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Dopaminergic Neuromodulation in Short-term Sensitization

by an Aversive Chemical Stimulus in Larval Zebrafish (*Danio rerio*)

A thesis submitted in partial satisfaction

of the requirements for the degree Masters of Science

in Physiological Sciences

by

Yuqi Ma

2020

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2020

ABSTRACT OF THE THESIS

Dopaminergic Neuromodulation in Short-term Sensitization

by an Aversive Chemical Stimulus in Larval Zebrafish (*Danio rerio*)

by

Yuqi Ma

Master of Science in Physiological Sciences

University of California, Los Angeles, 2020

Professor David L Glanzman, Chair

Zebrafish (*Danio rerio*) hold immense promise for the study of learning and memory. Several attractive properties characterize this vertebrate animal model. For instance, zebrafish possesses a relatively reduced neural circuitry, which facilitates the drawing of causal relationships between changes on the behavioral scale to changes on the cellular-molecular level. They can also be affected by and absorb pharmacological agents in bath, and undergo learning and memory paradigms as early as 5 days post-fertilization (dpf). In particular, following a brief introduction of a noxious stimulus, allyl isothiocyanate (mustard oil, MO), 5-6 dpf zebrafish exhibit short-term sensitization. This memory has been demonstrated behaviorally through increased thigmotaxis and locomotion. However, the neuromodulatory mechanisms behind this non-declarative, non-associative form of memory remains to be elucidated. Exploiting this

animal model's strengths, we placed zebrafish under high-throughput pharmacological dissections with dopamine-receptor antagonists SCH-23390, eticlopride hydrochloride, haloperidol, and L-745,870 trihydrochloride in order to determine the role of dopamine and delineate receptor subtype-specific influences. These blockades have not only confirmed the role of dopamine, but also have demonstrated a differential involvement of the receptor subtypes within the two behaviors. Nonetheless, further investigations are required in order to determine the influence of other neuromodulators in the establishment of MO-induced short-term sensitization.

The thesis of Yuqi Ma is approved.

Mark Arthur Frye

Stephanie Ann White

David L Glanzman, Committee Chair

University of California, Los Angeles

2020

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Introduction

Zebrafish, or *Danio rerio*, has become a popular learning and memory animal model in recent decades. They are translucent in their larval stage, and in combination with their genetic tractability, permit non-invasive optogenetic manipulation *in vivo* (1-2, 9, 11-12). Zebrafish also represent a mechanistically reduced neural system in an evolutionarily conserved vertebrate animal model, which facilitates the drawing of causal relationships between changes on the neuronal to changes on the behavioral level. In addition, they can readily absorb chemicals directly placed in bath, and coupled with a large clutch size, this model enables high throughput protocols, simplifying pharmacological manipulations (3-6, 13, 17, 24). Finally, this vertebrate has a rapid developmental maturation time: at 5 days post fertilization (dpf), zebrafish can reliably undergo learning and memory experiments, including short- and long-term habituation, a form of non-associative, non-declarative memory (31, 33).

More recently, short-term sensitization, another form of non-associative learning, was demonstrated in larval zebrafish. This memory is characterized by a state of fear or anxiety as a result of an exposure to an arousing stimulus (20, 32). In our lab, we used the noxious stimulus allyl isothiocyanate (mustard oil, MO) (unpublished data). This molecule binds to TRPA1, a transient receptor potential channel found on trigeminal sensory and Rohon-Beard neurons innervating the skin (29). Following exposure, zebrafish exhibited increased thigmotaxis, a measurement of anxiety (30). Animals in a state of fear will also freeze or attempt to escape (21). Hence, we also observed fish increase in locomotor activity after presentation of MO.

How this noxious stimulus induces sensitization, however, is unknown. We, therefore, set to identify the neuromodulators that may be involved in the two behavioral changes in order to better understand the underlying circuitry. There are three common neuromodulators implicated

in the induction of memory: dopamine, serotonin, and noradrenaline (20, 22, 28, 29, 33). Although one or a combination of modulators could be involved, we began our investigations with dopamine. Its receptor consists of five subtypes. D1 and D5 are under the D1-class of receptors, which localize to the dopamine-receptive (postsynaptic) cell. They are coupled to $G\alpha_{s/olf}$, which activate adenylyl cyclase, leading to increased neural activity. In contrast, D2, D3, and D4 are under the D2-class of receptors, which are located on both the dopaminergic (presynaptic) and target (postsynaptic) neurons. They are coupled to $G\alpha_{i/o}$, which inhibit adenylyl cyclase and decrease neural activity. This permits a negative feedback mechanism that adjusts dopamine release in response to a neuron's own neurotransmission (3-5, 23, 38).

Dopamine has been demonstrated to increase anxiety and locomotion in various animal models, including zebrafish (17, 19, 38, 39, 44). In particular, knock out of the dopamine transporter (DAT) in zebrafish juveniles and adults has been shown to increase anxiety, as measured by increased bottom-dwelling and thigmotaxis. In addition, blocking D1 receptors, but not D2 receptors, minimized these behaviors in wildtype fish (19). In locomotion, on the other hand, both classes of receptors are involved (17-19, 38, 39).

We, therefore, sought to determine whether dopamine is also involved in MO-induced sensitization and delineate the specific receptor subtypes. Through direct bath application, we introduced SCH-23390, eticlopride hydrochloride, haloperidol, and L-745,870 trihydrochloride, which have the strongest affinity to D1/D5, D2/D3, D2, and D4 receptor subtypes, respectively (6, 24, 27). Based on previous literature, we hypothesized that D1-class, but not D2-class receptors are involved in the increase in thigmotaxis, whereas both subtypes play a role in the increase in locomotion.

Materials and Methods

Animals

Tupfel long fin wild-type (TLWT) zebrafish were stored in tanks and bred in breeding chambers in the UCLA aquatic vivarium in established, optimized male to female ratios. Fertilized eggs were collected the following day in 75 mL petri dishes filled with E3 medium (5 mM NaCl, 0.33 mM MgCl2, 0.33 mM CaCl2, 0.17 mM KCl, 10–5% methylene blue, pH 7.2) and placed in an incubator set at 28.5°C. Any sick or dead embryos were removed upon cleaning and returned to the incubator daily. 5 dpf fish were removed for experiments on the sixth day after collection.

