain at 96-Hours Post-Egg-Lay. The means and standard deviations of the difference between the 1st and 30th tap of each strain's probability, duration, and speed when worms are 96-hours old. Green cells indicate a significant difference (i.e., $p < 0.05$) whereas empty (white) cells indicate no significant difference (i.e., $p > 0.05$, n.s.) on a Dunnett's post-hoc corrected analysis between the response-component of that mutant strain and wild type control (i.e., N2). N = minimum 14 plates per strain across three days of testing. To see the complete response curves for each of these strains, consult Figure 4.

Strain	Probability		Duration		Speed	
	M	*SD*	*M*	*SD*	*M*	*SD*
N2	0.599	0.111	2.275	0.822	0.0675	0.0367
tbh-1(n3247)	0.621	0.0785	2.629	0.444	0.0918	0.0330
tbh-1(ok1196)	0.481*	0.134*	2.487	0.403	0.0489	0.0349
tdc-1(n3420)	0.711*	0.0765*	1.926	0.580	0.0910	0.0643
tdc-1(n3419)	0.654	0.150	1.858	0.303	0.0672	0.0349
tdc-1(ok941)	0.450**	0.145**	2.146	0.568	0.0314	0.0344

* $p < .05$. ** $p < 0.01$.

3.3 Effects of Tyramine and Octopamine on Response Duration and Speed at 72-Hours Post-Egg-Lay

Having demonstrated the effects of tyramine and octopamine on response-components at the standard age of tap habituation testing in our lab (i.e., 96-hours), the next question to address was the impact of these monoamines on the same measures in younger worms. There are several reasons why this question was pertinent based on existing literature; larval *C. elegans* produce only a fifth of the amount of octopamine synthesized by adult worms by net weight, which strongly suggests that octopamine and it's biosynthetic precursor tyramine play unique roles in adult behavior (Horvitz et al., 1982). This chemical concentration corresponds to the proportional amount of a gene being transcribed within the worm itself. The capacity of an organism to make a protein corresponding to a specific gene can be represented using Fragments Per Kilobase Million (FPKM), which indicates the normalized proportion of times the indicated unique sequence of base pairs has been identified within the transcriptome of an animal at any given moment using RNA-seq. *tbh-1* and *tdc-1* drastically increase their expression amount following the fourth and final molting of *C. elegans*. N2 worms that have had no treatment that would affect gene expression nearly double their expression of *tbh-1* between larval stage 4 and newly molted young adulthood (~ 56-hours), with 13.3 FPKM and 81.5 FPKM, respectively. Similarly, the median relative expression of the *tdc-1* loci for these same animals is 18.2 FPKM during larval stage 4 and 66.3 FPKM during young adulthood (Gerstein et al., 2010). The expression profiles for both genes continue to increase throughout adulthood as well. The dramatic increase in expression profiles for both monoamines suggests that they may play differential roles in behaviors across the lifespan of the animal. The earlier time point of testing was performed on three day old worms, or young adulthood, because this is the earliest time

point that worms will reliably reverse to tap and therefore the earliest that they can habituate in their tap withdrawal response (Chiba & Rankin, 1992). In addition, work by Timbers et al. (2012) examined habituation in worms ranging from 72-hours of age to 120-hours of age and found that the younger the worms, the shallower the habituation of response probability. To test whether tyramine and octopamine play a role in this developmental change in habituation *tbh-1* and *tdc-1* were tested at a younger age. In this experiment all mutant and wild type worms were given 30 taps at a 10-second ISI and the average response probability, duration and speed to all 30 taps and the degree of response-decrement were once again assessed between all strains, but this time the worms were tested 72-hours after their eggs were laid.

Similar to worms that had been tested at 96-hours, the one-way ANOVAs of the total response averages for all strains tested (i.e., N2, *tbh-1[n3247]*, *tbh-1[ok1196]*, *tdc-1[n3420]*, *tdc-1[n3419]*, and *tdc-1[ok914]*) across each strain at 72-hours was significant across all response measures; probability: F (5, 92) = 3.26, p = 0.0094, duration: F (5, 92) = 8.112, $p < 0.0001$, and speed: F (5, 92) = 13.37, $p < 0.0001$. However, while some strains individually differed wild type, there were no genotypes in which all the alleles significantly differed from N2 on any of the response-components. The Dunnett's post hoc tests on average response probability across all taps indicated that none of the alleles were significantly different from N2 (Table 3; Figure 5G). The Dunnett's post hoc tests on the average response duration had one individual allele (i.e., *tbh-1[ok1196]*) that differed from N2 while all other alleles were non-significantly different, including: *tbh-1(n3427)*, *tdc-1(n3420)*, *tdc-1(n3419)*, and *tdc-1(ok914)* (Table 3; Figure 5H). Similarly, while the Dunnett's post hoc comparison identified that the average response speed of *tbh-1(n3427)* and *tdc-1(n3419)* significantly differed from that of N2, the remaining alleles of these genotypes (i.e., *tbh-1[ok1196]*, *tdc-1[n3420]*, *tdc-1[ok914]*) were non-

significantly different (Table 3; Figure 5I). While there were no consistently significant differences between all of the genotypes and N2 across any of the response measures at 72-hours, it is interesting to note that the direction of difference is the same as the consistently significant differences found at 96-hours although generally smaller.

Similar to the response-decrement analyses at 96-hours, the one-way ANOVAs of the degree of response-decrement to tap at 72-hours on every strain together (i.e., N2, *tbh-1[n3247]*, *tbh-1[ok1196]*, *tdc-1[n3420]*, *tdc-1[n3419]*, and *tdc-1[ok914]*) revealed significant differences across all response-components; probability: F (5, 92) = 4.966, p = 0.0005, duration: F (5, 92) = 16.83, $p < 0.0001$, and speed: F (5, 92) = 8.516, $p < 0.0001$. Differences in tap habituation between wildtype and each genotype reached statistical significance more frequently in young adult worms than in old adult worms. While the Dunnett's post hoc comparisons in response-decrement of probability at 72-hours were significant between N2 and one *tdc-1* allele (i.e., *tdc-1[ok914]*), none of the other alleles of this genotype or any alleles of *tbh-1* were significantly different (Table 4; Figure 5J). The Dunnett's post hoc comparisons of duration response-decrement revealed that both alleles of *tbh-1* were significantly larger than that of N2, while only two of the *tdc-1* alleles (i.e., *tdc-1[n3420]*, and *tdc-1[n3419]*) were significantly different and the remaining allele (i.e., *tdc-1[ok914]*) was non-significant (Table 4; Figure 5K). The Dunnett-corrected post hoc comparisons of the response-decrement on speed at 72-hours identified that all three alleles of *tdc-1* were significantly smaller than that of N2, while both of the alleles of *tbh-1* had response-decrements that were non-significantly different (Table 4; Figure 5L). Thus, the only consistent strain differences for degree of response-decrement at 72-hours of age were between N2 and the two alleles of *tbh-1* for duration of the response and N2 and between N2 and the three *tdc-1* alleles for response speed.

The consistently larger response-decrement in duration between *tbh-1* strains and N2 at 72-, but not 96-, hours post-egg-lay suggest that the habituation of response duration may be most sensitive to tyramine over expression in three-day-old worms. On a similar note, the response-decrements in speed being consistently significantly smaller between *tdc-1* mutants and N2 worms at 72-, but not 96-, hours post-egg-lay might indicate that the habituation of response speed is most impacted by the presence of tyramine and/or octopamine in young adult worms.

Figure 5. Genetic Dissection of Response-Components Among Three-Day-Old Worms. The strains indicated below show significant effects for all three response measures of reversal-response to tap (ordinary one-way ANOVA across strains for both the average response to all response measures and response decline from the 1st to 30th taps). '*' indicates $p < 0.05$, '**' indicates $p \leq 0.01$, '***' indicates $p \leq 0.001$, '****' indicates $p \leq 0.0001$, and 'n.s.' indicates $p < 0.05$ on a Dunnett's post hoc analysis. A-C) The three response-components' tap-habituation curves of both alleles of *tbh-1* vs N2 at 72-hours. D-F) The three response-components' tap-habituation curves of all three alleles of *tdc-1* vs N2 at 72-hours. G-I) No consistent difference between the average of each strain across all 30 taps against that of N2 on any response measure. H-J) Both strains of *tbh-1* have a larger response-decrement in duration of reversal, and all three strains of *tdc-1* have a significantly smaller response-decrement in response speed compared to N2.

