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The control of crawling movement by interneuron circuits of the *Drosophila* larva

Mary Sweet
College of Saint Benedict/Saint John's University

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Mary Sweet

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Approved by:

Jennifer Schaefer

Assistant Professor of Biology

Barbara May

Associate Professor of Biology

Elizabeth Wurdak

Professor of Biology

William Lamberts

Chair, Department of Biology

Tony Cunningham

Director, Honors Thesis Program

I. Introduction

Humans are only beginning to study the deep complexities of the brain and the nervous system. Though there are ideas about the neuronal basis for human emotion and behavior and a basic understanding of what happens on a cellular and molecular level, we have yet to achieve great understanding of how neural circuits are interconnected and work together. There has been a recent movement with the creation of the Brain Activity Map (BAM) to go beyond our current molecular and biochemical understanding of the brain and to start mapping the real time interactions of neural circuits in the brain in millisecond resolution. This will bring us closer to understanding how the brain operates via perception, action, memories, thoughts, and consciousness and has the potential to elucidate neural networks involved in brain disorders like schizophrenia, autism, and epilepsy (Alivisatos *et al.* 2013).

Neural circuits are made up of groups of neurons, which transmit information through electrical and chemical signals. Neurons are organized into circuits in order to effectively process specific kinds of information together (Purves *et al.,* 2001). Some neural circuits such as those of the retina (Baccus *et al.,* 2008; Eggers & Lukasiewicz, 2000) and those of the cerebellum that are involved in the control of movement (Robinson, 1995; Gilman, 1994) have been well mapped. However, many neural circuits like those responsible for complex cognition and control of breathing, as well as the crawling circuit in the fruit fly *Drosophila melanogaster,* are not yet well understood.

The nervous system of most animals is comprised of two main parts: the central nervous system (CNS) and the peripheral nervous system (PNS). In vertebrates, the CNS includes the brain and the spinal cord, but in many invertebrates it is comprised of ganglia, groups of nerve

cell bodies. The PNS in both vertebrates and invertebrates consists mainly of nerves connected to the CNS or ganglia. The neurons that make up the CNS and PNS are organized into circuits that deliver information. There are nerve cells that carry information both towards and away from the CNS: afferent, or sensory, neurons carry information to the CNS while efferent, or motor, neurons carry information away from it. Whereas afferent and efferent neurons carry information for long distances in the peripheral nervous system, interneurons act locally within the central nervous system to relay information between sensory neurons and motor neurons within a given circuit (Purves *et al.,* 2001).

Locomotion in general relies on a dynamic relationship between three components: the CNS, PNS, and muscles (Hughes & Thomas, 2007). Sensory feedback from the PNS aids in providing instant-by-instant adjustments to the rhythmic pattern that underlies locomotion, which is created by central pattern generators (CPGs) (Hughes & Thomas, 2007). CPG circuits of neurons are located within the CNS and are responsible for coordinating the activation of motoneurons, which control movements. The organization of CPGs in humans is still unclear, but their roles in locomotion, feeding, and breathing are essential (McCrea & Rybak, 2008; Lyengar *et al.*, 2011).

The human brain is comprised of approximately 100 billion neurons connected through trillions of synapses, which makes the study of behavior rather complicated and difficult. To further understand the human brain it is necessary to study model organisms with many fewer neurons, synapses, and a generally reduced complexity. Model organisms like the fruit fly *Drosophila* are often used to simplify the study of the nervous system. *Drosophila* is valuable in that powerful genetic tools are available for experimental modification of the nervous system.

The larval stage is particularly advantageous because its motor behaviors have a stereotypical, simple output meaning it can be easily quantified (Jones 2009; Lyengar et al. 2011). Additionally, with only 10,000 neurons, the larval stage is one order of magnitude simpler than the adult fruit fly (Louis et al. 2012).

