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Submission

2. PI Contact Record

Please provide information about the Principal Investigator.

Principal Investigator	Fetcho, Joseph
Protocol Application Number	2009-0084
Document Type	Full Review
PI	Fetcho, Joseph
Net ID	JRF49
Primary Role	Principal Investigator
Organization	Arts And Science / Neurobiology And Behavior
Position	Professor
Preferred Contact Method (Phone, Email, Cell, etc.)	Email
Campus Phone	254-4341
Email(xxxx@xxxxx.xxx)	JRF49@cornell.edu

Please review and update person's campus address information. City, state, zip and country are only required if above address is off the main campus in Ithaca.

Address 1	
Address 2	
City	
State	
Zip	
Country	Usa

3. Protocol Information

Title, Type, Non-Scientific Abstract &Emergency Contact

Please provide a title for this protocol.

Identify the protocol type.

In the abstract space provided below:
* Briefly describe the major goals of the protocol, the research question to be answered or, in the case of a teaching or demonstration protocol, a brief description of the objectives, and why the animal model you chose is the

appropriate model.
* Write in non-technical language. For example, how would you describe your work to a group of high school students? All abbreviations/acronyms should be defined on first use.
* Be clear and concise; several sentences to one or two short paragraphs, up to 200-250 words, should be adequate.

(If a teaching protocol, provide course number and title.)
Neurobiology of zebrafish
Research
This proposal involves experiments to study the neurobiology of sleep and control of movements in zebrafish. This protocol also involves experiments to study stress defense and endurance in zebrafish following transcriptional activation of antioxidant response (AR), using a small-molecule technique termed GAIN (detailed procedure described elsewhere). Sleep: Sleep and sleep states are fundamental not only to human life, but to every animal with a nervous system. Surprisingly, it is still not clear why they are so important. One compelling idea is that there are global shifts in the strengths of synaptic connections and excitability during sleep that act to keep synaptic function and neuronal excitability in a range where synapses and excitability of neurons can change relative to one another to allow for learning. If this does not happen, network function and behavior, whether in a worm or a human, degrade, leading ultimately to death. Such thinking about an important role of homeostatic mechanisms is moving to the fore in neuroscience, but what is needed to test hypotheses about global patterns of change in synapses and excitability is a model system and tools that allow us to monitor single synapses and neurons broadly in the living brain. We propose to develop and apply optical and electrophysiological tools that allow us to examine patterns of scaling of synapses and excitability in the transparent larval zebrafish model where we can monitor

these regularly and non-invasively over time during sleep and wakefulness. We will use these to directly test whether global resetting occurs during sleep. If sleep really involves such rescaling, the implications would be major, not only for a basic understanding of sleep, something that we should understand by now, but also for trying to restore functional states when sleep is impaired as a result of sleep disorders.

Motor control:

The hindbrain and spinal cord are critical for the control of movements. The proposed work attempts to reveal principles of organization of the circuits for movement in hindbrain and spinal cord. The structure of the circuits will be studied in transgenic fish with different neuronal classes labeled with fluorescent marker; the function and connectivity of the circuits will be studied by calcium imaging or by patch electrophysiology. We expect that the work will reveal a ground plan underlying the function of motor circuits of fish that reflects the pattern in vertebrates more generally.

Adult zebrafish imaging:
In order to test the possibility that the long wavelength 3 photon microscopy recently invented in Chris Xu s laboratory at Cornell can be used to image adult zebrafish, we plan to evaluate it by doing some adult imaging. This will set the stage for studying structure and function thought a vertebrate brain in a non-invasive way.

Provide the name of the person to contact in case of emergency associated with this protocol.
Joseph R. Fetcho
Business Hours Phone #:
607 254 4341
Non-Business Hours Phone#:
607 257 1877
Notes

Attachments List

File Spec	Description	Created

4. Collaboration with Live Animals and Outside Organizations

For each collaborating organization, please provide the information requested below. For guidance, refer to IACUC Policy 350 : Research with Collaborating Organizations or click here for information on procedures .

Will you be collaborating using live animals with an outside organization? If yes, please complete the Collaboration Form and attach below.	NO

Attachments List

File Spec	Description	Created

5. Scientific Design

Research Protocol: Please explain the scientific design(s) of the studies covered by this protocol. For each experiment (or type of experiment), clearly identify treatment and control groups. For studies involving multiple treatments or sequential procedures, clearly outline the project time line. Use scientifically correct language, but avoid jargon.
Procedural details involving animals including non-surgical procedures, (e.g. blood collection, behavioral training, administration of substances or test compounds, breeding, tumor induction etc.) surgical procedures and care, and restraints are covered in Section 13.7. (and its subsections) and should be omitted here.
Avoid including details of experiments or procedures that have no impact on animal use or animal welfare. Do include the rationale and need for the use of tissues *in vitro*.
Attach a figure or a table in a file, if necessary, but do not exceed 2 pages.

Teaching or Demonstration Protocols: Briefly describe the exercises that will involve the use of animals and how this relates to the goals of the course. Attach a course outline or syllabus to this protocol application. Applications without an attached syllabus will not be reviewed.

Design
Motor control: Objective 1: To determine if the neurons in the transmitter stripes connect topographically to those in other stripes. Hypothesis: Neurons at the same dorsoventral position and thus the same age connect to those in other stripes at the same dorsoventral location and age. Experiments: Channelrhodopsin will be expressed in a stripe and neurons in that stripe will be activated by light while recording from a cell in another stripe to assess patterns of connectivity. Objective 2: To examine how the position, morphology and projection patterns of individual neurons within hindbrain stripes change as neurons migrate out of the stripes to form nuclei. Hypothesis: Neurons connect to others in stripes and then migrate, while maintaining connections, to form nuclei. Experiments: Transient transgenic labeling with membrane targeted fluorescent proteins along with targeted injections into stripes will be used to examine the structure of neurons and their patterns of projections. The neurons will also be tracked during their development. Objective 3: To explore basic electrophysiological properties of neurons located at different positions within the stripes. Hypothesis: The input resistance, and hence to some extent the excitability, of a neuron maps onto its time of differentiation and its location within a stripe. Experiments: The input resistance and firing properties of neurons at different locations within a stripe will be examined by targeted patch recording from neurons at different places in the stripes. Connectivity between stripes will be examined by using light activation of neurons or laser perturbation of connections and electrophysiological recording from candidate postsynaptic neurons. Objective 4: To explore when connections form onto neurons

that migrate a long way and whether that migration is critical for function.

Hypothesis; Neurons develop connections early and then migrate to their later positions. The migration itself is not critical for proper connectivity.

Experiments: The connectivity and activity of neurons in the facial motonucleus will be studied during development in normal fish and in mutant lines without migration to assess how the connectivity and function is tied to migration.

Objective 5: To determine if transneuronal transport of CRE recombinase can be used to map which neurons are connected to targeted neuron.

Hypothesis: Transneuronal transport of CRE will lead to recombination and expression of a fluorescent protein in a floxed transgenic line and the presence of that protein will allow the identification of the connected neurons.

Experiments: CRE recombinase coupled to transneuronally transported proteins such a TTC and WGA will be injected into fish to label neurons. Transport of the construct across synapses will lead to visualization of the connected cells.

Sleep:
Objective 1: To examine changes in the level of synaptic proteins and receptors during sleep and wakefulness with optical methods.

Hypothesis: We will test the hypothesis that the strength of synapses is systematically scaled down during sleep.

Experiments: We will make transgenic animals with fluorescently tagged synaptic proteins and image these at different time during the day and night and in sleep deprived larvae (sleep deprivation is accomplished by tank vibration or electrical stimuli) to observe whether scaling occurs.

Objective 2 : To test synaptic scaling electrophysiologically.

Hypothesis: The strength of synapses will change systematically during sleep, with excitatory synapses weakening broadly.

Experiments: We will use light activation of neurons and patch recording at different times to directly measure the strengths of connections between neurons to observe how they change during the day and night.

Objective 3: To examine changes in the excitability of neurons during sleep and wakefulness.

Hypothesis: The level of excitability of neurons will change systematically during sleep.

Experiments: We will use light activated ion channels, in vivo calcium imaging, and electrophysiology to examine how the ability to activate particular neurons changes during sleep and wakefulness.

Imaging into adult zebrafish with three photon microscopy:

Objective: To look through an intact living zebrafish brain.

Hypothesis: We will be able to noninvasively image structure and function through the entire brain of an adult zebrafish with three photon microscopy.

Experiments: Zebrafish 1-4 months of age with fluorescently tagged neurons will be imaged through the head (either totally intact, or initially with the brain exposed) to test the imaging depth of three photon microscopy to open the possibility of non invasive structural and functional studies in adult zebrafish. The work is primarily focused on development of the imaging technology, with some initial biological studies of the structural and functional organization of hindbrain already being studied in larval fish (e.g. objective 2 above, but with the imaging pushed even later in life).

Attachments List

File Spec	Description	Created

6. Animal Use Assurance

Please provide assurance that the activities in this protocol are justifiable uses of animals by checking any of the following statements which apply.

Check all statements that apply to this protocol:
These experiments have not been done before.
Yes
Previously performed experiments were inconclusive.
These experiments extend our knowledge.
Yes
Animals will be used for teaching, demonstration, breeding or other non-experimental purposes.

7. Field Studies

Studies Conducted with Wild Animals in Their Natural Habitat.

Please provide the following information about animal work that will be conducted with wild animals in their natural habitat. You must describe in sections 13.8 and 13.11 of this protocol, a contingency plan including possible treatments or euthanasia if any animals are injured. If you will be administering any substances or drugs, (including C02) for euthanasia, please add them to section 13.10. Please follow ACUP 718 Safety Guidelines for Field Studies.

Does this protocol involve studies conducted with wild animals in their natural habitat?	NO
If yes, answer the following:	
Please provide a brief description of the field site.	
Please confirm that	

all applicable permits will be obtained prior to starting the field work.	
ACUP 718 Safety Guidelines for Field Studies must be followed. If you are not able to adhere to this ACUP, please describe any deviations and explain why those changes are necessary.	
Please describe the Personal Protective Equipment (PPE) that will be used. Please **attach** any additional SOPs or documentation available.	

