THE UNIVERSITY of WISCONSIN MADISON

Graduate School Animal Care and Use Committee
Closed Session – December 12, 2011

Present (voting): Brinkmann, Capuano, Colman, Fine, Lindstrom, Smith, Stuffed, Ziegler

Present (nonvoting): Boehm, McEntee

Guests: Lisa Krugner-Higby, [redacted]

Absent:

Approval of Closed Session Minutes of November 14, 2011

Brinkmann/Smith moved to approve the Closed Session Minutes of November 14, 2011, with minor editorial modifications. The vote was unanimous.

Protocol Review

L00443-0-11-11 (New) — rhesus macaque (120)

Effects of Early Experience on the Development of Anxiety and its Neural Substrate

Dr. Capuano introduced Dr. Krugner-Higby as the Senior Program Veterinarian for the College of Letters and Science (L&S) ACUC, explaining that this protocol is also reviewed by that committee because some of the animals used in the study will be housed at [redacted] Ms. Boehm noted that this protocol was not pre-reviewed by the Wisconsin National Primate Research Center (WNPRC) veterinarian. Ms. McEntee noted starting in January 1st, 2012, the L&S ACUC will require a veterinary pre-review for all protocols.

Dr. Lindstrom asked if there has been any discussion about the type of extreme experiments of this study. Dr. Krugner-Higby said yes, and said that she feels the creation of nursery-raised infant non-human primates (NHP) is severe because of what it can do to young animals. Dr. Capuano said this type of research continues to be carried out at other NHP research centers, but acknowledged that this type of research has not occurred at UW-Madison since the 1980s. Dr. Lindstrom asked if this is an established animal model for this work, and asked if separation from the mothers could be half as long as is proposed. Dr. Krugner-Higby said the PI will say that his lab has gone as far as they can within "normal" anxiety range and the research needs to be carried out past that range. Dr. Capuano said that the PI is trying to compare mother-reared animals to nursery-reared animals at a specific developmental stage. He said that he is unsure if the ACUC has the right to tell a PI not to do their research because the research may cause harm. The ACUC frequently approves protocols that will have adverse effects on animals. Dr. Krugner-Higby said the difference is in other studies of pathogenesis (such as SIV) specific therapeutic or preventative endpoints can be identified and reached, but in these studies endpoints are less clear, noting the behavioral damage to the animals from this type of study is all ready well-known. Dr. Krugner-Higby said that she has read both of the grants listed on this protocol and neither of the grants describe the creation of nursery-reared infants in the specific aims nor in the Vertebrate Animal Sections. She said PI knows that he will have to inform his program officers of this explicit proposal. Dr. Capuano said he believes that a new grant has been submitted to cover that aspect of work. Extensive discussion ensued. It was noted that the PI is trying to learn what is different about the brains of young anxious NHPs in order to eventually develop therapies to treat anxious children and

adults. Dr. Capuano said the PI over the past year has tested every NHP infant for the anxious phenotype to identify candidate animals for his work, and again stated he is not sure if the ACUC should question NIH-approved scientific research. Dr. Krugner-Higby noted the request for the creation of nursery-reared infants has not in fact been approved. Dr. Smith noted that this study is basic science, but the hypotheses and goals are not clearly noted in the protocol. He added that the proposed deprivation is not necessarily troubling, but it is the fact that the PI has not explained it well in this protocol in terms he can understand. Dr. Lindstrom agreed. Ms. Boehm asked if these NHPs infants are purpose-bred, do the fathers of the infants need to be accounted for? Dr. Capuano will check with Dr. Welter.

Discussion ensued. Lindstrom/Smith moved to:
- defer protocol G00443
- require a WNPRC veterinary pre-review prior to submitting a rewrite from ACUC questions (see attached)
- call a special joint meeting of the Graduate School and College of Letters and Science ACUCs and invite the PI and his lab staff to this meeting to answer committee questions.

The vote was unanimous. It was suggested that the PI present a brief presentation of history of his research at the joint committee meeting. Dr. Capuano thanked the committee for the thorough protocol review discussion.

