



University of
Massachusetts
Amherst

Modular Laboratory Exercises to Analyze the Development of Zebrafish Motor Behavior

Item Type	Article
Authors	McKeown, Kelly Anne;Downes, Gerald B.;Hutson, Lara D.
DOI	10.1089/zeb.2008.0564
Download date	2026-03-15 08:29:01
Link to Item	https://hdl.handle.net/20.500.14394/3801

Zebrafish in Education

Modular Laboratory Exercises to Analyze the Development of Zebrafish Motor Behavior

Kelly Anne McKeown,¹ Gerald B. Downes,¹ and Lara D. Hutson²

Abstract

The embryonic zebrafish is an excellent research model to examine the neural networks that coordinate locomotive behavior. It demonstrates robust locomotive behavior early in development, its nervous system is relatively simple and accessible compared to mammalian systems, and there are mutants available with specific molecular and motor deficits. We have developed a series of four exercises that provide students with a basic understanding of locomotive behavior development, nervous system organization, development of neurotransmitter responsiveness, and genetics. The first two exercises can be performed in one 3-h laboratory period, and the third and fourth exercises, which build on the first two, can be completed in one or two subsequent periods. In the first exercise, students observe and quantify two distinct behaviors that characterize different developmental stages, spontaneous movement, and touch-evoked tail coiling. In the second, the students use a pharmacological approach to determine if the neurotransmitter glycine is required for the embryo to perform each behavior. In the third, they use simple lesions to assess whether the brain is required for each type of behavior. In the fourth, the students examine *bandoneon*, a zebrafish motility mutant that has a glycine receptor defect, by observing its behavior during spontaneous movement and touch-evoked tail coiling, performing lesions, and applying pharmacological drugs. These exercises are readily adaptable, such that portions can be omitted or expanded to examine other neurotransmitter systems or later stages of locomotive behavior development.

Introduction

LOCOMOTIVE BEHAVIOR IN VERTEBRATES, such as walking or swimming, relies upon neural networks in the brain and spinal cord. The embryonic zebrafish has several features that have made it an increasingly popular research system to examine how these networks develop and function.¹⁻³ Zebrafish embryos demonstrate robust locomotive behavior, develop rapidly and external to the mother, and are amenable to pharmacological drug application. Further, there are mutants available with specific locomotor behavior deficits. In the series of laboratory exercises that we describe here, these characteristics are leveraged to illustrate how the nervous system develops and controls locomotive behavior.

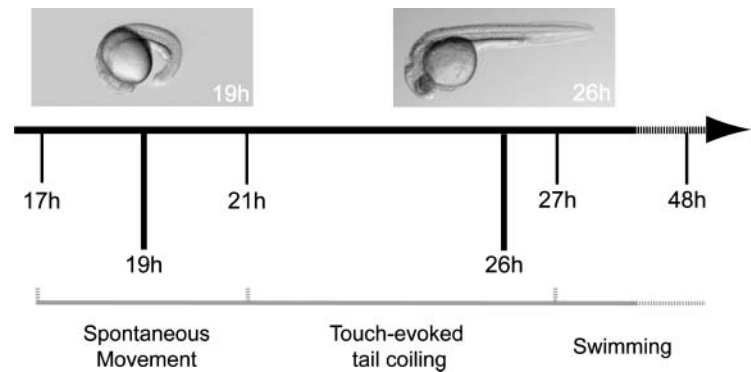
The development of locomotion in vertebrates is composed of a characteristic sequence of behaviors.⁴ Zebrafish embryos

progress rapidly through these behaviors (Fig. 1). The first behavior observed, beginning at 17 hours postfertilization (hpf), is spontaneous movement, during which embryos repeatedly perform slow, alternating tail coils. Beginning at 21 hpf, embryos first react to touch and demonstrate rapid tail coils in response to touch stimuli. Finally, at 27 hpf, the embryo begins to exhibit swimming behavior, in which it can move itself forward by at least one body length. In subsequent stages of development, embryos move faster and acquire different swimming behaviors, such as burst swimming and beat-and-glide swimming.^{5,6} These different behaviors reflect the development of locomotor networks in the brain and spinal cord. Using simple lesions, pharmacological drug application, and a locomotor mutant, this lab investigates the role of the brain and the neurotransmitter glycine in the first two behaviors, spontaneous movement and touch-evoked tail coiling.

¹Molecular and Cellular Biology Program, Department of Biology, University of Massachusetts, Amherst, Massachusetts.

²Department of Biology, Williams College, Williamstown, Massachusetts.