Pharmacology

We induced sensitization in zebrafish by exposing them to 10 µM allyl isothiocyanate (mustard oil, MO). To block dopamine receptor subtypes, we administered the following antagonists: 1 and 5 µM SCH-23390 (D1/D5), 20 µM haloperidol (D2), 20 µM eticlopride hydrochloride (D2/D3), and 1 µM L-745,870 trihydrochloride (D4). MO was supplied by Sigma (St. Louis, MO), SCH-23390 by Cayman Chemicals (Ann Arbor, MI), eticlopride hydrochloride and L-745,870 by Tocris (Bristol, UK), and haloperidol by SC Biotechnology (Santa Cruz, CA).

Thigmotaxis Protocol

For each experimental trial, we acclimated 20 zebrafish in a small petri dish (50 mm diameter/12 ml volume) containing a dopamine receptor antagonist dissolved in either DMSO (dimethyl sulfoxide) or E3 (embryo water) for 1 hour. In particular, SCH-23390, eticlopride, and L-745,870 were dissolved in E3, whereas haloperidol was in DMSO. To ensure the results were due to the antagonists and not E3 or DMSO, we acclimated fish in their respective solvents for control trials. Following acclimation, we exposed them to MO (experimental) or E3 (control) for

30 seconds and washed out the drugs for 1 minute. We immediately transferred the fish into a larger petri dish (138 mm diameter/100 ml volume) and photographed their location 30 min later. Thus, we had four conditions: DMSO/E3-E3, DMSO/E3-MO, antagonist-E3, and antagonist-MO (Fig. 1A).

We analyzed images on ImageJ software (National Institutes of Health, Bethesda, MD, USA). For each of the 20 fish in a trial, we calculated its distance from the edge of the plate. We then took the average of these distances and designated this as n = 1. Unpaired *t*-tests were run and graphs were generated using GraphPad Prism version 8.4.2 (464) for Mac OS X, GraphPad Software, San Diego, California USA, www.graphpad.com (Fig. 1C).

Locomotion Protocol

Similar to our thigmotaxis protocol, we acclimated a zebrafish in a small petri dish (36 mm diameter/14 ml volume) containing a dopamine receptor antagonist dissolved in either DMSO or E3 for 31 min for each experimental trial; control trials were in their respective solvents. In the last minute, we recorded their baseline movements using a high-speed camera at 240 frames per second (fps) (pretest). Following the recording, we exposed fish to MO (experimental) or E3 (control) for 30 seconds and washed out the drugs for 30 seconds. Finally, we recorded their movements 4.5 minutes later (5 minutes after the end of MO exposure) for 1 minute (posttest) (Fig. 1B).

We analyzed these recordings on ImageJ, noting the coordinates of the fish for every second. We subsequently calculated the distances between these coordinates and took the sum of these distances. This was done for both pre- and posttests and the changes in distance traveled (post-pre) were compared between conditions. Unpaired *t*-tests were run and graphs were

generated using GraphPad Prism version 8.4.2 (464) for Mac OS X, GraphPad Software, San Diego, California USA, www.graphpad.com (Fig. 1D).

Results

Role of D1/D5 receptors in MO-induced increase in thigmotaxis and locomotion

To determine whether D1/D5 receptors were involved in MO-induced anxiety, we bathed zebrafish in either 1 or 5 μM of the antagonist, SCH-23390, before MO exposure and observed their resulting levels of thigmotaxis. There were no significant differences between fish that received only MO (E3-MO: n=20, 9.03±0.66 mm) and those supplemented with either 1 or 5 μM of SCH-23390 (SCH-MO(1 μM): n=20, 7.78±0.64 mm; SCH-MO(5 μM): n=20, 10.89±0.88 mm) (Fig. 2A). There were also no significant differences between fish bathed in embryo water (E3-E3: n=20, 11.19±0.69 mm) and those in the antagonists (SCH-E3(1 μM): n=20, 10.98±0.95 mm; SCH-E3(5 μM): n=20, 11.19±0.77 mm), indicating that SCH-23390 alone did not alter thigmotaxis levels (Fig. 2B). Therefore, the data suggests that D1/D5 receptors are not involved in the induction of MO-mediated anxiety.

Similarly, we acclimated zebrafish in SCH-23390 to ascertain whether these receptors are involved in MO-induced increase in locomotion. Because neither concentration of the antagonist significantly changed thigmotaxis levels, we opted for the higher concentration when testing locomotion. We observed a significant decrease in in the distance traveled between fish given MO (E3-MO: n=12, 249.22±32.44 mm) and those supplemented with SCH-23390 (SCH-MO(5 μM): n=12, 95.33±43.07 mm; unpaired *t*-test $p < 0.01$) (Fig. 2C). There were also no significant differences between fish bathed in E3 (E3-E3: n=12, -25.00±27.11 mm) and those in the antagonist (SCH-E3(5 μM): n=12, 31.59±28.62 mm) (Fig. 2D). Therefore, in contrast to the

thigmotaxis circuitry, D1/D5 are involved in the induction of MO-mediated increase in locomotion.

Role of D2 and D3 receptors in MO-induced increase in thigmotaxis and locomotion

To deduce the role of D2 and D3 receptors, we utilized two antagonists: eticlopride, which blocks both receptors, and haloperidol, which targets only D2. When compared to fish exposed to only MO (E3-MO: n=20, 7.69±0.66 mm), the former drug significantly decreased thigmotaxis (Eti-MO: n=20, 10.52±1.07 mm; unpaired *t*-test $p < 0.05$) (Fig. 3A). Likewise, eticlopride alone decreased thigmotaxis compared to fish in embryo water (E3-E3: n=20, 10.13±0.071 mm; Eti-E3: n=20, 18.64±1.10 mm; unpaired *t*-test $p < 0.0001$) (Fig. 3B). This indicates that the decrease in thigmotaxis seen in the Eti-MO condition may be due to a block of D2/D3 receptors within the anxiety circuit itself, rather than receptors responding to a MO-induced release of dopamine. Therefore, we cannot conclude whether the D2 or D3 receptors are involved in the induction of MO-mediated anxiety based on this drug. However, blocking D2 receptors with haloperidol did not significantly alter thigmotaxis levels in fish when exposed to either MO (DMSO-MO: n=20, 6.88±0.71 mm; Halo-MO: n=20, 7.78±0.64 mm) (Fig. 2C) or E3 (DMSO-E3: n=20, 11.91±0.77 mm; Halo-E3: n=20, 11.45±0.77 mm) (Fig. 2D). Hence, the D2 receptor is not involved, but the D3 receptor is still a candidate.