Reports on Recent Semiannual Inspections

Ms. McEntee said that no inspections have occurred since the meeting last month.

Spring 2012 Semiannual Inspection Sign-up

Ms. McEntee explained the Spring 2012 Semiannual Inspections have been scheduled and she passed around a sign-up sheet (see attached).

Program Assessment Update

Ms. McEntee said there is no program assessment update to report.

Senior Program Veterinarians Reports

Dr. Stoffel said that he received a verbal self-report from a [redacted] PI. He explained that the lab performs their own animal husbandry, and nobody from this lab performed animal health checks on December 5th or 6th. He said that he and the PI are further investigating this incident. He said that he will request the PI submit a written self-report. He said that the animals experienced no adverse effects from this event.

Dr. Capuano reported that ceiling renovations have started in room [redacted] of [redacted]

Dr. Capuano requested that the correct-by-date be extended for the repair of the floors in rooms [redacted] and [redacted] of [redacted] He said that WNPRC has received funds to replace these floors and are currently in the process of obtaining quotes from flooring companies as per UW-Madison policies. He said WNPRC hopes to have the repairs completed June 29th, 2012. Discussion ensued and the committee accepted the request to extend the proposed correct-by-date.

Dr. Capuano reminded the committee that humidity levels frequently drop below 30% in the animal holding rooms at [redacted] and [redacted] due to the cold dry weather. He said that the rooms are being closely monitored and no animals appear to be exhibiting adverse clinical signs due to low humidity. He said that WNPRC staff will continue to monitor closely and will report back to the ACUC if any adverse event occurs. He said the Edstrom Watchdog system is being installed at [redacted] and this system may give the ACUC a better idea of what the true humidity is in each room, because staff are currently using handheld devices to monitor temperature and humidity. He noted that Physical Plant staff are in the process of determining whether they are capable of raising the overall room humidity in animal areas of [redacted]

Dr. Capuano reported that on November 13th, 2011 the ART assigned to do feedings in the [redacted] nursery failed to perform their duties at 7:00 p.m. He said this ART's failure resulted in two infants having no access to food until 7:00 a.m. the next morning. He said the infants suffered no adverse consequences from the missed feeding. He said the ART involved was reprimanded by her supervisor and has been disqualified from nursery duties for 3 months. He said that any future failure to execute scheduled responsibilities will result in a formal disciplinary action involving human resources and union personnel.

Dr. Capuano reported that on November 19th, 2011 a three-day old infant marmoset was reported as missing from its cage. He said that no major husbandry activities (e.g. cage sanitation) had occurred in this room between the animal's birth and November 19th, 2011. The ART who cleaned the room that morning when the infant was reported missing did not confirm the presence of three infants. He said the ART rinsed the room and left. The breeding coordinator entered the room and noted the missing infant. The breeding coordinator went to get the ART and they both searched for evidence of the missing infant but found none. Dr. Capuano said the infant may have been cannibalized by its family, which is not uncommon with litters with more than two infants, and remains could have possibly been washed down the floor drain. Ms. Fine asked if this ART is reliable. Dr. Capuano said that this employee is usually only in the marmoset area on weekends. Ms. Boehm said that she checked this ART's records of husbandry back to March and all the records were consistent. Dr. Capuano said the ART was counseled on failing to verify the number of infants. He said renovation of the floor drain covers are in process of having smaller gauge mesh installed to ensure that body remnants cannot be easily lost in that way in the future. Discussion ensued, and the committee was satisfied that all appropriate steps have been taken.

Report from All Campus Animal Planning and Advisory Committee (ACAPAC)

Dr. Capuano said there was no report from the ACAPAC December meeting.

Other Business

Dr. Capuano called for other business for closed session. Hearing none, Smith moved to adjourn into open session. Brinkmann seconded. The vote was unanimous.

