Members Present:

J. Bales  S. Henderson  M. Lucas
T. Brabb  C. Hotchkiss  J. Muster
E. Clark  P. Lang  M. Newman
S. Cunningham  S. Libby  J. Sullivan
D. Fitts

Members Absent:

A. Burich  C. Goodwin  L. Kinman
C. Carrier  M. Gough  A. Leache
J. Clark  C. Grue  J. Stoloff

* D. Fitts alternate for M. Tetrick.

Opening Business:

The IACUC Chair called the meeting to order at 2:30pm.

Protocol Review

Dr. Gire, 4356-01 “Neural Circuit Mechanisms of Oder Localization”

A member of the IACUC gave a brief introduction to the protocol explaining that the purpose of the research was to examine the mouse olfactory system to understand how interactions between the olfactory cortex and other brain regions control sensory processing, to understand the role that specific types of neurons play in odor processing, and to understand how these mechanisms are involved in natural behaviors such as odor localization. The results of these studies will provide new information about how the brain processes sensory information during natural behaviors, which is applicable to human psychiatric disorders involving deficits in sensory integration, such as schizophrenia and autism spectrum disorders.

The studies outlined in the protocol involve intracranial injections of adeno-associated virus to express light-activated proteins in specific brain regions or types of neurons. The activity of these neurons is then monitored through in vivo imaging (via multiphoton microscopy through an implanted cranial window to directly visualize the brain) or through an implanted neural activity recording array, while animals perform behavioral tasks including odor localization and odor discrimination. It is important to note that implantation of the cranial window and implantation of the neural array are done in separate studies, never in the same animal, however, all mice are implanted with a nasal cannula to accurately monitor sniffing behavior during the tasks, and a headpost for restraint.

The sensitive nature of the imaging and recording procedures requires mice to be restrained via an implanted head post for the duration of the behavioral experiments, up to 2 hours per day for a maximum of 2 total months of training and testing. Mice are first acclimated to the restraint apparatus for 2 days, then they are placed on a water-restriction schedule of 1 ml per day. On the following day, they return to the restraint apparatus where they learn they can receive water from a spout located within the head restraint area. For 3-4 days they are head-restrained while they receive water from the spout, gradually making the mice accustomed to receiving reward whenever they are head restrained.

After habituation, the mice are trained to associate a target stimulus (either a specific odor or the odor location) with reward. The total amount of water a given mouse will receive per training day is 1 ml. Each training and testing period lasts up to 14 days, and a given mouse may undergo up to 4 14-day periods (for a total of 2 months of training).
A veterinarian summarized the concerns that prompted a Full Committee Review. It was explained that many of the techniques in the protocol are techniques that are common in non-human primate neuroscience protocols, but at least at the UW, there has not been a mouse neuroscience protocol like the one proposed. However, the methods proposed are approved at multiple other US institutions, and there are many publications based on that work.

There are a few reasons why the protocol is being discussed in a full committee review. The protocol contains some fairly invasive surgical procedures, including placement of a cranial window, a nasal cannula, a neural recording array, and a head post. Other than the nasal cannula; however, these are all surgeries that other groups perform in mice here at UW, and appropriate analgesia is being provided. The other reasons for discussing this in full committee are the use of water deprivation to motivate the mice to perform the behavioral task, and the use of prolonged conscious restraint, which is necessary for the behavioral task and associated imaging. The Guide specifically lists physical restraint, food and water restriction, and multiple major survival surgery as topics that are “special considerations for IACUC review” because of animal welfare considerations.

The issue of water restriction was addressed first. The maximum water restriction in this protocol is 14 consecutive days of 1 ml/day, which is roughly 20% of what the mice would drink free choice. This is used as a motivator for performing an odor discrimination task. Compared to food restriction, water restriction is better-tolerated in rodents, which (up to a point) can adapt physiologically and behaviorally to scarce water in order to minimize fluid losses. These adaptations include a significant decrease in food intake, which, combined with the low water intake from water restriction, can lead to more than 20% weight loss in less than two weeks of water restriction. Mice under this type of water restriction require frequent monitoring, which is built into the proposed protocol. Water restriction is established in the literature on sensory discrimination, but may not be the only option. Using only a positive motivator, such as sugar water, without water restriction could potentially be sufficient for mice to perform the described behavior tasks, though this is not known. Dr. Gire’s scientific question depends on olfactory discrimination; therefore behavioral rewards with an odor detectable by mice could confound the results. Before resorting to water restriction, the group will determine whether mice can learn the task with a positive motivator only. However, both water deprivation and non-deprivation behavioral motivators are included as possibilities in the protocol as written.