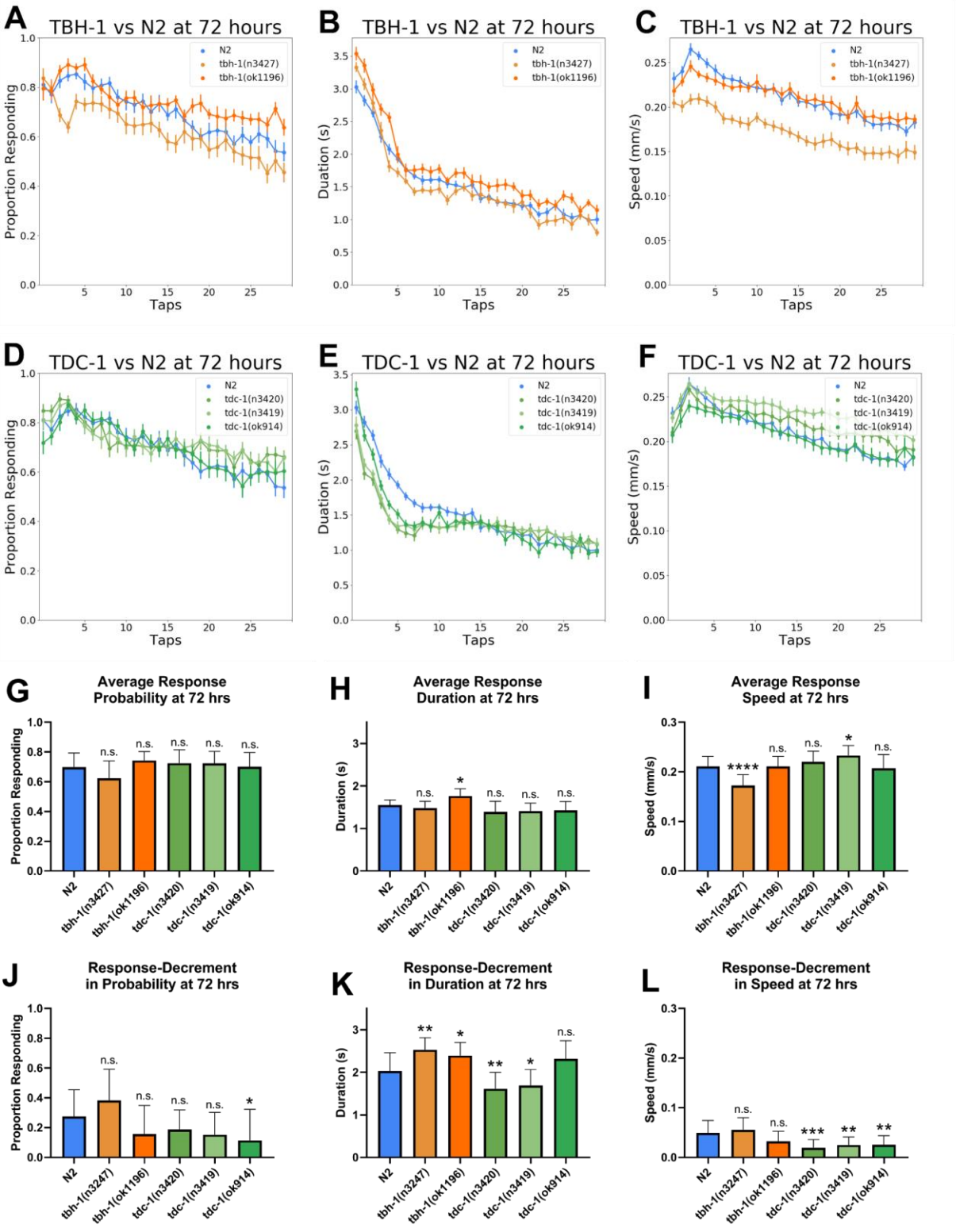

A
TBH-1 vs N2 at 72 hours
Proportion Responding
Taps
N2
tbh-1(n3427)
tbh-1(ok1196)
B
TBH-1 vs N2 at 72 hours
Duation (s)
Taps
C
TBH-1 vs N2 at 72 hours
Speed (mm/s)
Taps
D
TDC-1 vs N2 at 72 hours
tdc-1(n3420)
tdc-1(n3419)
tdc-1(ok914)
E
TDC-1 vs N2 at 72 hours
F
TDC-1 vs N2 at 72 hours
G
Average Response Probability at 72 hrs
H
Average Response Duration at 72 hrs
I
Average Response Speed at 72 hrs
J
Response-Decrement in Probability at 72 hrs
K
Response-Decrement in Duration at 72 hrs
L
Response-Decrement in Speed at 72 hrs
n.s.
*
**
***
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Table 3. The Means and Standard Deviations of Average Response-Component Measures for Each Strain at 72-Hours Post-Egg-Lay. The means and standard deviations of each strain's probability, duration, and speed across all 30 taps when worms are 72-hours old. Green cells indicate a significant difference (i.e., $p < 0.05$) whereas empty (white) cells indicate no significant difference (i.e., $p > 0.05$, n.s.) on a Dunnett's post hoc corrected analysis between the response-component of that mutant strain and wild type control (i.e., N2). N = minimum 14 plates per strain across three days of testing. To see the complete response curves for each of these strains, consult Figure 5.

Strain	Probability		Duration		Speed	
	M	*SD*	*M*	*SD*	*M*	*SD*
N2	0.699	0.0947	1.522	0.121	0.211	0.0200
tbh-1(n3247)	0.623	0.118	1.485	0.156	0.172****	0.022****
tbh-1(ok1196)	0.743	0.0604	1.762*	0.174*	0.211	0.0205
tdc-1(n3420)	0.725	0.0907	1.391	0.249	0.220	0.0216
tdc-1(n3419)	0.723	0.0819	1.411	0.185	0.233*	0.0204*
tdc-1(ok941)	0.700	0.0977	1.429	0.058	0.207	0.0281

* $p < .05$. **** $p < 0.0001$.

Table 4. The Means and Standard Deviations of Average Response-Decrements Between 1st and 30th Tap for all Response-Components of Each Strain at 72-Hours Post-Egg-Lay. The means and standard deviations of the difference between the 1st and 30th tap of each strain's probability, duration, and speed when worms are 72-hours old. Green cells indicate a significant difference (i.e., $p < 0.05$) whereas empty (white) cells indicate no significant difference (i.e., $p > 0.05$, n.s.) on a Dunnett's post-hoc corrected analysis between the response-component of that mutant strain and wild type control (i.e., N2). Of the green cells, only those whose text is red is considered to have consistent significant differences from N2. N = minimum 14 plates per strain across three days of testing. To see the complete response curves for each of these strains, consult Figure 5.

Strain	Probability		Duration		Speed	
	M	*SD*	*M*	*SD*	*M*	*SD*
N2	0.275	0.180	2.031	0.428	0.0492	0.0251
tbh-1(n3247)	0.382	0.210	2.528**	0.287**	0.0555	0.0244
tbh-1(ok1196)	0.157	0.191	2.393*	0.309*	0.0322	0.0204
tdc-1(n3420)	0.187	0.132	1.611**	0.388**	0.0193***	0.0165***
tdc-1(n3419)	0.152	0.151	1.687*	0.376*	0.0248**	0.0163**
tdc-1(ok941)	0.114*	0.209*	2.316	0.430	0.0250**	0.0185**

* $p < .05$. ** $p < 0.01$. *** $p < 0.001$.

3.4 Effects of Tyramine and Octopamine Across Adulthood

Previous work by Timbers et al. (2013) identified that the rate of habituation is age dependent. Wild type worms were tested in the same tap habituation paradigm that was used in this thesis - 30 taps spaced 10-seconds apart - at four different adult timepoints. Timbers et al. (2013) found that older worms habituated more deeply than younger adult worms in response probability, but that reversal distance was not affected by age. My work with wild-type worms replicated these findings, with worms tested at 72-hours having significantly shallower habituated levels (as indicated by final response to tap) than worms tested at 96-hours of age. Further replication of these findings demonstrated that while the speed of wild-type reversal increased as the animal aged, these differences failed to reach significance (Timbers et al., 2013). Timbers et al. (2013) utilized these findings to conclude that the changes in tap habituation curves were the result of differences in response probability, but not other response-components.

3.4.1 Effects of Age on Response Probability

The habituation curves for N2 (Figure 6A), for each of the two *tbh-1* alleles (Figure 6B, C), and the three alleles of *tdc-1* (Figure 6D-F) for response probability at 72- and 96-hours of age replicated the results that were described for wild type worms in Timbers et al. (2013) indicated for N2 worms at these time points, with initial responses being roughly the same across age groups but worms at 96-hours post-egg-lay habituating much more deeply than their younger counterparts.

3.4.1.1 Statistical Analyses of Average Response Probability Across Age Groups

A two-way ANOVA to examine the effects of age and strain on the average probability of responding to tap was conducted on data for every strain examined in Experiments 1 and 2 (i.e., N2, *tbh-1[n3247]*, *tbh-1[ok1196]*, *tdc-1[n3420]*, *tdc-1[n3419]*, and *tdc-1[ok914]*). There was a statistically significant interaction between the age of the worms at testing and the strain being tested on the average response probability, F (5, 177) = 3.874, p = 0.0023, as well as a significant main effect of the allele, F (5, 177) = 380.1, $p < 0.0001$, and the age of the worms, F (5, 177) = 7.79, $p < 0.0001$, on the total average response probability. Put another way, this means that the differences in average response probability depends on the strain and the age of the worms being considered. All strains tested showed significantly different average response probability between 72- and 96-hour old worms ($p < 0.05$) when assessed using a Šidák post hoc test (Figure 6G). A review of the means indicated that the average of the probability of responding across all 30 taps was greater in 72-hour old worms than 96-hour old worms.

3.4.1.2 Statistical Analyses of Amount of Decrement in Response Probability

A two-way ANOVA to examine the effects of age and strain on the amount of response-decrement between the 1st and 30th tap for response probability was conducted on the data for all strains represented in Figure 6 A-F, (i.e., N2, *tbh-1[n3247]*, *tbh-1[ok1196]*, *tdc-1[n3420]*, *tdc-1[n3419]*, and *tdc-1[ok914]*). There was a statistically significant interaction between the age of the worms at testing and the strain being tested on the response-decrement in probability, F (5, 177) = 4.33, p = 0.0010, as well as a significant main effect of the allele, F (5, 177) = 9.010, $p < 0.0001$, and the age of the worms, F (5, 177) = 278.3, $p < 0.0001$, on the response-decrement of response probability. To control for Type I error rate across the six comparisons, we used a Šidák

adjustment. All strains tested had significantly different response-decrements in probability between 72- and 96-hour old worms ($p < 0.05$) (Figure 6H). A review of the means indicated that every strain had larger response-decrement differences at 96-hours of age than at 72.

3.4.2 Effect of Age on Response Duration

The average duration response curves for N2 worms at 72- and 96-hours of age are very similar (Figure 7A), as are the average response duration curves for the two alleles of *tbh-1* (Figure 7B, C), while the response duration curves for each of the three alleles of *tdc-1* (Figure 7D-F) appear to have steeper rates of habituation in this response measure, as indicated by the response durations to the final 2/3rds of the taps.

3.4.2.1 Statistical Analyses of Average Response Duration Across Age Groups

The two-way ANOVA comparing the effect of age and strain on average reversal duration for every strain in this experiment (i.e., N2, *tbh-1[n3247]*, *tbh-1[ok1196]*, *tdc-1[n3420]*, *tdc-1[n3419]*, and *tdc-1[ok914];* Figure 7A-F) revealed a significant effect of age, F (5, 177) = 45.63, $p < 0.0001$, and strain, F (5, 177) = 28.76, $p < 0.0001$, on average response probability, and a significant interaction between age and strain, F (5, 177) = 3.281, $p = 0.0074$. In the Šidák corrected analyses between the different age groups organized by strain, the wild type strain and both alleles of *tbh-1* were not significantly different across the age groups tested for average response duration. Interestingly, all three alleles of *tdc-1* had significantly different average response durations ($p < 0.05$) with 72-hour-old worms showing significantly longer duration reversals than 96-hour-old worms (Figure 7G). Thus, the average response duration is significantly different depending on both the age and the strain being considered, with only the alleles of *tdc-1* having statistically significant differences between the ages tested.

