

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)  
**CALIFORNIA STATE UNIVERSITY, BAKERSFIELD**  
9001 Stockdale Highway, Bakersfield, CA 93311-1099

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*MINUTES OF MEETING*  
*Friday, 05 October 2007*  
*Stockdale Room*

**Members Present:**

**Scientific Concerns:** David Germano, Todd McBride, Steve Suter

**Non-Scientific Concerns:** Andrew Troup, Liora Gubkin

**Community Issues:** Eugene Couture, Debby Kroeger

**Safety and Risk Management:** David Beadle

**Veterinarian:** Mylon Filkins

**Ex-Officio:** Robert Horton

**Visitors:**

Isabel Sumaya, Psychology Department, for Protocols 04-01 & 07-05

Joe McFaddin, Biology Student, for Protocol 07-01

Adam Grimes, Biology Student, for Protocol 07-03

Matthew Leon, Psychology Department, for Protocol 07-04

MandyLi May, Psychology Student, for Protocol 07-05

Carol Raupp, Psychology Department, member of the public

Meeting was called to order by Chair Steve Suter at 1 PM.

**I. ANNOUNCEMENTS:**

- A. New Member.** Dr. Eugene Couture, clinical neuropsychologist in private practice, was introduced as a new community member of the IACUC.
- B. AVP for GRASP.** Dr. Robert Horton, Interim AVP for Grants, Research, and Sponsored Programs and Ex-Officio member of the IACUC was introduced.
- C. Re-Ordering of Events.** A new order of events for the meeting was distributed, prompted by scheduling conflicts of several investigators on the agenda.
- D. Facility Inspection.** The Animal Facility will be inspected from 2:00 - 2:30 PM midway through the meeting.
- E. Form for Posting.** Member and contact info on the posted form for registering concerns about animal use needs to be updated and was circulated for current telephone info.

**II. PREVIOUS MINUTES:**

Kroeger moved and Filkins seconded, a motion to approve the minutes for the IACUC meeting of 08 June 2007. The motion was approved 8-0.

### III. OLD BUSINESS:

#### A. Adverse Event Reporting

Federal reports, which will be required at some point, require reporting of adverse events, which are basically events that are not part of the protocol that adversely affect animal subjects. Therefore the CSUB IACUC needs a mechanism for adverse event reporting by investigators. A modified form, adapted from one used by Oregon State University, was proposed. The form will be attached to IACUC authorization and renewal letters. A section at the end of the form will be added for the investigator to affirm "no adverse events" if that is the case, to be filed at the end of each year the protocol is in effect.

Filkins moved the above, seconded by Couture. The motion was approved 8-0.

### IV. NEW BUSINESS:

#### A. Review of Proposed Modifications of Active Protocols

##### 1. Modification of Protocol 04-01 (Sumaya, Psychology) "Circadian Effects of Melatonin on Extrapyrimal Side Effects in Rats Treated with Typical and Atypical Antipsychotics"

The investigator summarized. The aged animals in this protocol offer a chance to examine "clock genes" as a function of age in rats, which hasn't been studied. At present rats are returned to the colony to be observed across normal aging. This proposal is to extract brains from some of the animals at the end of the current Protocol 04-01 activities, freeze the brains, preserve them, and then carry out genetic analyses using assay methods to be determined. Therefore, this modification comes down to a proposed alteration in the disposition of the animals at the end of the protocol.

Questions followed:

**[Q = question A = answer C = comment]**

**Q:** How will you obtain the brains? **A:** Rats will be euthanized by decapitation using a guillotine, which the investigator already has.

**Q:** Why not just let them age in the colony if interested in old brains and then obtain the brain when the animal dies? **A:** The cause and nature of the animal's death could comprise the biochemical data needed to conduct the genetic assay.

**Q:** What is the trade name for thiothixene? **A:** I don't remember.

**Q:** How many animals are being used in Protocol 04-01? **A:** The protocol is authorized for  $n = 144$ , but the research is going more slowly than anticipated so it appears that about 30 to 40 animals will complete the protocol.

**Q:** Will the guillotine decapitation be done following anesthesia? **A:** No, it must be straight decapitation to get the brain without possible chemical alterations from the anesthesia.