FIG. 1. Embryonic zebrafish motor behaviors. A time-line and important time-points are shown. Lateral views of embryos are depicted at top during the two stages that are the focus of these laboratory exercises. The first three behaviors that zebrafish embryos perform are indicated below. Although each behavior occurs primarily during a particular time span, the transition period between stages consists of a mixture of the two behaviors, which could generate some confusion for the students, so may be best avoided. These exercises can be expanded to also examine swimming behavior, which is easily studied at 48 hpf.



Roles of hindbrain and spinal cord in locomotor behavior

Compared to mammalian systems, the embryonic zebrafish hindbrain and spinal cord is relatively simple, with fewer cells and fewer axon tracts than mammalian systems.^{7,8} The hindbrain, composed of seven segments or rhombomeres, is known to integrate sensory information and initiate locomotive behavior.^{9,10} A major component of the zebrafish hindbrain is the roughly 50 reticulospinal neurons, whose axons project into the spinal cord to regulate spinal cord network activity. These axons begin to grow into the spinal cord at 22 to 24 hpf, well after spontaneous movement and touch-evoked tail coiling has begun.¹¹ In the embryonic zebrafish spinal cord, systematic efforts to identify neuronal classes revealed only motor neurons, seven types of interneurons, and Rohon-Beard sensory neurons.^{12–14} One study examined if the hindbrain is required to perform spontaneous movement, touch-evoked tail coiling, and swimming, by performing simple lesions and observing the behavior of tails from which brain input has been removed.¹⁵ Using these “spinalized” preparations, it was found that spinal cord net-

works can mediate each of these behaviors independent of the hindbrain, although swimming behavior is more difficult to elicit. The lesion component of this lab exercise will recapitulate aspects of this study to demonstrate these findings.

Glycine is essential for aspects of locomotive behavior development

Glycine, classically thought of as an inhibitory neurotransmitter, plays a central role in the locomotor networks in the hindbrain and spinal cord.^{16–18} In several different vertebrate preparations, it has been shown to provide inhibition within the hindbrain to control reticulospinal neuron output to the spinal cord. Within the spinal cord, glycine has been found to coordinate the alternating left and right rhythms that are essential for locomotor behavior^{4,19} (Fig. 2). Glycine exerts these effects through its receptor, a pentameric ligand-gated ion channel that selectively fluxes chloride ions (Fig. 3). Since glycine is important in both hindbrain and spinal cord networks, interrupting glycine receptor function disrupts motor behavior. Pharmacological blockade of glycine receptors with