Within the locomotion circuit, the opposite may be true. Fish presented with eticlopride followed by MO swam nearly as far as those exposed to only MO (E3-MO: n=12, 163.25±36.89 mm; Eti-MO: n=12, 128.96±34.13 mm) (Fig. 2E). Similarly, the change in movement of fish acclimating in E3 also did not differ with those in the antagonist (E3-E3: n=12, 13.79±16.22 mm; Eti-E3: n=12, 5.17±5.51 mm) (Fig. 2F). However, some fish exposed to eticlopride did not

move in either pre- or posttest recordings, indicating that this drug may block D2/D3 receptors within the locomotion circuit, rather than receptors directly responding to a MO-induced release of dopamine (Table 4). Therefore, we cannot exclude the involvement of D2 and D3 receptors in MO-induced increase in locomotion based on this experiment. On the other hand, fish in haloperidol and MO did exhibit significantly decreased locomotion compared to those given only MO (DMSO-MO: n=12, 185.49±42.46 mm; Halo-MO: n=12, 12.51±13.92 mm; unpaired *t*-test $p < 0.001$) (Fig. 2G). There were also no significant off-target effects (DMSO-E3: n=12, -26.87±27.05 mm; Halo-E3: n=12, 0.62±41.22 mm) (Fig. 2H), which is confirmed by the pre- and posttest values (Table 6). Thus, D2 receptors are involved, but D3 receptors still need confirmation.

Role of D4 receptors in MO-induced increase in thigmotaxis and locomotion

Finally, to determine whether D4 receptors were involved in MO-induced anxiety, we bathed zebrafish in L-745,870. With this antagonist followed by MO, we observed a significant difference compared to those in MO alone (E3-MO: n=20, 6.64±0.62 mm; Eti-MO: n=20, 8.87±0.92 mm; unpaired *t*-test $p < 0.05$) (Fig. 4A). There were also no significant off-target drug interactions ((E3-E3: n=20, 11.76±0.62 mm; L-745,870-E3: n=20, 10.77±0.76 mm) (Fig. 4B).

Similarly, L-745,870 induced a significant decrease in locomotion following MO exposure compared to control (E3-MO: n=12, 405.93±52.19 mm; L-745,870-MO: n=12, 152.67±36.36 mm; unpaired *t*-test $p < 0.001$) (Fig. 4C). In addition, this antagonist did not alter levels of activity when presented alone (E3-E3: n=12, 24.43±22.58 mm; L745,870-E3: n=12, -10.74±55.39 mm) (Fig. 4D). Therefore, D4 receptors are involved in both the increase in anxiety and locomotion in response to MO.

Discussion

In summary, our experiments demonstrate that D4 receptor subtypes are involved in the MO-induced increase in thigmotaxis, whereas D1/D5 and D2 are not. In contrast, D1/D5, D2, and D4 are all involved in the MO-induced increase in locomotion. However, the role of D3 receptors remains inconclusive for both circuits.

This poses an interesting insight. Based on a previous study, we had originally predicted that D1/D5 would be involved in MO-induced anxiety, but our results show the opposite (19). This could be attributed to differences in age, where their study used juvenile and adult fish, whereas our experiments used only larval. Perhaps more importantly, the drug concentrations also differed. They administered 10 μM of SCH-23390 in order to block D1/D5 receptors, but we only provided 1 and 5 μM. In fact, a different study acclimated adult zebrafish in various concentrations from 0-3 μM and found no changes in anxiety levels (39). Therefore, our non-significant results could be attributed to our low concentrations, which may not have sufficiently blocked the receptors. Nonetheless, 5 μM did yield significant differences in the locomotion paradigm. Why different behaviors may require different concentrations of receptor antagonists is a point for further investigation.

In fact, our locomotion results did match our predictions. This confirms the role of D1-class receptors, which provide excitatory input and thus, increases locomotion (3, 18, 38). D2-class receptors, as aforementioned, have both pre- and postsynaptic localizations. This enables a biphasic response to dopamine, where low concentrations of the modulator will preferentially bind to the presynaptic receptor and decrease further neurotransmitter release. As a result, postsynaptic D2-class receptors are less activated, which can lead to increased locomotion. At high concentrations, dopamine will bind to the postsynaptic receptor, which will inhibit cell

activity and decrease locomotion (3). This was validated by a blockade of D2-class receptors with amisulpride and haloperidol (17, 38). Similar to dopamine, both these drugs have differential binding dependent on concentration. In particular, 5.5 μM of haloperidol decreased, whereas 50 μM increased locomotion. Our study used a concentration in between these extremes. To follow up, we can replicate our experiments with their concentrations in order to better distinguish between pre- or postsynaptic D2 receptor input in the MO-induced increase in locomotion. If 5.5 μM can block this increase, then the presynaptic receptor may be the one involved. Likewise, we can recapitulate D4 receptor blockades with a high concentration of L-745,870, which may further increase locomotion. This would entail that MO triggers a release of a small concentration of dopamine, which would decrease neurotransmitter release onto the postsynaptic receptor, resulting in increased neural activity and eventually, increased locomotion. Therefore, increased locomotion could be due to not only increased D1-class receptor excitatory input, but also decreased D2-class receptor inhibition. A similar argument can be made for increased anxiety (Fig. 5A).

The role of the D3 receptor, however, remains unknown due to off-target drug interactions. In the presence of eticlopride alone, zebrafish demonstrated decreased thigmotaxis, which may be explained by the accompanying lack of movement. Alternatively, D3 receptors could be involved in general anxiety. However, in all other antagonists, they were able to maintain some level of locomotion. This may be explained by the distribution of the receptor subtypes. While D2-class receptors are found throughout the brain and spinal cord, D1-class receptors are localized to diencephalon, hypothalamus, and hindbrain (4, 5, 23) (Fig. 5B). Therefore, SCH-23390 had minimal changes on locomotion, while eticlopride, which blocks both D2 and D3 subtypes, had the greatest effect.

Nonetheless, there is one main limitation to this study: these drugs were delivered through bath application, which could block receptors anywhere throughout the body. To achieve region-specific administration, we can utilize photolytic compounds. Through spinal cord injections, we can deliver a retrograde dye coupled to a caged dopamine receptor antagonist into the brain and direct UV light to various nuclei (7, 10). To gain more precision, we can also develop zebrafish lines that express channelrhodopsin (ChR) within dopaminergic neurons in order to selectively activate cell clusters (2, 26). These transgenic fish will also express GCaMP6 in order to determine which cells are activated in response to the dopamine release (1, 41). Alternatively, we can express halorhodopsin (NpHR) in order to deactivate dopaminergic neuron subsets during MO exposure (2).