Drosophila larval crawling is based on repetitive motor patterns where the muscles in each segment contract and relax in a wave-like peristaltic pattern that propagates from the posterior of the organism to the anterior in order to propel the organism in forward movement (Hughes & Thomas 2007). In larvae, a CPG is presumably responsible for generating this peristaltic, rhythmic locomotion (Hughes & Thomas 2007; McCrea & Ryback 2008). While CPG networks have been studied in other organisms like lamprey (Grillner, 2006), lobster (Marder & Bucher, 2007), and leech (Friesen & Krristan, 2007), the neural basis of the CPG in crawling *Drosophila* has yet to be studied extensively (Inada et al., 2011; Rickert et al., 2011). As a result, there is little information on which interneurons are required for larval locomotion in *Drosophila* (Suster et al., 2003). Studying CPG circuits is useful not only in understanding the systems involved in movement but also for understanding circuit function in general. Because vertebrates also utilize CPGs to generate repetitive movements, findings in *Drosophila* may eventually be applied to our understanding of the human nervous system.

Drosophila has historically been a well-studied organism and as such there are well-developed tools for analyzing nervous system function. The transgenic GAL4-UAS system (Brand & Perrimon 1993) allows manipulation of gene expression in controlled groups of cells. The system is composed of two parts. The first part is the GAL4 gene that is placed downstream of a tissue-specific genomic enhancer, and it encodes the yeast transcriptional activator GAL4. The

second part is the UAS (Upstream Activating Sequence) that is the enhancer to which GAL4 binds to initiate gene transcription. This two-part system of the GAL4 "driver" and the UAS "target" is useful for investigating neural function because it allows for genes under UAS control to be expressed only in cells that contain GAL4 (Berni et al. 2010). Currently, there are upwards of 10,000 GAL4 lines, many of which are available for order from stock centers, that have been screened for expression in specific tissues in various stages of development, making GAL4-UAS manipulation relatively simple and accessible (Hodge, 2009). This system has allowed the shift from morphological studies to functional ones meaning that we now have the tools to manipulate neural circuits rather than simply study their structure and organization (Meinertzhagen et al. 2009).

It is possible to either activate or deactivate neuronal groups using the GAL4-UAS system, and in this study they were deactivated using the UAS target EKO or "electrical knockout." The EKO line encodes a modified hyperactive potassium channel that electrically inactivates neuron activity (White et al., 2001). It can then be assessed if specific neural subsets have an effect on crawling behavior in *Drosophila*.

The GAL4 driver lines were chosen for their supposed connection to neuronal development and crawling behavior in *Drosophila* (Vömel & Wegener 2008; Suster et al. 2003). GAL4 driver lines as described in Table 1 in the Supplemental Materials were chosen to inhibit specific subtypes of neurons. Specifically, the interneuron populations involved in the crawling circuit of larvae were being investigated. Little is known about which populations of interneurons play a role in crawling behavior, so inhibiting expression of groups of interneurons and quantifying the effect on locomotion will help to understand which groups are important

within the crawling circuit. Particularly, cholinergic, dopaminergic, serotonergic, glutamatergic, and tyraminergic interneurons were tested for their involvement in larval crawling.

A heat shock approach was utilized to supplement the GAL4-UAS system with temporal control of the deactivation of subsets of neurons. It is a genetically encoded tool that takes advantage of temperature sensitive ion channels or vesicle trafficking proteins to manipulate membrane potentials or neurotransmitter releases (Berni et al. 2010). *Shibire* is a gene that encodes dynamin, a protein involved in synaptic release (Kitamoto, 2001). *Shibire*^{ts} encodes a temperature sensitive form of the protein. At the restrictive temperature, production of dynamin stops and the pool of vesicle proteins needed to transport neurotransmitters rapidly depletes (Jones, 2009; Lyengar et al. 2011). This allows for conditional inactivation of interneuron subpopulations.

Under the non-temperature sensitive (normal) GAL4-UAS system, the fruit flies grew and developed with the chosen groups of neurons deactivated, which presented a possibility that homeostatic mechanisms would compensate for deficiencies by normalizing neuronal activity levels through long term regulation of other protein expression levels. The heat shock approach allowed for a relatively immediate deactivation prior to observing the crawling behavior, which was advantageous in that it was possible to observe rapid behavioral consequences without the possibility of developmental homeostasis. Utilizing this rapid silencing technique eliminated the possibility of the larvae adapting to the change over time (Lyengar et al. 2011). A representative GAL4 line, the positive control *OK371-GAL4,* was chosen to cross with UAS-shibire^{ts} in order to examine the possibility of developmental homeostasis camouflaging behavioral effects of neuronal deactivation.