Holly McEntee
IACUC Administrator

Approved by [illegible]

Protocol Review

L00443-0-11-11 (New) rhesus macaque (120)

Effects of Early Experience on the Development of Anxiety and its Neural Substrate

Review questions:
Administrative Review:
No questions.

Reviewer 1:
Q11a – This response is too general and uses technical terms (some examples: genotyping, amygdala, hippocampus, deep RNA sequencing, DNA methylation, neuroplasticity genes) that would not be understandable by a lay person with a high school reading level. The editorial tone in places ("We have developed the best...") is inappropriate. The use of citations in this section is not appropriate.
Q11b – Given that the monkeys will be deprived of maternal contact, these experiments should be carefully and thoroughly justified. The present approach is too cursory and general. For example, what does "mechanistic work" mean? Please be as specific as possible. Overly general statements like "No petri dish..." are not helpful.
Q11b – I suggest that Dr. Lindstrom evaluate the statistical approach.
Q13 – Grant 2 needs a funding source (presumably NIH).
Q15 – It is stated that [redacted] will train [redacted] but [redacted] is not identified anywhere in this table. The sentence describing [redacted]'s role does not make sense.
Q16b – The 99-A approval number is missing.
Q17a – This section is difficult to evaluate for two major reasons: 1. The specific hypotheses being addressed are not well described. 2. The description of the experiments is lacking key details: A) How old are animals when testing starts? B) The testing procedures need to be described in more detail particularly with regard to what is being tested and how the data will be obtained/recorded. C) "Special cages", "playroom", "test apparatus", " special primate cage"; these need to be described in more detail. D) The husbandry, species, and use of the snake should be included in this protocol. E) Blood sample sizes and sampling frequency should be specified. As written, one gets the impression that the maximum allowable blood volume will be removed each time. F) What are the criteria for administering or withholding analgesia post CSF sampling in the younger monkeys? What is the expected sampling interval? G) "Isotonic fluids" should be specified, e.g., 0.9% saline, lactated Ringer's, etc.
Q21/Q24-30 – Terminal perfusion should be described as a surgical procedure and all relevant questions answered.

Reviewer 2:
Q9c – The following language should be used to answer this question – "The [redacted] Colony Records Unit keeps complete colony records of all manipulations and drug treatments, as well as major and minor surgeries, performed on the monkeys through their lifetimes. This data is reviewed by the principal investigator, project director, and [redacted] veterinary staff before assigning monkeys to this protocol. This facilitates multiple usage of all monkeys when possible, and ensures that previous use will not compromise an animal's health and the proposed research. With PI and veterinarian consent, the animals may receive blood draws for other protocols while assigned to this protocol."
Q11a – A portion of this answer is in lay language but the latter half of the second paragraph and a large portion of the third paragraph contain technical language that needs to be simplified and undefined abbreviations. Please simplify or explain the technical terms, especially regarding the specific brain areas, and define abbreviations like HPA.
Q13 – Please provide a funding source for grant #2
Q16b – The entry for the radioactive material that will be utilized does not appear to be completed. No OBS-2 number is provided.
Q16c – The following standard [redacted] language should be utilized – "As described in [redacted] SOP 5.01, all personnel entering animal areas of the [redacted] must wear personal protective equipment (PPE) consisting of long-sleeved area-dedicated scrubs/coveralls, rubber boots or area dedicated shoes covered with waterproof disposable shoe covers, a hair cover, a standard surgical mask, two pairs of latex or nitrile

gloves, and a face shield. Forearm protection (disposable lab coat or sleeves) is highly recommended when having direct contact with a non-human primate.

Q16c – Animals are kept in facilities designed for housing of macaques. Sanitation is performed according to [redacted] SOP 2.01. Only experienced personnel are allowed to perform the required duties in the animal housing area.

Q16c – Potentially infectious nonhuman primate tissue samples must be disposed of as per [redacted] SOP 5.05. Syringes, needles, and other sharp items are disposed of in biohazard sharps containers and any non-sharps items such as gloves, gauze, etc. which come into contact with animal body fluids are disposed of in standard biohazard bags. All biological materials, including tissues and used culture media, are handled as per BSL2 recommendations, and after use, are discarded into biohazard bags and autoclaved.