3.4.2.2 Statistical Analyses for Amount of Decrement in Response Duration

Interestingly, the effects of strain on response-decrement in duration were not dependent on the age that the worms were tested. The two-way ANOVA comparing the effect of age (i.e., 72- and 96-hours post-synchronization) and strain (i.e., N2, *tbh-1[n3247]*, *tbh-1[ok1196]*, *tdc-1[n3420]*, *tdc-1[n3419]*, and *tdc-1[ok914]*) on response-decrement on duration revealed a significant effect of strain, F (5, 177) = 16.92, $p < 0.0001$, but not age, nor an interaction between age and strain. In line with this, the Šidák post hoc test revealed no significant differences in response-decrement for any strain when compared to that same strain tested at a different age ($p < 0.05$) (Figure 7H).

3.4.3 Effects of Age on Response Speed

The average speed response curves were very similar across age groups not only for N2 worms (Figure 8A), but also for both alleles of *tbh-1* (Figure 8B, C) as well as all three *tdc-1* alleles (Figure 8D-F).

3.4.3.1 Statistical Analyses of Average Response Speed Across Age Groups

The effects of strain on the average response speed was not dependent on the age of the worm. The two-way ANOVA evaluating the effects of age and strain of the worms (i.e., N2, *tbh-1[n3247]*, *tbh-1[ok1196]*, *tdc-1[n3420]*, *tdc-1[n3419]*, and *tdc-1[ok914]*) on average response speed across age identified a significant effect of strain, F (5, 177) = 20.4, $p < 0.0001$, but not age, nor an interaction between strain and age. None of the Šidák adjusted post hoc comparisons identified significant changes in average response speed between the worms tested at 72-hours and worms tested at 96-hours post-egg-lay ($p < 0.05$) (Figure 8G). These data indicate that any

potential differences between strains tested was independent of the age at which the animal was tested.

3.4.3.2 Statistical Analyses of Amount of Decrement in Response Speed Across Age Groups

The response-decrements for response speed were similar between 96- and 72-hour-old worms for most of the strains tested (Figure 8H). The two-way ANOVA comparing the effect of age and strain on response-decrement in reversal speed for all strains tested across time (i.e., N2, *tbh-1[n3247]*, *tbh-1[ok1196]*, *tdc-1[n3420]*, *tdc-1[n3419]*, and *tdc-1[ok914]*) revealed a significant effect of age, F (5, 177) = 45.82, p < = 0.0001, and strain, F (5, 177) = 7.369, p < 0.0001, on average response-decrement in speed, and a significant interaction between age and strain, F (5, 177) = 4.238, p = 0.0012. These significant main effects and interactions did not translate to any consistently significant differences across alleles between the two ages being tested in this response-component ($p < 0.05$) (Figure 8H).

Thus, the response-decrements across ages showed remarkably similar response-decrements between the time points in the genotypes tested. Extrapolating from this, the wild-type age-dependent phenotypes in response-decrements between first and final tap do not appear to have contributions from tyramine or octopamine.

Figure 6. Age-Dependent Differences in Response Probability. The habituation curves of response probability of each strain vs themselves for A) N2, B) *tbh-1(n3247),* C) *tbh-1(ok1194),* D*) tdc-1(n3420),* E) *tdc-1(n3419),* and F) *tdc-1(ok914)* between worms that were tested at 72- and 96-hours post-egg-lay. G) The results of a post hoc Šidák's multiple comparisons test for the average response probability across all 30 taps following a significant two-way ANOVA of all strains. H) The results of a Šidák corrected post hoc comparison after an ordinary two-way ANOVA ($p < 0.05$) for average responses or response-decrements between 1st and 30th tap of each strain at each timepoint. '****' indicates $p \leq 0.0001$, and 'n.s.' indicates $p < 0.05$.

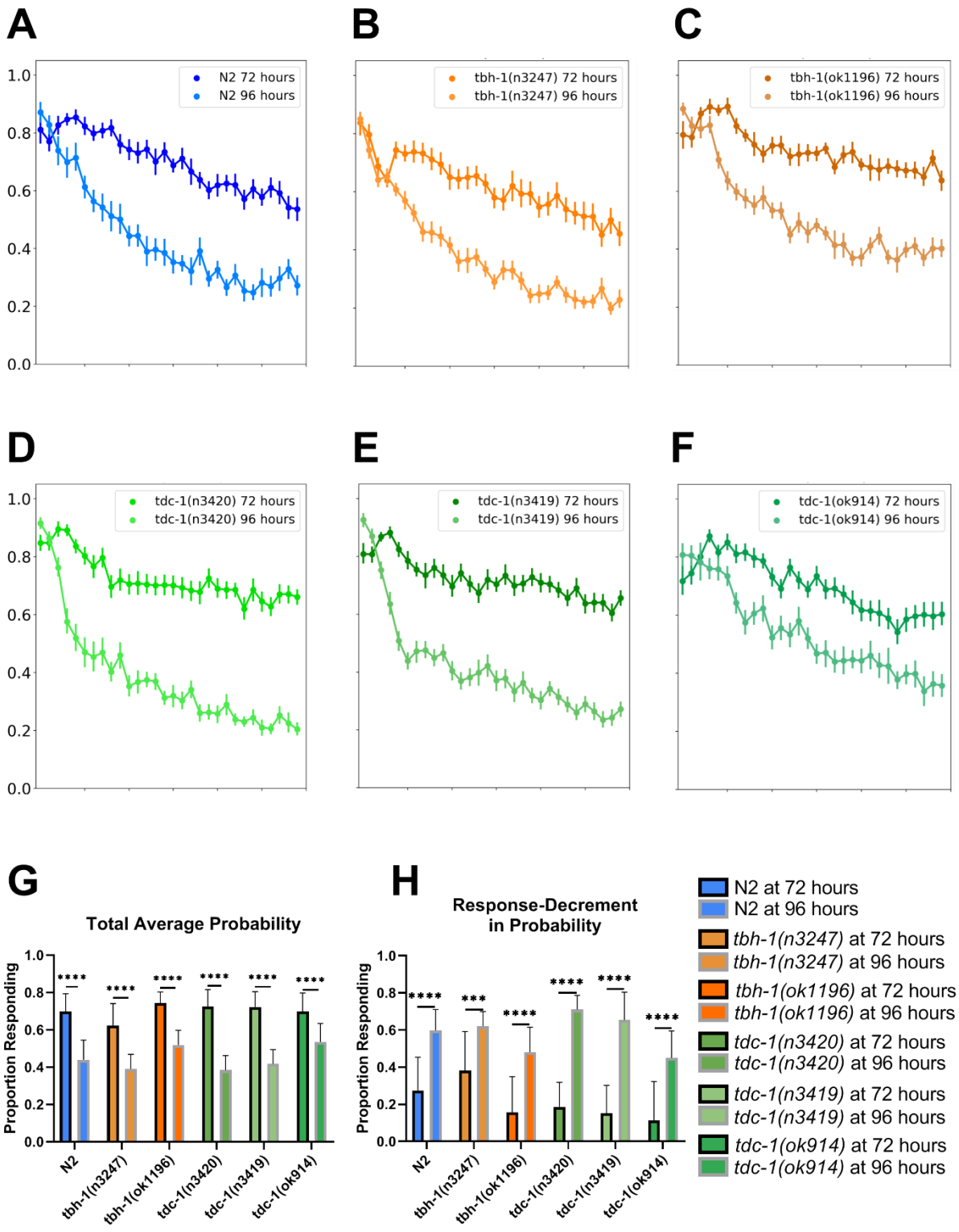

A
B
C
D
E
F
N2 72 hours
N2 96 hours
tbh-1(n3247) 72 hours
tbh-1(n3247) 96 hours
tbh-1(ok1196) 72 hours
tbh-1(ok1196) 96 hours
tdc-1(n3420) 72 hours
tdc-1(n3420) 96 hours
tdc-1(n3419) 72 hours
tdc-1(n3419) 96 hours
tdc-1(ok914) 72 hours
tdc-1(ok914) 96 hours
1.0
0.8
0.6
0.4
0.2
0.0
G
Total Average Probability
H
Response-Decrement in Probability
Proportion Responding
****
***
N2
tbh-1(n3247)
tbh-1(ok1196)
tdc-1(n3420)
tdc-1(n3419)
tdc-1(ok914)
N2 at 72 hours
N2 at 96 hours
tbh-1(n3247) at 72 hours
tbh-1(n3247) at 96 hours
tbh-1(ok1196) at 72 hours
tbh-1(ok1196) at 96 hours
tdc-1(n3420) at 72 hours
tdc-1(n3420) at 96 hours
tdc-1(n3419) at 72 hours
tdc-1(n3419) at 96 hours
tdc-1(ok914) at 72 hours
tdc-1(ok914) at 96 hours

Figure 7. Age-Dependent Differences in Response Duration. The habituation curves in the response duration of each strain vs themselves for A) N2, B) *tbh-1(n3247),* C) *tbh-1(ok1194),* D*) tdc-1(n3420),* E) *tdc-1(n3419),* and F) *tdc-1(ok914)* between worms that were tested at 72- and 96-hours post-egg-lay. G) The results of a post hoc Šidák's multiple comparisons test for the average response duration across all 30 taps following a significant two-way ANOVA of all strains. H) The results of a Šidák corrected post hoc comparison after an ordinary two-way ANOVA ($p < 0.05$) for response-decrements between 1st and 30th tap of each strain at each timepoint. '*' $p < .05$., '***' $p < 0.001$, '****' indicates $p \leq 0.0001$, and 'n.s.' indicates $p < 0.05$.