**Q:** Could you use CO<sub>2</sub> to euthanize and then decapitate? **A:** Prefer not to.

**Q:** How will you store the brain tissue? **A:** Hopefully in the Science Stockroom freezer, but permission for that has not been worked out.

**Q:** Where will you do the brain slices? **A:** At CSUB or send to Northwestern, where she does research in the summer. Not sure she can get the brain slicing operation up and running soon enough here and is positive that the work could be done at Northwestern.

**Q:** Do you have experience working with methylbutane? **A:** Yes, at Northwestern keeping brains frozen in 2-methylbutane/dry ice.

**Q:** Describe the method of preservation. **A:** A bed of methylbutane and dry ice is prepared and then the brain is added and dry ice is packed around it until it is moved to a freezer.

**Q:** Is this done with special ventilation or under a fume hood? **A:** No, this procedure has been done in the open.

**C:** Probably OK as 2-methylbutane gives off very little vapor.

**Q:** Is there some kind of back-up or alarm system to make sure the brain specimens aren't lost in case of electrical failure or failure of the freezer unit itself? **A:** At Northwestern there was an alarm on the freezer. There is some kind of alarm perhaps tied to a call-out procedure here, but not clear how that works.

**C:** The freezer circuit should be added to a list of critical circuits known to Facilities Management to avoid damage to specimens due to some routine disabling of the freezer circuit.

**Q:** Are there adrenaline effects to brain tissue with decapitation? **A:** That depends on how well the technique is carried out.

**Q:** Where will the decapitations be carried out? **A:** In the prep room with the large sink where the operant chambers are. A station will be set up for getting the body into a container, the brain removed, and preserved.

**Q:** How quickly will the brain be removed after decapitation? **A:** Within a few minutes. Tool used will be a bone cutter, etc. Rat skulls are not thick.

**Q:** You will do the decapitation and brain removal? **A:** Yes.

**Q:** Any student involvement? **A:** No.

**Q:** How does the guillotine work? **A:** There is a top and bottom knife and the top knife is weighted. There is a head restraint mechanism.

**Q:** What is the shelf life for use of the brain tissue, once in storage? **A:** This could be years if the preservation is done properly. It also can depend upon the type of assay procedure to be done on the brain specimen.

**Q:** When do you plan to carry out the euthanization, brain removal, and storage procedures? **A:** Realistically this will have to wait until December when the investigator is not teaching.

**Q:** How will the anti-psychotic drugs be stored? **A:** In a locked cabinet.

**Q:** And the syringes? **A:** The needles are also stored in a locked cabinet.

**Q:** Are the dosages to be used possibly harmful to humans. **A:** No.

When there were no further questions from the IACUC on the proposal to modify Protocol 04-01, questions and comments were solicited from the visitor, Dr. Raupp. After the PI had been excused, Raupp asked the following questions, which were then posed by the IACUC Chair to the PI when she had been called back to the meeting and Dr. Raupp had been excused. Feedback from the IACUC was provided to Dr. Raupp after the PI had been excused.

**Q:** Is pain and suffering added to the protocol by the use of the restraints required by the guillotine? **A:** The restraint involved is similar to that used to pick up and inject an animal.

**Q:** Justify the use of decapitation as euthanasia. There is evidence that consciousness can remain for minutes following decapitation. **A :** The issue is not about whether decapitation is a desirable means of euthanasia, but whether the scientific needs of the investigation justify and out-weigh the pain and suffering of the animal subjects, which is the bottom line in all IACUC deliberations. Most, but not all, members of the IACUC believe that decapitation is justified in this protocol based on the above utilitarian reasoning.

The PI and the visitors were excused and the IACUC deliberated in executive session. There was a motion to conditionally approve modification of Protocol 04-01: Couture moved, Germano seconded. Approved 7-1. The conditions were to modify Protocol 04-01 to include the information listed below. The protocol would then be distributed to IACUC members to affirm successful revision. This information was announced when everyone had returned.