These experiments should also be expanded to test other neuromodulators. Although the anxiety circuit has not been entirely delineated, the habenula has been shown to regulate the release of serotonin and dopamine and may be modulated by noradrenaline itself (14, 40). In addition, while dopamine is known to target locomotion regulatory centers, including the posterior tuberculum, pretectum, and spinal cord, so has noradrenaline (25, 35, 36). Lastly, all three molecules also target the nucleus of the medial longitudinal fasciculus (nMLF), a set of midbrain reticulospinal neurons important for swim posture and speed (Fig. 5B) (8, 25, 35, 36, 41). Altogether, this will provide a more well-rounded depiction of neuromodulation in order to eventually decipher the circuitry behind MO-induced short-term sensitization.

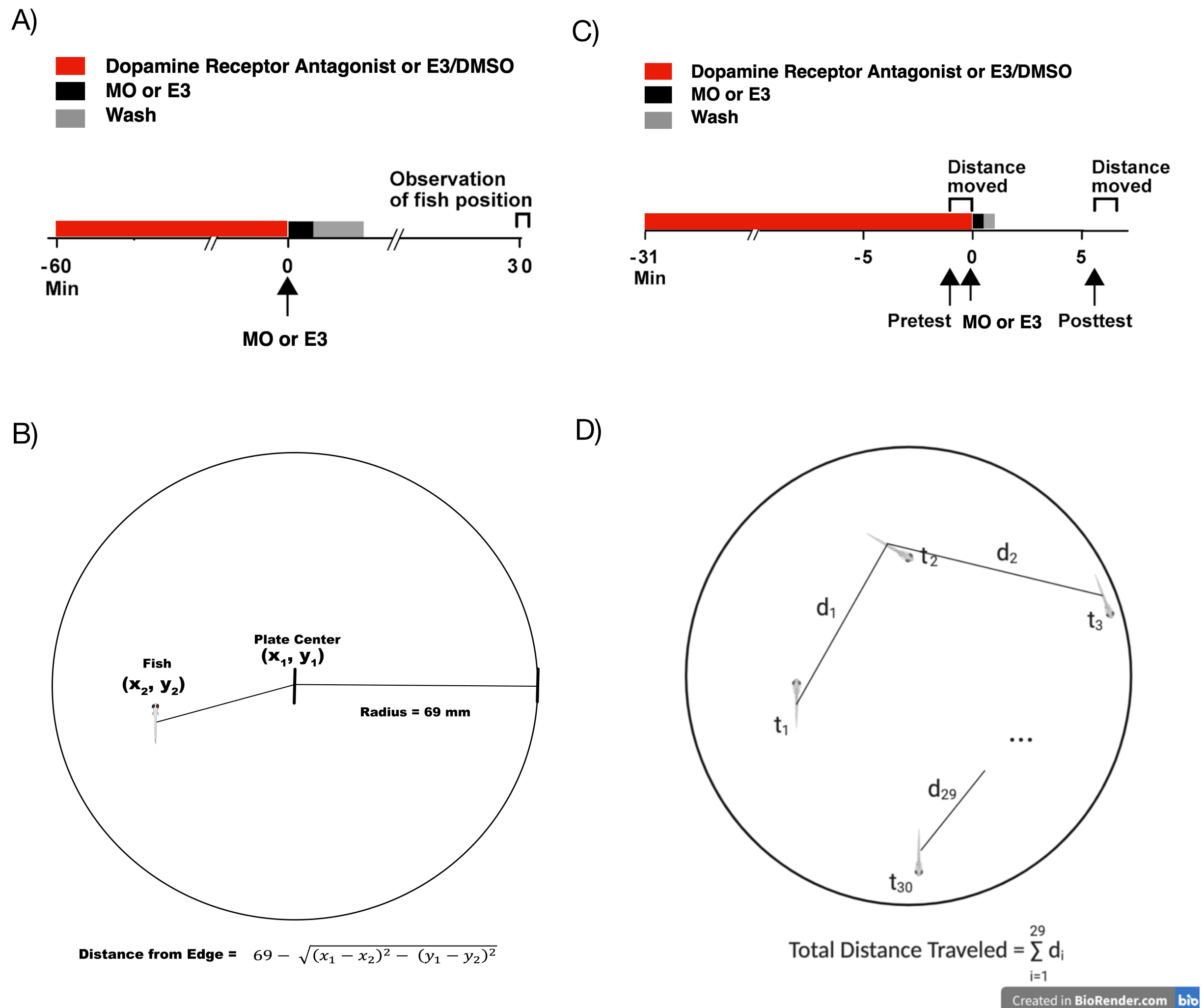

Figure 1: Pharmacological Dissection Methods
A) Timeline for thigmotaxis behavioral paradigm. For each trial, we acclimated 20 zebrafish in a small petri dish (50 mm diameter/12 ml volume) containing either a dopamine receptor antagonist (experimental) or E3/DMSO (control) for 1 h. Following acclimation, we exposed them to MO (experimental) or E3 (control) for 30 s and washed out the drugs for 1 min. We immediately transferred the fish into a larger petri dish (138 mm diameter/100 ml volume) and captured their location 30 min later.
B) Schematic diagram of the thigmotaxis distance-from-edge calculation.
C) Timeline for locomotion behavioral paradigm. For each trial, we acclimated a zebrafish in a small petri dish (36 mm diameter) containing either a dopamine receptor antagonist (experimental) or E3/DMSO (control) for 31 min. In the last minute, we recorded their baseline movements (pretest). Following the recording, we exposed fish to MO (experimental) or E3 (control) for 30 s and washed out the drugs for 30 s. We recorded their movements 4.5 min later (posttest).
D) Schematic diagram of the locomotion distance-moved calculation. (This image was created with BioRender.com.)