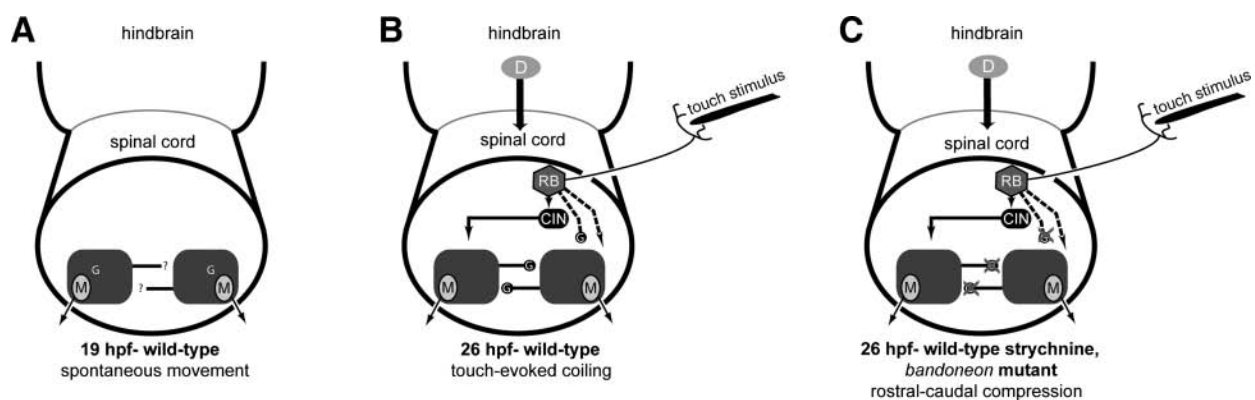


FIG. 2. The spinal cord during spontaneous movement and touch-evoked tail coiling. These schematics, adapted from Downes and Granato (2006),¹⁵ model neural networks in the hindbrain and spinal cord. (A) At 19 hpf there is little input from the hindbrain. Local circuits in the spinal cord (boxes) coordinate output to motor neurons (M), which innervate skeletal muscle. The neurotransmitter glycine (G) modulates spinal cord output, but its role is not entirely clear. (B) Touch-evoked tail coiling is mediated by Rohon-Beard (RB) neurons, which have projections in the skin. RB neurons make synaptic connections onto commissural interneurons (CIN), which project across the spinal cord to stimulate contralateral activity. RB cells also provide indirect ipsilateral excitation (dashed arrow) and glycinergic inhibition (dashed line with circle). Local spinal cord circuits provide glycinergic reciprocal inhibition (line with circle). Descending input from the hindbrain (D) is also being established around this stage. (C) Strychnine-treated wild-type and *beo* mutant embryos have impaired glycine receptor function, which diminishes ipsilateral and reciprocal inhibition (X).

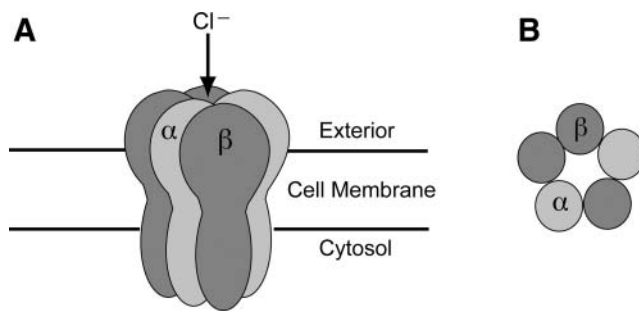


FIG. 3. The glycine receptor is a heteropentameric ligand-gated ion channel. **(A)** A lateral view of a glycine receptor is shown. The α and β subunits are indicated as well as the location of the pore (arrow), which selectively passes chloride ions. The β subunit, which is mutated in *beo* mutants, binds to scaffolding proteins inside of the cytosol (not shown) to help localize the receptor to synaptic regions. **(B)** A view from the outside of the cell shows the organization of the glycine receptor subunits and the channel pore. The glycine and strychnine binding sites reside at the interface between α and β subunits.^{16–18}

the competitive antagonist strychnine causes severe muscle convulsions, and mutation of glycine receptor subunits can result in hereditary motor disorders in humans, horses, cattle, mice, and zebrafish.^{16,20,21}

In zebrafish embryos, bath application of pharmacological drugs that target the glycine receptor alters motor behavior differently depending on the stage of development. Application of strychnine during touch-evoked tail coiling disrupts the alternating muscle contractions that constitute the alternating tail coils. Instead, the embryos compress along the rostral-caudal axis, like an accordion, due to simultaneous muscle contraction of the left and right sides.^{15,22} Application of glycine at these stages results in paralysis, caused by global activation of glycine receptors. Exposing embryos to these drugs earlier in development, during spontaneous movement, generates different behavior. Instead of causing accordion-like behavior, strychnine decreases the rate of spontaneous tail coils, while glycine application at this stage also leads to paralysis. The cellular and molecular mechanisms behind this finding are still unclear, but the different responses to strychnine at 19 and 24 hpf likely reflect the different roles of glycine during these two phases of locomotor network development.

Examination of a locomotor mutant reinforces the observations obtained using pharmacological drugs. *bandoneon* (*beo*) is a mutant recovered from large-scale mutagenesis screens to identify genes essential for locomotor behavior.²² In subsequent studies, it was found to harbor a mutation in *glrb2*, a glycine receptor subunit required for its proper subcellular localization.²³ Consistent with a disruption in glycinergic neurotransmission, *beo* mutants perform accordion-like compressions indistinguishable from wild-type embryos treated with strychnine during the touch-evoked tail coiling phase of development.²² Moreover, *beo* mutants are resistant to glycine application, and they continue to move at glycine concentrations that paralyze wild-type embryos. *beo* mutants do not show a motor defect during the spontaneous movement phase of locomotor behavior, indicating that the *glrb2* subunit is not required at this stage of development.

We have developed a series of laboratory exercises that use a combination of lesion analysis, pharmacological drug application, and *beo* mutants to illustrate how the spinal cord and the neurotransmitter glycine play essential roles in locomotive behavior development. These exercises are modular, and different combinations can be used that fit within one or more 3-h time periods. These exercises can also be tailored to individual interests and can be expanded to examine additional neurotransmitter pathways or later stages of development. The main learning objectives of this lab are

1. to observe and quantify differences in behavior,
2. to explore the neural underpinnings of behavior,
3. to perform statistical analysis of quantitative data, and
4. to develop skills that can be used for an inquiry-based approach in testing other neurotransmitters/stages of development.