Second, background on prolonged restraint in mice was provided. Because mice are prey species, restraint stress may be more significant in this species than in non-prey species. Prolonged restraint stress is used as a mouse model for depression, and generally in this field of research the restraint consists of placing the mouse in a 50 ml conical tube with holes drilled into it for airflow. Studies based on this model have looked at increases in stress hormones during the course of chronic restraint. Mice chronically restrained in this manner also have been shown to have altered inflammatory cytokine profiles, and this chronic restraint has been used to induce recrudescence of latent viral infections, thus demonstrating an immunosuppressive effect. The IACUC member was not aware of any publications characterizing the degree of stress associated with chronic restraint using a head post, but she thought it was clear that there is some stress involved with this procedure. However, she did not see an alternative or an obvious refinement to the conscious restraint for this particular study, since the mice must be still and awake in order to perform the behavioral task and for imaging. Just to review, the mice will be restrained via headpost for up to 2 hours a day for one month. While the head is restrained, the mice are still able to walk on a treadmill apparatus and groom, so the restraint is not as restrictive as the conical tube model.

The most fundamental question to consider in this FCR is the balance between the knowledge to be gained from the research and animal welfare considerations based on the combination of the surgeries, water deprivation, and chronic prolonged conscious restraint. The PI has been amenable to refinements of the protocol, e.g., during the revision process, in response to committee member concerns, he amended the protocol to include trying positive reinforcement prior to water deprivation.

The PI was present via phone to answer any questions raised by the IACUC.

Motion: A motion to approve the protocol and add it to veterinary monitoring was made and seconded.
Discussion: An IACUC member asked if the animals are able to groom while restrained. Dr. Gire replied that they can’t put their paws up to their ears but they can groom their faces. A member commented that the PI indicated that the experiments go for approximately 57 minutes but have asked that they be approved to go for up to two hours and asked the PI to explain. The PI replied that it’s up to the mouse to stop. They will continue to do the task until they are no longer interested. Once they stop doing the task, usually around 57 minutes, they stop the experiment and the mice are released from the restraint.

The Committee discussed the treadmill procedure and a picture of the restraint device was passed around to the Committee.

A member asked if there had been any issues associated with this study when the PI did them at his previous institution. The PI replied that the main problem was that when they were teaching the mice to do a complicated task, sometimes the mice would not learn the task and so they were not assigned to that experiment.

The Committee discussed the concerns associated with the water deprivation and the criteria for removal from water deprivation.

Vote on the Motion: The protocol was approved and placed on veterinary monitoring with 10 members voting in favor and 3 members opposed.

Approval of the September 18, 2014 IACUC Meeting Minutes

The IACUC Chair called for the approval of the minutes.

Motion: A motion for approval of the Meeting Minutes was made and seconded.

Discussion: None

Vote on the Motion: The minutes were approved with 11 members voting in favor and 2 members abstaining.

Attending Veterinarian’s Report:

Facility issues:

Humidity: Two decentralized rodent rooms reported low humidity in September. No health effects were noted.

Temperature: On August 12, 2014, the temperatures in a decentralized room used for imaging and may also be used to house rodents or birds was unstable, reaching a high of 81°F. and a low of 67°F. This was corrected. No health effects were noted.

On September 5 through September 8, 2014 a decentralized rodent room had a low temperature of 67.5°F (just outside the accepted range). This was reported to Facility Services and was corrected. No health effects were noted.

In April/May of 2014, and again this past week, one decentralized rat housing room reached temperatures outside the Guide (highest temp 81-82). This room was just centralized this week, and Facility Services has reduced the temperature.

Protocol Monitoring:
There are 18 protocols on the veterinary monitoring program. We added protocol 2110-03 to the veterinary monitoring program during review of their significant change. They were adding numerous IP injections of steroid hormones in oil and we were concerned about discomfort related to the repeated IP oil administration. Thirteen protocols involve surgery or anesthesia. Two protocols involved tumor monitoring. The remaining three protocols involve unusual procedures.

**Office of Animal Welfare Director’s Report**

**Copy to Committee Report**
Since the September 18, 2014 meeting there were 75 items that were sent to the IACUC. There were 5 that were urgent and were sent via email and the rest were posted on Catalyst. There were 3 new projects, 19 renewals of which, 7 were 3-year renewals requiring a complete Project Review Form. There were also 53 significant or minor changes. The IACUC has completed its approval process for most of these items, although some are awaiting final approve due to holds for items such as EH&S approval, OH review or revisions from PI’s.

**Liaison Trend Report**
September – 51 meetings occurred out of 83 (32 didn’t occur because of non-responsiveness or postponement). 98% no issues; 7 administrative fixes, 5 amendments and 4 protocols archived.

**Response to PI Letters**

- Response to letter of Advisement regarding the extended administration of ethanol-rich diet from 8 to 12 weeks. Investigator acknowledged error and has modified the protocol.

- Response to letter of Counsel regarding animals being left in hallway unattended. Investigator acknowledged error and has revised procedures that animals are placed immediately in the surgery suite or adjacent room and this has been communicated to all lab personnel.

- Response regarding mortality in fish population due to improper cleaning procedures. Updated procedure to eliminate the risk of future injury and procedure was reviewed with all personnel in the lab.

- Response regarding the use of topical anesthetic in place of systemic analgesia in a mouse procedure. Topical anesthetic has been removed from the lab and systemic analgesia has been emphasized by placing signs in visible places to remind everyone of the proper use and administration. Also reviewed analgesic protocol with all lab personnel.

- Response regarding unapproved administration of tamoxifen – meeting was held and logical way of dividing the protocol was agreed to. This is in progress.

