## Medical College of Georgia Animal Use Protocol Form

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Institutional Animal Care and Use Committee - “The IACUC is charged by the federal government to review all research, teaching, and diagnostic projects using live vertebrate animals. IACUC approval is required prior to the procurement of animals and the commencement of activities.” Submit electronically to [jwhitloc@mcg.edu](mailto:jwhitloc@mmcg.edu). Do not send or mail a signed copy at this time. The veterinary staff of Laboratory Animals Services can and will pre-review your protocol before submission to the committee.

|  |  |
| --- | --- |
| Principal Investigator: **Almira Smith** | Degree/Academic Rank: **Asst. Prof.** |
| Telephone Number: **706.721.8000** | Fax Number: **706.434.8000** |
| Department: **Neurology, SCNS**  Campus Address: **CB 3704**  Email Address: **asmith@mcg.edu** | Anticipated starting date: **Oct. 1st, 2008** |
| Title(s) of this project: (Include all titles on applications for external funding):  **Individual differences in acquisition and consolidation of emotional memories in a rat model of Post-Traumatic Stress Disorder.** | Funding Source(s):   |  | | --- | | MCM MCG |   Is or has this project been funded?   |  |  | | --- | --- | |  | Yes. | | X | No |   If Yes, what is the Grant number?   |  | | --- | |  | |
| Does this protocol accurately reflect what is in the grant proposal?   |  |  | | --- | --- | |  | Yes. | |  | No | |
| Animal Emergency Contact: (name & **after hours** phone number): **Almira Smith 520-820-2137 (cell)** | |

**Check one:**

|  |  |
| --- | --- |
| X | New Protocol (Continue to question 1) |
|  | 3-Year Renewal (Answer questions below) |

* What is the protocol number this is replacing:

|  |
| --- |
|  |

* Do you presently have any live animals under this number?

|  |  |
| --- | --- |
|  | Yes. |
|  | No |

If Yes, how many? (This amount will be subtracted from the total number of animals you request)

|  |
| --- |
|  |

As this is a 3-year renewal the IACUC requests that you provide a very brief progress report on the outcome of the work carried out under the previous 3-year approval. (Type in the highlighted area below)

|  |
| --- |
| **Explain here:** |

1. Please provide a brief statement, in LAY TERMINOLOGY, understandable by someone with a high school

education, with no acronyms or scientific jargon, outlining the objectives and specific aims of the study. Describe the   
 relevance of the study to advancing scientific knowledge and/or benefits of the study to human and/or animal health.   
 **(Note: A scientific abstract from grant application using highly technical terms is NOT acceptable)**

|  |
| --- |
| **Exposure to a traumatic experience, either with or without physical harm, can cause long-lasting changes in stress hormone levels and in psychological wellbeing. Typical Post-Traumatic Stress Disorder (PTSD) symptoms include unwanted daytime flashbacks and nightmares, increased startle, generalized anxiety, and often co-morbid depression. It is currently not understood how PTSD develops and why it affects some people and not others. We are investigating such questions in an animal model that replicates key features observed in PTSD patients, such as elevated startle, generalized anxiety and difficulty to suppress fear responses, once the trauma-associated cues no longer predict an aversive event. We are studying whether rats predisposed to show PTSD-behaviors after experiencing a traumatic event are learning about the event differently than rats that cope well with the identical trauma. We are also studying how traumatic memories are consolidated into long-term memory in the immediate aftermath of the trauma (first 12 hrs) by examining the expression of learning-related genes. To reveal which rats have a predisposition to develop PTSD-like we assess their startle and generalized anxiety after one stressful event (exposure to a simulated predator). Then, to study how traumatic memories are acquired and stored, we collect their brains at different times after a second stressful event, footshock in a new place.** |

2. Animal Procedures: Describe in narrative form, using LAY TERMINOLOGY, understandable by someone with

a high school education, no acronyms or scientific jargon, the experimental procedures and manipulation that will

be performed on the animals (not scientific rationale). *[Be brief and specific in describing the animal procedures.*