Materials and Methods

Zebrafish resources

Information about zebrafish husbandry, breeding, staging, egg collection, and common chemical solutions can be found in *The Zebrafish Book*.²⁴ *The Zebrafish Book* and a wealth of information about zebrafish can be obtained from the zebrafish community website, <http://zfin.org>. Additional information can be found on the zebrafish education website, <http://zfic.org>.

Obtaining embryos

For this laboratory exercise, zebrafish adults should be maintained and bred in a nearby facility. Virtually any wild-type strain can be used to obtain wild-type embryos. The *beo* line is available from ZIRC, the Zebrafish International Resource Center (<http://zebrafish.org>). Heterozygous *beo* adults should be acquired and bred to obtain homozygous mutant embryos.

Instructor preparation

Embryos should be collected, and the fertilized eggs sorted and transferred into large 100-mm Petri dishes filled with E3. Roughly 160 embryos should be collected for each group of students the day before the lab. The embryos should be staged and sorted so that each student group has approximately 80 embryos at 19 hpf and 80 embryos at 26 hpf for the lab period. These two time points were chosen because 19 hpf is peak for the spontaneous tail coiling, and by 26 hpf the response to touch is evident with little spontaneous movement. However, embryos at any age between 17 and 20 hpf can be used to demonstrate spontaneous movement, and embryos between 26 and 30 hpf can be used to demonstrate response to touch. Embryo development can be slowed by placing them at room temperature, instead of 28.5°C. The following calculation can be used to estimate the developmental stage according to the temperature in which it is raised:

$$H_T = h / (0.055T - 0.57),$$

where H_T is the hours of development at temperature T , and h = the hours of development to reach the stage at 28.5°C.²⁵

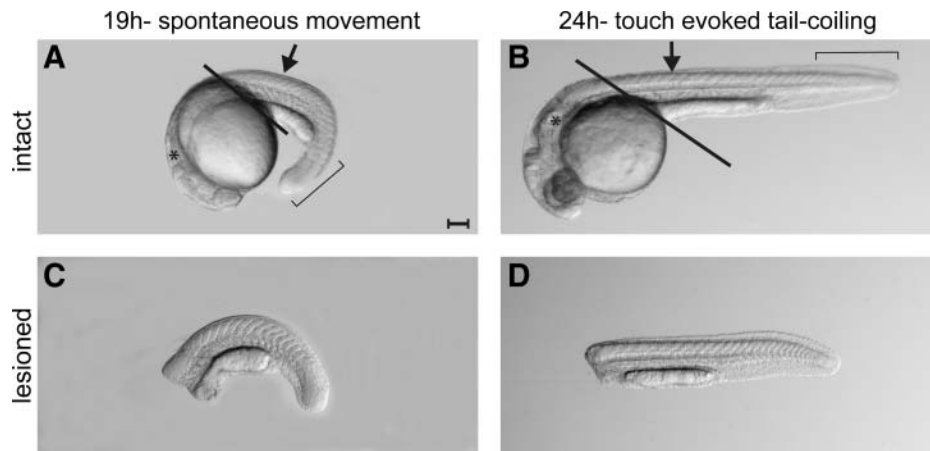


FIG. 4. Intact embryos and isolated tails at 19 and 26 hpf. (A, B) Lateral views are shown of live, intact embryos at both of the stages that are the focus of these laboratory exercises: spontaneous movement at 19 hpf and touch-evoked tail coiling at 24 hpf. The ear placode (*) is indicated as a landmark. The arrows indicate the area where the touch stimuli should be applied. The embryos should be lesioned within the first few somites (line). The brackets indicate the portion of the tail that should be removed to facilitate drug uptake. The scale bar in (A) is 0.1 mm. All panels were taken at the same magnification. (C, D) The corresponding isolated tails are displayed below.