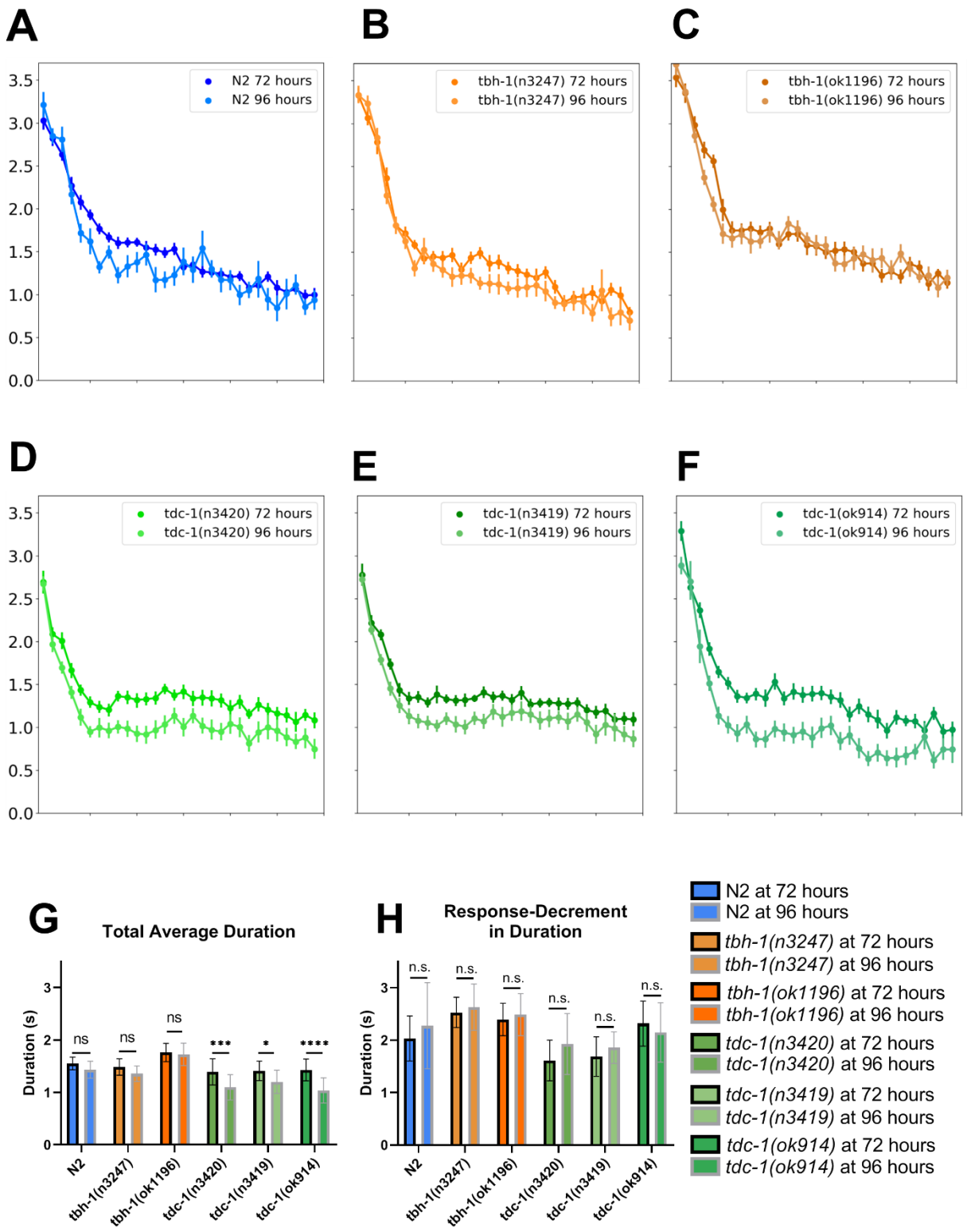
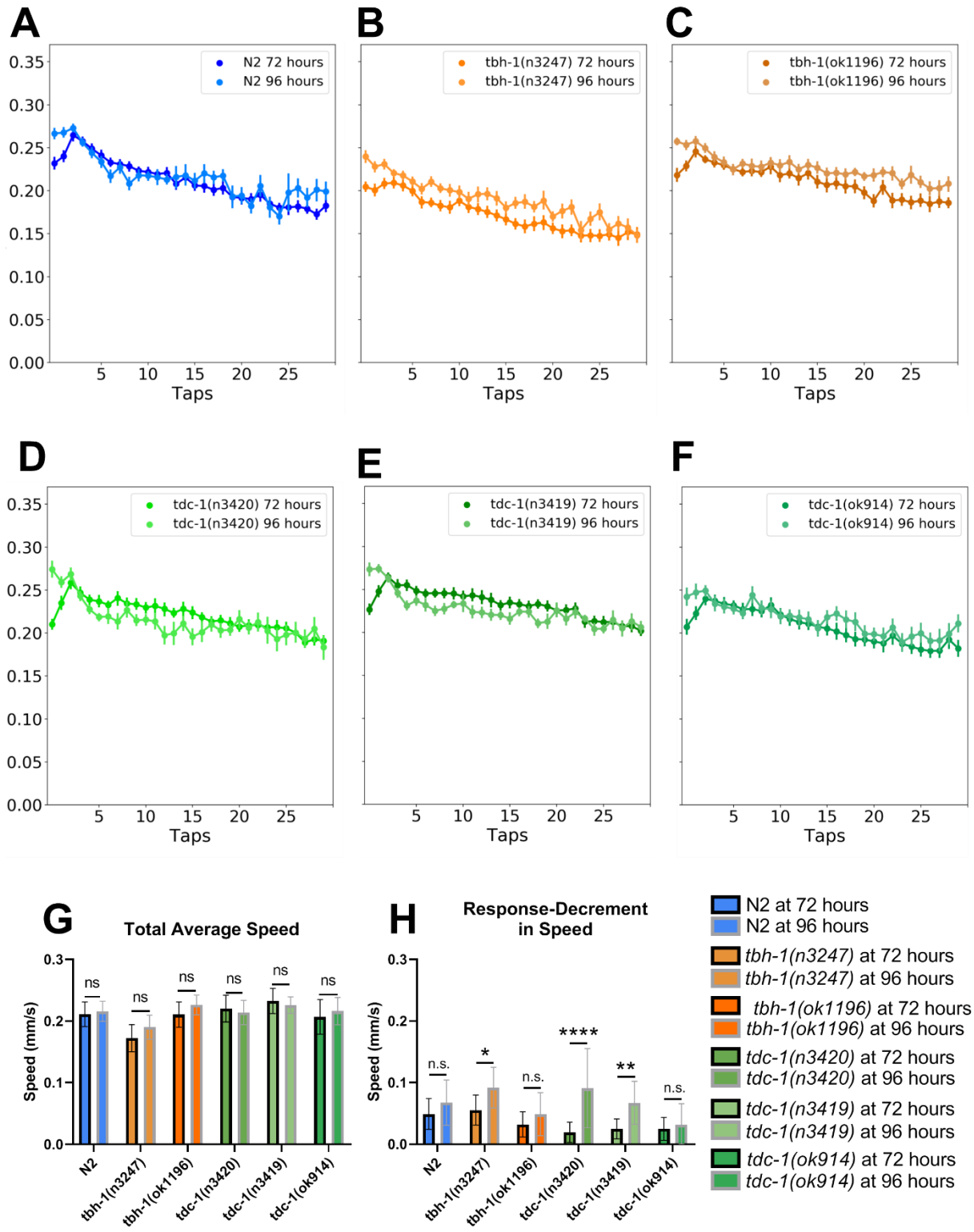

A
N2 72 hours
N2 96 hours
B
tbh-1(n3247) 72 hours
tbh-1(n3247) 96 hours
C
tbh-1(ok1196) 72 hours
tbh-1(ok1196) 96 hours
D
tdc-1(n3420) 72 hours
tdc-1(n3420) 96 hours
E
tdc-1(n3419) 72 hours
tdc-1(n3419) 96 hours
F
tdc-1(ok914) 72 hours
tdc-1(ok914) 96 hours
G
Total Average Duration
Duration (s)
ns
ns
ns
***
*
****
N2
tbh-1(n3247)
tbh-1(ok1196)
tdc-1(n3420)
tdc-1(n3419)
tdc-1(ok914)
H
Response-Decrement in Duration
Duration (s)
n.s.
N2 at 72 hours
N2 at 96 hours
tbh-1(n3247) at 72 hours
tbh-1(n3247) at 96 hours
tbh-1(ok1196) at 72 hours
tbh-1(ok1196) at 96 hours
tdc-1(n3420) at 72 hours
tdc-1(n3420) at 96 hours
tdc-1(n3419) at 72 hours
tdc-1(n3419) at 96 hours
tdc-1(ok914) at 72 hours
tdc-1(ok914) at 96 hours

Figure 8. Age-Dependent Differences in Response Speed. The habituation curves of response speed of each strain vs themselves for A) N2, B) *tbh-1(n3247)*, C) *tbh-1(ok1194)*, D*) tdc-1(n3420)*, E) *tdc-1(n3419)*, and F) *tdc-1(ok914)* between worms that were tested at 72- and 96-hours post-egg-lay. G) The results of a post hoc Šidák's multiple comparisons test for the average response speed across all 30 taps following a significant two-way ANOVA of all strains. H) The results of a Šidák corrected post hoc comparison after an ordinary two-way ANOVA ($p < 0.05$) for response-decrement between 1st and 30th tap of each strain at each timepoint. '*' $p <$ .05, '**' $p < 0.01$, '****' indicates $p \leq 0.0001$, and 'n.s.' indicates $p < 0.05$.


A
N2 72 hours
N2 96 hours
B
tbh-1(n3247) 72 hours
tbh-1(n3247) 96 hours
C
tbh-1(ok1196) 72 hours
tbh-1(ok1196) 96 hours
D
tdc-1(n3420) 72 hours
tdc-1(n3420) 96 hours
E
tdc-1(n3419) 72 hours
tdc-1(n3419) 96 hours
F
tdc-1(ok914) 72 hours
tdc-1(ok914) 96 hours
Taps
G
Total Average Speed
Speed (mm/s)
ns
H
Response-Decrement in Speed
n.s.
*
****
**
N2
tbh-1(n3247)
tbh-1(ok1196)
tdc-1(n3420)
tdc-1(n3419)
tdc-1(ok914)
N2 at 72 hours
N2 at 96 hours
tbh-1(n3247) at 72 hours
tbh-1(n3247) at 96 hours
tbh-1(ok1196) at 72 hours
tbh-1(ok1196) at 96 hours
tdc-1(n3420) at 72 hours
tdc-1(n3420) at 96 hours
tdc-1(n3419) at 72 hours
tdc-1(n3419) at 96 hours
tdc-1(ok914) at 72 hours
tdc-1(ok914) at 96 hours

Chapter 4: Conclusion

4.1 Discussion

The first objective of this research, as examined by Experiments 1 and 2, was to investigate the role of the biogenic amines tyramine and octopamine on tap habituation. Because there are many genes related to these two amines expressed in the tap circuit, I hypothesized that they might play a major role in this simple form of plasticity. The results did not support this hypothesis and these biogenic amines play an unexpectedly minor role in tap habituation.