In this experiment, three controls were used: a wild type Oregon R that does not contain the GAL4-UAS sequences and two positive controls, *OK371-GAL4 x UAS-EKO* and *OK371-GAL4 x UAS-shi^{ts}* (see above) where many, but not all, of the motor neurons have been silenced. Deactivation of motor neurons should alter crawling behavior. Therefore, this positive control ensured that the GAL4-UAS system was working and that enough GAL4 was being made to effectively initiate gene transcription and decrease neuronal activity levels. Five parameters were measured in each animal: average speed, wave frequency, wave duration, turning behavior, and stopping time (Hughes & Thomas 2007).

II. Materials and Methods

Fly Culture

Room temperature conditions were used to culture all larvae. All cultures were fed using Instant Drosophila Medium Formula 4-24 from Carolina Biological Supply Company with 5-10 grains of supplemental dry active yeast. All tests were conducted using 3rd instar larvae, which occur at approximately 90 hours of age after the egg is laid (Lyengar et al. 2011).

Fly Stocks

All fly strains were obtained from the Bloomington Stock Center (http://flystocks.bio.indiana.edu) except *shi^{ts}* which was generously provided by Toshi Kitamoto. Wild type Oregon R larvae and F1 generation larvae from GAL4-UAS crosses were used for analysis. Descriptions of each line can be seen in Table 1 in the Supplemental Materials.

Larval Locomotion

To monitor larval locomotion, petri dishes were used as an arena where the larvae could crawl. A substrate of 6% agar, 2% sucrose, 2% acetic acid and 90% water was made to provide a soft, slick surface for crawling. Food coloring was added until the substrate obtained a dark green color to offset the color of the larvae when filming. The substrate was poured into petri dishes (~1cm thick) and allowed to set and cool. Petri dishes were stored at room temperature and were no longer used once they became visibly desiccated.

In each trial, five to twelve 3rd instar larvae were chosen and transported from their vials to the arena on the tip of a paintbrush. They were placed in the center of the arena and allowed to adjust to the new environment (~90 seconds). Larvae that crawled to the edge of the arena during this time were transferred back to the center using the tip of the paintbrush.

To record locomotion, suggestions were taken from the creators of CTRAX (ctrax.sourceforge.net) for how to set up video recording. Videos were filmed in a dark room with a Fiber-Lite® High Intensity Illuminator Series 180 as a source of light. The two 150W bulbs were situated above the petri dish but below the camera interface 17cm above the table. Bulbs were not pointed directly at the petri dish but instead off to the sides on either side so that the glare of the lights was not reflected in the substrate. To reduce glare as much as possible and give the highest quality of light, light shades were removed to expose the bulb, and bulbs were covered with one layer of Kimwipes. A Canon Vixia HF R300 digital camcorder was positioned directly above the petri dish 20cm from the table so that the entire dish would fit within the camera's field of view. Between trials, the substrate was wiped clean to remove residues.

Larvae in each trial were recorded for 60-90 seconds at room temperature and promptly observed under the Leica EZ4 dissecting microscope.

Temperature-sensitive inactivation of neuronal subsets was performed as follows. After an initial recording, the petri dish containing the *OK371-GAL4 x UAS-shi^{ts}* and control *UAS-shi^{ts}* larvae was placed in a Precision brand Low Temperature Illuminated Incubator Model 818 preset at 30.0°C (10 minutes). Following this treatment the larvae were immediately filmed again as previously described. They were then monitored to ensure recovery.