1. Present a detailed rationale explaining the scientific value of the research -- summarizing existing knowledge with respect to clock genes, clock genes and aging, and specifically research on the above with rats. Then argue the benefits of the proposed research in terms of how it may potentially extend this knowledge.
2. Provide a detailed description of how the brains will be extracted, including use of the guillotine, extraction of the brain, and preservation of the brain.

3. Describe the gene assay methods and justify their potential value in terms of the rationale presented for the protocol modification.
4. Describe the methods of storage, including safety measures taken to ensure the integrity of the brain specimens. If the brains are to be shipped, describe the method of packing and shipping in terms of integrity of the brain specimens.

## **B. Review of New Protocols**

1. **Protocol 07-01** (Joe McFaddin, Biology Student) "Investigation and Analysis of Home Ranges and Diet of the Blunt-Nosed Leopard Lizard (BNLL), *Gambelia sila*, in Differing Habitats within Kern County"

The investigator summarized. Scat will be analyzed to make inferences about diet in relation to prey densities. Animals will be captured and then released at the capture site following scat analysis. This species is fully protected.

Questions followed:

**[Q = question A = answer C = comment]**

**Q:** Is there any mortality in the capture procedure? **A:** No.

**Q:** What will get analyzed? **A:** Eating habits.

**Q:** Prey species will be identified how? **A:** Visual analysis of items in the scat like insect parts.

**Q:** Is there any risk of getting cited by Fish and Game during this research? **A:** No. McFaddin will be working under Germano's permit.

**Q:** Is Germano at risk via this research? **A:** No and neither is CSUB, based on Germano's reputation as the leading expert on this species.

When there were no further questions from the IACUC on Protocol 07-01, questions and comments were solicited from the visitor, Dr. Raupp. After the PI and faculty mentor had been excused, Raupp asked the following questions, which were then posed by the IACUC Chair to the PI when he had been called back to the meeting and Dr. Raupp had been excused. Feedback from the IACUC was provided to Dr. Raupp after the PI had been excused.

**Q:** Will the lizards be fed during their two-day stay away from their habitat? Is fasting OK with lizards? **A:** Yes. These animals often go a week without eating.

**Q:** What are the effects of the radio implant in terms of survival and breeding? **A:** Minimal effects, because this is a tiny glass bead that is a passive signaling device.

**Q:** What's it like for a lizard to die from stress? **A:** This happens very rarely and it is heat stress. They basically roll over and die.

The PI, faculty mentor, and the visitors were excused and the IACUC deliberated in executive session. There was a motion to approve Protocol 07-01: McBride moved, Troup seconded. Approved 6-0, with Germano abstaining. The information was announced when everyone had returned.

**2. Protocol 07-02** (David Germano, Biology) "Is Growth, Reproduction, and Population Demographics of the Desert Box Turtle (*Terrapene ornate luteola*) Affected by Global Climate Change?"

The investigator summarized. He is taking sabbatical to return to same location where specimens were studied in 1986 and 1998. Growth will be measured and radiography will be done to see if females are bearing eggs. Specimens will be returned to point of collection within 24 hours. Some will get radio transmitters to make them easy to find again. Interest is in looking at growth as a function of temperature across time with reference to global warming.

Questions followed:

**[Q = question A = answer C = comment]**

**Q:** Although you're licensed in California, will there be problems in the other two states involved? **A:** Maybe, will check and deal with the necessary permitting.

**Q:** How will the radio be attached? **A:** Glued on.

**Q:** Can you take a film badge and have it checked afterwards to verify your safety from having used the portable radiography device? **A:** Yes, will do.

The PI and the visitor were excused and the IACUC deliberated in executive session. There was a motion to approve Protocol 07-02: Couture moved, Beadle seconded. Approved 6-0, with Germano abstaining. The information was announced when everyone had returned.

**3. Protocol 07-03** (Adam Grimes, Biology Student) "Evaluation of the Hybrid Zone of *Gambelia sila* and *Gambelia wislizenii* and Its Management Implications"

The investigator summarized. There is a lot of blunt-nosed [endangered species] and long-nosed [not endangered species] hybridization. Tissues will be obtained by toe-clipping for genetic analysis to determine the extent of hybridization in a specific location of interest where Cal Trans, who is funding the research, wants to build a road.