Materials required for the laboratory exercise

Small (60×15 mm) and large (100×15 mm) Petri dishes (many scientific suppliers, e.g., VWR [West Chester, PA] cat. nos. 25384-090 and 25384-070)

Dumont forceps #5 (Fine Science Tools [Foster City, CA] cat. no. 11250-20)

Razor blades (e.g., VWR cat. no. 55411-050) or scalpel blades (e.g., Fine Science Tools cat. no. 10010-00)

Surgical blade holder (optional) (e.g., Fine Science Tools cat. no. 10060-13)

Dissecting stereomicroscope (many scientific suppliers)

Straight Probe (Fine Science Tools cat. no. 10140-01)

Plastic transfer pipettes (Fisher [Pittsburg, PA] cat. no. 13-711-7)

Stopwatch/timer

E3 (5 mM NaCl, 0.17 mM KCl, 0.33 mM CaCl₂, 0.33 mM MgSO₄)

1× Ringer's solution (116 mM NaCl, 2.9 mM KCl, 1.8 mM CaCl₂, 5 mM HEPES)

50 mM Glycine (EMD BioSciences [Gibbstown, NJ] cat. no. EM-4810) in Ringer's solution

20 mM Strychnine (Sigma-Aldrich [St. Louis, MO] cat. no. 50532-256) in Ringer's solution

4% Tricaine (Ethyl 3-aminobenzoate methanesulfonate salt, pH to 7.4; Sigma-Aldrich cat. no. A5040)

bandoneon (*beo*) embryos

Wild-type embryos

Laboratory Exercise

Student preparation for lab

Depending on the level of the student, there are several scientific articles directly relevant to this lab exercise. Granato *et al.* (1996) outline the screen in which the locomotive mutants were characterized.²² Brustein *et al.* (2003) give a nice overview on development of motor behavior in the zebrafish.¹ Hirata *et al.* (2005) characterize *beo* as a glycine

receptor mutation.²³ Lastly, Downes and Granato (2006) examine supraspinal input and its role in glycine-mediated behavior.¹⁵

Laboratory activities

The lab is divided into the following four exercises. In Exercise 1, students are responsible for dechorionating and staging the embryos. The students are then introduced to spontaneous tail coiling at 19 hpf and touch-evoked tail coiling at 26 hpf. The following exercises build upon these two different stages of motor behavior. Exercise 2 focuses on the use of pharmacologic drugs, glycine and strychnine, to elucidate the role of glycine in generating these two different motor behaviors. In Exercise 3, the students remove the head and analyze spinalized preparations to examine the distinct roles of the brain and spinal cord during 19 and 26 hpf (Fig. 4). Finally, Exercise 4 uses *beo*, a mutant known to have a mutation in the glycine receptor, as another tool to examine the role of glycine in these two motor behaviors. As previously stated, these are modular exercises, each of which builds on previous one(s). Exercise 1 can be performed alone or in combination with 2, and as long as both 1 and 2 have been completed, either Exercise 3 or 4 (or both) can be done. Exercises 1 and 2 can be performed in one 3-h laboratory period, while Exercises 3 and 4 can each be done in one or more additional laboratory periods. We recommend that students work in groups of two to four. See Appendix for a step-by-step approach to each of the exercises.

Notes for the instructor about analysis

We typically have students tabulate all data using Excel, either in the lab or later, entering the data that were recorded in their laboratory notebook. Using Excel, they can calculate the average, standard deviation, and standard error of the mean from five embryos from each group. These data can also be graphed. The primary goals of each exercise are to compare

differences in spontaneous activity and touch-evoked tail-coiling between the different stages of development (Exercise 1); compare wild-type, drug-treated embryos with wild-type untreated embryos (Exercise 2); compare spinalized preparations with nonlesioned embryos (Exercise 3); and compare *beo* mutants with wild-type embryos (Exercise 4). For 19 hpf embryo data, the students should tabulate the frequency of tail coiling for control wild-type embryos (C), wild-type treated with strychnine (S), wild-type treated with glycine (G), and *beo* mutants. To examine the response to touch, a table can be made that indicates a positive or negative response as well as any comments on the qualitative nature of the touch response (e.g., rostral-caudal compressions) at 19 and 26 hpf for all conditions (C, S, G, spinalized for 26 hpf, and *beo*). The students can then include tables and graphs in a formal lab report. We typically recommend that the students write lab reports in the style of a research article, including an introduction summarizing motor behavior development in zebrafish; a detailed methods section; a results section with figures, tables, and graphs; a discussion of their data in the context of the neural circuitry of zebrafish embryos and previously published results; and references. Statistical analysis could also be performed on the data if the students have a background in statistics. ANOVA or a Student's *t*-test can be used for comparison with wild type. The instructor or the students may also pool data from the whole class as a basis for comparison with individual group data to demonstrate the power of large sample sizes. The students could also be prompted to use the following questions as guidelines in writing up their lab reports:

Exercise 1

How do spontaneous movement and touch-evoked motor behavior change through development?
What are the defining features of zebrafish locomotive behaviors at 19 and 26 hpf?