Attachments List

File Spec	Description	Created

8. Custom Antibodies from Outside Sources

Please indicate if custom antibodies will be made in animals from material you provide to an outside source for this protocol. If so, additional information will be required. Note: Antibodies available "off the shelf" are not considered "custom". If you are producing antibodies on this protocol, please add the information to section 13.7.2 instead of completing this section. For guidance, see IACUC Policy 360 : Obtaining and Using Custom Antibodies

Will custom antibodies be made in animals from material you provide to an outside source?	NO

9. Use of Hazardous Agents

Please indicate if any hazardous materials will be administered to animals.

Hazardous Agents include:
Biohazardous agents: infectious agents, toxins, recombinant or synthetic nucleic acid molecules (r/sNA), viral vectors, human/primate tissues, fluids or cells. If using Biohazardous agents you must have prior approval by the Institutional Biosafety Committee. For more information go to IBC.
Hazardous chemicals: acute toxicants, teratogens, mutagens, carcinogens, antineoplastic compounds.
Radioactive agents: radioactive isotopes or an irradiator (Cs137).

Also indicate if wild caught mammals, pregnant sheep/newborn lambs or calves under 30 days of age will be handled, as these animals may pose an increased risk for rabies, Coxiella burnetii or Cryptosporidium parvum, respectively. If you answer yes to any of the questions in this section, answer yes to the question about using hazardous agents in Section 13.1 Species Information. For more information about these agents or risks, contact EH

Will **biohazardous** or **radioactive agents** be administered to animals? Do not answer yes for the use of imaging equipment (for example, x-ray, CT, MRI) since this information will be captured in *Section 13.7.2 Non-Surgical Procedures.*	YES
Will **hazardous** chemicals be administered to animals?	No
Will wild caught mammals, pregnant sheep/newborn lambs, or calves under 30 days of age be handled?	No

Occupational Health Information

For information of Animal Biosafety Levels (ABSL), go to Institutional Biosafety Committee and click on *Summary of Recommended Biosafety Levels for Activities in Which Experimentally or Naturally Infected Vertebrate Animals are Used*.

AUHSP Risk Category	Low
Highest Animal Biosafty Level (ABSL)	ABSL 2
Occupational Health Comments	

9.1 Hazardous Agent Use Information

Please provide the information requested. More detailed information about the use of hazardous agents will be collected later on in the protocol application.

*All researchers working with rDNA and biohazardous materials must secure IBC approval by submitting a Memorandum of Understanding and Agreement (MUA) or amending an approved MUA to include the biohazardous material listed in this protocol prior to approval of the IACUC protocol. To submit or amend an MUA for use of rDNA or biohazardous materials listed go to IBC or contact the IBC Administrator at 255-7219 or email IBC.

**All work involving the use of an irradiator (Cs137) or radioactive isotopes needs approval by Environmental Health and Safety (EHprior to approval of the IACUC protocol. For more information, please contact EHor call 607-255-8200.

Do you have Institutional Biosafety Committee (IBC) approval?	YES	MUA #	16051
Will an Irradiator (Cs137) be used to irradiate animals?	NO	If yes, please enter the location of the irradiator.	
Will radioactive isotopes be administered to animals?	NO	If yes, please list the location (building and room number) where it will be used.	

10. Animal Use Question

Please confirm the use of live vertebrates in this protocol.

Confirm live vertebrates will be used in this protocol.	YES

12. Animal Transportation

Please complete this section if Cornell-owned animals or free-ranging wildlife will be transported between different buildings or locations. Do not include transportation of client owned animals. If animals will be transported on public roads or out of state, principal investigators must ensure that transportation complies with USDA regulations (USDA Transportation Regulations; State Office Contacts; Record Keeping Requirements for Research Facilities). Transportation must also follow guidelines set by the IACUC. If animals are to be transported between facilities on the Cornell University campus, or on local roads for short distances (i.e. less than 1 hour traveling time), see ACUP 547: Animal Transport Outside Animal Facilities.

Will animals be transported on this protocol?	Yes
Will you be using an ACUP?	No
f YES, reference the ACUP for Animal Transportation you will be following and provide specific information (e.g. vehicle, caging, duration) relevant to your transportation of animals. If NO, describe the transportation method in detail.	The eggs/embryos/larvae are less than a few millimeters long and are in moved in a small petri dish. Adults are moved in a plastic container of water.
Give origin, destination, frequency and reason(s) for transport:	Animal facility to lab for imaging etc.

13. Animal Species List

Please list all species proposed for use in this protocol. On this page, you may add, copy, delete or edit .

Click on the select button next to the particular species you wish to edit(add or review detailed information), copy or remove.

Species	# of Req Animals
Fish- Zebra Fish	94950

13.1 Species Information

Please provide detailed information about the species as well as the total number of animals of this species to be used in the **36 month period** covered by this protocol. If adding a species, please select one from the drop down list. If species is not available, choose Other and list. The numbers of animals requested should correspond to those in 13.12 and 13.13.
Based on your answers to the questions related to species activities, you may be asked to provide detailed information in the subsections that follow. You will find the list of pertinent pages in the Table of Contents on the left hand side of your screen.

Species	Fish- Zebra Fish
If you selected 'other' above, name the species.	
Total number of requested animals (all years of protocol):	94950
species-specific zoonoses information sheet	Fish &Amphibians - Zoonosis Information Sheet
If information about specific zoonoses is not available here , or if you will not be able to implement	

the control measures described in the information documents, please describe how you will reduce the risk of zoonoses. Otherwise state NA.	
I have reviewed and will share the appropriate information about reducing the risk of zoonotic diseases with all protocol participants (including students), and I will incorporate suitable measures to reduce the risk of exposure.	YES

Species Activities

Answer "YES" to all that apply to this species. Answering "YES" will open pages to collect additional information.

Will non-surgical procedures (i.e. blood collection, tissue collection, etc.) be performed?	Yes
Will surgeries be performed on this species?	Yes
Will animals be subject to chemical or prolonged (greater than 30 minutes) physical restraints?	Yes
Will animals be bred on this protocol?	YES

Will biohazardous agents, radioactive agents, or hazardous chemicals be administered, or will wild caught mammals, pregnant sheep/newborn lambs, or calves under 30 days of age be handled?	Yes
Will drugs or any substances other than hazardous agents be administered to this species?	Yes
Will animals be euthanized?	Yes
If no euthanasia is selected or not all the animals will be euthanized, what will be done with animals after the experiment or project is completed?	

13.2 Justification For Choice of Species

Please explain the choice of the specific species. If a non-animal procedure is available as an alternative, explain why it is not appropriate. More on What the IACUC expects.

Species Name	Fish- Zebra Fish

Justify choice of a specific species of animal.
Embryonic and larval zebrafish are transparent, so we can use optical methods to study nervous system structure and function. Genetic approaches are also possible in zebrafish, making them the most powerful model system for combining optical approaches with genetics. Most of our experiments involve embryonic or larval fish early in their development and still living off yolk. Adults are used for non-invasive

structural and functional imaging experiments and for breeding to produce the larvae.

13.3 Species Source

Please identify the source from which you will be procuring this species. If the animals are privately owned, attach an **Owner Consent Form**. Protocols using privately owned animals will not be approved without an Owner Consent Form. If you are transferring animals from another protocol, please provide the originating protocol number and follow procedures outlined in Policy 430 Tracking Animal Use Including Animal Transfer Across Protocols. An animal that has had major survival surgery on one protocol cannot be transferred to another protocol for another major survival surgery. More on What the IACUC expects.

Principal Investigator	Fetcho, Joseph
Protocol Application Number	2009-0084
Document Type	Full Review
Species Name	Fish- Zebra Fish
Please identify source(s)of animals (e.g. vendor, breeder, donor, bred in-house, other institutuon, or transfered from another protocol).	Bred in house or from other laboratories studying zebrafish.
Does this study involve privately owned animals?	No
If these are privately owned animals, what will be done to them that is different from usual care practices?-More on What the IACUC expects.	

Attachments List

File Spec	Description	Created

13.4 Cornell Housing Location Information

Please identify all **Cornell sites** where animals of this species will be housed for more than 12 hours. **Do not include a quarantine facility as housing**. If a housing location is not listed in the drop down menu, it is not an IACUC approved housing facility and must be inspected by the IACUC prior to use. If you are requesting a facility not listed in the drop down menu, choose "Other Facility Not Listed", provide details in the box marked "Provide "Other" housing location", and contact IACUC-inspections@cornell.edu for guidance.
When a housing location is added, you will be asked for detailed information for each housing location.

Species Name	Fish- Zebra Fish

Click on the select box to remove a housing location. To edit or view a listed location click on the Facility Name.

Facility Name
Corson-Mudd

13.4.1 Housing Location Information

Please identify all **Cornell sites** and type of housing (cage, etc) where animals of this species will be housed for more than 12 hours. Do not include a quarantine facility as housing. If a housing location is not listed in the drop down menu, it is not an IACUC approved housing facility and **must be inspected** by the IACUC prior to use. If you are requesting a facility not listed in the drop down menu, choose "Other Facility Not Listed", provide details in the box marked "Provide "Other" housing location", and contact IACUC-inspections@cornell.edu for guidance.

Species Name	Fish- Zebra Fish
Facility Name	Corson-Mudd
Provide "Other" housing location.	
Type of housing: Check all that apply.	
Cage	
Tank	Yes
Stall	
Pasture	

Pen	
Other	
"Other" Details	

13.5 Enrichment and/or Exercise

For some species, group housing is considered environmental enrichment. Please indicate whether group housing is used and if not, please justify single housing. For all species, animal enrichment will be provided as part of routine animal care. Links to Animal Care and Use Protocols (ACUP) for enrichment can be found at ACUP. If you are requesting a change from standard enrichment (for example: providing extra enrichment, or withholding or limiting enrichment), please explain.

Species Name	Fish- Zebra Fish
Will all animals of this species be group housed with other members of this species?	YES
If no, provide justification.	
Are you requesting a change from standard enrichment?	NO
If yes, describe and justify.	
Additional comments	NA

13.6 Clinical Care, Quarantine, Acclimatization and Daily Care

If the proposed animal care deviates from recommended guidelines as described in the Guide or the Ag Guide, please complete the Exemption section 13.14.