Q16c – Potentially infectious nonhuman primate tissue samples must be transported per [redacted] SOP 5.06. Specifically, the sample must first be placed in a biohazard-labeled plastic bag accompanied by enough absorbent material to contain the total volume of tissue and then must be placed inside a sealable, unbreakable biohazard labeled container."

Q17a (Study Overview) – It would be helpful to the reviewers if the PI could provide a general idea of what one animal will be subjected to while assigned to this protocol. In other words, how many time might an animal undergo all of the separate tests listed in the flow chart of the sequence of testing and how will a decision be made on when euthanasia occurs.

Q17a (Study Overview) – When animals are moved from the nursery at 3-5 months of age and relocated to the general colony, will they be moved with their existing partner(s)?

Q17a (Study Overview) – Please amend the following sentence "[redacted] veterinary staff will assess the animal for fitness" to "[redacted] veterinary and behavioral management staff will assess the animal for fitness."

Q17a (Study Overview) – At what age will the experimental subjects first be utilized in the list of tests described (Human Intruder Paradigm, Observation of Social Behavior in Test Cage and/or Playroom, Response to Conspecifics Test, etc.)?

Q17a (Response to Conspecifics Test) – What action will be taken if aggression or even wounding occurs during this test?

Q17a (Snake Exposure Test) – This description is somewhat confusing. The first paragraph states that the animal will be placed "in the test apparatus" while the second paragraph states that "during the snake exposure test, animals will be placed in the special primate cage." Are the "test apparatus" and the "special primate cage" two different things? Please resolve.

Q17a (Blood and CSF Sampling) – [redacted] guidelines for blood collection need to be added here also. The standard [redacted] wording is as follows:

"Blood will be obtained using a vacutainer system or needle and syringe, from the femoral vein or alternatively, the saphenous vein and pressure will be used to assure hemostasis following the draw. The amount of blood obtained from each draw will be based on the [redacted] blood volume calculations in SOP 4.01 [animal's body weight (kg) x 60 = estimated total volume of blood]. The allowable volume is 20% of estimated blood volume per 30 days. Animals being bled at maximal level may require fluid replacement therapy and clinical monitoring. A [redacted] veterinarian will be contacted for any single blood draw greater than 10% of blood volume and for animals being drawn chronically at maximal levels to assess clinically and provide clinical supportive care and clinical monitoring. This may include fluid replacement and periodic CBCs. This volume will be included in the total monthly volume. The CBC will be available to the veterinary staff for evaluation, and they will determine the need for further monitoring and/or iron supplementation."

Q17a (Blood and CSF Sampling) – The PI should also state why the blood and CSF samples are being collected.

Q17a (Blood and CSF Sampling) – The information about the ketoprofen dose is stated twice in this section. Please resolve.

Q17a (PET Imaging) – A better description than "18F" needs to be provided for the tracer.

Q17a (PET Imaging) – The description of the imaging of animals less than 2 months of age and less than 8 months of age is redundant. The PI should work with the [redacted] and [redacted] Veterinary personnel to amend this language so that it is consistent and flexible.

Q17a (PET Imaging) – The PI should provide a brief statement explaining why PET imaging is being performed.

Q17a (MRI) – The language in this section needs be amended to coincide with what we have learned recently during the pilot imaging sessions with the young animals performed on G00181.
Q17a (MRI) – The PI should provide a brief statement explaining why MRI imaging is being performed.
Q17b – The PI should communicate with [redacted] regarding the [redacted]s current language on use of the table-top restraint device.
Q17b – The section entitled "Primate Collar" should be amended to state that this method will only be used at the [redacted] Laboratory.
Q18 – Please describe the potentially adverse effects of nursery rearing on an animals psychological and physiological condition.
Q19 – The phrase "Animals that require ketamine or dexmedetomidine anesthesia" should be change to "Animals that require injectable anesthesia."
Q24/Q31 – *The surgical section of this* protocol needs to be completed because terminal perfusion is considered a surgical procedure.