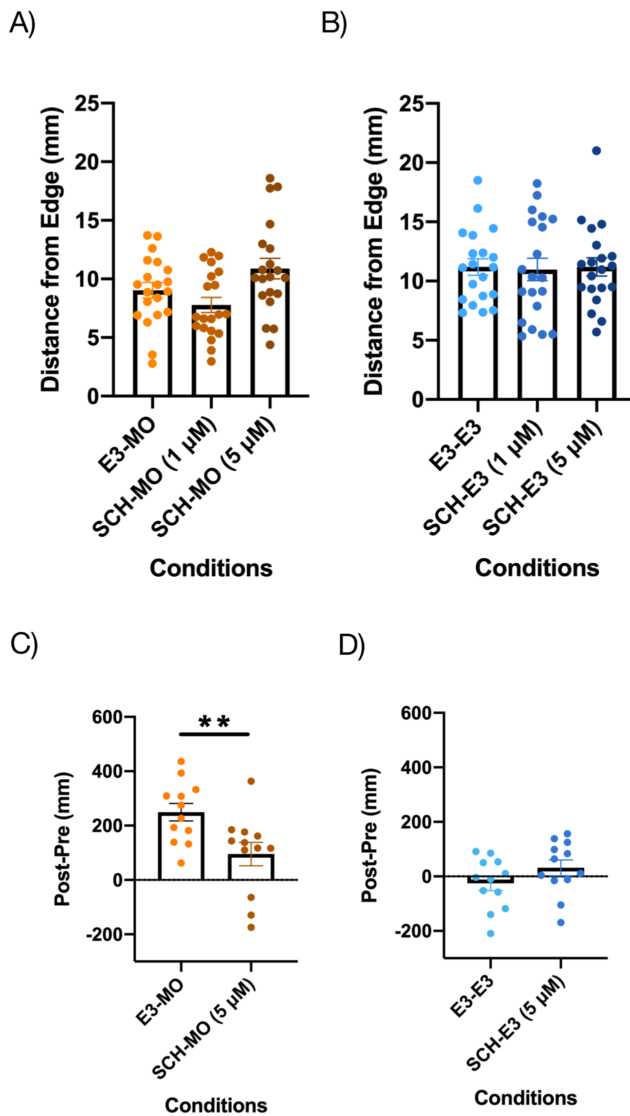

Figure 2: Role of D1/D5 receptors in thigmotaxis and locomotion.
(A) Blockade of D1/D5 receptors did not disrupt the MO-induced increase in thigmotaxis, a measurement of anxiety (E3-MO: n=20, 9.03±0.66 mm; SCH-MO(1 µM): n=20, 7.78±0.64 mm; SCH-MO(5 µM): n=20, 10.89±0.88 mm).
(B) There were no significant off-target drug interactions in response to SCH-23390 in the thigmotaxis behavioral paradigm (E3-E3: n=20, 11.19±0.69 mm; SCH-E3(1 µM): n=20, 10.98±0.95 mm; SCH-E3(5 µM): n=20, 11.19±0.77 mm).
(C) Antagonism of D1/D5 receptors disrupted the MO-induced increase in locomotion (E3-MO: n=12, 249.22±32.44 mm; SCH-MO(5 µM): n=12, 95.33±43.07 mm; unpaired *t*-test $p < 0.01$).
(D) There were no significant off-target drug interactions in response to SCH-23390 in the locomotion behavioral paradigm (E3-E3: n=12, -25.00±27.11 mm; SCH-E3(5 µM): n=12, 31.59±28.62 mm).

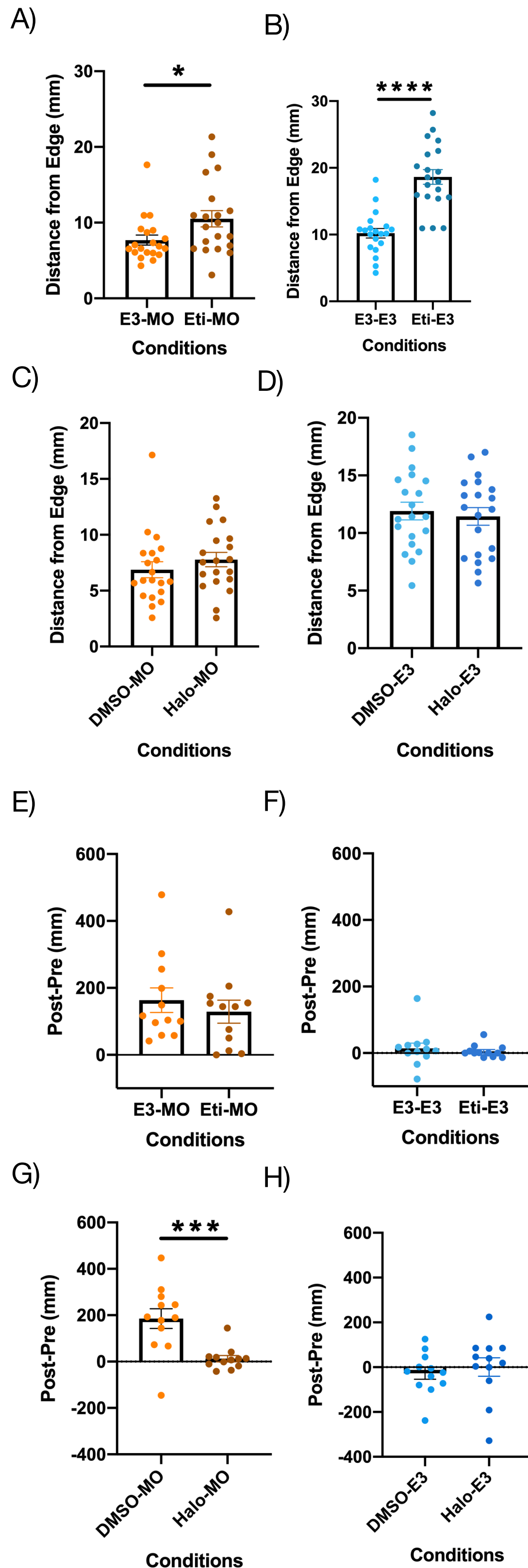

Figure 3: Role of D2 and D3 receptors in thigmotaxis and locomotion.
(A and B) The D2/D3 receptor antagonist, eticlopride, decreased thigmotaxis in both the MO and E3 conditions (E3-MO: n=20, 7.69±0.66 mm; Eti-MO: n=20, 10.52±1.07 mm; unpaired *t*-test $p < 0.05$) (E3-E3: n=20, 10.13±0.071 mm; Eti-E3: n=20, 18.64±1.10 mm; unpaired *t*-test $p < 0.0001$).
(C) Antagonism of D2 receptors by haloperidol did not interfere with the MO-induced increase in thigmotaxis (DMSO-MO: n=20, 6.88±0.71 mm; Halo-MO: n=20, 7.78±0.64 mm).
(D) There were no significant off-target drug interactions in response to haloperidol in the thigmotaxis behavioral paradigm (DMSO-E3: n=20, 11.91±0.77 mm; Halo-E3: n=20, 11.45±0.77 mm).
(E) Antagonism of D2/D3 receptors by eticlopride did not disrupt the MO-induced increase in locomotion (E3-MO: n=12, 163.25±36.89 mm; Eti-MO: n=12, 128.96±34.13 mm).
(F) Although *changes* in movement did not differ, many fish in the eticlopride condition did not move at all (E3-E3: n=12, 13.79±16.22 mm; Eti-E3: n=12, 5.17±5.51 mm) (Table 4). Therefore, there were off-target drug interactions.
(G) The D2 receptor antagonist, haloperidol, blocked the MO-induced increase in locomotion (DMSO-MO: n=12, 185.49±42.46 mm; Halo-MO: n=12, 12.51±13.92 mm; unpaired *t*-test $p < 0.001$).
(H) There were no significant off-target drug interactions in response to haloperidol in the locomotion behavioral paradigm (DMSO-E3: n=12, -26.87±27.05 mm; Halo-E3: n=12, 0.62±41.22 mm).