Species Name	Fish- Zebra Fish
CARE Veterinarians	Yes
CVM Amb &Prod Med in Consultation with CARE Veterinarians	
Other Veterinarians with CARE Letter of Agreement	

If "Other..", list the Veterinarians on the Letter of Agreement	
Select the appropriate ACUP used for quarantine. If quarantine is not required, or if you are not following an ACUP, please make the appropriate selection.	509 Animal Acquisition, Receiving, and Acclimation
If you are following quarantine procedures that are not covered in an ACUP , please describe them.	Any fish or eggs received from external sources are kept in a separate quarantine fish rack with independent water supply. Eggs are raised to adulthood there, fish are bred and eggs laid in our facility are then bleached and raised in the main non-quarantine facility. Only bleached eggs laid here and bleached by us go into our main fish rooms.
ACUP 509	
Will ACUP 509 be used as a guideline?	Yes
Describe any specialized acclimatization procedures not covered in ACUP 509. If none, or if acclimatization is not required, state NA.	NA
If you will be following any specialized care procedures(e.g.special caging, water, feed, etc.) please describe. If not, please answer NA.	NA

13.7 Sequential List of Procedures

Provide a **sequential list and timeline** of all experimental or instructional procedures (Non-Surgical, Surgical Procedures, Restraints) to be performed on the animals of this species, and any and all animal endpoints to be used in the study. Do not provide details of these procedures here, as they will be requested in subsequent sections. Do not describe in vitro procedures performed on tissues after removal from animals.

Species Name	Fish- Zebra Fish

Sequential list and Timeline of All procedures
Motor control: Objective 1: Electrophysiology together with light activation (less than 7 day embryos). These are one time, terminal experiments with the embryos euthanized at the end of the experiment. Objective 2: Imaging - (less than 7 day embryos) .Embryos will be imaged non-invasively once a day for the 7 days and then euthanized. Objective 3: Electrophysiology and light activation - (less than 7 days embryos). These are one time, terminal experiments with the embryos euthanized at the end of the experiment. Objective 4: Imaging and electrophysiology (less than 7 days embryos). These are one time, terminal experiments with the embryos euthanized at the end of the experiment. Objective 5: Imaging (less than 7 days embryos). After labeling, fish will be imaged once a day for 1 -7 days and then euthanized. Sleep: Objective 1: Imaging (all non-invasive up to 10 days old); some with sleep deprivation and behavioral imaging. Larvae will be imaged twice in 24 hours, for each of 3- 4 days and then euthanized at the end. Objective 2 Electrophysiology and light activation (less than 7 days embryos). The electrophysiological experiments are one time experiments and the larvae will be euthanized at the end. Objective 3: Imaging and light activation (all non-invasive up to 10 day old). The larvae will be tested twice a day for 3-4 days and then euthanized. Adult zebrafish imaging: Objective: To image through an intact living zebrafish brain.

Imaging - 1. anesthetize in MS222 or local Bupivicaine, 2. sometimes then paralyze with bungarotoxin, 3. embedded in agar. The preceding steps take place over 10 minutes or so. 4. Image the neurons in the fish over the course of an hour or less typically, but sometimes up to two hours. 5. If the fish was simply anesthetized and imaged the fish is sometimes removed from the agar and retained for future imaging. 6. in terminal experiments the fish are euthanized after imaging. Note thatthe larvae can respire through their skin, so the paralysis does not kill the larvae.

Patch Electrophysiology: 1. anesthetize in MS222 or local Bupivicaine, 2. paralyze with bungarotoxin, he preceding steps take about 10 minutes or so. 3. Record with patch electrodes through small opening into the larvae. The embryos/larvae are very small - just about a maximum of 4 millimeters long, so the procedures are not conventional surgical procedures. The recording typically lasts about 1 to two hours maximum per animal. 4. Larvae are euthanized after the recording. Alldone on embryos less than 7 days.

Sleep deprivation by electrical stimulation or vibration of the tank. 1. individual fish are placed in a tank 2. When movement ceases, an indicator of entry into a sleep state, the tank is vibrated or a shock is applied to keep the fish from entering sleep. 3. The deprivation lasts a day or two, followed by neuronal imaging and/or behavioral analysis of sleep recover after deprivation.

Behavior of larvae is imaged with high speed or regular video cameras, usually in home tanks or small petri dishes. Imaging is typically down by 1. placing the fish in a small 35 millimeter petri dish with tank water, 2. imaging either spontaneous behavior or responses to vibration, touch by a probe, electrical stimuli, or a squirt of water in experiments lasting 1-3 hours.

Light activation of neurons by expressing the protein channelrhodopsin Neurons are labeled with DNA constructs by injecting them into the single cell embryo or by electroporating them onto anesthetized embryos/larvae and neurons are then with blue light flashes to active the cells.

More specifically. 1. Eggs are collected at laying. 2. DNA constructs are injected at the single cell stage. 2. fish are raised until 4-7 days. 3. Fish are paralyzed and embedded in agar. Neurons in the fish are activated by flashing light onto labeled cells via a microscope while patch recording or imaging other nerve cells. The experiments last about 2-3 hours. 4. larvae are euthanized after the experiment.

Breeding: Approach 1: 1. Fish are moved into separate tanks with false grid bottoms in the late afternoon. 2. Egg laying hopefully occurs the next morning and eggs are harvested by moving the fish back to their home tank and collecting the eggs under the false bottom. In some cases male and female fish are separated by a clear partition which is pulled out in the morning to obtain more precise timing of the breeding event and egg laying.
Approach 2: 1. Small inserts with a gridded lid and a plastic plant on top are placed in the home tanks in late afternoon. 2. The next day the fish lay eggs over the insert which fall through the grid. Removal of the inert allows for collection of the eggs laid in it.

PCR Testing: In order to identify carriers of genetic modifications (mutants in the migration goals), adult zebrafish will be anesthetized and a few scales removed, or a small portion of the tail fin clipped for tissue for PCR testing. The fish fully recover from this procedure.

Adult zebrafish imaging: For imaging through the head, anesthetize the fish in MS222, place in holder with perfusion device and stabilizing agar, with MS222 in perfusion fluid and then image. If an opening in the head is required, then that will be made after MS222 anesthesia, local bupivacaine anesthesia will be applied to the wound edges, and the fish will be placed in the holder as in a with continued MS222 anesthesia via gill perfusion water. Fish will be euthanized after imaging with the skull opened and returned to housing if imaged under anesthesia without opening the skull. For functional imaging, the fish is anesthetized with MS222 and then paralyzed with a retro-orbital injection of pancuronium bromide (2 micrograms per milligram of fish), and perfused over the gills during imaging. Imaging is done in the intact fish with local bupivacaine anesthesia of the surface of the

head through which the imaging is done in case of local heating by the illumination light. Fish health is monitored by gill color and/or central blood flow (which can be visualized in some imaging conditions).

Attachments List

File Spec	Description	Created

13.7.1 Procedure Location

Please list ALL procedure locations for this species. You may add or remove a particular location on this page, and you will select a location on the following page. Procedure locations must be approved by the IACUC before use.

Species Name	Fish- Zebra Fish

To remove a procedure location, click the select box next to the location and then click the remove button.

Facility Name	Room #
Corson-Mudd	E107
Corson-Mudd	E109
Corson-Mudd	Procedure_Room
Corson-Mudd	W105
Corson-Mudd	W106
Corson-Mudd	W112
Other Facility Not Listed	OTHER

13.7.1.1 Procedure Location Information

Please select the facility and room combination where procedures will be performed on this species. If procedures are being performed in a vivarium, you do not need to select a specific room number, but instead you will select "Procedure_Room" (for example, Weill Hall Barrier/Procedure_Room). If you are working at a field site, please select "Field Study Site/FIELDSTUDYSITE". If you do not find the appropriate facility in the dropdown lists below, contact the IACUC office.

Species Name	Fish- Zebra Fish
Facility Name	Corson-Mudd
Facility Room Number	E107

13.7.1.1 Procedure Location Information

Please select the facility and room combination where procedures will be performed on this species. If procedures are being performed in a vivarium, you do not need to select a specific room number, but instead you will select "Procedure_Room" (for example, Weill Hall Barrier/Procedure_Room). If you are working at a field site, please select "Field Study Site/FIELDSTUDYSITE". If you do not find the appropriate facility in the dropdown lists below, contact the IACUC office.

Species Name	Fish- Zebra Fish
Facility Name	Corson-Mudd
Facility Room Number	E109

13.7.1.1 Procedure Location Information

Please select the facility and room combination where procedures will be performed on this species. If procedures are being performed in a vivarium, you do not need to select a specific room number, but instead you will select "Procedure_Room" (for example, Weill Hall Barrier/Procedure_Room). If you are working at a field site, please select "Field Study Site/FIELDSTUDYSITE". If you do not find the appropriate facility in the dropdown lists below, contact the IACUC office.

Species Name	Fish- Zebra Fish
Facility Name	Other Facility Not Listed
Facility Room Number	OTHER

13.7.1.1 Procedure Location Information

Please select the facility and room combination where procedures will be performed on this species. If procedures are being performed in a vivarium, you do not need to select a specific room number, but instead you will select "Procedure_Room" (for example, Weill Hall Barrier/Procedure_Room). If you are working at a field site, please select "Field Study Site/FIELDSTUDYSITE". If you do not find the appropriate facility in the dropdown lists below, contact the IACUC office.

Species Name	Fish- Zebra Fish
Facility Name	Corson-Mudd
Facility Room Number	Procedure_Room

13.7.1.1 Procedure Location Information

Please select the facility and room combination where procedures will be performed on this species. If procedures are being performed in a vivarium, you do not need to select a specific room number, but instead you will select "Procedure_Room" (for example, Weill Hall Barrier/Procedure_Room). If you are working at a field site, please select "Field Study Site/FIELDSTUDYSITE". If you do not find the appropriate facility in the dropdown lists below, contact the IACUC office.

Species Name	Fish- Zebra Fish
Facility Name	Corson-Mudd
Facility Room Number	W105

13.7.1.1 Procedure Location Information

Please select the facility and room combination where procedures will be performed on this species. If procedures are being performed in a vivarium, you do not need to select a specific room number, but instead you will select "Procedure_Room" (for example, Weill Hall Barrier/Procedure_Room). If you are working at a field site, please select "Field Study Site/FIELDSTUDYSITE". If you do not find the appropriate facility in the dropdown lists below, contact the IACUC office.