The genes responsible for producing the biogenic amines tyramine and octopamine and receptors to bind them are heavily expressed within the neural circuitry responsible for the TWR. For this reason, in Experiments 1 and 2 I examined the roles of tyramine and/or octopamine in the habituation to tap within *C. elegans* by comparing the tap habituation curves of worms null in tyramine/octopamine biosynthesis with that of wild-type. Additionally, the expression profiles of these biosynthetic precursors vastly increase across the lifespan of *C. elegans*. Because of this, in Experiment 3 I compared the tap habituation of tyramine and/or octopamine null worms as well as wild type worms against themselves at two early adult timepoints, 3 and 4 days (~adolescent and early middle age) after their eggs were laid, to examine if these biogenic amines contribute to the age-dependent changes of wild-type tap habituation.

Additionally, although habituation is a relatively simple form of learning, the components of this behavioral response are not and there are many potential measurable variables. There are a number of different ways to statistically assess habituation in *C. elegans* research, in this study I chose to compare the average response values for each response-component to all tap stimuli as well as the amount of response-decrement between the first and final taps to reveal potential

differences in overall responsiveness and degree of habituation or decrement of response-components, respectively, for response probability, duration, and speed of reversal following tap as the data (McDiarmid et al 2020) suggests that these components have unique biological underpinnings.

Experiment 1, in which I compared the tap habituation of *tbh-1*, *tdc-1*, and wild type worms at 96-hours post-egg-lay (i.e., the standard developmental time point at which tap habituation experiments are performed in our lab), identified that all three alleles of *tdc-1* have significantly lower average response duration than wild type worms (Table 1, Figure 4H). This means that either tyramine or octopamine, both of which *tdc-1* mutants lack, contribute to the length of time that four-day old wild type worms reverse in response to time. The impact of tyramine and/or octopamine on duration in absence of other behavioral components is notable because duration of the TWR is an uncommon mutant phenotype (McDiarmid et al., 2017).

The goal of Experiment 2 was to elucidate the differences between *tbh-1*, *tdc-1*, and wild-type worms at 72-hours post-egg-lay (i.e., the youngest time-point at which tap-habituation can be reliably produced in *C. elegans* and the worms normally express less of both of these biogenic precursors). As with four-day old worms, the average response in duration of all three *tdc-1* strains were smaller than that of wild type at this time point, however these differences failed to reach significance (Table 3, Figure 5H). Notably, there were several response-components in which the response-decrements of all null alleles of a gene consistently differed significantly from N2 at 72-hours post-egg-lay (Figure 5K, L). The response-decrement in response duration revealed that both alleles of *tbh-1* had significantly larger decrements in response duration than wild type worms (Figure 5K). *tdc-1* mutants, which produce neither monoamine, tended to have smaller decrements in response (although these results were not consistently significant) (Figure

5K). Because *tbh-1* mutants produce no detectable amounts of octopamine while producing larger amounts of unconverted tyramine than wild-type levels, the larger response-decrement between first and final tap when compared to wild type may be the result of an absence of octopamine or over expression of tyramine. Together, these results indicate that either the presence or overabundance of tyramine result in deeper habituation to tap. On another note, all three alleles of *tdc-1* had significantly smaller response-decrements in response speed when compared to wild type, which suggests that either octopamine or tyramine plays a role in the depth of the habituation of response speed in wild type worms (Table 4, Figure 5L).

Animals generate complex patterns of behavior across development. Experiments 1 and 2 identified a time-dependent effect wherein the decrement of the average response duration of *tdc-1* mutants at 72-hours of age (Figure 5H) showed significant differences from N2 in older worms in average response duration, (Figure 4H). Response-decrements in duration and speed were only consistently significantly difference in worms tested at younger timepoints (Figure 5K, L) but not in these same alleles when tested at 96-hours of age (Figure 4K, L). These age-mediated differences support the hypothesis outlined in Goal 3 of the experiment, in that the differential expression of tyramine/octopamine play age-related roles in behavior throughout adulthood, and additionally suggest the possibility that there might be different critical periods at which these monoamines contribute to the behavior of wild type worms.

Experiment 3 identified the ways tyramine or octopamine affected the general deepening of habituation curves in animals tested at two different timepoints (i.e., 72- and 96-hours post-egg-lay) by comparing the habituation curves of different groups within each strain against themselves at both time points. The wild type controls in these experiments replicated the results first identified by Timbers et al. (2013), in which average response probability, (Figure 6G), but

not response duration (Figure 7G) or speed (Figure 8G), significantly differed between worms tested at 72- and 96-hours post-egg-lay. For the most part, the alleles of each gene consistently followed these patterns of significant differences (i.e., average response probability) or lack thereof (i.e., average response duration and speed) between these two time points that are portrayed in wild type worms. The notable exception to this is in average response duration, in which worms tested at 96-hours post-egg-lay responded to tap for an average of significantly less time than worms tested at 72-hours of age across all three alleles of *tdc-1* while the differences across age groups in wild type worms was not significantly different (Figure 7G). Consistent with Timbers et al. (2013), the response distance of wild type worms in my experiments were not significantly different between these timepoints, but significant difference in response duration was identified between wild type worms at 72- and 120-hours of age. The presence of consistently significant differences between *tdc-1* worms at this comparatively short age difference may suggest that *tdc-1* worms grow faster than wild type worms and have the body length of wild time worms at 120-hours of age (i.e., when the rate of habituation is steeper than that of worms tested at 72-hours of age). If size or developmental difference was the reason that for the average response duration differences identified here, then *tdc-1* worms would need to be larger than wild type at 96-hours post-egg-lay. Indeed, research by Yemini et al. (2013) identified that *tdc-1* mutants were longer than wild type worms at this time point. It should be noted that because the synchronization method in the Yemini et al. (2013) paper relied on stage matching rather than age matching, it is possible that the worms used in their studies were a range of ages and therefore difficult to extrapolate to acute differences in early adulthood. Because *tdc-1*, but not *tbh-1* mutants or wild type worms, had significant differences in the rate of habituation between time points it seems plausible that whatever biological mechanisms that

contribute to similar average response duration between 72- and 96-hours of age in wild type worms are dependent on the presence of tyramine and/or octopamine, but that an overexpression of tyramine does not affect this phenotype. Future experiments, which will be described later, will elucidate the degree to which each of these biogenic amines contribute to the age-related phenotype.

4.2 Limitations

While there were some notable exceptions, the total effect of tyramine and octopamine on tap habituation was surprisingly low. The reason these monoamines were being investigated in the context of tap habituation was because of the ample expression of their receptors and production of the chemicals themselves within this circuit (Figure 3). The action of biogenic amine receptors in the tap habituation has profound phenotypes on tap habituation when studied in the context of dopamine, which has only a single receptor expressed in this circuit, in contrast to the many tyramine receptors in this circuit (Kindt et al., Sanyal et al., 2004).

Any phenotypes discovered in *tdc-1* null worms may be the result of both tyramine and octopamine, or just one of these amines while the other is not impactful. Any *lack* of observed phenotypes may be the result of potential antagonistic roles between tyramine and octopamine. Indeed, tyramine and octopamine have had noted antagonistic interactions in other behaviors, including locomotion. The individual impacts of tyramine and octopamine might be more striking than what was reported with these paradigms, but further research is needed to identify these potential impacts.

4.3 Future Directions

This lack of profound difference in tyramine/octopamine null worms may simply be the result of minimal impact of these neurotransmitters in tap habituation, but this is difficult to

confirm given the limitations of our experimental design. Because tyramine is the precursor to octopamine in the same biosynthetic pathway (Figure 1), these results (or lack thereof) in *tbh-1* null worms could be the phenotypic output of an overexpression of tyramine rather than the absence of octopamine.

One way around this might be placing biologically relevant amounts of pharmacological tyramine or octopamine on the plates of worms expressing null *tbh-1* and *tdc-1* alleles together (*tbh-1;tdc-1* double). Similar to worms that are null in just *tdc-1*, *tdc-1;tbh-1* double mutants would be null in both tyramine and octopamine. However, exogenous tyramine could be applied to the testing plates housing these double mutants without being converted into octopamine. Any differences between *tdc-1;tbh-1* + exogenous tyramine and N2 would theoretically be due to only the absence of octopamine. Similarly, the application or absence of exogenous octopamine to the double mutant would theoretically produce phenotypes that result only from the absence of tyramine.

Additionally, the individual contributions of tyramine and octopamine could be experimentally deduced by testing mutants null in the octopamine receptor *octr-1*. While still creating the same amounts of tyramine and octopamine relative to wild type worms, octopamine would be unable to bind directly to any neurons in the tap habituation neural circuitry. Therefore, any statistically significant differences between wild type and *octr-1* mutants would be the result of octopamine specifically. One could similarly also create a mutant that is null in all four tyramine receptors, because all four known *C. elegans* tyramine receptors are expressed at some point in the tap habituation neural circuitry, and the differences between these mutants and wild type worms in tap habituation would be the result of tyramine alone.

In addition to being tested at 72- or 96-hours post-egg-lay, there are potential benefits in observing the trends of the duration and speed phenotypes that were noted across a larger difference in age. I would test N2 and *tdc-1;tbh-1* strains with both +/- exogenous tyramine and octopamine at 120-hours post-egg-lay in this new paradigm. It would be interesting to see whether the age-dependent changes of phenotypes in worms null in tyramine and/or octopamine would continue their trajectories in older worms.

Another possibility to consider is that octopamine is considered an invertebrate counterpart to noradrenaline/norepinephrine that in mammals plays a role in regulating response vigor, i.e. how persistent an animal will be in responding (Liu et al., 2019). It is interesting to speculate whether response duration is a measure of response persistence, in which case this could be the foundation of experiments relating the effects of octopamine on habituation with results on response persistence in mammals with depleted noradrenaline.

4.4 Contributions to Tap Habituation

The focus of my thesis was to expand on our knowledge of behavioral impact of two monoamines whose' receptors are broadly expressed in the tap habituation circuits. I have achieved this goal in several ways. Firstly, I identified that worms null in both tyramine and octopamine have an age-dependent effect on average response duration in isolation of other response-components or other measures of habitation. The isolation of this impact is notable because of its specificity; very few genes affect just response duration (McDiarmid et al., 2017). This means that either of these neurotransmitters could be contributing to the biological mechanisms unique to this response-component, and that knowledge may be useful in further research elucidating these independent molecular pathways.