Videos and Analysis

Videos were acquired at 29 frames per second (1280 x 720 pixel resolution) in the compressed mp4 format setting. Videos were analyzed manually using iMovie version 8.0.6. Video parameters were designed to analyze the videos using CTRAX, an open-source software developed by Branson *et al* that allows for tracking the individual positions and orientation of many moving organisms to provide a quantitative output of behavior. Currently, all videos have been decompressed using VirtualDub (http://www.virtualdub.org/) and converted to an AVI format suitable for analysis using CTRAX. The software also utilizes MatLab's Statistics and Image Processing Toolboxes (http://www.mathworks.com/products/matlab/) in order to fix errors in CTRAX tracking and analyze behavioral patterns and statistics for each individual larva. Video analysis using CTRAX may be performed in the future.

The data was statistically analyzed by calculating 95% confidence ranges and performing T-tests in order to evaluate whether significant differences exist between wild type results, control results, and the rest of the crosses.

III. Results

Crosses were analyzed according to five quantitative parameters: speed, wave duration, wave frequency, the number of directional changes >40 degrees, and the amount of time spent stopping. Future video analysis using CTRAX will produce additional parameters with which to analyze data. Five animals were observed for data for each cross, except in noted cases where larvae were too immobilized to gather data. The temperature sensitive control, *OK371-GAL4 x UAS-Shi^{ts}* was affected to the extent that data for all parameters except directional changes could not be quantified due to the immobility of the larvae. Larvae were also observed for qualitative characteristics that were not represented quantitatively such as stopping time of individuals and behavior of colliding larvae. Two supplementary videos are provided, one of the wild type and one of *6793 x UAS-EKO.*

Speed

Larval movement was calculated in mm/sec for representative time frames when the larvae were consistently moving in a straight line and does not include time spent stopped. Data is shown in Figure 1a. Wild type larvae speed was calculated at about 1.55 mm/sec. Because of the large number of unmoving larvae, data for *6793 x UAS-EKO* only includes three samples (see discussion). While the positive control could not be calculated as explained previously, the *UAS-Shi^{ts}* used as a control for the temperature sensitive cross and the *OK371-GAL4 x UAS-Shi^{ts}* as monitored before the temperature treatment exhibited similar speeds to the wild type at 1.39 and 1.44 mm/sec respectively. Though three crosses did not exhibit significantly different speeds than the controls, ranging from 1.24 mm/sec for *C380-GAL4, cha-GAL80 x UAS-EKO* to

1.36 mm/sec for *27637 x EKO*, six were significantly different: *OK371-GAL4 x UAS-Shi^{ts}* (no data), *OK371-GAL4 x UAS-EKO (p=0.0038), 6793 x UAS-EKO (p=0.0001), 7009 x UAS-EKO (p=0.0001), 36494 x UAS-EKO (p=0.0001)*, and *C380-GAL4 x UAS-EKO (p=0.0002).* All had speeds significantly less than that of the wild type ranging from 0.39 mm/sec for *6793 x UAS-EKO* to 1.09 mm/sec for *OK371-GAL4 x UAS-EKO*.

Wave frequency

Wave frequency was defined as the number of waves completed in a given amount of time. Data is shown in Figure 1b. For the wild type, wave frequency was calculated to be about 1.08 waves/sec. This is slightly higher than the other two controls (*UAS-Shi^{ts}* at 1.00 waves/sec and *OK371-GAL4 x UAS-Shi^{ts}* before temperature treatment at 0.97 waves/sec) but not significantly different. Because of the large number of unmoving larvae, data for *6793 x UAS-EKO* only includes four samples. Similar to speed, there were seven crosses with wave frequencies significantly different than the controls: *OK371-GAL4 x UAS-Shi^{ts}* (no data), *OK371-GAL4 x UAS-EKO (p=0.0034), 6793 x UAS-EKO (p=0.0001), 7009 x UAS-EKO (p=0.0004), 24635 x UAS-EKO (p=0.0038), 36494 x UAS-EKO (p=0.0001),* and *C380-GAL4, cha-GAL80 x UAS-EKO (p=0.0039).* All had wave frequencies significantly lower than the wild type, with the lowest at 0.30 waves/sec (*6793 x UAS-EKO)*. It should be noted that wild type wave frequency in this experiment was significantly slower than previous studies with larvae crawling (Hughes & Thomas, 2007) where it was found to average 1.6 waves/sec, but this difference could be due to differences in lighting or crawling surfaces, both of which were controlled throughout the experiment.