Species Name	Fish- Zebra Fish
Facility Name	Corson-Mudd
Facility Room Number	W106

13.7.1.1 Procedure Location Information

Please select the facility and room combination where procedures will be performed on this species. If procedures are being performed in a vivarium, you do not need to select a specific room number, but instead you will select "Procedure_Room" (for example, Weill Hall Barrier/Procedure_Room). If you are working at a field site, please select "Field Study Site/FIELDSTUDYSITE". If you do not find the appropriate facility in the dropdown lists below, contact the IACUC office.

Species Name	Fish- Zebra Fish
Facility Name	Corson-Mudd
Facility Room Number	W112

13.7.2 Non-Surgical Procedures

Please indicate the non-surgical procedures (definitions) you will conduct.

Species Name	Fish- Zebra Fish

* Questions asked on protocols initiated prior to 9/2011. When updating protocol, please select a more descriptive option from the list.

Procedure Name
X- Other Non-Surgical Procedure NOT in LIST
Behavioral Observation/Studies/Testing
Collection of Oocytes
Collection of Tissue using Fin Clips/Scales
Imaging:Other

13.7.2.1 Non-Surgical Procedure Information

Provide detailed information pertaining to the following procedures. More on What the IACUC expects. For more information on procedures, please refer to the appropriate ACUP

Species Name	Fish- Zebra Fish
Behavioral Observation/Studies/Testing	
Describe	The movements of embryos/larvae are imaged with a high speed (1,000 frame per second) camera or with a regular video camera. The imaging is done in the home tank or by moving the embryos/larvae to a small petri dish to reduce the required field of view. Images are then analyzed with automated software to track movement patterns. For sleep deprivation, Larvae will be deprived of sleep by weak electrical stimuli (approximately 2 Volts) applied through the water containing the animal, as in other similar studies from the Stanford sleep lab.(Yokogawa et al., Plos Biology Vo. 5 issue 10 e277). Our plan is to deprive the larvae for 1 -2 days and then examine the synapses and their plasticity subsequently. Alternatively we will vibrate the tank to deprive the larvae of sleep.

	These larvae are from 5-8 days of age.
Describe special care or monitoring.	the sub 4 millimeter embryos and larvae are carefully moved with small pipettes to avoid damaging them which would impact on behavior. Larvae that show disrupted movements are euthanized, as treating individual larvae is not feasible given the size.
Collection of Oocytes	
Describe	Adult males and female fish are placed in a breeding tank in late afternoon. This tank has a mesh false bottom. The fish breed in the morning and the eggs fall through the mesh to avoid having the adults eat them. Fish are removed after laying and the eggs collected from the tank. Alternatively, an insert tray with a mesh lid is placed in the home tank and the fish lay eggs over it in the morning. The insert is removed and the eggs collected. The second approach can be used when the exact timing of the egg laying is not needed. The first approach uses breeding tanks in which males and females can be separated until morning and then the partition removed to get more precisely timed breeding.
Collection of Tissue using Fin Clips/Scales	
Describe	In order to identify carriers of genetic modifications (mutants in the migration goals), adult zebrafish will be anesthetized and a few scales removed, or a small portion of the tail fin clipped for tissue for PCR testing. The fish fully recover from this procedure.
Imaging:Other	
Describe	Embryos and larvae are stabilized by embedding in low melting point agar. They obtain oxygen through their skin at embryonic and larval stages, and so remain healthy even in agar (as assessed by blood flow which is visible in the transparent larvae. The embryos/larvae are then imaged with either a confocal or multi photon microscope to view neurons in the nervous system, through the intact, transparent body and head. Fish may be imaged non-invasively no more

	than twice daily for 3-4 days or once a day for 7 days. For light activation of neurons embedded larvae expressing a light activated protein are placed on a microscope and the labeled neurons are flashed with 2 millisecond light pulses to produce action potentials in the cells. Embryos and Larvae for imaging range from about 24 hours to 8 days of age. Anesthetized adults will be held in agar, perfused over the gills with anesthetic solution for structural imaging and imaged through the skull with three photon microscopy. For functional Imaging they are anesthetized, then paralyzed with pancuronium bromide (2 micrograms per milligram fish), followed by agar restraint and noninvasive functional imaging with local bupivacaine applied to the region of the head through which the light penetrates to mitigate any potential distress from heating by the light. We estimate that the entire procedure will last 1 to 4 hours, depending on the extent of the brain imaged, with the time increasing as the optical tools are perfected. They will be euthanized if there is any evidence of poor health such as inability to move properly after imaging, or returned to a separate tank in the fish facility if they recover well from anesthesia.
X- Other Non-Surgical Procedure NOT in LIST	
Describe procedure.	Patch recording: A small glass pipette is brought into contact with the cell body of a neuron and sealed onto the cell. the seal is then ruptured to allow physiological recording of the membrane potential of the neuron. This is done in the larvae with a tiny opening to access the nervous system. The recording typically lasts one-two hours. Light activation of neurons. An intact larval fish

	with neurons labeled with channelrhodopsin is held in agar under a fluorescent microscope and illuminated with a micromirror device that allows selective illumination of individual neurons, which are flashed with light pulses of typically 2-3msec to activate a neuron or several illuminated neurons. Patch recording and light activation are done in 4-6 day old larvae.

13.7.3 Surgery

Please list the surgical procedures being performed in this protocol. Add, edit (by clicking on its name) or remove a particular surgical procedure. Detailed information will be required on the following page.

Species Name	Fish- Zebra Fish			
Select	Surgical Procedure	Survival	Non Survival	Duration
23	Craniotomy	No	Yes	3-10 minutes
1072	few 100micron sized opening for patch recording in a 4 mm larvae	No	Yes	1-2 hours for entire patch recording experiment

Surgical Procedure - Multiple Major Surgeries

Be sure to answer the Yes/No question below.

Definitions:
Major surgery is one which penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic function, or involves extensive tissue dissection or transection. The IACUC will determine whether a surgery is "major" or "minor" on a case-by-case basis, particularly in regard to laparoscopic surgery. An important consideration in the determination is the potential for pain and post-op complications.

Multiple major survival surgery is defined as more than one major surgical procedure from which the animal is allowed to recover. Multiple major survival surgeries in a single animal are acceptable only if they are essential components of the research project and are scientifically justified. Cost saving alone is NOT an adequate reason for performing multiple major survival surgeries.

Note: Surgeries that are considered routine husbandry or are clinically indicated (e.g., emergency C-section) do not count as major survival surgeries and do not

need to be described in the protocol. Consult a CARE veterinarian for guidance.

A single animal may not undergo multiple major survival surgeries on more than one protocol.

Will multiple major survival surgeries be performed on an individual animal?	NO
If yes, please provide scientific justification. Otherwise state N/A.	
Number of Major Survival Surgeries per animal	0
Minimum number of days between major survival surgeries on an individual animal. Enter a whole number.	0

13.7.3.1 Surgical Procedure

Please describe the planned surgical procedure. Select the most appropriate surgical procedure from the drop down list. If you will be performing multiple surgical procedures, add additional procedures. ACUPs pertaining to surgeries can be found at the CARE website. More on What the IACUC expects.For assistance in completing this page, contact a CARE veterinarian .

Species Name	Fish- Zebra Fish
Surgery:	
Type of surgery to be performed	Craniotomy
If z-Other is selected, then please provide the type of surgery that will be performed.	
Survival Surgery?	No
	Yes

Non-survival Surgery?	
Pre-Op Procedures:	
Identify the ACUP_____ being used for pre-operative procedures.	110
Describe surgical site preparation.	dry the area and apply local anesthetic - bupivicaine
Anesthesia:	
Maximum duration of anesthetic	1-2 hours
If a neuromuscular agent will be used, please justify, and describe how will anesthesia levels (which may be masked) be monitored.	
Are you using CUHA Anesthesia Services or CARE Veterinary Services (ACUP 806)?___	No
********** ********** **********	* * *If NO, please answer the next 4 questions.* * *
Identify ACUP being used as a guide for Anesthesia.	110
Please list drugs used for anesthesia but provide dosing details in 13.10. Also provide specific details not specified in the CARE SOP.	MS222 bupivicaine
	Dawnis Chow or Joe Fetcho

Who will perform and/or supervise anesthesia?	
How will anesthetic depth be measured?	movement cessation and unresponsivess to a gradual tail compression with a forceps (tail pinch)
Surgical Care:	
Identify the ACUP_____ being used for surgical care.	210
Describe the surgical procedure, including site of incision and additional details of the surgical procedure not specified in the ACUP.	A hole is made in the skull with a forceps and small portions are removed by breaking small pieces or by cutting with a microscissors. The skull is cartilage, so not very hard. Fish are euthanized after the experiment.
Who will perform and/or supervise surgery?	Dawis Chow or Joe Fetcho
Duration of surgery	3-10 minutes
Wound closure	not applicable
Post Op Care:	
Identify the ACUPs_____ being used for Post Operative Care	
Please list analgesics but provide dosing details in 13.10. Also describe additional details of the postoperative care not specified in the ACUP. Include specifics on the frequency of observation;	

identity of the responsible individual(s); and the detection and management of postoperative complications during work hours, weekends, and holidays.	
If applicable, when will sutures be removed?	

Surgical Locations

Select location(s) where surgery is performed. If not on list, return to page 13.7.1 and add.

Surgical Location	Room Number
Corson-Mudd..E107	E107
Corson-Mudd..E109	E109
Corson-Mudd..Procedure_Room	Procedure_Room

13.7.3.1 Surgical Procedure

Please describe the planned surgical procedure. Select the most appropriate surgical procedure from the drop down list. If you will be performing multiple surgical procedures, add additional procedures. ACUPs pertaining to surgeries can be found at the CARE website. More on What the IACUC expects.For assistance in completing this page, contact a CARE veterinarian .