Additional Reviewers:
Q8 – In the "Method of transport & precautions, if any" column – the facilities mentioned as having PPE and exposure kits is inconsistent. For example, the first row describes PET Scans at [redacted] and [redacted] but in the last column of the row, you talk about PPE and exposure kits at [redacted] which is irrelevant. This inconsistency occurs in three out of the four rows.
Q12 – You also need to include the 20 mothers of the nursery-reared animals. In Q17a, it sounds like you are pairing the subjects with other subjects so you don't need 40 cagemates for them.
Q16a.2 – Some of the procedures listed do not cause more than momentary or slight pain or distress, such as arterial catheterizations, blood collections, PET scans, and MRIs. They should be removed from this list.
Q16c – First paragraph, hair covers are missing.
Q17a – How and where will the snake be housed and cared for? Who will be responsible for its husbandry?
Q17a – In the Study Overview section, the second paragraph says, "Mother reared infants will be weaned from their dams at approximately 6-12 months of age and relocated in the general colony where they will be pair housed with another mother reared subject." In Q12 you have accounted for 20 cage mates for these subjects, which seems unnecessary since you are pair housing them with other research subjects.
Q17a – In the Study Overview section, the third paragraph says, "At approximately 3-5 months of age, animals will be relocated to the general colony and at approximately 6-12 months of age will be pair housed with a novel nursery/peer reared animal." At 3-5 months of age, who will they be paired with, the animal(s) they were nursery reared with? Also, see comment above about pair housing with other research subjects so cagemates aren't necessary.
Q17a – In the Study Overview section, before the two boxes, it says, "Subjects will receive the following sequence of testing." At what age will this testing begin? In some areas of Q17a, it sounds like this testing could begin before the infants are two months old, but it isn't more specific than that.
Q17a – In the Study Overview section, after the two boxes, it lists all of the tests to be done. Only two of them do not indicate how many times per year the tests could be done (Observation of Social Behavior in Test Cage and/or Playroom test and MR Imaging Procedure). Please add this information. Also, in the Blood and CSF Sampling test, it says "In accordance with RARC Guidelines" – this should say as per [redacted] SOP 4.01.
Q17a – In the Human Intruder Paradigm section, will a human be present for the whole 20-90 minutes of the test, and if so, will they just stand in the room looking at and away from the animal?
Q17a – In the Observation of Social Behavior in Test Cage and/or Playroom section – is the playroom included in Q8?
Q17a – In the Blood and CSF Sampling section, it says, "Blood and CSF will be sampled in relation to the Human Intruder paradigm, PET imaging or at baseline." What does this mean… will the samples be collected before, during or after these procedures/tests? Also, at the end of the last sentence, it reads as if 20% of blood volume may be collected once monthly, when really this should read that 20% can be collected over the course of one month.
Q17a – In the PET Imaging section, it says, "Their behavioral response to one or more conditions of the human intruder paradigm…." What does this mean – two or more humans will be used, or two different types of tests will be used? Please clarify. Please specify how long the tracer uptake period is. Please

change the administration of atropine sulfate to MAY rather than WILL because later in this description you say that the young animals MAY get it. Please specify how you will "safely transfer animals to the scanner." The explanation you give at the beginning of the MRI section would be appropriate.
Q17a – In the Histology section, you say the description of transcardiac perfusion is included in Q28, but it isn't. It definitely needs to be. In addition, the remainder of the surgery section will need to be completed, and the response to Q7 changed.
Q17b – should collar placement for the mothers be included in Q17a?
Q18 – Why are the animals less than 8 months of age specifically referenced when it is described that all animals receive ketoprofen after CSF collection?
Q18 – Why are both of these sentences included... they seem redundant.
Q35a – The wrong SOP is referenced for Nursery Rearing... Should be SOP 1.16, not 7.03.