Wave Duration

Larvae move in a characteristic peristaltic wave fashion and, as a result, their wave movements can be easily measured. Wave duration was defined as the time from the contraction at the tail to contraction at the head and excludes the between-wave pause interval. Data are shown in Figure 1c. The wild type and two negative controls had durations of about 0.40 seconds while others that were not significantly different had averages between 0.30 and 0.40 seconds (*27637 x UAS-EKO, C380-GAL4 x UAS-EKO*). Six crosses had wave durations varying significantly from the wild type: *24635 x UAS-EKO* had 0.53 seconds *(p=0.0009)*, *OK371-GAL4 x UAS-EKO (p=0.0001)* had nearly double the wild type at 0.75 *(p=0.025)*, and *6793 x UAS-EKO (p=0.0001), 7009 x UAS-EKO (p=0.0001)*, and *C380-GAL4, cha-GAL80 x UAS-EKO (p=0.0001)* more than doubled the wild type with 1.12, 1.30, and 1.21 seconds respectively.

Turning

Larval turning of the head greater than 40 degrees was counted for all larvae throughout the 30-second video clip except when turning was the direct result of collision with the walls of the petri dish. Data are shown in Figure 1d. The number of turns was fairly consistent for the wild type and negative controls but varied greatly within the crosses. Wild type and controls averaged about 2 turns/30sec/larva. One cross demonstrated significant displays of excessive turning: *27637 x UAS-EKO* averaged 6.17/30sec/larva *(p=0.0059)*. Two others averaged significantly lower than the wild type: *24635 x UAS-EKO* with 0.67/30sec/larva *(p=0.03)*, and *36494 x UAS-EKO* with zero turns *(p=0.0031)*.

Stopping

Many of the crosses, even those that appeared to be crawling normally, would stop for substantial amounts of time, which neither the wild type nor the negative controls tended to do. Stopping time did not include animals stopped as a result of running into petri dish walls. Most notably, *27637 x UAS-EKO,* which did not show significant differences in speed or wave functions, had larvae that spent large amounts of time still in the arena (data not shown). *A*ll but one individual stopped during the 30-second time frame for an average of 57% of the time per larva, with one larva actually stopped (but still moving its head) during the entire period (data not shown). *6793 x UAS-EKO* had an average of 62% of the time stopped, with four larvae never moving enough to quantify other parameters (data not shown). Others displaying similar behavior include *C380-GAL4 x UAS-EKO* with larvae stopped for 61%, 15%, and 72% of the time; *C380-GAL4, cha-GAL80 x UAS-EKO* with larvae stopped for 44%, 7%, 5%, and 63% of the time; and *24635 x UAS-EKO* with larvae stopped for 45% and 50% of the time. Additionally, the *OK371-GAL4 x UAS-Shi^{ts}* after the rise in temperature had three unmoving larvae during the 30-second period with all the larvae spending an average of 72% of the time stopped.

Qualitative Observations

In the *27637 x UAS-EKO* film, there are larvae that turn in circles. Additionally, many larvae can be observed implementing anterior to posterior peristaltic waves independent of interaction with other larvae or running into walls. This behavior was also noted when observing *6793 x UAS-EKO* and *7009 x UAS-EKO* under the dissecting microscope. It was also noted from the microscope observations that in comparison to the wild type *24635 x UAS-EKO* had unusual

peristaltic waves where the wave towards the back of the larva was irregular. Lastly, *C380-GAL4 x UAS-EKO* and *7009 x UAS-EKO* both had instances of larvae running into each other in a way that suggested they had not sensed that other larvae were near. They would run into each other and then quickly pull back as if they were not expecting the other larvae to be there.

IV. Discussion

Results for crosses with individuals that stopped or remained immobile throughout the videos (*GAL4-OK371 x UAS-shi^{ts}, 6793 x UAS-EKO, 24635 x UAS-EKO, 27637 x UAS-EKO, C380-GAL4, cha-GAL80 x UAS-EKO* and *C380-GAL4 x UAS-EKO*) are underrepresented in that unmoving larvae could only be analyzed quantitatively when they started moving again.