Species Name	Fish- Zebra Fish
Surgery:	
	few 100micron sized opening for patch recording in a 4 mm larvae
If z-Other is selected, then please provide the type of surgery that will be performed.	
Survival Surgery?	No
	Yes

Non-survival Surgery?	
Pre-Op Procedures:	
Identify the ACUP_____ being used for pre-operative procedures.	
Describe surgical site preparation.	Note: Note: We only do surgery on embryos and larvae. The embryos/larvae are very small - at most 4 millimeters long, so this is not surgery in the conventional sense and far from major surgery. The surgery involves using an etched tungsten pin to make a very small (roughly 200 micron) opening in the head after local bupivacaine application. As I understand it, embryos and larvae of fish and frogs that are not free feeding are exempt from the usual guidelines. We nonetheless attempt to treat the embryos and larvae as one would an adult and use anesthetics where it is feasible, except where the small size precludes such treatment. Invasive procedures are done on embryos 7 days or younger.
Anesthesia:	
Maximum duration of anesthetic	1-2 hours
If a neuromuscular agent will be used, please justify, and describe how will anesthesia levels (which may be masked) be monitored.	Movement of the embryos/larvae cannot during the electrical recording would not allow for the recordings, so they are paralyzed with bungarotoxin or curare. We can determine if there is any distress because the electrical recordings allow us to monitor activity in the nervous system. The embryos/larvae at this stage have barely any forebrain, so any awareness of pain is unlikely. The recordings are from motor nerves innervating axial muscles, so we can determine whether the larvae is attempting to move as it normally would (intermittent swimming bouts).
Are you using CUHA Anesthesia Services or CARE Veterinary Services (ACUP 806)?___	No
********** ********** **********	* * *If NO, please answer the next 4 questions.* * *

Identify ACUP being used as a guide for Anesthesia.	
Please list drugs used for anesthesia but provide dosing details in 13.10. Also provide specific details not specified in the CARE SOP.	MS222
Who will perform and/or supervise anesthesia?	Lab staff
How will anesthetic depth be measured?	sensory responsiveness to a touch (bupivacaine, MS222) as well as cessation of movement (MS22).
Surgical Care:	
Identify the ACUP_____ being used for surgical care.	
Describe the surgical procedure, including site of incision and additional details of the surgical procedure not specified in the ACUP.	The embryos/larvae are very small - at most 4 millimeters long, so this is not surgery in the conventional sense. The surgery involves using an etched tungsten pin to make a very small (roughly 200 micron) opening in the head or body after MS 222 or local bupivacaine anesthesia.
Who will perform and/or supervise surgery?	lab members
Duration of surgery	1-2 hours for entire patch recording experiment
Wound closure	terminal experiments, not closed, but too small to do anything to close it.
Post Op Care:	

Identify the ACUPs being used for Post Operative Care	
Please list analgesics but provide dosing details in 13.10. Also describe additional details of the postoperative care not specified in the ACUP. Include specifics on the frequency of observation; identity of the responsible individual(s); and the detection and management of postoperative complications during work hours, weekends, and holidays.	terminal experiments.
If applicable, when will sutures be removed?	

Surgical Locations

Select location(s) where surgery is performed. If not on list, return to page 13.7.1 and add.

Surgical Location	Room Number
Corson-Mudd..E107	E107
Corson-Mudd..E109	E109
Corson-Mudd..Procedure_Room	Procedure_Room

13.7.4 Chemical or Prolonged Restraints

Please describe any chemical, or prolonged (greater than 30 minutes) physical restraints used on animals in this protocol. Detailed information on each restraint will be required on the following page.

If using Chemical Restraint, list the drugs used in the "Administered Substances" section.

Do not include anesthesia/sedation as a chemical restraint when used in conjunction with a surgical procedure.

Do not include brief physical restraint (less than 30 minutes) of animals either manually or with devices such as rodent restraint devices, head gates, leashes, halters, used for examination, collection of samples, and other experimental manipulations.

Species Name	Fish- Zebra Fish

Click on the select box to remove a restraint. To edit or view a listed restraint click on the Restraint Type.

Select	Restraint Type	Restraint Duration
9	Chemical Restraint	Up To 4 Hours

13.7.4.1 Restraint Types

Please describe physical and chemical restraints used in this protocol. Do not include anesthetics or analgesics used in conjunction with surgicial procedures.

Restraint Locations

Select location(s) in which animals are restrained. If not on the list, return to page 13.7.1 and add the location.

Restraint Location	Room Number
Corson-Mudd..E107	E107
Corson-Mudd..E109	E109
Corson-Mudd..Procedure_Room	Procedure_Room

Species Name	Fish- Zebra Fish
Restraint Type	Chemical Restraint
If you have selected "z-Other", please name the type of restraint being	

used.	
Duration of restraint and frequency of observation	Up To 4 Hours
What is the purpose for this restraint (for example, what procedures are done during restraint)?	needed for imaging neurons in vivo
If a neuromuscular blocking agent will be used, please justify. Also describe how anesthesia levels (which may be masked) will be monitored.	The embryos/larvae must not move during experiments or we cannot record form the neurons.These are embryonic or larval fish which are at ages where what little evidence there is for any cognition in fish is not evident (the forebrain is barely developed). Fish are 7days or less old, except for some non-invasive imaging procedures in which we image older larvae to look at neuronal structure or image activity). These are up to 8 days old. In paralyzed adults we will image non-invasively with local bupivacaine on the top of the head to mitigate any heating effects from the light. We also monitor for any tissue damage with the imaging to be sure that light intensities are not high enough to cause damage that could lead to pain if there were no local anesthetic. Imaging in larvae lasts about 1 hour. Imaging in adults is still under development, but will last about 1-4 hours. Typically individual animals are imaged only once, except in cases where we look at the time course of differentiation of neurons, when we image at several time points during development (prior to hatching) and once a day after hatching.
How is the animal acclimated to prolonged physical restraint?	NA

13.7.5 Breeding Information

Please provide information concerning the breeding program in this protocol.

Species Name	Fish- Zebra Fish
Will a breeding colony be maintained?	YES
Will genetically modified animals be bred?	YES
Describe the breeding strategy to be used, (such as monogamous pairings, timed-mating, trio or harem breeding). If you are breeding rodents you must follow the breeding practices outlined in ACUP 513 Rodent Husbandry and Breeding or describe how cage density will be managed in breeding cages with multiple litters.	Breeding: Approach 1: 1. Fish are moved into separate tanks with false grid bottoms in the late afternoon. 2. Egg laying hopefully occurs the next morning and eggs are harvested by moving the fish back to their home tank and collecting the eggs under the false bottom. In some cases male and female fish are separated by a clear partition which is pulled out in the morning to obtain more precise timing of the breeding event and egg laying. Approach 2: 1. Small inserts with a gridded lid and a plastic plant on top are placed in the home tanks in late afternoon. 2. The next day the fish lay eggs over the insert which fall through the grid. Removal of the inert allows for collection of the eggs laid in it.

13.8 Discomfort, Distress or Pain

Discuss discomfort, distress or pain that is more than slight or momentary and may result from procedures, injuries or conditions induced to animals while on this protocol. Examples include injuries to wild animals during field studies, tumor inductions, surgery, infectious or spontaneous disease studies, extreme food/environmental manipulations and particular transgenic phenotypes. Include a discussion of **humane intervention-points**. An intervention-point is a time point during an experiment which commands a specific action to prevent or minimize discomfort, distress or pain. **If death is to be used as an end-point of the study, provide scientific justification.** If you need help to set intervention-points, see ACUP 402 or contact a CARE veterinarian.. Please note that the AWAR defines a painful procedure as one "that would reasonably be expected to cause more than slight or momentary pain or distress in a human to which the procedure is

applied."

Species Name	Fish- Zebra Fish

List any pain, discomfort, and distress (which is more than momentary) that animals are expected to experience as a result of the procedures. If no pain or distress beyond momentary is expected, respond with NA.
As described in other sections, application of a very tiny (the larvae are only 4 mm or so long) amount of bupivicaine to the head at the site of insertion of pipettes is used to minimize pain/distress. However, our physiology is all done on embryos/larvae less than 7 days old. No pain or distress other than momentary expected in imaging experiments with in larvae or imaging in adult fish, as anesthesia will be used for anything invasive. Most experiments will be performed on zebrafish larvae that are less than 7 days old. Larvae at this stage have not developed pain sensitivity. No pain or distress other than momentary is expected during in imaging experiments in adults as it is non-invasive and we provide local anesthesia and monitor for any tissue damage due to imaging light. Fish will be anaesthetized prior to tail fin clipping and all imaging experiments.
For each potentially painful/distressful procedure (beyond momentary), describe the monitoring plan. Include information on the following: - period of time for monitoring (number of hours or days post-procedure); -frequency of monitoring (e.g. every 2/4/6/8 hours, or every 1 to 2 days); -specific health and/or behavioral abnormalities which will be monitored; (e.g. weight loss, ambulation, lack of appetite, etc.). *Do not repeat any information provided in the Surgical Procedure (13.7.3) section of this protocol.*
We observe for altered behavior or movements.
Beginning with the earliest humane intervention-point, list the criteria for each point at which you will intervene, and describe what will be done to prevent or relieve unnecessary distress and/or pain to an animal. *(e.g. At 10% weight loss you will give fluids/moist food; at 20% you will euthanize)* . See ACUP 402 for guidance.

Do not repeat any information provided in the Surgical Procedure (13.7.3) section of this protocol.
We will euthanize any adults or larvae that show altered behavior or movement patterns, either after manipulation (like pipetting of larvae) or in our adult housing tanks.

13.9 Hazardous Agents

Please list all the hazardous materials that will be administered. Hazardous materials are biological, chemical, and radiological agents that pose a risk to humans or animals. If wild caught mammals will be handled, list "rabies virus frequent risk" or "rabies virus infrequent risk" (http://www.cdc.gov/rabies/specific_groups/travelers/pre-exposure_vaccinations.html), or if handling pregnant sheep/newborn lambs, list "Coxiella burnetii" or if handling calves under 30 days of age, list "Cryptosporidium parvum".

Hazardous Agents include:

Biohazardous agents: infectious agents, toxins, recombinant or synthetic nucleic acid molecules (r/sNA), viral vectors, human/primate tissues, fluids or cells. If using Biohazardous agents you must have prior approval by the Institutional Biosafety Committee. For more information go to IBC.

Hazardous chemicals: acute toxicants, teratogens, mutagens, carcinogens, antineoplastic compounds.

Radioactive agents: radioactive isotopes or an irradiator (Cs137)

Add, edit (by clicking its name) or remove hazardous agents here. Detailed information will be required on the following page. All work involving the use of an irradiator (Cs137) or radioactive isotopes must have prior approval by Environmental Health and Safety (EHFor more information, please contact EHor call 607-255-8200.