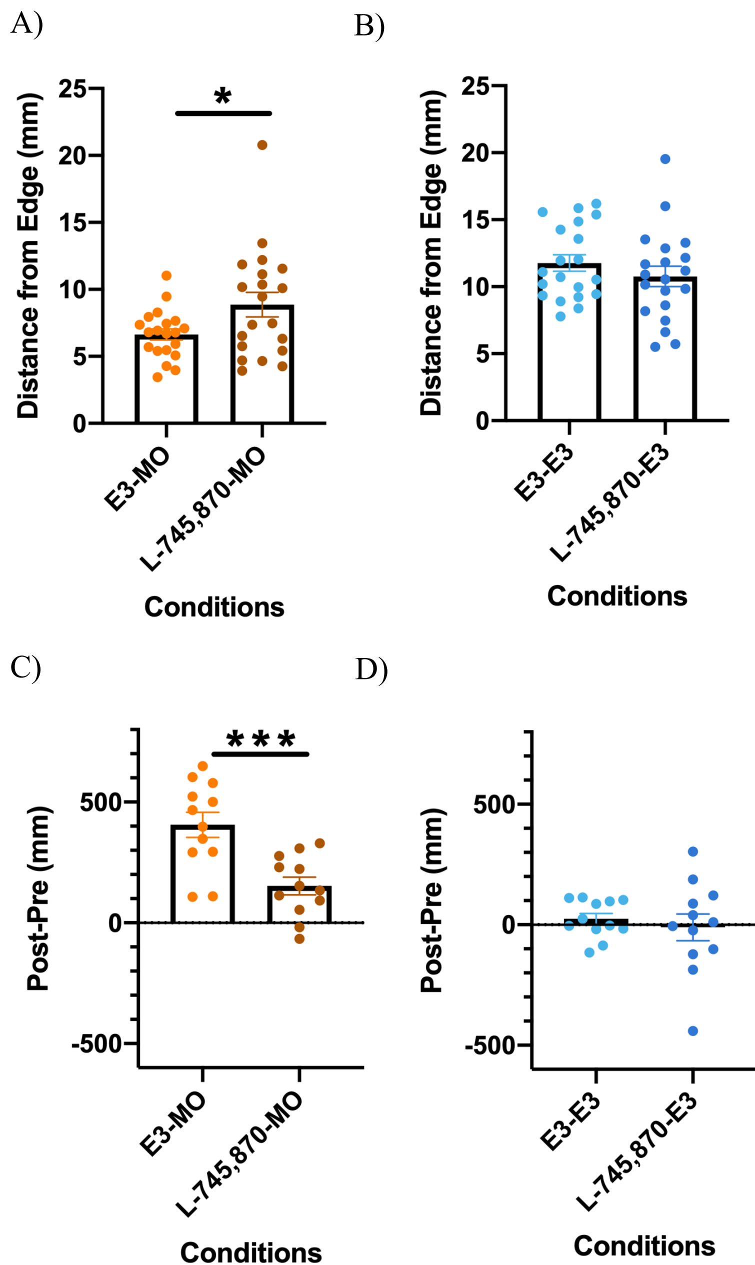

Figure 4: Role of D4 receptors in thigmotaxis and locomotion.
(A) Antagonism of D4 receptors by L-745,870 disrupted the MO-induced increase in thigmotaxis (E3-MO: n=20, 6.64±0.62 mm; Eti-MO: n=20, 8.87±0.92 mm; unpaired *t*-test $p < 0.05$).
(B) There were no significant off-target drug interactions in response to L-745,870 in the thigmotaxis behavioral paradigm (E3-E3: n=20, 11.76±0.62 mm; L-745,870-E3: n=20, 10.77±0.76 mm).
(C) Blockade of D4 receptors disrupted the MO-induced increase in locomotion (E3-MO: n=12, 405.93±52.19 mm; L-745,870-MO: n=12, 152.67±36.36 mm; unpaired *t*-test $p < 0.001$).
(D) There were no significant off-target drug interactions in response to L-745,870 in the locomotion behavioral paradigm (E3-E3: n=12, 24.43±22.58 mm; L745,870-E3: n=12, -10.74±55.39 mm).