Questions followed:

**[Q = question A = answer C = comment]**

**Q:** Is there danger to the animal of toe-clipping? **A:** No and Germano is permitted to do this. There is minimal blood supply. Loss of one toe does not affect locomotion.

**Q:** Really going to use flour to stop bleeding? **A:** No, will use an appropriate coagulant.

**Q:** There are safety issues with persons in the wild alone. Can work in pairs? **A:** That's not practical, but will make sure to have the researcher file a travel plan before going into the field.

**Q:** Is there risk of infection from the toe clipping? **A:** Very little -- infection is rare in reptiles.

**Q:** What kind of tools will you use for the toe-clipping? **A:** Small cuticle scissors or nail clippers.

**C:** Make sure to clean the instrument so as not to contaminate. **A:** Will do.

**Q:** Have there been effects of the recent forest fire in the study region? **A:** No.

The PI, faculty mentor, and the visitor was excused and the IACUC deliberated in executive session. There was a motion to approve Protocol 07-03: Filkins moved, Kroeger seconded. Approved 6-0, with Germano abstaining. The minor conditions indicated above will be communicated in the authorization letter. The information was announced when everyone had returned.

**4. Protocol 07-04** (Matthew Leon, Psychology) "Psychology Course 301L: Principles Of Learning Laboratory"

The investigator summarized. This protocol was previously Sumaya's but now Leon is teaching PSYC 301L. The sequence of lab assignments was summarized week by week.

Questions Followed:

**[Q = question A = answer C = comment]**

**Q:** Do the students do the HCUAS training? **A:** They do Modules 1 and 2.

**Q:** Do these activities take all quarter to complete? **A:** They are finished by about week 6 or 7. Then they analyze and discuss data. There is a lecture session once per week.

**Q:** Can this lab be set up as a collaborative activity with one rat per pair of students? **A:** It's better individually, for example each student making choices during shaping.

**Q:** Wouldn't students learn about the choices in pairs? Also, how about using software stimulations of operant conditioning? **A:** Has thought about "Sniffy" for P301L, but has not used it himself. Live animals are better and cannot replace experience of student seeing an actual live animal learn.

**Q:** Is the course taught every quarter? **A:** It's taught two quarters/year. Animals are sacrificed afterward. [Note: IACUC Chair points out that they are typically moved to other protocols afterward.]

**C:** Although the protocol indicates that rats will be euthanized via sodium pentobarbital overdose at the end of the protocol, in fact CO2 euthanasia is accepted and used if animal bodies are to serve as food for species at the FACT facility. Also, animals from this protocol are to be re-cycled into other protocols whenever possible.

When there were no further questions from the IACUC on Protocol 07-04, questions and comments were solicited from the visitor, Dr. Raupp. After the PI had been excused, Raupp asked the following questions, which were then posed by the IACUC Chair to the PI when he had been called back to the meeting and Dr. Raupp had been excused. Feedback from the IACUC was provided to Dr. Raupp after the PI had been excused.

**Q:** One reason this protocol was not approved when presented by Dr. Sumaya is that the IACUC wanted to see Dr. Leon's background and experience. We have not heard this yet. **A:** [Note: these had been summarized in the written protocol submitted, which was not available to the visitor]. The PI has been involved in laboratory activities with rats continuously since the early 1990s when he was an undergraduate at UCLA. In graduate school he was involved in electrode implants and stimulation and many learning procedures, including during his post-doc.

**Q:** Enrollments in P301L were very small last year, so that there were more animals ordered than were needed. Can this be corrected, given the IACUC guideline to use as few animals as possible? **A:** Last year animals were not ordered separately for P301L. Actual enrollments will be taken into account when ordering this year and the timing of ordering will be set up to allow this.

**Q:** In the past animals at CALM in a zoo setting were sometimes used for P301L assignments. Can this be done? **A:** Hasn't thought about that, but is open to that possibility. **C:** There are possible safety issues associated with this in terms of communicable diseases and injuries from biting.