Exercise 2

How do strychnine treatment and glycine treatment affect behavior?
Are these results consistent with each other?
Relate your findings to the zebrafish neural circuitry.
What were your controls and what do they control for?

Exercise 3

What do lesions tell us about spontaneous and touch-evoked movement?

Exercise 4

How does the *beo* mutant behavior compare to the behavior of strychnine-treated embryos?
What is the result of treating *beo* mutants with glycine? Explain.
What were your controls and what do they control for?

Expected results

In Exercise 1, students should observe spontaneous tail coiling from embryos at 19 hpf, which decreases in frequency after 21 hpf. The response to touch is evident only after 21 hpf.

We recommend using embryos 26 hpf or later since much of the spontaneous coiling has stopped, which makes it easier to distinguish the touch response. In Exercise 2, application of strychnine at ~19 hpf results in a decrease in the number of tail coils per minute compared to the control. Application of glycine leads to paralysis at both 19 and 26 hpf. However, the response to strychnine at 26 hpf differs from that at 19 hpf. The strychnine-treated embryos exhibit the accordion phenotype, a rostral-caudal compression along the length of the embryo, in response to touch. In Exercise 3, spinalized embryos at both time points exhibit a similar phenotype to the nonlesioned embryos, indicating that supraspinal input is not required for these two behaviors. However, the reliability of the response to touch at 26 hpf may be slightly diminished. In Exercise 4, the *beo* mutant exhibits an accordion-like phenotype similar to that of strychnine-treated embryos after 24 hpf. Application of glycine has little effect on the *beo* mutant, reflecting the defect in the glycine receptor subunit.

Discussion

The overarching goal of this series of laboratory exercises is to teach students to think about the neural basis of simple behaviors in zebrafish embryos, with an emphasis on the role of the neurotransmitter glycine in mediating these behaviors. Exercises 1 and 2, which can be done together in a single 3-h laboratory period, can achieve these goals. These exercises require the students to, at minimum, make and record observations, quantify behavior, and interpret the results of pharmacological manipulations. Depending on the level of sophistication required by the instructor in analysis and interpretation, the students may also perform statistical analysis of their quantitative data. Exercises 3 and 4 teach simple surgical techniques and an appreciation for the power of genetic mutants. Several simple models have been used to describe the neural basis of locomotor behavior in the zebrafish,^{1,15} and students at many levels should be able to analyze their results in the context of these models.

The authors have collectively performed these experiments several times, with and without labs full of students. While we have not performed any sort of assessment of their impacts on student learning, our feedback has been uniformly positive. In our experience this is one of the most straightforward, enjoyable, and successful teaching labs we have administered.

While these exercises give students an opportunity to think critically, perform quantitative analysis, and compare their results with the published models of locomotor circuitry, it is becoming increasingly clear that to really engage our students and teach them how to ask the right questions (and then seek out the answers to those questions) it is critical that we provide them with inquiry-based learning opportunities.²⁶ An important goal of this lab is to give students the skills that they need to design experiments of their own, such as studying swimming, a more complex behavior that is observed beginning around 27 hpf, or examining the roles of other neurotransmitters at these and other stages of development.

Acknowledgments

We thank members of the Downes Lab and Dr. John Nambu for their critical reading of the manuscript. We also

thank Dr. Michael Granato for his contribution in establishing these laboratory exercises.

Disclosure Statement

No competing financial interests exist.