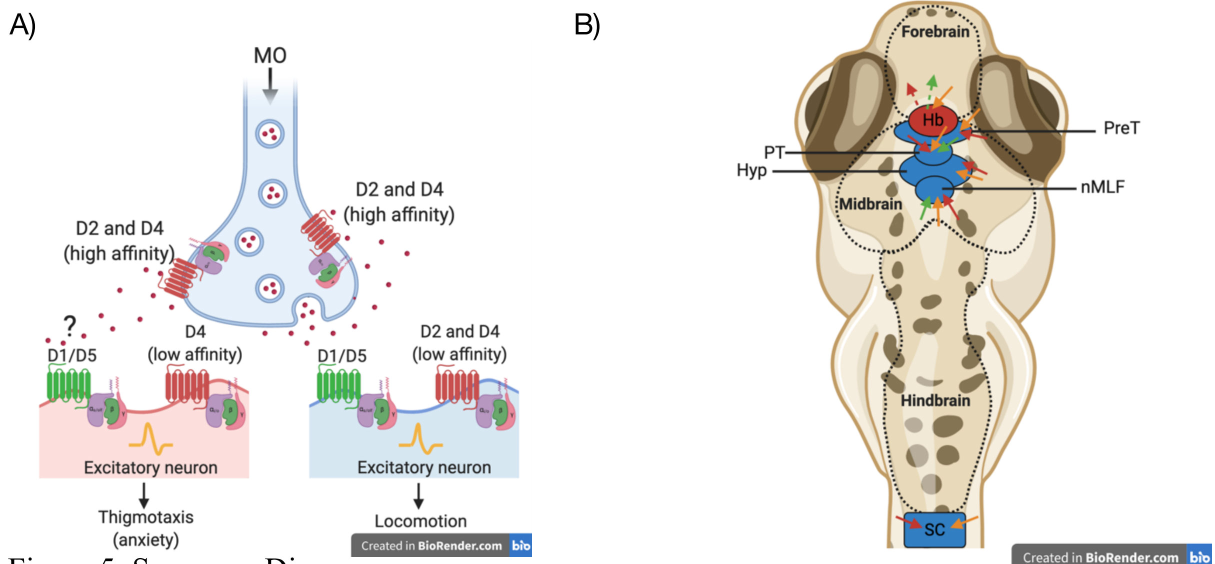

Figure 5: Summary Diagram.
(A) Possible pathway based on our pharmacological dissection. The red cell represents neurons in the anxiety circuit, whereas blue represents those in the locomotion circuit. Note: This model assumes the effect of D2/D4 receptors outweigh that of D1/D5.
(B) Important anxiety (red) and locomotion (blue) centers within the larval zebrafish brain and their respective neuromodulatory inputs and outputs. The brain is oriented from rostral to caudal. Arrow colors designate the following neuromodulators: dopamine (red), noradrenaline (orange), serotonin (green); solid arrows indicate direct, whereas dashed lines are indirect inputs/outputs. Hb: habenula, PreT: pretectum, PT: posterior tuberculum, Hyp: hypothalamus, nMLF: nucleus of the medial longitudinal fasciculus, SC: spinal cord. (These images were created with BioRender.com.)

Table 1: Distance from edge values (mm) for zebrafish in SCH-23390 conditions.

E3-E3	E3-MO	SCH-E3 (1uM)	SCH-MO (1uM)	SCH-E3 (5uM)	SCH-MO (5uM)
10.35	8.39	12.28	9.47	11.60	12.60
8.74	13.64	17.25	12.28	9.50	10.71
14.08	13.72	14.99	11.85	9.43	18.59
16.14	9.54	9.05	11.98	15.15	10.08
12.39	10.75	5.50	6.61	14.81	10.09
12.01	11.45	5.50	5.98	11.93	4.39
18.52	6.93	10.98	7.01	7.25	8.73
12.33	8.94	5.91	10.21	8.41	17.75
11.13	11.60	9.13	6.98	14.44	14.69
9.74	2.79	10.29	5.83	11.28	11.26
13.85	10.15	18.24	2.97	13.04	8.87
7.94	8.05	7.88	5.34	9.34	10.17
7.36	12.61	9.12	4.78	21.01	8.60
8.46	9.51	15.24	11.46	12.09	17.86
14.45	3.54	10.35	5.58	11.43	5.74
11.42	8.87	14.56	10.62	10.43	8.05
7.33	6.29	5.34	6.77	9.50	13.00
7.54	7.18	6.48	9.36	6.59	5.78
8.89	9.77	15.46	6.58	5.70	10.01
11.18	6.90	16.00	3.90	10.97	10.78

Table 2: Pretest and posttest values for zebrafish in SCH-23390 conditions.

Conditions	Pretest (mm)	Posttest (mm)	Posttest-Pretest (mm)	Conditions	Pretest (mm)	Posttest (mm)	Posttest-Pretest (mm)
E3-E3	225.57	168.13	-57.45	**SCH-E3**	28.18	13.00	-15.19
	123.19	113.31	-9.87		107.17	170.93	63.76
	129.25	11.42	-117.83		176.59	164.75	-11.84
	237.74	185.26	-52.49		82.10	220.64	138.54
	320.32	110.99	-209.34		15.48	114.47	98.99
	347.76	207.91	-139.85		153.63	236.89	83.27
	282.98	367.96	84.98		216.63	111.63	-104.99
	342.43	352.94	10.50		198.31	202.23	3.92
	315.68	365.94	50.26		384.10	395.01	10.90
	107.51	103.81	-3.70		218.37	49.17	-169.19
	255.20	308.61	53.41		408.46	533.22	124.76
	287.62	379.02	91.40		314.85	470.98	156.12
E3-MO	169.46	477.23	307.77	**SCH-MO**	186.53	12.01	-174.52
	225.87	452.77	226.90		185.42	56.40	-129.02
	14.12	407.39	393.26		67.19	204.66	137.46
	186.16	248.77	62.61		39.50	155.79	116.29
	134.20	273.65	139.45		131.57	316.41	184.84
	159.44	292.07	132.63		133.59	250.81	117.23
	337.71	519.81	182.10		248.20	409.83	161.63
	117.21	449.80	332.59		85.20	262.00	176.80
	278.51	553.54	275.03		241.45	177.52	-63.93
	136.93	572.85	435.92		207.73	351.22	143.49
	362.11	671.56	309.45		249.00	358.97	109.96
	334.44	527.38	192.94		13.54	377.26	363.72

Table 3: Distance from edge values (mm) for zebrafish in eticlopride conditions.

E3-E3	E3-MO	Eti-E3	Eti-MO
10.05	4.31	25.72	10.79
10.79	7.88	24.75	16.66
10.45	8.71	15.60	11.56
10.16	10.97	22.54	8.18
18.22	8.99	20.23	18.98
9.37	6.58	19.40	13.17
11.24	6.90	19.68	21.33
10.77	6.06	28.20	17.25
8.71	17.63	10.97	10.97
10.97	10.97	10.97	10.97
15.30	5.98	24.10	6.50
13.37	6.08	17.94	9.99
7.70	5.02	16.76	6.95
5.30	6.58	15.39	8.20
8.10	5.75	18.58	3.08
10.11	7.34	17.45	7.51
4.27	9.17	15.95	6.57
10.64	5.33	22.01	6.38
10.68	6.53	10.94	6.01
6.47	7.07	15.71	9.44

Table 4: Pretest and posttest values for zebrafish in eticlopride conditions.