Species Name	Fish- Zebra Fish

Select	Hazardous Agent	Type	Amount Administered	Route	Other ROA	Hazard Shed by Animals	Class/ Biosafety Level
137	Alpha bungarotoxin	Toxin	Embryos/larvae Immersed In 1mg/ml Until Paralyzed; 2-5min	IMMERSION		NO	Toxin
293	Curare	Toxin	Immersion In 3mg/ml For 1-Min Until Movement Stops	IMMERSION		NO	Neurotoxin
535	RNA	Biological	Microinjection Of 1 Nanoliter	OTHER	Microinjection of	NO	NC

			Of Rna (1.2 Mg/ml)		single-celled embryo		
82	Ultraviolet Light	Radiation	Very Low Dose Exposure; 5 Min Of 365 Nm (0.3 Mw/cm2)	OTHER	Light illumination	NO	Irradiation
2619	z-Other	Zz-other	2 Micrograms Per Milligram Fish Injected Retroorbitally	OTHER	injected retroorbitally	NO	

13.9.2 Hazardous Agents Information

Provide information about the hazardous materials administered to animals and the conditions for their administration. For additional assistance in completing any part of this section, contact Environmental Health &Safety (255-8200 or ehs@cornell.edu) or CARE (253-4378) or CARE@cornell.edu).

Species Name	Fish- Zebra Fish
Hazardous Agent	Ultraviolet Light
Category	Radiation
If Z-Other, provide name of hazardous agent	
Class/Biosafety Level	Irradiation
Dose, frequency, and duration of administration	Very Low Dose Exposure; 5 Min Of 365 Nm (0.3 Mw/cm2)
Will the agent or its derivatives be excreted or shed by inoculated or treated animals?	NO
Route of administration (check all applicable)	OTHER
Other Route (specify)	Light illumination

Hazard Handling

In this section, describe aspects of hazardous agent handling.

Pharmaceutical-grade substances should be used, or Investigators are expected to provide a scientific justification for the use of non-pharmaceutical grade substances. Many hazardous agents are not available as pharmaceutical grade, and under these circumstances, "Not Available" is an acceptable justification. To determine if substances are pharmaceutical grade, search the FDA databases, the Orange Book or the Green Book. For further assistance, please contact CARE staff. See ACUP 413 for more information and for examples of acceptable justifications.

Is this a pharmaceutical grade substance?
Not applicable
If No, please provide a scientific justification for using a non-pharmaceutical grade. If a hazardous agent is not available as pharmaceutical grade, "Not Available" is an acceptable justification. Any deviations from ACUP 413 must also be included here.
MSDS or other reference information about the agent (provide a web link or attach a file below if appropriate). Please use the ChemWatch SDS Library through Cornell EHS.
Please see attached
Provide a short description of the relevant characteristics (e.g., hazardous properties, LD50, health effects for humans)of the hazardous agent.
Long term exposure of UV directly on skin and eyes is harmful. Long term exposure may cause premature aging of the skin and cancer. Direct illumination in eyes can result in long term injury to eyes.
Describe any expected clinical signs for inoculated or treated animals.
No changes are expected
Select the appropriate Animal Biosafety Procedure (ABP) that describes administration of the agent, handling of infected or treated animals, and control measures to be used. If you have minor deviations from the ABP, please describe those deviations below. If an ABP is not available or cannot be used without major changes, please use this SOP template to develop an appropriate SOP and attach the completed document to this section.
No Animal Biosafety Procedure Available or Applicable

If applicable, please describe any minor deviations from the ABP you have selected above, and explain why those changes are needed.
No changes are needed. Brief exposure with low frequency UV light
I have reviewed the Animal Biosafety procedure or SOP related to the use of this hazard with animals, will share the requirements of the ABP or SOP with all protocol participants (including students) and will ensure that the procedures described in the ABP or SOP are followed.
YES

Attachments List

File Spec	Description	Created
2009-0084_12_FZEB_82_0001_Tech Bulletin 103 A92086-7.pdf		01/23/2020
2009-0084_12_FZEB_82_0001_Tech Bulletin 103 A92086-7.pdf		01/23/2020

13.9.2 Hazardous Agents Information

Provide information about the hazardous materials administered to animals and the conditions for their administration. For additional assistance in completing any part of this section, contact Environmental Health &Safety (255-8200 or ehs@cornell.edu) or CARE (253-4378) or CARE@cornell.edu).

Species Name	Fish- Zebra Fish
Hazardous Agent	Alpha bungarotoxin
Category	Toxin
If Z-Other, provide name of hazardous agent	
Class/Biosafety Level	Toxin
Dose, frequency, and duration of administration	Embryos/larvae Immersed In 1mg/ml Until Paralyzed; 2-5min
Will the agent or its derivatives be excreted or shed by inoculated or treated animals?	NO
	IMMERSION

Route of administration (check all applicable)	
Other Route (specify)	

Hazard Handling

In this section, describe aspects of hazardous agent handling.

Pharmaceutical-grade substances should be used, or Investigators are expected to provide a scientific justification for the use of non-pharmaceutical grade substances. Many hazardous agents are not available as pharmaceutical grade, and under these circumstances, "Not Available" is an acceptable justification. To determine if substances are pharmaceutical grade, search the FDA databases, the Orange Book or the Green Book. For further assistance, please contact CARE staff. See ACUP 413 for more information and for examples of acceptable justifications.

Is this a pharmaceutical grade substance?
No
If No, please provide a scientific justification for using a non-pharmaceutical grade. If a hazardous agent is not available as pharmaceutical grade, "Not Available" is an acceptable justification. Any deviations from ACUP 413 must also be included here.
Not available
MSDS or other reference information about the agent (provide a web link or attach a file below if appropriate). Please use the ChemWatch SDS Library through Cornell EHS.
See attached
Provide a short description of the relevant characteristics (e.g., hazardous properties, LD50, health effects for humans)of the hazardous agent.
Neuromuscular blocking agent, binds to acetlycholine receptors, Considered hazardous on inhalation, ingestion or injection. Lethal dose, Intraperitoneal; Rodent - mouse 150 ug/kg.
Describe any expected clinical signs for inoculated or treated animals.
embryonic and larval fish... not really applicable, except to monitor health via blood flow.

Select the appropriate Animal Biosafety Procedure (ABP) that describes administration of the agent, handling of infected or treated animals, and control measures to be used. If you have minor deviations from the ABP, please describe those deviations below. If an ABP is not available or cannot be used without major changes, please use this SOP template to develop an appropriate SOP and attach the completed document to this section.
New SOP Attached Below
If applicable, please describe any minor deviations from the ABP you have selected above, and explain why those changes are needed.
I have reviewed the Animal Biosafety procedure or SOP related to the use of this hazard with animals, will share the requirements of the ABP or SOP with all protocol participants (including students) and will ensure that the procedures described in the ABP or SOP are followed.
YES

Attachments List

File Spec	Description	Created
2009-0084_12_FZEB_137_0001_Bungarotoxin SOP.doc		01/23/2020
2009-0084_12_FZEB_137_0001_Bungarotoxin SOP.doc		01/23/2020
2009-0084_12_FZEB_137_0001_MSDS Bungarotoxin good one.pdf	MSDS Bungarotoxin	01/23/2020
2009-0084_12_FZEB_137_0001_MSDS Bungarotoxin good one.pdf	MSDS Bungarotoxin	01/23/2020

13.9.2 Hazardous Agents Information

Provide information about the hazardous materials administered to animals and the conditions for their administration. For additional assistance in completing any part of this section, contact Environmental Health &Safety (255-8200 or ehs@cornell.edu) or CARE (253-4378) or CARE@cornell.edu).

Species Name	Fish- Zebra Fish

Hazardous Agent	Curare
Category	Toxin
If Z-Other, provide name of hazardous agent	
Class/Biosafety Level	Neurotoxin
Dose, frequency, and duration of administration	Immersion In 3mg/ml For 1- Min Until Movement Stops
Will the agent or its derivatives be excreted or shed by inoculated or treated animals?	NO
Route of administration (check all applicable)	IMMERSION
Other Route (specify)	

Hazard Handling

In this section, describe aspects of hazardous agent handling.

Pharmaceutical-grade substances should be used, or Investigators are expected to provide a scientific justification for the use of non-pharmaceutical grade substances. Many hazardous agents are not available as pharmaceutical grade, and under these circumstances, "Not Available" is an acceptable justification. To determine if substances are pharmaceutical grade, search the FDA databases, the Orange Book or the Green Book. For further assistance, please contact CARE staff. See ACUP 413 for more information and for examples of acceptable justifications.

Is this a pharmaceutical grade substance?
YES
If No, please provide a scientific justification for using a non-pharmaceutical grade. If a hazardous agent is not available as pharmaceutical grade, "Not Available" is an acceptable justification. Any deviations from ACUP 413 must also be included here.
MSDS or other reference information about the agent (provide a web link or attach a file below if appropriate). Please use the ChemWatch SDS Library through Cornell EHS.
http://www.chemcas.com/msds/cas/msds44/8063-06-7.asp
Provide a short description of the relevant characteristics (e.g., hazardous properties, LD50, health effects for humans)of the hazardous agent.
neuromuscular blocker, LD50 rodents 140ug/kg IV injection, lowest published lethal dose in humans 735 ug/kg (route unknown)
Describe any expected clinical signs for inoculated or treated animals.
see SOP attached
Select the appropriate Animal Biosafety Procedure (ABP) that describes administration of the agent, handling of infected or treated animals, and control measures to be used. If you have minor deviations from the ABP, please describe those deviations below. If an ABP is not available or cannot be used without major changes, please use this SOP template to develop an appropriate SOP and attach the completed document to this section.
New SOP Attached Below
If applicable, please describe any minor deviations from the ABP you have selected above, and explain why those changes are needed.
I have reviewed the Animal Biosafety procedure or SOP related to the use of this hazard with animals, will share the requirements of the ABP or SOP with all protocol participants (including students) and will ensure that the procedures described in the ABP or SOP are followed.
YES

Attachments List

File Spec	Description	Created
2009-0084_12_FZEB_293_0001_Curare SOP.doc		01/23/2020
2009-0084_12_FZEB_293_0001_Curare SOP.doc		01/23/2020

13.9.2 Hazardous Agents Information

Provide information about the hazardous materials administered to animals and the conditions for their administration. For additional assistance in completing any part of this section, contact Environmental Health &Safety (255-8200 or ehs@cornell.edu) or CARE (253-4378) or CARE@cornell.edu).