Interneurons in locomotion

All crosses observed displayed abnormal behavior in comparison to the wild type, either quantitatively or qualitatively. The cross that behaved the most differently from the wild type was *6793 x UAS-EKO* (see Figure 1). This cross prevents expression in all cholinergic neurons, and the lack of these interneurons severely inhibited the larvae's ability to move or perform stereotypical behaviors. Thus, the neurotransmitter acetylcholine, made by cholinergic neurons, appears to play an integral role in crawling behavior. This result has been previously noted in studies with *C. elegans* (Rand, 2007).

Similarly, preventing the activity of dopaminergic and serotonergic interneurons in *7009 x UAS-EKO* effectively inhibited the larvae's ability to crawl. However, *27637 x UAS-EKO,* which is a promoter for serotonin receptor 1b*,* failed to effect the same results, suggesting one of two

things: (1) serotonergic interneurons are not essential to crawling behavior, but are necessary for sensory input (as discussed below), or (2) serotonergic interneurons are important in crawling, but receptor 1B does not have a role in crawling. It is likely that serotonin does play a role in crawling as it has been previously shown that it, along with dopamine, it is vital for the crawling and swimming behaviors of *C. elegans* and the leech (Vidal-Gadea *et al.* 2011).

It has been previously shown through immunofluorescence and antibody labeling that tyramine and octopamine signaling are necessary for larval locomotion. This experiment was a morphological study concerned with characterizing single neural cells, and it showed that a small set of about 40 octopaminergic and tyraminergic neurons within the ventral nerve cord are sufficient to trigger normal locomotion in larvae (Selcho *et al.*, 2012). Our results support this study in that we have shown that lack of some tyramine interneurons slows down both the speed and the wave frequency of larval locomotion.

Because *C380-GAL4 x UAS-EKO* also prevents activity of some cholinergic neurons in addition to motor neurons, it was expected that the larvae would display similar results to that of *6793 x UAS-EKO.* While there was a reduction in crawling speed, wave functions appeared to be unaffected (Figures 1a, 1b, 1c). It is speculated that *OK371-GAL4 x UAS-EKO* was able to at least partially compensate for loss of function, as demonstrated by the shibire^{ts} experiments discussed below, and perhaps this also occurred with *C380-GAL4 x UAS-EKO.* A future cross of *C380-GAL4* with *UAS-shi^{ts}* would help to clarify the results.

Developmental homeostasis

Though *OK371-GAL4 x UAS-EKO* had significantly decreased motor function (see Figure 1), this deficiency was increased through the use of a temperature-sensitive cross, which displayed severe crawling inhibition. Therefore, this study demonstrates that it is possible for larvae to compensate throughout their development for deficiencies by normalizing neuronal activity. Specifically, this case of developmental homeostasis occurred in glutamatergic neurons in the ventral nerve cord that are involved in motor function (Mahr & Aberle, 2006). In agreement with our results, it has been previously suggested that inhibiting neurons during development could cause larvae to partially compensate for the loss (Kitamoto, 2001). However, a similar study using a tetanus toxin construct (TeTxLC) inhibiting neurotransmitter release instead of an electrical knockout (EKO) as the target line saw no change in phenotype between the *UAS-TeTxLC* targets and the *UAS-shi*^{ts} targets (Hughes & Thomas, 2007; Suster *et al.*, 2002). Because TeTxLC is similar to using EKOs, it was hypothesized that results between the knockout lines and the temperature sensitive one would not be significantly different. It may be that larvae can normalize the effects of EKOs better than TeTxLC. A comparative study would help determine which method might be the most useful in future studies.