Secondly, the age-dependent increase or decrease in different measurements of the same behavior (e.g., *tdc-1* compared to N2 at 72-, but not 96-, hours when just considering response-decrement) hinted at potential periods of sensitivity for these biogenic amines in the biological mechanisms unique to each response-component. This supported our hypothesis that the varying expression levels of tyramine and octopamine may differentially impact the same behavioral measurement.

The age-specific neuromodulatory effects suggested by this thesis joins the growing body of research that has implicated other biogenic amines in stage-specific neuromodulatory effects on behavior. One example of this is how worms deficient in serotonin have roaming speeds similar to that of wild type worms at larval stage 1 but are faster in all subsequent stages, even though other components of roaming (e.g., fraction of time spent roaming) was consistently increased or indistinguishable across every developmental timepoint (Stern et al., 2017).

While the age-dependent effects of tyramine and octopamine on habituation of response duration reported in this thesis support our original hypothesis of age-dependent effects of tyramine and octopamine on behavior, the results presented here are surprisingly few and far between. The expression of tyramine and octopamine receptors in so many of the neurons in the neural circuitry of the tap withdrawal response suggested that they had profound roles in the behaviors resulting from these neurons, but the massive expression didn't appropriately reflect impacts on tap habituation. It will be interesting to discover what role(s) they do have in *C. elegans* behavior.

Much more work is needed to continue this research in order to identify the individual contributions of these neurotransmitters in the pathways underlying tap habituation; however,

this contribution highlights that differences do exist and moves us a critical step closer to understanding the mechanism of habituation in *C. elegans*.

Bibliography

Abdi, H. (2007). The Bonferonni and Šidák Corrections for Multiple Comparisons. In N. J. Salkind (Ed.), *Encyclopedia of Measurement and Statistics* (Vol. 1, pp. 103–107). Sage Publications, Inc. https://doi.org/10.4135/9781412952644.n60

Alkema, M. J., Hunter-Ensor, M., Ringstad, N., & Horvitz, H. R. (2005). Tyramine functions independently of octopamine in the *Caenorhabditis elegans* nervous system. *Neuron*, *46*(2), 247–260. https://doi.org/10.1016/j.neuron.2005.02.024

Ballestriero, F., Nappi, J., Zampi, G., Bazzicalupo, P., Schiavi, E. Di, & Egan, S. (2016). *Caenorhabditis elegans* employs innate and learned aversion in response to bacterial toxic metabolites tambjamine and violacein OPEN. *Nature Publishing Group*. https://doi.org/10.1038/srep29284

Barstead, R., Moulder, G., Cobb, B., Frazee, S., Henthorn, D., Holmes, J., Jerebie, D., Landsdale, M., Osborn, J., Pritchett, C., Robertson, J., Rummage, J., Stokes, E., Vishwanathan, M., Mitani, S., Gengyo-Ando, K., Funatsu, O., Hori, S., Imae, R., … Zapf, R. (2012). Large-scale screening for targeted knockouts in the *caenorhabditis elegans* genome. *G3: Genes, Genomes, Genetics*, *2*(11), 1415–1425. https://doi.org/10.1534/g3.112.003830

Ben Arous, J., Laffont, S., & Chatenay, D. (2009). Molecular and sensory basis of a food related two-state behavior in *C. elegans*. *PLoS ONE*, *4*(10), 7584. https://doi.org/10.1371/journal.pone.0007584

Bendesky, A., Tsunozaki, M., Rockman, M. V., Kruglyak, L., & Bargmann, C. I. (2011). Catecholamine receptor polymorphisms affect decision-making in *C. elegans*. *Nature*, *472*(7343), 313–318. https://doi.org/10.1038/nature09821

Bentley, B., Branicky, R., Barnes, C. L., Chew, Y. L., Yemini, E., Bullmore, E. T., Vértes, P. E., & Schafer, W. R. (2016). The Multilayer Connectome of *Caenorhabditis elegans*. *PLoS Computational Biology*, *12*(12), 1005283. https://doi.org/10.1371/journal.pcbi.1005283

Bettinger, J. C., & McIntire, S. L. (2004). State-dependency in *C. elegans*. *Genes, Brain and Behavior*, *3*(5), 266–272. https://doi.org/10.1111/j.1601-183X.2004.00080.x

Bianchi, L., Kwok, S. M., Driscoll, M., & Sesti, F. (2003). A potassium channel-MiRP complex controls neurosensory function in *Caenorhabditis elegans*. *Journal of Biological Chemistry*, *278*(14), 12415–12424. https://doi.org/10.1074/jbc.M212788200

Boulin, T., & Hobert, O. (2012). From genes to function: The *C. elegans* genetic toolbox. In *Wiley Interdisciplinary Reviews: Developmental Biology* (Vol. 1, Issue 1, pp. 114–137). NIH Public Access. https://doi.org/10.1002/wdev.1

Branicky, R., & Schafer, W. R. (2009). Tyramine: A New Receptor and a New Role at the Synapse. In *Neuron* (Vol. 62, Issue 4, pp. 458–460). Cell Press. https://doi.org/10.1016/j.neuron.2009.05.005

Brenner, S. (1974). THE GENETICS OF *CAENORHABDITIS ELEGANS*. *Genetics*, *77*(1).

Byrne, J. H. (1982). Analysis of synaptic depression contributing to habituation of Gill-withdrawal reflex in Aplysia californica. *Journal of Neurophysiology*, *48*(2), 431–438. https://doi.org/10.1152/jn.1982.48.2.431

Cai, S. Q., Wang, Y., Park, K. H., Tong, X., Pan, Z., & Sesti, F. (2009). Auto-phosphorylation of a voltage-gated K channel controls non-associative learning. *EMBO Journal*, *28*(11), 1601–1611. https://doi.org/10.1038/emboj.2009.112

Carnell, L., Illi, J., Hong, S. W., & McIntire, S. L. (2005). The G-protein-coupled serotonin receptor SER-1 regulates egg laying and male mating behaviors in *Caenorhabditis elegans*. *Journal of Neuroscience*, *25*(46), 10671–10681. https://doi.org/10.1523/JNEUROSCI.3399-05.2005

Chalfie, M., Sulston, J. E., White, J. G., Southgate, E., Thomson, J. N., & Brenner, S. (1985). The neural circuit for touch sensitivity in *Caenorhabditis elegans*. *Journal of Neuroscience*, *5*(4), 956–964. https://doi.org/10.1523/jneurosci.05-04-00956.1985

Chao, M. Y., Komatsu, H., Fukuto, H. S., Dionne, H. M., & Hart, A. C. (2004). Feeding status and serotonin rapidly and reversibly modulate a *Caenorhabditis elegans* chemosensory circuit. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(43), 15512–15517. https://doi.org/10.1073/pnas.0403369101

Chase, D. L., Pepper, J. S., & Koelle, M. R. (2004). Mechanism of extrasynaptic dopamine signaling in *Caenorhabditis elegans*. *Nature Neuroscience*, *7*(10), 1096–1103. https://doi.org/10.1038/nn1316

Churgin, M. A., McCloskey, R. J., Peters, E., & Fang-Yen, C. (2017). Antagonistic serotonergic and octopaminergic neural circuits mediate food-dependent locomotory behavior in *Caenorhabditis elegans*. *Journal of Neuroscience*, *37*(33), 7811–7823. https://doi.org/10.1523/JNEUROSCI.2636-16.2017

Colbert, H. A., & Bargmann, C. I. (1995). Odorant-specific adaptation pathways generate olfactory plasticity in *C. elegans*. *Neuron*, *14*(4), 803–812. https://doi.org/10.1016/0896-6273(95)90224-4

Donnelly, J. L., Clark, C. M., Leifer, A. M., Pirri, J. K., Haburcak, M., Francis, M. M., Samuel, A. D. T., & Alkema, M. J. (2013). Monoaminergic orchestration of motor programs in a complex *C. elegans* behavior. *PLoS Biology*, *11*(4), e1001529. https://doi.org/10.1371/journal.pbio.1001529

Ezak, M. J., & Ferkey, D. M. (2010). The *C. elegans* D2-like dopamine receptor DOP-3 decreases behavioral sensitivity to the olfactory stimulus 1-Octanol. *PLoS ONE*, *5*(3), e9487. https://doi.org/10.1371/journal.pone.0009487

Flavell, S. W., Pokala, N., Macosko, E. Z., Albrecht, D. R., Larsch, J., & Bargmann, C. I. (2013). Serotonin and the neuropeptide PDF initiate and extend opposing behavioral states in *C. Elegans*. *Cell*, *154*(5), 1023–1035. https://doi.org/10.1016/j.cell.2013.08.001

Gerstein, M. B., Lu, Z. J., Van Nostrand, E. L., Cheng, C., Arshinoff, B. I., Liu, T., Yip, K. Y., Robilotto, R., Rechtsteiner, A., Ikegami, K., Alves, P., Chateigner, A., Perry, M., Morris, M., Auerbach, R. K., Feng, X., Leng, J., Vielle, A., Niu, W., … Waterston, R. H. (2010). Integrative analysis of the *Caenorhabditis elegans* genome by the modENCODE project. *Science*, *330*(6012), 1775–1787. https://doi.org/10.1126/science.1196914

Gray, J. M., Hill, J. J., & Bargmann, C. I. (2005). A circuit for navigation in *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(9), 3184–3191. https://doi.org/10.1073/pnas.0409009101

Gürel, G., Gustafson, M. A., Pepper, J. S., Robert Horvitz, H., & Koelle, M. R. (2012). Receptors and other signaling proteins required for serotonin control of locomotion in *Caenorhabditis elegans*. *Genetics*, *192*(4), 1359–1371. https://doi.org/10.1534/genetics.112.142125

Hare, E. E., & Loer, C. M. (2004). Function and evolution of the serotonin-synthetic bas-1 gene and other aromatic amino acid decarboxylase genes in Caenorhabditis. *BMC Evolutionary Biology*, *4*, 24. https://doi.org/10.1186/1471-2148-4-24

Harris, J. D. (1943). Habituatory response decrement in the intact organism. *Psychological Bulletin*, *40*(6), 385–422. https://doi.org/10.1037/h0053918