Species Name	Fish- Zebra Fish
Hazardous Agent	RNA
Category	Biological
If Z-Other, provide name of hazardous agent	
Class/Biosafety Level	NC
Dose, frequency, and duration of administration	Microinjection Of 1 Nanoliter Of Rna (1.2 Mg/ml)
Will the agent or its derivatives be excreted or shed by inoculated or treated animals?	NO
Route of administration (check all applicable)	OTHER
Other Route (specify)	Microinjection of single-celled embryo

Hazard Handling

In this section, describe aspects of hazardous agent handling.

Pharmaceutical-grade substances should be used, or Investigators are expected to

provide a scientific justification for the use of non-pharmaceutical grade substances. Many hazardous agents are not available as pharmaceutical grade, and under these circumstances, "Not Available" is an acceptable justification. To determine if substances are pharmaceutical grade, search the FDA databases, the Orange Book or the Green Book. For further assistance, please contact CARE staff. See ACUP 413 for more information and for examples of acceptable justifications.

Is this a pharmaceutical grade substance?
No
If No, please provide a scientific justification for using a non-pharmaceutical grade. If a hazardous agent is not available as pharmaceutical grade, "Not Available" is an acceptable justification. Any deviations from ACUP 413 must also be included here.
Not available
MSDS or other reference information about the agent (provide a web link or attach a file below if appropriate). Please use the ChemWatch SDS Library through Cornell EHS.
Not hazardous
Provide a short description of the relevant characteristics (e.g., hazardous properties, LD50, health effects for humans)of the hazardous agent.
Not hazardous
Describe any expected clinical signs for inoculated or treated animals.
No symptoms expected
Select the appropriate Animal Biosafety Procedure (ABP) that describes administration of the agent, handling of infected or treated animals, and control measures to be used. If you have minor deviations from the ABP, please describe those deviations below. If an ABP is not available or cannot be used without major changes, please use this SOP template to develop an appropriate SOP and attach the completed document to this section.
No Animal Biosafety Procedure Available or Applicable
If applicable, please describe any minor deviations from the ABP you have selected above, and explain why those changes are needed.
I have reviewed the Animal Biosafety procedure or SOP related to the use of this hazard with animals, will share the requirements of the ABP or SOP with all protocol participants (including students) and will ensure that the procedures

described in the ABP or SOP are followed.
YES

Attachments List

File Spec	Description	Created

13.9.2 Hazardous Agents Information

Provide information about the hazardous materials administered to animals and the conditions for their administration. For additional assistance in completing any part of this section, contact Environmental Health &Safety (255-8200 or ehs@cornell.edu) or CARE (253-4378) or CARE@cornell.edu).

Species Name	Fish- Zebra Fish
Hazardous Agent	z-Other
Category	Zz-other
If Z-Other, provide name of hazardous agent	pancuronium bromide
Class/Biosafety Level	
Dose, frequency, and duration of administration	2 Micrograms Per Milligram Fish Injected Retroorbitally
Will the agent or its derivatives be excreted or shed by inoculated or treated animals?	NO
Route of administration (check all applicable)	OTHER
Other Route (specify)	injected retroorbitally

Hazard Handling

In this section, describe aspects of hazardous agent handling.

Pharmaceutical-grade substances should be used, or Investigators are expected to provide a scientific justification for the use of non-pharmaceutical grade

substances. Many hazardous agents are not available as pharmaceutical grade, and under these circumstances, "Not Available" is an acceptable justification. To determine if substances are pharmaceutical grade, search the FDA databases, the Orange Book or the Green Book. For further assistance, please contact CARE staff. See ACUP 413 for more information and for examples of acceptable justifications.

Is this a pharmaceutical grade substance?
yes
If No, please provide a scientific justification for using a non-pharmaceutical grade. If a hazardous agent is not available as pharmaceutical grade, "Not Available" is an acceptable justification. Any deviations from ACUP 413 must also be included here.
MSDS or other reference information about the agent (provide a web link or attach a file below if appropriate). Please use the ChemWatch SDS Library through Cornell EHS.
see attachment
Provide a short description of the relevant characteristics (e.g., hazardous properties, LD50, health effects for humans)of the hazardous agent.
The biggest concern at the levels used here is eye irritation should it get in the eyes.
Describe any expected clinical signs for inoculated or treated animals.
paralysis
Select the appropriate Animal Biosafety Procedure (ABP) that describes administration of the agent, handling of infected or treated animals, and control measures to be used. If you have minor deviations from the ABP, please describe those deviations below. If an ABP is not available or cannot be used without major changes, please use this SOP template to develop an appropriate SOP and attach the completed document to this section.
New SOP Attached Below
If applicable, please describe any minor deviations from the ABP you have selected above, and explain why those changes are needed.
see attached SOP
I have reviewed the Animal Biosafety procedure or SOP related to the use of this hazard with animals, will share the requirements of the ABP or SOP with all protocol participants (including students) and will ensure that the procedures

described in the ABP or SOP are followed.
YES

Attachments List

File Spec	Description	Created
2009-0084_12_FZEB_2619_0001_Fetcho Biological Toxins SOP_02_07_2018.pdf		01/23/2020
2009-0084_12_FZEB_2619_0001_Pancuronium bromide SDS.pdf	Pancuronium bromide SDS	01/23/2020
2009-0084_12_FZEB_2619_0001_Pancuronium bromide SOP.pdf	Pancuronium bromide SOP	01/23/2020

13.10 Administered Substances

Please add all appropriate substances you are administering to this species by clicking on "Add Substance". To edit a substance, click on the substance name. Detailed information will be required on the following page. To remove a substance, first click the "Select" box, and then click on "Remove Substance".

Examples of substances to list here are: experimental compounds, antibiotics, analgesics, anesthetics, euthanasia agents (including CO2), exogenous hormones and sedatives. Substances listed as hazardous agents should be listed in the separate "Hazardous Agents" section, and not listed here.

Use of Controlled substances requires appropriate NYS licensing and DEA permits. CARE and the Cornell pharmacy cannot provide controlled substances for research purposes. For questions about controlled substances contact EH Please consult CARE staff or the IACUC staff if you have questions regarding the inclusion of a substance in this section. Investigators are expected to use pharmaceutical-grade substances whenever they are available, even in terminal procedures. The IACUC can approve the use of non-pharmaceutical grade substances on an individual basis only after reviewing the scientific justification. Please see ACUP 413 for more information, and for examples of appropriate justifications.

Species Name	Fish- Zebra Fish

Select	Agent	Type	Pharm Grade	Drug Dosage	Route of Administration	Frequency of Administration	Admin Reason
16	Bupivacaine	Anesthetic	Yes	0.25%	TOPICAL	Once For 1-2 Hours	anesthetic
39	Tricaine Methanesulfonate (MS222)	Anesthetic	Yes	0.03%	IMMERSION	Once	anesthetic, euthanasia (overdose)

13.10.2 Administered Substance Information

Please provide information on all substances used in this protocol. For assistance in determining species-appropriate dosages, contact a CARE veterinarian.

Pharmaceutical-grade substances should be used, or Investigators are expected to provide a scientific justification for the use of non-pharmaceutical grade substances. To determine if substances are pharmaceutical grade, search the FDA databases, the Orange Book or the Green Book. For further assistance, please contact CARE Staff. You must follow guidelines in ACUP 413 for the use of non-pharmaceutical grade substances. Any deviations from the ACUP must also be included below. See ACUP 413 for more information and for examples of acceptable justifications.

Species Name	Fish- Zebra Fish
Substance Name	Bupivacaine
Type	Anesthetic
If Z-Other, provide name of substance	
Reason for Administration.	anesthetic
Dose	0.25%
Route of administration	TOPICAL
Frequency and duration of Administration	Once For 1-2 Hours
Is this a pharmaceutical-grade substance? (Answer NA if using CO2 as a euthanasia agent.	Yes
If NO, please provide a scientific justification for the use of this non-pharmaceutical grade substance. Any deviations from ACUP 413 must also be included here.	Used on embryonic and larval fish in terminal experiments

13.10.2 Administered Substance Information

Please provide information on all substances used in this protocol. For assistance in determining species-appropriate dosages, contact a CARE veterinarian.

Pharmaceutical-grade substances should be used, or Investigators are expected to provide a scientific justification for the use of non-pharmaceutical grade substances. To determine if substances are pharmaceutical grade, search the FDA databases, the Orange Book or the Green Book. For further assistance, please contact CARE Staff. You must follow guidelines in ACUP 413 for the use of non-pharmaceutical grade substances. Any deviations from the ACUP must also be included below. See ACUP 413 for more information and for examples of acceptable justifications.

Species Name	Fish- Zebra Fish
Substance Name	Tricaine Methanesulfonate (MS222)
Type	Anesthetic
If Z-Other, provide name of substance	
Reason for Administration.	anesthetic, euthanasia (overdose)
Dose	0.03%
Route of administration	IMMERSION
Frequency and duration of Administration	Once
Is this a pharmaceutical-grade substance? (Answer NA if using CO2 as a euthanasia agent.	Yes
If NO, please provide a scientific justification for the use of this non-pharmaceutical grade substance. Any deviations from ACUP 413 must also be included here.	used on fish embryos and larvae as well as adults

13.11 Euthanasia

Please list the euthanasia methods used for this species. Multiple methods per species may be added when appropriate. Add, edit (by clicking on method name) or remove a method. Detailed information will be required on the following pages. For acceptable euthanasia methods see ACUP's or the AVMA Guidelines for the Euthanasia of Animals.

Species Name	Fish- Zebra Fish
Select	Euthanasia Method
20	M S 222 Overdose

13.11.1 Euthanasia Information

Please describe the euthanasia procedures for this species. You may use one of the Animal Care and Use Procedures (ACUPs) on Euthanasia for guidance on the procedure.

Species Name	Fish- Zebra Fish
Euthanasia Method	M S 222 Overdose

How will you assure the animal will not revive? For example: use a secondary method of euthanasia or monitor for cessation of heart beat and respiration for at least 3 minutes.
For embryos less than 3 days old: MS22 followed by dilute Sodium Hypochlorite. for 4-7 days old, MS222 until 20 mins after opercular movement For breeding adults, MS222 until 20 mins after opercular movement

Describe procedures for the euthanasia of animals. Include instructions for tissue collection, refrigeration, notification, etc.