Sensory feedback in locomotion

Larvae with wave durations longer than that of the wild type, wave frequencies slower than that of the wild type (see Figure 1a and 1b), or larvae that appeared to be unnecessarily running into each other without caution could be having locomotion problems related to sensory feedback. Previous studies have suggested that sensory deprivation disrupts normal

locomotion because excessively slow, tight, and exaggerated contraction waves indicate the need for sensory feedback during crawling. Sensory neurons could be providing feedback to the CPG as the wave progresses from posterior to anterior, thus aiding in the locomotion process (Hughes & Thomas, 2007). To our knowledge none of the lines studied label sensory neurons, but there is a possibility they could be. Determining exactly which neurons are being labeled will be important in understanding if sensory neurons are necessary for normal crawling behavior.

Also, *24635 x UAS-EKO* and *36494 x UAS-EKO* both exhibited turning behaviors that were below the average frequency for the wild type (Figure 1d). This could mean that they are not displaying the normal sensory behavior characterized by wild type larvae, which have forward peristaltic waves interspersed by normal, but stereotypic, actions of head turning (Suster *et al.*, 2002). Both an increase and a decrease of this usual behavior suggests problems with the sensory nervous system, as highlighted above, but absence of the behavior suggests a disconnect between the sensory and motor systems and an inability to be alert to the surrounding environment.

V. Conclusion

A summary of the data can be found in Table 2. According to the data collected in this experiment, all the interneurons observed appear to have a role in larval locomotion although specific effects on crawling behavior differ. Additional analysis with CTRAX may reveal other trends or behavioral patterns that could solidify these results. Further study of movement in

Drosophila could investigate the roles of developmental homeostasis and sensory feedback, as well as imaging of the neuron populations used in the study.

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VII. Supplemental Materials

Table 1. Fly strains descriptions.

Name/Stock Number	GAL 4 Driver Lines	Generated By
6793	Cholinergic neurons	Salvaterra and Kitamoto, 2001
7009	Dopaminergic and serotonergic neurons	Haywood et al., 2002
24635	Many, but not all, glutamatergic neurons of the larva	Saxton, 2007
27637	Serotonin receptor 1B promoter	Sehgal, 2009
36494	A large subset of ventral nerve cord and motor cord tyraminergic neurons	Stowers, 2011
OK371	5 types of dorsomedial motoneurons (including ventral nerve cord motoneurons and neuronal clusters in the brain)	Mahr A & Aberle H 2006
C380	Some motor and interneurons (including cholinergic)	Subhabrata Sanyal
C380cha	Some motor and interneurons (non-cholinergic)	Subhabrata Sanyal
Ore-R	Wild type	
Name/Stock Number	**UAS Target Lines**	**Generated By**
EKO	UAS responder that expresses a genetically modified Shaker K^+ channel (EKO channel) in targeted cells	White et al, 2001
UAS-shi^{ts}	Shibire temperature-sensitive	Kitamoto, 2001