Conditions	Pretest (mm)	Posttest (mm)	Posttest-Pretest (mm)	Conditions	Pretest (mm)	Posttest (mm)	Posttest-Pretest (mm)
E3-E3	0.00	0.00	0.00	Eti-E3	0.00	0.00	0.00
	265.36	231.84	-33.52		15.29	31.13	15.84
	218.14	222.38	4.24		7.91	8.83	0.92
	31.37	58.13	26.76		12.12	34.02	21.91
	281.11	304.21	23.10		13.43	0.00	-13.43
	269.57	287.02	17.45		0.00	0.00	0.00
	291.49	298.11	6.61		0.00	0.00	0.00
	0.64	164.51	163.87		11.07	0.00	-11.07
	296.31	328.79	32.48		0.00	55.51	55.51
	261.80	252.57	-9.23		5.72	11.47	5.75
	138.02	59.81	-78.21		0.00	0.17	0.17
	0.18	12.12	11.94		13.88	0.33	-13.55
E3-MO	317.23	516.45	199.22	Eti-MO	0.00	76.04	76.04
	313.20	370.98	57.78		7.94	20.25	12.31
	308.98	350.33	41.35		4.48	54.78	50.31
	241.85	342.19	100.33		0.00	0.00	0.00
	175.81	477.98	302.17		46.33	201.25	154.92
	12.42	268.30	255.89		0.00	427.49	427.49
	83.93	200.52	116.60		0.00	144.12	144.12
	277.47	381.11	103.64		10.01	13.69	3.68
	0.50	59.14	58.64		45.65	199.73	154.09
	0.52	478.70	478.19		0.23	205.63	205.39
	225.44	321.94	96.50		4.08	148.37	144.29
	0.00	148.69	148.69		0.00	174.82	174.82

Table 5: Distance from edge values (mm) for zebrafish in haloperidol conditions.

DMSO-E3	DMSO-MO	Halo-E3	Halo-MO
18.52	8.75	7.79	12.50
11.42	5.67	8.11	6.47
15.06	4.89	17.01	13.26
12.44	3.59	14.36	9.48
13.45	9.79	11.54	11.35
11.19	4.55	7.42	6.69
11.43	10.24	8.66	8.40
15.67	5.70	13.03	7.80
8.35	7.73	10.27	2.56
7.54	8.37	13.25	3.24
9.03	4.37	14.44	5.83
8.14	7.48	13.29	5.41
17.34	6.66	12.23	8.91
9.70	5.90	5.66	6.66
14.52	5.93	7.77	7.67
10.20	2.58	13.77	4.98
14.62	5.81	6.61	11.20
13.54	17.14	16.60	9.66
5.44	8.35	12.06	6.03
10.56	3.99	15.06	7.55

Table 6: Pretest and posttest values for zebrafish in haloperidol conditions.

Conditions	Pretest (mm)	Posttest (mm)	Posttest-Pretest (mm)	Conditions	Pretest (mm)	Posttest (mm)	Posttest-Pretest (mm)
DMSO-E3	117.95	119.19	1.25	Halo-E3	248.97	294.97	46.00
	103.10	228.82	125.72		341.43	13.32	-328.12
	66.22	25.92	-40.30		335.13	274.79	-60.34
	169.74	251.79	82.05		10.08	95.59	85.51
	114.55	14.90	-99.65		215.13	218.65	3.52
	209.53	186.02	-23.52		107.87	193.52	85.65
	228.30	274.03	45.73		72.17	155.65	83.49
	119.39	100.79	-18.61		77.89	96.92	19.03
	318.36	238.92	-79.45		1.09	0.59	-0.51
	330.52	258.38	-72.15		201.04	9.48	-191.56
	189.86	184.19	-5.67		190.50	230.33	39.84
	251.32	13.42	-237.90		218.09	443.05	224.95
DMSO-MO	206.63	451.73	245.10	Halo-MO	38.21	56.86	18.66
	70.05	259.65	189.59		79.22	120.46	41.25
	188.21	381.15	192.94		38.77	51.03	12.26
	310.56	383.48	72.92		54.03	11.99	-42.04
	172.62	350.92	178.30		160.02	174.08	14.05
	189.60	470.40	280.81		37.01	26.81	-10.20
	94.43	405.01	310.58		94.48	75.91	-18.57
	243.42	310.89	67.47		331.84	330.32	-1.52
	479.59	333.77	-145.82		14.40	21.10	6.70
	86.66	533.48	446.82		95.99	59.10	-36.89
	177.47	419.99	242.52		30.43	175.26	144.83
	227.66	372.27	144.61		36.15	57.74	21.60

Table 7: Distance from edge values (mm) for zebrafish in L-745,870 conditions.

E3-E3	E3-MO	L-E3	L-MO
15.38	5.41	6.62	10.36
10.19	7.44	13.28	3.93
12.04	6.92	16.02	13.46
9.22	7.94	11.83	10.09
15.57	3.96	10.15	6.31
9.35	6.80	5.72	7.47
9.98	5.07	8.62	4.25
14.27	11.02	19.53	11.87
11.96	8.28	9.84	20.79
10.72	7.37	11.68	9.47
7.78	3.46	8.18	10.17
15.87	9.47	10.90	4.65
16.19	7.09	12.87	4.71
11.10	6.78	7.48	11.55
8.90	4.29	9.71	12.20
13.58	5.92	10.55	5.76
9.45	7.65	11.19	6.53
10.53	5.71	13.54	7.36
8.38	6.70	5.50	11.13
14.86	5.47	12.15	5.44

Table 8: Pretest and posttest values for zebrafish in L-745,870 conditions.

Conditions	Pretest (mm)	Posttest (mm)	Posttest-Pretest (mm)	Conditions	Pretest (mm)	Posttest (mm)	Posttest-Pretest (mm)
E3-E3	13.15	116.22	103.07	L-E3	451.65	10.80	-440.86
	252.09	247.38	-4.72		479.70	378.20	-101.51
	113.40	95.53	-17.88		449.55	443.58	-5.97
	9.50	34.06	24.56		318.80	295.63	-23.16
	365.39	461.95	96.56		515.70	555.83	40.13
	145.62	257.28	111.66		534.77	348.09	-186.68
	14.03	10.38	-3.65		29.63	217.55	187.92
	141.91	228.41	86.50		197.64	75.68	-121.96
	124.25	237.46	113.22		198.40	209.69	11.29
	212.65	126.95	-85.71		264.54	351.78	87.24
	226.32	210.80	-15.52		51.27	354.64	303.37
	341.33	226.44	-114.89		236.37	357.63	121.26
E3-MO	135.19	602.33	467.14	L-MO	423.77	477.98	54.21
	13.18	410.74	397.56		437.81	551.66	113.85
	12.52	122.13	109.61		0.00	329.25	329.25
	9.42	658.23	648.81		520.73	454.02	-66.71
	15.38	516.13	500.75		583.41	718.12	134.71
	10.84	533.21	522.37		460.09	442.01	-18.08
	100.75	395.46	294.71		200.47	477.75	277.28
	162.23	509.57	347.34		197.52	427.49	229.97
	48.97	652.67	603.70		351.51	504.91	153.40
	19.53	311.96	292.44		367.43	590.89	223.46
	14.29	592.93	578.64		0.00	308.56	308.56
	264.61	372.65	108.04		170.54	262.67	92.12

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