Harris, G., Mills, H., Wragg, R., Hapiak, V., Castelletto, M., Korchnak, A., & Komuniecki, R. W. (2010). The monoaminergic modulation of sensory-mediated aversive responses in *Caenorhabditis elegans* requires glutamatergic/peptidergic cotransmission. *Journal of Neuroscience*, *30*(23), 7889–7899. https://doi.org/10.1523/JNEUROSCI.0497-10.2010

He, F. (2011). Common worm media and buffers. *BIO-PROTOCOL*, *1*(7). https://doi.org/10.21769/bioprotoc.55

Hills, T., Brockie, P. J., & Maricq, A. V. (2004). Dopamine and glutamate control area-restricted search behavior in *Caenorhabditis elegans*. *Journal of Neuroscience*, *24*(5), 1217–1225. https://doi.org/10.1523/JNEUROSCI.1569-03.2004

Hobson, R. J., Hapiak, V. M., Xiao, H., Buehrer, K. L., Komuniecki, P. R., & Komuniecki, R. W. (2006). SER-7, a *Caenorhabditis elegans* 5-HT7-like receptor, is essential for the 5-HT stimulation of pharyngeal pumping and egg laying. *Genetics*, *172*(1), 159–169. https://doi.org/10.1534/genetics.105.044495

Horvitz, H. R., Chalfie, M., Trent, C., Sulston, J. E., & Evans, P. D. (1982). Serotonin and octopamine in the nematode *Caenorhabditis elegans*. *Science*, *216*(4549), 1012–1014. https://doi.org/10.1126/science.6805073

Huberty, C. J., & Morris, J. D. (1989). Multivariate analysis versus multiple univariate analyses. In *Psychological Bulletin, 105*(2), 302–308. https://doi.org/10.1037/0033-2909.105.2.302

Hukema, R. K., Rademakers, S., & Jansen, G. (2008). Gustatory plasticity in *C. elegans* involves integration of negative cues and NaCl taste mediated by serotonin, dopamine, and glutamate. *Learning and Memory, 15*(11), 829–836. https://doi.org/10.1101/lm.994408

Jorgensen, E. M., Kaplan, J. M., Chase, D. L., & Koelle, M. R. (2007). Biogenic amine neurotransmitters in *C. elegans*. *WormBook*, The *C. elegans* Research Community, WormBook, https://doi.org/10.1895/wormbook.1.132.1

Kindt, K. S., Quast, K. B., Giles, A. C., De, S., Hendrey, D., Nicastro, I., Rankin, C. H. H., & Schafer, W. R. (2007). Dopamine mediates context-dependent modulation of sensory plasticity in *C. elegans*. *Neuron*, *55*(4), 662–676. https://doi.org/10.1016/j.neuron.2007.07.023

Kratsios, P., Kerk, S. Y., Catela, C., Liang, J., Vidal, B., Bayer, E. A., Feng, W., De La Cruz, E. D., Croci, L., Giacomo Consalez, G., Mizumoto, K., & Hobert, O. (2017). An intersectional gene regulatory strategy defines subclass diversity of *C. Elegans* motor neurons. *ELife*, *6*. https://doi.org/10.7554/eLife.25751

Kudlow, B. A., Zhang, L., & Han, M. (2012). Systematic analysis of tissue-restricted miRISCs reveals a broad role for MicroRNAs in suppressing basal activity of the *C. elegans* pathogen response. *Molecular Cell*, *46*(4), 530–541. https://doi.org/10.1016/j.molcel.2012.03.011

Lee, R. Y. N., Sawin, E. R., Chalfie, M., Horvitz, H. R., & Avery, L. (1999). EAT-4, a homolog of a mammalian sodium-dependent inorganic phosphate cotransporter, is necessary for glutamatergic

neurotransmission in *Caenorhabditis elegans*. *Journal of Neuroscience*, *19*(1), 159–167. https://doi.org/10.1523/jneurosci.19-01-00159.1999

Leifer, A. M., Fang-Yen, C., Gershow, M., Alkema, M. J., & Samuel, A. D. T. (2011). Optogenetic manipulation of neural activity in freely moving *Caenorhabditis elegans*. *Nature Methods*, *8*(2), 147–152. https://doi.org/10.1038/nmeth.1554

Lemieux, G. A., Cunningham, K. A., Lin, L., Mayer, F., Werb, Z., & Ashrafi, K. (2015). Kynurenic acid is a nutritional cue that enables behavioral plasticity. *Cell*, *160*(1–2), 119–131. https://doi.org/10.1016/j.cell.2014.12.028

Li, W., Feng, Z., Sternberg, P. W., & Xu, X. Z. S. (2006). A *C. elegans* stretch receptor neuron revealed by a mechanosensitive TRP channel homologue. *Nature*, *440*(7084), 684–687. https://doi.org/10.1038/nature04538

Li, W., Kang, L., Piggott, B. J., Feng, Z., & Xu, X. Z. S. (2011). The neural circuits and sensory channels mediating harsh touch sensation in *Caenorhabditis elegans*. *Nature Communications*, *2*(1), 315. https://doi.org/10.1038/ncomms1308

Liu, H., Qin, L. W., Li, R., Zhang, C., Al-Sheikh, U., & Wu, Z. X. (2019). Reciprocal modulation of 5-HT and octopamine regulates pumping via feedforward and feedback circuits in *C. Elegans*. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(14), 7107–7112. https://doi.org/10.1073/pnas.1819261116

Loer, C. M., & Kenyon, C. J. (1993). Serotonin-deficient mutants and male mating behavior in the nematode *Caenorhabditis elegans*. *Journal of Neuroscience*, *13*(12), 5407–5417. https://doi.org/10.1523/jneurosci.13-12-05407.1993

McDiarmid, T. A., Yu, A. J., & Rankin, C. H. (2018). Beyond the response-High throughput behavioral analyses to link genome to phenome in *Caenorhabditis elegans*. *Genes, Brain, and Behavior*, *17*(3), 1–14. https://doi.org/10.1111/gbb.12437

Mendel, J. E., Korswagen, H. C., Liu, K. S., Hajdu-Cronin, Y. M., Simon, M. I., Plasterk, R. H. A., & Sternberg, P. W. (1995). Participation of the protein G_o in multiple aspects of behavior in *C. elegans*. *Science*, *267*(5204), 1652–1655. https://doi.org/10.1126/science.7886455

Mills, H., Wragg, R., Hapiak, V., Castelletto, M., Zahratka, J., Harris, G., Summers, P., Korchnak, A., Law, W., Bamber, B., & Komuniecki, R. (2012). Monoamines and neuropeptides interact to inhibit aversive behaviour in *Caenorhabditis elegans*. *EMBO Journal*, *31*(3), 667–678. https://doi.org/10.1038/emboj.2011.422

Movson, A., & Seung, S. (2012). *Connectomics: Sebastian Seung vs. Tony Movshon* [Video]. YouTube. https://www.youtube.com/watch?v=q4KrhDZQ088

Nai, Q., Dong, H. W., Linster, C., & Ennis, M. (2010). Activation of α1 and α2 noradrenergic receptors exert opposing effects on excitability of main olfactory bulb granule cells. *Neuroscience*, *169*(2), 882–892. https://doi.org/10.1016/j.neuroscience.2010.05.010

Nagel, G., Brauner, M., Liewald, J. F., Adeishvili, N., Bamberg, E., & Gottschalk, A. (2005). Light activation of Channelrhodopsin-2 in excitable cells of *Caenorhabditis elegans* triggers rapid behavioral responses. *Current Biology*, *15*(24), 2279–2284. https://doi.org/10.1016/j.cub.2005.11.032

Niacaris, T., & Avery, L. (2003). Serotonin regulates repolarization of the *C. elegans* pharyngeal muscle. *Journal of Experimental Biology*, *206*(2), 223–231. https://doi.org/10.1242/jeb.00101

O'Hagan, R., Chalfie, M., & Goodman, M. B. (2005). The MEC-4 DEG/ENaC channel of *Caenorhabditis elegans* touch receptor neurons transduces mechanical signals. *Nature Neuroscience*, *8*(1), 43–50. https://doi.org/10.1038/nn1362

Osuna-Luque, J., Rodríguez-Ramos, Á., Gámez-del-Estal, M. M., & Ruiz-Rubio, M. (2018). Behavioral mechanisms that depend on dopamine and serotonin in *Caenorhabditis elegans* interact with the antipsychotics risperidone and aripiprazole. *Journal of Experimental Neuroscience*, *12*, 1-11. https://doi.org/10.1177/1179069518798628

Packham, R., Walker, R. J., & Holden-Dye, L. (2010). The effect of a selective octopamine antagonist, epinastine, on pharyngeal pumping in *Caenorhabditis elegans*. *Invertebrate Neuroscience*, *10*(1), 47–52. https://doi.org/10.1007/s10158-010-0107-9

Pirri, J. K., & Alkema, M. J. (2012). The neuroethology of *C. elegans* escape. *Current Opinion in Neurobiology*, *22*(2), 187–196. https://doi.org/10.1016/j.conb.2011.12.007

Li, C., Timbers, T. A., Rose, J. K., Bozorgmehr, T., McEwan, A., & Rankin, C. H. (2013). The FMRFamide-related neuropeptide FLP-20 is required in the mechanosensory neurons during memory for massed training in *C. elegans*. *Learning and Memory*, *20*(2), 103–108. https://doi.org/10.1101/lm.028993.112

Raizen, D. M., Zimmerman, J. E., Maycock, M. H., Ta, U. D., You, Y. J., Sundaram, M. V., & Pack, A. I. (2008). Lethargus is a *Caenorhabditis elegans* sleep-like state. *Nature*, *451*(7178), 569–572. https://doi.org/10.1038/nature06535

Rankin, C. H., Beck, C. D., & Chiba, C. M. (1990). *Caenorhabditis elegans*: A new model system for the study of learning and memory. *Behavioural Brain Research*, *37*(1), 89–92. https://doi.org/10.1016/0166-4328(90)90074-O

Rankin, C. H., & Broster, B. S. (1992). Factors affecting habituation and recovery from habituation in the nematode *Caenorhabditis elegans*. *Behavioral Neuroscience*, *106*(2), 239–249. https://doi.org/10.1037/0735-7044.106.2.239

Rankin, C. H., & Carew, T. J. (1987). Development of learning and memory in Aplysia. II. Habituation and dishabituation. *Journal of Neuroscience*, *7*(1), 133–143. https://doi.org/10.1523/jneurosci.07-01-00133.1987