References

1. Brustein E, Saint-Amant L, Buss RR, Chong M, McDearmid JR, Drapeau P. Steps during the development of the zebrafish locomotor network. *J Physiol Paris* 2003;97:77–86.
2. Fetcho JR, Bhatt DH. Genes and photons: new avenues into the neuronal basis of behavior. *Curr Opin Neurobiol* 2004; 14:707–714.
3. Lewis KE, Eisen JS. From cells to circuits: development of the zebrafish spinal cord. *Prog Neurobiol* 2003;69:419–449.
4. Roberts A. Early functional organization of spinal neurons in developing lower vertebrates. *Brain Res Bull* 2000;53:585–593.
5. Saint-Amant L, Drapeau P. Time course of the development of motor behaviors in the zebrafish embryo. *J Neurobiol* 1998;37:622–632.
6. Buss RR, Drapeau P. Synaptic drive to motoneurons during fictive swimming in the developing zebrafish. *J Neurophysiol* 2001;86:197–210.
7. Kimmel CB, Powell SL, Metcalfe WK. Brain neurons which project to the spinal cord in young larvae of the zebrafish. *J Comp Neurol* 1982;205:112–127.
8. Fetcho JR, Liu KS. Zebrafish as a model system for studying neuronal circuits and behavior. *Ann NY Acad Sci* 1998; 860:333–345.
9. Gahtan E, Baier H. Of lasers, mutants, and see-through brains: functional neuroanatomy in zebrafish. *J Neurobiol* 2004;59:147–161.
10. Moens CB, Prince VE. Constructing the hindbrain: insights from the zebrafish. *Dev Dyn* 2002;224:1–17.
11. Mendelson B. Development of reticulospinal neurons of the zebrafish. I. Time of origin. *J Comp Neurol* 1986;251:160–171.
12. Bernhardt RR, Chitnis AB, Lindamer L, Kuwada JY. Identification of spinal neurons in the embryonic and larval zebrafish. *J Comp Neurol* 1990;302:603–616.
13. Downes GB, Waterbury JA, Granato M. Rapid *in vivo* labeling of identified zebrafish neurons. *Genesis* 2002;34:196–202.
14. Hale ME, Ritter DA, Fetcho JR. A confocal study of spinal interneurons in living larval zebrafish. *J Comp Neurol* 2001; 437:1–16.
15. Downes GB, Granato M. Supraspinal input is dispensable to generate glycine-mediated locomotive behaviors in the zebrafish embryo. *J Neurobiol* 2006;66:437–451.
16. Betz H, Kuhse J, Schmieden V, Laube B, Kirsch J, Harvey RJ. Structure and functions of inhibitory and excitatory glycine receptors. *Ann NY Acad Sci* 1999;868:667–676.
17. Betz H, Laube B. Glycine receptors: recent insights into their structural organization and functional diversity. *J Neurochem* 2006;97:1600–1610.
18. Rajendra S, Lynch JW, Schofield PR. The glycine receptor. *Pharmacol Ther* 1997;73:121–146.
19. Grillner S. The motor infrastructure: from ion channels to neuronal networks. *Nat Rev Neurosci* 2003;4:573–586.
20. Andermann F, Andermann E. Startle disorders of man: hyperekplexia, jumping and startle epilepsy. *Brain Dev* 1988; 10:213–222.
21. Legendre P. The glycinergic inhibitory synapse. *Cell Mol Life Sci* 2001;58:760–793.
22. Granato M, van Eeden FJ, Schach U, Trowe T, Brand M, Furutani-Seiki M, *et al.* Genes controlling and mediating locomotion behavior of the zebrafish embryo and larva. *Development* 1996;123:399–413.
23. Hirata H, Saint-Amant L, Downes GB, Cui WW, Zhou W, Granato M, *et al.* Zebrafish bandoneon mutants display behavioral defects due to a mutation in the glycine receptor [beta]-subunit. *Proc Natl Acad Sci USA* 2005;102:8345–8350.
24. Westerfield M. *The Zebrafish Book: A Guide for the Laboratory Use of Zebrafish (Danio rerio)*, 3rd ed. Eugene, Oregon: University of Oregon Press, 1995.
25. Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF. Stages of embryonic development of the zebrafish. *Dev Dyn* 1995;203:253–310.
26. Loucks-Horsley SOS. *Inquiry and the National Science Education Standards: A Guide for Teaching and Learning*. Washington DC: National Academic Press, 2000.

Address reprint requests to:

Gerald B. Downes, Ph.D.

Molecular and Cellular Biology Program

Department of Biology

University of Massachusetts

Amherst, MA 01003

E-mail: gbdownes@bio.umass.edu

Lara D. Hutson, Ph.D.

Department of Biology

Williams College

Williamstown, MA 01267

E-mail: lara.d.hutson@williams.edu

Appendix

EXERCISE 1: Staging zebrafish embryos and quantifying normal behavior

- a) Dechorionate embryos using fine forceps to gently pull away the chorion from the embryo. Ensure that the embryos are not damaged, and allow them to sit for 20 min before recording tail coils/min.

Instructor Note: This may also be done before lab if time is limited.

- b) Determine which embryos are 19 and 26 hpf (Fig. 1).
- c) Select at least 40 undamaged dechorionated embryos at each stage.
 - i. Transfer five wild-type embryos at 19 hpf to another small Petri dish. For each of these five embryos, record the age and number of tail coils per minute in your laboratory notebook or, if computers are available, in an Excel spreadsheet.

Instructor Note: Three embryos instead of five can be used if short on time. Each group's data can be pooled at the end to increase the sample size.