The PI and the visitors were excused and the IACUC deliberated in executive session. There was a motion to approve Protocol 07-04 with several conditions to be communicated in the authorization letter: Filkins moved, Beadle seconded. Approved 7-0. The information was announced when everyone had returned. The conditions were:

1. Order rats for P301L guided by the actual registration in order to minimize animals used in accordance with IACUC principles.
2. Animals acquired and used for P301L are to be re-cycled to other protocols if possible. CO2 euthanasia is acceptable and will be used if animal bodies are to be used as food for the raptors at the FACT facility.



**5. Protocol 07-05** MandyLi May, Psychology Student) "Effects of Cage Size and Environmental Enrichment on Rats"

The investigator summarized. This study will look at effects of environmental enrichment and cage size on reproduction in rats.

Questions followed:

**[Q = question A = answer C = comment]**

**Q:** Looking at anticipated litter sizes and reproductive rates, this would lead to a large number of rats. What are you going to do with all those rats? **A:** Pups will be taken out at 4 weeks of age. Resulting rats will be used to create a breeding colony or rats. Only the initial 20 pairs will breed. Females will have separate male partners across the 6-month project period.

**Q:** So, how many litters do you anticipate per female rats. **A:** Probably two to three litters per female.

**Q:** Still, that's a lot of rats. How many rats will the room hold? **A:** About 200. [IACUC members believe that room capacity is rated more like 120 rats.] The rats resulting from the breeding could supply other protocols, including the needs of the PSYC 301 laboratories [Protocol 07-04].

**C:** You will need to figure out how to deal with the large number of rats resulting from this breeding protocol.

**Q:** You state the dimensions of the two cage sizes involved. Will the larger cages replace existing cages in the colony? How will you get them onto the racks? **A:** The larger cages have not been seen yet. This protocol would start fresh when the Animal Facility is empty. The large cages will be used only for this protocol.

**Q:** Has establishing a breeding colony in the Animal Facility been discussed with anyone? **A:** No.

**Q:** Is there existing research on this topic? **A:** Only correlational studies in zoos. Cage size has not been manipulated.

**Q:** What will the breeding animals be fed? **A:** The normal rat chow consumed in the Animal Facility.

When there were no further questions from the IACUC on Protocol 07-05, questions and comments were solicited from the visitor, Dr. Raupp. After the PI had been excused, Raupp asked the following questions, which were then posed by the IACUC Chair to the PI when she had been called back to the meeting and Dr. Raupp had been excused. Feedback from the IACUC was provided to Dr. Raupp after the PI had been excused.

**Q:** What is going to happen to the rats created in this breeding experiment? The investigators mention "pruning down" -- what is meant by this? **A:** There won't be any

"pruning down" or euthanizing of offspring. The protocol will be set up to end when capacity of the Animal Facility and/or number of animals that can be housed and used for other protocols is reached.

**Q:** The investigators mention infant mortality. What is the rate and what are the causes? **A :** Infant mortality in the natural or zoo setting is due to stress that is almost never tied to specific sources and, more often in rodents, competition for food as selection pressure on litter sizes greater than the food available.

The PI and the visitors were excused and the IACUC deliberated in executive session. There was a motion to conditionally approve Protocol 07-05: Germano moved, Troup seconded. Approved 7-0. The conditions were:

1. *The IACUC does not authorize breeding any animals for Protocol 07-05 that then must be euthanized without participation in other protocols, except in emergencies or other adverse events.* The protocol must be revised to specify termination in terms of relevant capacity indices rather than time -- six months. Specify termination in meaningful terms involving number of rats, number of cages, allowable animals in the Animal Facility, and number of animals that can be accommodated by other protocols.
2. If creation of a breeding colony in the Animal Facility continues to be offered as a rationale in support of this protocol, then sufficient evidence to indicate that a breeding colony will be supported by CSUB must be presented. In addition, description of what protocols could be supplied by CSUB-bred rats, rather than those obtained from established commercial breeders, must be included.

**V. AREAS OF CONCERN:** [none]

**VI. NEXT MEETING:**

Friday, 25 January, 2008 – meeting room to be announced.

**VII. ADJOURNMENT:**

There being no further business, the meeting was adjourned at 4:30 PM. Filkins moved/McBride seconded. 7-0.

Respectfully Submitted,

**Steve Suter, Professor of Psychology, Secretary for the IACUC**