**Noncompliance**

- Bat Sample Collection - Ecology study designed to understand the relationship among morphology, behavior and performance in small mammals. Interested in their feeding ecology and evolution, and use a variety of techniques to generate a comprehensive understanding of the causes and consequences of the morphological and behavioral diversity of bats and other mammals. Their approved protocol permits the capture of small mammals (including bats), morphological measurements, observations of feeding behavior, measure the force of their bite, collect feces for dietary analyses, and implant Passive Integrative Transponders (PIT) tags to monitor bats at roosting sites. They are also collecting 3mm tissue biopsies in bat wings which was not approved on the protocol. Investigator thought it was covered under tissue collection. This non-compliance has been reported to OLAW.

**Motion**: A motion to send the PI a letter of counsel, including a request for a reply explaining how they will prevent future reoccurrences of this nature was made and seconded.
Discussion: A member of the IACUC commented that there were a number of bats involved in the noncompliance. A main concern was that the samples were collected and then the bats were released so the health of the animals was not monitored.

Vote on the Motion: The motion was approved unanimously with 13 members voting in favor.

- Extended MRI - This project is designed to look at the biodistribution of nanoparticles in the brain to see if they are viable vehicles for administering anticancer therapeutics. The protocol involves anesthesia and MRI to observe the distribution of the particles. The initial study done to evaluate this was approved for a 4 hour MRI evaluation and was extended beyond the approved anesthetic duration. There was some confusion based on the wording of the protocol that the actual MRI anesthetic duration of 4 hours only covered the MRI procedure and did not account for the induction, transport to/from the MRI and recovery of the animal. There are no immediate plans to repeat this procedure and the protocol will be modified if it is to be used in the future. This non-compliance has been reported to OLAW.

Motion: A motion to send a letter of counsel to the PI and the individual that was present for the MRI and require a reply explaining how they will prevent a reoccurrence of this nature was made and seconded.

Discussion: None

Vote on the Motion: The motion was approved unanimously with 13 members voting in favor.

Housekeeping

- Information Requests: - All requests for information regarding animal use protocol or the animal use program should be forwarded to the University of Washington Office of Public Records and Open Meetings. If IACUC members have questions about this process they should contact OAW.

- Training:
  - On-line IACUC member training – less than a third of our members have completed the on-line course. A reminder including a link to the training site would be sent to the IACUC. Will be sending out a reminder and link to all members.

  Discussion: The Committee discussed the requirement for renewing their training.

  Motion: A motion to require training to be updated every 5 years was made and seconded.

  Vote on the Motion: The motion was approved with 12 members voting in favor and one member abstaining.

  The NWABR IACUC Regional Education Conference is scheduled for February 23, 2015. Registration information will be coming out towards the end of the year.

- Updates:
  - Electronic Database - Options for an electronic database for protocol submission, review and storage are being assessed. A Users Advisor Group is being established and volunteers are being recruited.
  - IACUC Sharepoint site – Relevant IACUC documents will be stored on this site and accessible to IACUC members.

Policies and SOP’s

- Polyclonal Antibody Policy
A veterinarian summarized the policy for the IACUC.

**Motion:** A motion to approve the policy as written was made and seconded.

**Discussion:** It was decided that the policy should be updated to say Complete Freund’s Adjuvant (CFA) instead of Freund’s Complete Adjuvant (FCA).

**Vote on the Motion:** The policy was approved unanimously with 13 members voting in favor.

- **Changes to Approved Animal Use Protocols**
  An IACUC member explained that the policy been developed by a subcommittee in response to the new OLAW guidance regarding changes to animal activities, specifically addressing how Significant Changes and other changes are defined, reviewed and approved. The Policy was summarized and it was explained that it was purposely drafted in such a way as to allow flexibility. When the method outlined in Item B is used, it is up to the veterinarian(s) who is responsible for reviewing and approving the Significant Change to make sure that the changes are within the parameters of the policy.

  **Motion:** A motion was made and seconded to approve the policy.

  **Discussion:**

  Regarding Section B of the policy:
  1) A member commented that he had sent comments to the Committee and he disagreed strongly with the way the policy was written.
  2) The Committee discussed creation of a name for the method outlined in section B.
  3) A veterinary designee list would be created to indicate who could act on behalf of section B of this policy.
  4) Significant Changes approved as part of this method would be sent to the Committee as part of the CTC process. Any member of the IACUC could call for a Full Committee review as part of the normal process.
  5) Additional information relevant to this policy would be presented at subsequent IACUC meetings.

  **Vote on the Motion:** The policy was approved with 9 members voting in favor, 2 members opposed and 2 members abstaining.

**Project Review Form**

The Director of OAW explained that the final revision has been sent to the IACUC for review. It was stressed that if a decision regarding the use of this form could not be made at the meeting, the project would not be pursued further.

**Motion:** A motion to approve the form was made and seconded.

**Discussion:** None

**Vote on the Motion:** The revised Project Review Form as approved with 12 members voting in favor and 1 abstention.

**Other Business**

The format and categories of the CTC Agenda were discussed.

**Closing Business:**

The meeting was brought to a close at 3:59 pm. The floor was opened to public comment.