- ii. Transfer five wild-type embryos at 26 hpf into another small Petri dish. Compare and contrast the spontaneous movement at 19 and 26 hpf.
- iii. Touch the 19 hpf and the 26 hpf embryos with a probe just above the yolk extension, and record whether or not they respond to touch (Fig. 4).

Instructor Note: The intent is to recognize that evoked movement is not detected in embryos earlier than 24 hpf.

EXERCISE 2: Application of pharmacologic drugs

Instructor Note: Strychnine is a poison. Reinforce laboratory safety when performing experiments with drugs. Students should be sure to wear gloves and safety goggles when handling the pharmacological drugs. After use, strychnine should be disposed of with hazardous waste.

- a) Label two sets of 15 small Petri dishes (one set for 19 hpf embryos and the second set for 26 hpf embryos): 5 for glycine treatment, labeled G1–G5; 5 for strychnine treatment, labeled S1–S5; and 5 for controls, labeled C1–C5.
- b) Half fill each dish C1–C5 with 1× Ringer’s solution.
- c) Half fill each S1–S5 with 200 μ M strychnine solution diluted in 1× Ringer’s solution.
- d) Half fill each dish G1–G5 with 500 mM glycine diluted in 1× Ringer’s solution.
- e) Cut the tips of the tails off of at least fifteen 19 hpf embryos and at least fifteen 26 hpf embryos, to facilitate penetration of the drugs.

Instructor Note: This should be done with embryos in a large Petri dish using a dissecting stereomicroscope. Ensure only the tip of the tail is cut off (Fig. 4). We do not typically use tricaine for head removal; however, it may be desirable in some cases. The effects of tricaine are rapidly reversible, so it is not likely to affect the outcome of the experiment, but we recommend testing this before doing the experiment in a classroom setting.

- f) Place one 19 hpf embryo, with the tip of the tail cut, into each dish (C1–C5, G1–G5, and S1–S5). Do the same for 26 hpf embryos.
- g) After 20 min, count and record the number of tail coils per minute for the 19 hpf embryos and record whether or not each of the 26 hpf embryos respond to touch.

EXERCISE 3: Head removal and analysis of spinalized preparations

Instructor Note: Lesioning should be performed after embryo staging, but at least 20 min before analysis.

- a) Before lesioning, add 40 μ L of 4% tricaine to a large Petri dish filled with 1× Ringer’s solution. This Petri

dish will be used to perform the lesions. Add 10 embryos to this dish and wait for the embryos to stop moving.

Instructor Note: The final concentration of tricaine should be between 0.0016% and 0.04%. It is important to add enough tricaine to ensure that the embryos do not move, but too much tricaine will lead to death.

- b) Using a razor blade or scalpel, remove the heads of 10 embryos at 19 hpf and another 10 at 26 hpf at the junction of the yolk ball and yolk extension as shown in Figure 4. The forebrain, midbrain, and hindbrain should be removed.

Instructor Note: The diagram depicting where the lesion should be made may be helpful (Fig. 4).

- c) Place the spinalized embryos (i.e., the tails) in a new Petri dish filled half way with 1× Ringer’s solution. After the embryos have recovered in 1× Ringer’s solution for 20 min, quantify and record movement by counting the number tail coils per minute for the 19 hpf embryos. For both 19 and 26 hpf spinalized embryos, record whether or not they respond to touch as well as any specific comments on the response.
- d) As a control, maintain five nonlesioned 19 and 26 hpf zebrafish in a separate dish with 1× Ringer’s solution.

Instructor Note: If Exercises 1 and 2 are performed on the same day, the embryos from Exercise 1 serve as controls.

- e) If time permits, the same pharmacological analysis in Exercise 2 can be done using spinalized preparations.

EXERCISE 4: Mutant analysis

- a) Place 80 embryos from a *beo* heterozygous cross in a large Petri dish filled with E3 and record the number of embryos exhibiting a rostral-caudal (accordion-like) compression of the tail at ~19 and ~26 hpf. Only *beo* homozygotes, approximately one quarter of the clutch, should demonstrate abnormal behavior.

*Instructor Note: This portion of the lab is a good place to review genetics. Also note that the *beo* phenotype is not exhibited until approximately 24 hpf.*

- b) Record and comment on the *beo* phenotype in response to touch at 26 hpf.
- c) Treat *beo* mutants with glycine as in Exercise 2 and explain the observation.

Instructor Note: The students should appreciate the concept that both the neurotransmitter and its receptor are necessary to generate a functional neural circuit.