Select an ACUP for Euthanasia to be followed (if not using an ACUP select NONE and describe the euthanasia procedure below).
306 Fish and Amphibian Euthanasia
If you are not following procedures that are covered in euthanasia ACUP's or the AVMA Guidelines for the Euthanasia of Animals, please describe the specifics of the euthanasia procedure and describe the rationale for selecting this method of euthanasia. Please also give assurance that it is the most humane method possible given the experimental constraints of the project. Contact a CARE veterinarian for further information on acceptable methods of euthanasia.

13.12 USDA Categories

Please enter the number of this species requested for the 36-month protocol approval period in each of the required categories. Please make sure that the numbers in this section correspond with those in pages 13.1 and 13.13. The sum of the numbers of animals in the Pain Level rows must equal your total requested number (as entered on page 13.1)

For reference the USDA Categories are defined
B = animal held for breeding only and not used in research
C = no pain or distress
D = alleviated pain or distress
E = unalleviated pain or distress

Species Name	Fish- Zebra Fish

This is a compilation of information that you have provided for this species on this protocol that may have an impact on the USDA pain category assignment.

Type	Procedure
NON SURGICAL	X- Other Non-Surgical Procedure NOT in LIST
NON SURGICAL	Behavioral Observation/Studies/Testing
NON SURGICAL	Collection of Oocytes
NON SURGICAL	Collection of Tissue using Fin Clips/Scales
NON SURGICAL	Imaging:Other
RESTRAINT	Chemical Restraint
SURGICAL	Craniotomy
SURGICAL	few 100micron sized opening for patch recording in a 4 mm larvae

Pain Level	# of Animals	Description
C	94730	No or minimum pain/distress
D	220	Alleviated Pain/distress

Total Number Requested for Species	94950

13.13 Justification For Number Requested

Indicate the rationale for the number of animals to be used in the space below. Address how you determined the number of animals required. Provide evidence that you have thought through how many animals you will need and will be using the minimal number of animals necessary. More on What the IACUC expects for Statistical Justifications and examples of Non-Statistical Justifications. Information may be provided in the form of a table or flow chart (please attach file). Be sure that numbers of animals correspond to those in previous sections. (Note: Approved numbers may not be exceeded without an amendment.)

Species Name	Fish- Zebra Fish

Use attached files for tables or charts.

Justify the number of animals requested.
Sleep numbers: The adult fish are used only for breeding to provide embryos and larvae. All of our experiments are done on embryos and larvae. The number of adults is estimated based upon maintaining our lines of wild type, mutant, and transgenic fish (on average 250 fish of each of 20 lines for 5000 fish for breeding). The embryos/larvae are used for the experiments. Inorder to generate the sample sizes for our experiments (about 15-20 per experiment). This estimate is based variance in prior data from similar types of analysis from which we have a sense for the statistical power of of this sample size. Please note that the situation with fish embryos is not like that with primates or mice because the fish lay many hundreds to of eggs, most of which must be euthanized, so the number we actually use for experiments is very small compared to the number they generate because of their reproductive strategy. In other words, using fewer embryos in the actual experiments does not reduce the total number of embryos used, which is controlled by the highly variable, but large number laid for the fish. We need to use about 300 embryos/larvae per week to get about 10 successful experiments. This allows us to complete about 25 experimental groups per year. The overall groups with respect to the objectives listed above include: Objective 1: 10 experimental groups, 5 for excitatory and 5 for inhibitory synapses in different brain regions.

Objective 2: 7 experimental groups in different brain regions

Objective 3: 8 experimental groups, in different brain regions.

Here is the basis for the estimates of embryos and larvae:
We assume that on average we use 300 embryos/larvae per week for these particular experiments. Since we need embryos at certain ages, we have to breed embryos each day even though only a fraction might be used in actual experiments (the rest are euthanized). This leads to 15600 total per year. Only a fraction of these (about 100 or less a week) are actually used to do the experiments. The rest are a byproduct of the prolific breeding process.

Of those used for these experiments each year, 70 are in pain category D (35 in Objective 2, 35 in Objective 3). These 70 animals have been placed in pain category D because they are 8 days post fertilization or older. The other 15,530 larval fish per year in the three objectives are in category C. The 5000 breeding fish are in category C.

Motor control numbers:
The adult fish are used only for breeding to provide embryos and larvae. Our experiments are done on embryos and larvae. The number of adults is estimated based upon maintaining our lines of wild type, mutant, and transgenic fish (on average 160 fish of each of 25 lines for 4000 fish for breeding).

The embryos/larvae are used for the experiments. In order to generate the sample sizes for our experiments (about 15-20 per experiment), we need to use about 250 embryos/larvae per week to get about 10 successful experiments. This allows us to complete about 25 experimental groups per year. The overall groups with respect to the objectives listed in the section for
motor control experiments include:

Objective 1: 4 experimental groups, one each for each ventral hindbrain stripe

Objective 2: 7 experimental groups, one for each glycine and glutamate stripe

Objective 3: 5 experiment groups, from sampling in two stripes at different dorso-ventral locations

Objective 4: 4 experimental groups each from wild type and three mutant lines

Objective 5: 5 experimental groups, testing different transneuronal constructs

The number of embryos and larvae to be used are necessarily broad estimates. Since the fish are bred and we cannot control how many eggs they lay (up to 50-100 per cross, but typically less) we cannot control the numbers precisely to provide precise numbers of embryos and larvae. In addition, we often inject DNA constructs to label neurons and only some of the larvae end up with labeled neurons in a manner that is hard to predict.

Here is the basis for the estimates of embryos and larvae: We assume that on average we use 250 embryos/larvae per week for these particular experiments. Since we need embryos at certain ages, we have to breed embryos each day even though only a fraction might be used in actual experiments (the rest are euthanized). This leads to 13000 total per year. Only a fraction of these (about 100 or less a week) are actually used to do the experiments. The rest are a byproduct of the prolific breeding process.

All 13,000 larvae in these objectives are in Category C. The 4000 breeding fish are in category C.

Imaging into adult zebrafish with three photon microscopy:

We will use approximately 150 adult fish for these experiments. This is an estimate based upon numbers needed for other technology development efforts, which involve lots of troubleshooting and evaluation of the technical imaging modifications. We place them in category D. The experiments are terminal

Summary of Animal Numbers:

Sleep Deprivation:
Adults: 5,000 for 3 years
Embryo/Larvae: 15, 600 per year x 3 years = 46, 800
Total: 5, 000 + 46, 800 = 51, 800

Motor Control:
Adults: 4,000 for 3 years
Embryo/Larvae: 13,000 per year x 3 years = 39,000
Total: 4,000 + 39, 000 = 43, 000

Adult imaging: 150

Grand total: 51, 800 (Sleep) + 43, 000 (Motor Control) + 150 (Adult imaging) = 94,950

Attachments List

File Spec	Description	Created

13.14 Exemptions from Standards of Care

Please complete all sections if you plan to deviate from the Guide for the Care and Use of Laboratory Animals (National Research Council, 2010). More on What the IACUC expects. Consult a CARE Veterinarian if you have questions. **A copy of this page from the Approved Protocol must be posted or available where animals covered by this exemption are housed.**

Species Name	Fish- Zebra Fish
Are you requesting an Exemption from Standards on this species ?	NO
If yes, please describe what you propose to do which deviates from the Guide for this	

species, and how long you plan to do this for a given animal.	
Give an estimate of the number of animals to be covered by this exemption.	
Explain and justify the need for this exemption.	
Is the proposed exemption likely to increase stress in the animals?	
If yes, explain your plan to relieve the distress associated with the deviation.	

14. Alternatives

This section needs to be filled out only for protocols classified as USDA category D and/or E.

More on What the IACUC expects. A reference librarian in the Veterinary Library is available to help prepare the required comprehensive search of the literature. To arrange a consultation, contact Reference Services at 607-253-3510 or vetref@cornell.edu.

Databases Searched (Provide a minimum of 2 databases)	
PubMed Web of Science	
Date of Search	01/15/2018
Search Period	1966-2018
Keywords (include the word "alternative(s)") or search strategy used	
motor control, interneurons, hindbrain, imaging plus cell culture, modelling, simulation, zebrafish, sleep, synapses, alternative and patch recording, 3 photon,	
Consultation with colleagues (include name, qualification, etc.)	
I am in constant interaction with members of the zebrafish community about the best practices with respect to	

experimentation. We originated many of the approaches used and so mostly people contact me to ask for advice, but as a result I am aware of most new approaches almost immediately.
Scientific meetings (seminars, focus groups, etc.)
Society for Neuroscience meeting, International Zebrafish Meeting.

Provide a narrative describing how information gathered from the analysis of alternatives (database searches, consultations, and/or scientific meetings) influenced the design of this protocol with respect to the "3Rs". (example:Alternatives to Animal Use)

Narrative
I found no alternatives to using live animals to address the questions posed in our work. Although there are models of what happens at the synaptic level during sleep, these models require the biological data that we plan to collect to evaluate them. Because there is no other place to get these data than from animals, we cannot REPLACE the animals used in the experiments. We are trying to use the most simple vertebrate model system (embryonic and larval zebrafish) to address our questions to avoid unnecessary use of more complex mammalian species. We have pioneered approaches to image neuronal activity and fluorescent proteins in intact animals. This REFINEMENT in approach avoids the need in many experiments for invasive surgery that might result in pain. The non-invasive imaging also has the beneficial consequence of a REDUCTION in the number of animals used, as we can often do noninvasive experiments in which the animal can survive.

15. Personnel List

Please name all personnel working on this project. All individuals working with animals are required to attend training provided by the IACUC. When creating a protocol, you must review the PI's personnel data. Be sure to review all personnel data when renewing a protocol.

Name	Role	Phone	Email Id	Campus	Organization	Department	Primary Contact	Requestor	Copy Requestor on All Email
Miller, Brian	PI Staff	254-4338	bjm15@cornell.edu		College of Vet Medicine	Dept of Clinical Sciences			
Fetcho, Joseph	Principal Investigator	254-4341	JRF49@cornell.edu		Arts And Science	Neurobiology And Behavior			