*However, it is not necessary to go into detail (in this section) regarding surgical procedures.*

|  |
| --- |
| **To allow us to pre-classify rats as prone to PTSD-like behaviors (PTSD-prone) and those that are not (PTSD-resistant), we first expose all rats to one stressful stimulus, a simulated predator made of a ball of cat hair. The next day we test them for the amplitude of their startle response to a loud noise and their generalized anxiety by testing whether they chose to go to a new place, high above the ground (Elevated Plus Maze, or EPM). PTSD-prone rats show higher startle and no time in the open arms of the EPM. A week later all rats experience a second traumatic event, footshock in a new place, and we collect their brains and blood at different times after this traumatic event to examine changes in stress hormones and gene expression in brain regions critical for emotional memory. Some rats are allowed to survive for up to a month and are tested in the shock place daily (no footshocks are delivered) to assess how quickly they learn that this place is now ‘safe’. Some may also be tested in a new place to assess generalized anxiety to novelty. To examine whether some known memory-impairing drugs may be beneficial in reducing the probability of developing PTSD-like behaviors, we will inject systemically cholinergic and adrenergic drugs an hour before the time when elevated levels of learning-related gene expression has been observed.** |

1. Animal Species (Check all that apply). Multiple species can be placed on the same protocol only if the

procedures and species distinction can be clearly assessed

|  |  |  |  |
| --- | --- | --- | --- |
| X | | Rat | |
|  | | Mouse | |
|  | | Pig | |
|  | | Dog | |
|  | | Rabbit | |
|  | | Nonhuman Primate | |
|  | | Hamster | |
|  | Other (Type the common name of species to be used below) | |
| **Explain here:** | | | |

A. SOURCE (check all that applies):

|  |  |
| --- | --- |
| X | Purchased from an approved vendor |
|  | Breeding Mice in-house (**breeding addendum must also be submitted**) |
|  | Breeding Rats in-house (**breeding addendum must also be submitted**) |
|  | Animals from another PI at MCG (A transfer between protocols form must be submitted) |
|  | Other (Type your response below) |
| **Explain here:** | |

4. Animal justification – Check all that apply.

A. Living animals are required for this study because:

|  |  |
| --- | --- |
| X | The complexity of the processes being studied cannot be duplicated or modeled in simpler systems i.e. computer or mathematical models |
| X | There is not enough information known about the processes being studied to design nonliving models |
| X | Preclinical studies in living animals are necessary prior to human use |
|  | Other (Type your response below) |
| **Explain here:** | |

B. This species has been selected because: - Check all that apply.

|  |  |
| --- | --- |
| X | A large database exists for this species allowing comparisons with previous data |
| X | This is the lowest sentient species that provides adequate size, tissue, or anatomy for the proposed study |
|  | This species provides a particularly good model for duplicating the human situation |
| X | Previous studies using this species formed the background of this project. |
|  | This species has the following unique features that make it the best choice available for this study because (explain below) |
| **Explain here:** | |

5. Enter minimum number of **animals needed for a three-year period** to obtain statistically valid results. If more than one species is being used enter a separate total for each species.

|  |  |
| --- | --- |
| **Species** | **Total for 3 years** |
| Rats | 576 |
|  |  |

1. Experimental Groups and Numbers

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Species** | **Strain** | **Number of Experimental/ Control Groups** | **Number of Animals per Group** | **Number of Times Procedure is Performed** | **Total Number of Animal Used** |
| Rats | SD or Wistar | 12 | 12 | 2 | 288 |
| Rats | Lewis | 12 | 12 | 2 | 288 |
|  |  |  |  |  |  |
|  |  |  |  |  |  |

1. Group sizes are expected to represent the minimum number of animals that are needed to achieve the goals of this study. The following are examples of acceptable ways to determining group sizes. Please check the ones used.

|  |  |  |
| --- | --- | --- |
| X | | Previous experience with the experiment has shown… (Explain below how previous experiments have led to current numbers). |
| X | | Information about variability of results. |
|  | | Power analysis. (Include the magnitude of expected change and explain how this was determined). |
| X | | These are best estimates until pilot studies are complete. |
|  | | Other. Explain below |
| **Explain here:** Previous experience with similar experiments has shown that a minimum of 10 animals per group is required to accurately approximate the mean of the behavioral scores with and without drugs. Additionally, a minimum of 6 animals per group are required to accurately evaluate either gene or protein levels. Because we will be examining both gene and protein changes, which need to be done on separate animals in the same behavioral groups, we will need at least 12 rats/ behavioral group. First we will evaluate changes in SD/Wistar rats which are known to show elevated corticosterone levels in response to stress and also show lower percent of PTSD-prone rats. In contrast, Lewis rats show a dampened corticosterone elevation and a larger percent of PTSD-prone rats. Thus, we will examine differences in the expression of learning-related proteins in the two species to evaluate whether similar mechanisms underlie predisposition to PTSD-like behaviors within and across strains which may give clues as to which genes or family of genes predispose rats to PTSD-like behaviors. | |