Rankin, C. H., Gannon, T., & Wicks, S. R. (2000). Developmental analysis of habituation in the nematode *C. elegans*. *Developmental Psychobiology*, *36*(4), 261–270. https://doi.org/10.1002/(SICI)1098-2302(200005)36:4<261::AID-DEV1>3.0.CO;2-7

Rankin, C. H., & Wicks, S. R. (2000). Mutations of the *Caenorhabditis elegans* brain-specific inorganic phosphate transporter eat-4 affect habituation of the tap-withdrawal response without affecting the response itself. *Journal of Neuroscience*, *20*(11), 4337–4344. https://doi.org/10.1523/jneurosci.20-11-04337.2000

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*(1), 181–191. https://doi.org/10.1111/j.1471-4159.2005.03180.x

Rex, E., Molitor, S. C., 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*(5), 1104–1115. https://doi.org/10.1111/j.1471-4159.2004.02787.x

Riddle, D., Blumenthal, T., & Meyer, B. (1998). Behavioral plasticity in the adult. In *C. Elegans II* (2nd ed., Ser. 33). New York, New York: Cold Spring Harbor Laboratory Press. ISBN: 978-087969532-3

Riley, S. P., Woodman, M. E., Holt, J., & Stevenson, B. (2017). Culture of *Escherichia coli* and Related Bacteria. *Current Protocols Essential Laboratory Techniques*, *15*(1), 4.2.1-4.2.30. https://doi.org/10.1002/cpet.17

Rogers, C. M., Franks, C. J., Walker, R. J., Burke, J. F., & Holden-Dye, L. (2001). Regulation of the pharynx of *Caenorhabditis elegans* by 5-HT, octopamine, and FMRF amide-like neuropeptides. *Journal of Neurobiology*, *49*(3), 235–244. https://doi.org/10.1002/neu.1078

Rose, J. K., & Rankin, C. H. (2001). Analyses of habituation in *Caenorhabditis elegans*. In *Learning and Memory* (Vol. 8, Issue 2, pp. 63–69). Cold Spring Harbor Laboratory Press. https://doi.org/10.1101/lm.37801

Sanyal, S., Wintle, R. F., Kindt, K. S., Nuttley, W. M., Arvan, R., Fitzmaurice, P., Bigras, E., Merz, D. C., Hébert, T. E., van der Kooy, D., Schafer, W. R., Culotti, J. G., & Van Tol, H. H. M. (2004). Dopamine modulates the plasticity of mechanosensory responses in *Caenorhabditis elegans*. *The EMBO Journal*, *23*(2), 473–482. https://doi.org/10.1038/sj.emboj.7600057

Sawin, E. R., Ranganathan, R., & Horvitz, H. R. (2000). *C. elegans* locomotory rate is modulated by the environment through a dopaminergic pathway and by experience through a serotonergic pathway. *Neuron, 26*(3), 619–631. https://doi.org/10.1016/S0896-6273(00)81199-X

Schafer, W. R. (2006). Neurophysiological methods in *C. elegans*: an introduction. *WormBook: The Online Review of C. Elegans Biology*. https://doi.org/10.1895/wormbook.1.111.1

Ségalat, L., Elkes, D. A., & Kaplan, J. M. (1995). Modulation of serotonin-controlled behaviors by G_o in *Caenorhabditis elegans*. *Science*, *267*(5204), 1648–1651. https://doi.org/10.1126/science.7886454

Serrano-Saiz, E., Poole, R. J., Felton, T., Zhang, F., De La Cruz, E. D., & Hobert, O. (2013). Modular control of glutamatergic neuronal identity in *C. elegans* by distinct homeodomain proteins. *Cell*, *155*(3), 659. https://doi.org/10.1016/j.cell.2013.09.052

Singh, K., Ju, J. Y., Walsh, M. B., DiIorio, M. A., & Hart, A. C. (2014). Deep conservation of genes required for both *Drosophila melanogaster* and *Caenorhabditis elegans* sleep includes a role for dopaminergic signaling. *Sleep*, *37*(9), 1439–1451. https://doi.org/10.5665/sleep.3990

Stephens, G. J., Johnson-Kerner, B., Bialek, W., & Ryu, W. S. (2010). From modes to movement in the behavior of *Caenorhabditis elegans*. *PLoS ONE*, *5*(11), 13914. https://doi.org/10.1371/journal.pone.0013914

Stern, S., Kirst, C., & Bargmann, C. I. (2017). Neuromodulatory Control of Long-Term Behavioral Patterns and Individuality across Development. *Cell*, *171*(7), 1649-1662.e10. https://doi.org/10.1016/j.cell.2017.10.041

Sulston, J., Dew, M., & Brenner, S. (1975). Dopaminergic neurons in the nematode *Caenorhabditis elegans*. *Journal of Comparative Neurology*, *163*(2), 215–226. https://doi.org/10.1002/cne.901630207

Sulston, J. E., Schierenberg, E., White, J. G., & Thomson, J. N. (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Developmental Biology*. Dev Biol. https://doi.org/10.1016/0012-1606(83)90201-4

Suzuki, H., Kerr, R., Bianchi, L., Frøkjær-Jensen, C., Slone, D., Xue, J., Gerstbrein, B., Driscoll, M., & Schafer, W. R. (2003). In vivo imaging of *C. elegans* mechanosensory neurons demonstrates a specific role for the MEC-4 channel in the process of gentle touch sensation. *Neuron*, *39*(6), 1005–1017. https://doi.org/10.1016/j.neuron.2003.08.015

Swierczek, N. A., Giles, A. C., Rankin, C. H., & Kerr, R. A. (2011). High-throughput behavioral analysis in *C. elegans*. *Nature Methods*, *8*(7), 592–602. https://doi.org/10.1038/nmeth.1625

Tao, J., Ma, Y. C., Yang, Z. S., Zou, C. G., & Zhang, K. Q. (2016). Octopamine connects nutrient cues to lipid metabolism upon nutrient deprivation. *Science Advances*, *2*(5). https://doi.org/10.1126/sciadv.1501372

Timbers, T. A., Giles, A. C., Ardiel, E. L., Kerr, R. A., & Rankin, C. H. (2013). Intensity discrimination deficits cause habituation changes in middle-aged *Caenorhabditis elegans*. *Neurobiology of Aging*, *34*(2), 621–631. https://doi.org/10.1016/j.neurobiolaging.2012.03.016

Tsalik, E. L., Niacaris, T., Wenick, A. S., Pau, K., Avery, L., & Hobert, O. (2003). LIM homeobox gene-dependent expression of biogenic amine receptors in restricted regions of the *C. elegans*

nervous system. *Developmental Biology*, *263*(1), 81–102. https://doi.org/10.1016/S0012-1606(03)00447-0

Tsui, D., & Van Der Kooy, D. (2008). Serotonin mediates a learned increase in attraction to high concentrations of benzaldehyde in aged *C. elegans*. *Learning and Memory*, *15*(11), 844–855. https://doi.org/10.1101/lm.1188208

Vidal-Gade, A., Topper, S., Young, L., Crisp, A., Kressin, L., Elbel, E., Maples, T., Brauner, M., Erbguth, K., Axelrod, A., Gottschalk, A., Siegel, D., & Pierce-Shimomura, J. T. (2011). *Caenorhabditis elegans* selects distinct crawling and swimming gaits via dopamine and serotonin. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(42), 17504–17509. https://doi.org/10.1073/pnas.1108673108

Waggoner, L. E., Zhou, G. T., Schafer, R. W., & Schafer, W. R. (1998). Control of alternative behavioral states by serotonin in *Caenorhabditis elegans*. *Neuron*, *21*(1), 203–214. https://doi.org/10.1016/S0896-6273(00)80527-9

Wang, D., Yu, Y., Li, Y., Wang, Y., & Wang, D. (2014). Dopamine receptors antagonistically regulate behavioral choice between conflicting alternatives in *C. elegans*. *PLoS ONE*, *9*(12), e115985. https://doi.org/10.1371/journal.pone.0115985

Weinshenker, D., Garriga, G., & Thomas, J. H. (1995). Genetic and pharmacological analysis of neurotransmitters controlling egg laying in *C. elegans*. *Journal of Neuroscience*, *15*(10), 6975–6985. https://doi.org/10.1523/jneurosci.15-10-06975.1995

White, J. G., Southgate, E., Thompson, J. N., & Brenner, S., (1986). The structure of the nervous system of the nematode *Caenorhabditis elegans*. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*, *314*(1165), 1–340. https://doi.org/10.1098/rstb.1986.0056

Wicks, S. R., & Rankin, C. H. (1995). Integration of mechanosensory stimuli in *Caenorhabditis elegans*. *Journal of Neuroscience*, *15*(3 II), 2434–2444. https://doi.org/10.1523/jneurosci.15-03-02434.1995

Wicks, S. R., & Rankin, C. H. (1996). The integration of antagonistic reflexes revealed by laser ablation of identified neurons determines habituation kinetics of the *Caenorhabditis elegans* tap withdrawal response. *J Comp Physiol A*, *179*(5), 675–685. https://doi.org/10.1007/BF00216131

Wicks, S. R., & Rankin, C. H. (1997). Effects of tap withdrawal response habituation on other withdrawal behaviors: The localization of habituation in the nematode *Caenorhabditis elegans*. *Behavioral Neuroscience*, *111*(2), 342–353. https://doi.org/10.1037/0735-7044.111.2.342

Wragg, R. T., Hapiak, V., Miller, S. B., Harris, G. P., Gray, J., Komuniecki, P. R., & Komuniecki, R. W. (2007). Tyramine and octopamine independently inhibit serotonin-stimulated aversive behaviors in *Caenorhabditis elegans* through two novel amine receptors. *Journal of Neuroscience*, *27*(49), 13402–13412. https://doi.org/10.1523/JNEUROSCI.3495-07.2007

Zhang, Y., Lu, H., & Bargmann, C. I. (2005). Pathogenic bacteria induce aversive olfactory learning in *Caenorhabditis elegans*. *Nature*, *438*(7065), 179–184. https://doi.org/10.1038/nature04216

Zheng, C., Diaz-Cuadros, M., & Chalfie, M. (2015). Hox Genes Promote Neuronal Subtype Diversification through Posterior Induction in *Caenorhabditis elegans*. *Neuron*, *88*(3), 514–527. https://doi.org/10.1016/j.neuron.2015.09.049