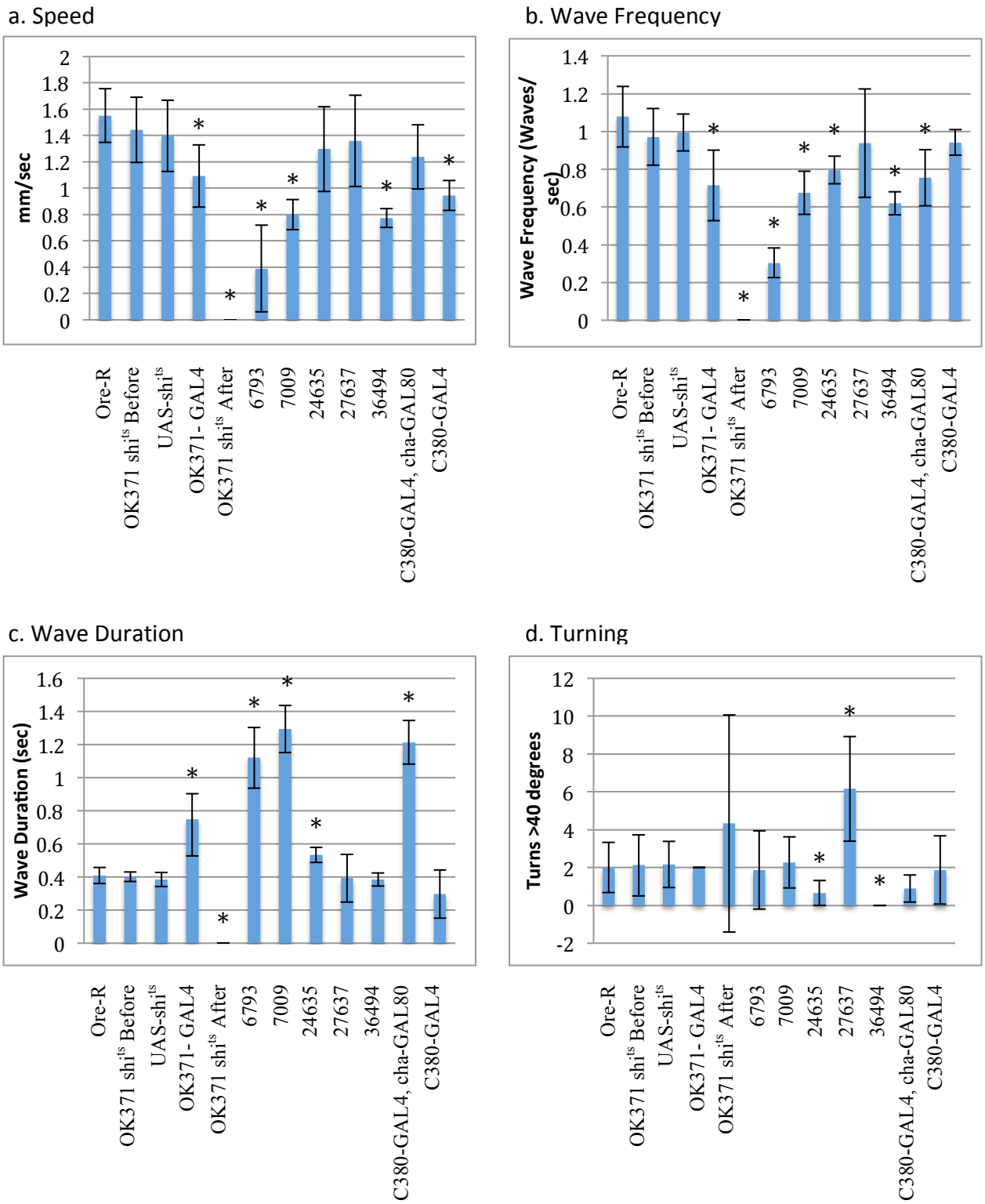

Figure 1. Crawling parameters of the larvae.

Parameters of crawling behavior were quantified from five individuals for each experiment. OK371-shi^{ts} Before indicates *OK371-GAL4 x UAS-shi^{ts}* before it underwent heat treatment, and OK371-shi^{ts} After indicates the same larvae immediately following treatment. OK371-GAL4 indicates the cross *OK371-GAL4 x UAS-EKO.* Error bars represent a 95% confidence range, and an asterisk (*) signifies significant difference from the wild type ($p < 0.05$).

Table 2. Summary of data

This table summarizes the data that is displayed in Figure 1 as well as other observations. The specific set of neurons targeted is listed under the line name. An 'X' indicates that the cross was significantly different from the wild type in the given parameters.

CONTROL	SPEED	WAVE FREQUENCY	WAVE DURATION	TURNING	QUALITATIVE OBSERVATIONS
ORE-R					
POSITIVE CONTROL					
OK371-GAL4 (Motor)	X	X	X		
KNOCKOUTS					
6793 (Cholinergic)	X	X	X		backward wave movement, 62% time spent stopped
7009 (Dopaminergic, Serotonergic)	X	X	X		backward wave movement, unexpected collisions
24635 (Glutamatergic)		X	X	X	irregular wave pattern, some spent up to 50% time stopped
27637 (Serotonergic)				X	backward wave movement, 57% time spent stopped
36494 (Tyraminergic)	X	X		X	
C380-GAL4, cha-GAL80 (Cholinergic)		X	X		some spent up to 63% time stopped
C380-GAL4 (Non-Cholinergic)	X				unexpected collisions, some spent up to 72% time stopped