1. Describe in detail all **non-surgical procedures** to be performed per species (If there are none please proceed to question 7). This description should allow the IACUC to understand the experimental course of an animal from its **entry into the experiment to the endpoint** of the study. Include groups/number of animals involved in each experiment; dose, route, volume, and frequency of administration for all injected substances.

|  |
| --- |
| **Explain here: All of the following procedures and drug administration have been previously approved as part of protocol # 06-03-789\*SS. However, we are using these procedures to address new scientific questions, therefore we are submitting a new protocol.**  Starting one day after their arrival in the animal facility, all animals will be handled for a week before start of behavioral training.  **Spatial exploration:** Animals will freely explore an apparatus (3-arm Y-maze or a 47x38x43 cm apparatus) for 5 minutes. Animals will not experience any pain.  **Elevated Plus Maze:** The plus-shaped EPM apparatus consists of four arms, each 50 cm long and 10 cm wide. One set of opposing arms is ‘closed’ by 30 cm tall opaque walls. The apparatus is elevated 60 cm above the floor. A rat will be placed in the center of the apparatus facing an open arm and will be allowed to explore freely for 5 min. The number of entries and time in each arm will be scored. EPM testing will be done in the PI lab, CB3705B. Rats will not experience pain, but some should become anxious while exploring the open arms of the EPM, because rats fear heights and open places (File, 1996). Animals will not experience any pain.  **Acoustic Startle:** Startle will be tested in a ventilated, sound-attenuated apparatus (SR-LAB, San Diego Instruments, San Diego, CA) with background noise level of 68 dB. Rats will be placed in a plastic tube resting on a platform and their movement will be detected by a piezoelectric accelerometer and recorded by the system software. After a 2 min acclimatization period, 15 acoustic startle stimuli will be presented (110 dB white noise, 40 ms, 30-45 s inter-trial interval). The startle amplitude will be recorded by the software. For the preliminary studies, Startle testing will be done in the Small Animal Behavioral Core. In the future, similar apparatus will be purchased by the PI and testing will be done in CB3710B. Rats will not experience pain.  **Simulated Cat Exposure:** The ‘simulated cat’ is a ball of cat hair (~15 cm in diameter) collected from a vaccinated domestic male cat housed at the residence of a friend of the PI. The cat will be combed and the hair will be stored in a glass jar until testing to maximally preserve the odor. The cat hair will be placed in one corner of a box (35x28x35 cm, covered with a transparent lid). A rat will be placed into the farthest corner and allowed to explore the apparatus freely for 5 min. Amount of time spent freezing, number of contacts with the ‘cat’ and time in the farthest quadrant will be scored. Control rats will be exposed to a similar apparatus, but instead of cat hair, they will face a cotton ball of the same size. Testing will be done in CB3705B. Rats will not experience pain, but will become anxious. Rats have innate fear of predators, including cat/cat odor (Blanchard and Blanchard, 1972; Zangrossi and File, 1992; Vazdarjanova et al., 2001). Rats will not experience pain.  **Contextual fear conditioning (CFC):** After 2 minutes in the Shock Arm of the Y-maze, rats will receive 2 brief (1 second-long, 30-60 seconds apart) footshocks through the steel plates on the floor. Previous experiments in our apparatus have suggested that footshock intensity of 1 mA is sufficient to induce strong long-term emotional memory. A minute after the second footshock, the animal will be removed from the Shock Arm. Some animals will be placed into a chamber with Isoflurane vapors and transported to be decapitated on a rodent guillotine (when deeply anaesthetized). Others will be returned to their home cage and killed at different times after CFC. Other rats will be tested for memory and extinction for several days by either placing them in the Shock Arm and assessing amount of time spent immobile (freezing), or by placing each animal in one of the “safe” arms and recording how long it takes them to enter into the arm where they received footshock and how much time they spend freezing. Immediately after the last testing, animals will be anesthetized with Isoflurane and decapitated.Some animals will also explore a novel place 25 min before the last test exposure to determine how well, at the cellular ensemble level, they can discriminate between the trauma-associated place and a novel place.  **Administering memory-impairing drugs:** Some rats will receive injections of either saline, propranolol (5 or 10 mg/kg, i.p.), nicotine (0-1.0 mg/kg, s.c.), a non-specific nicotinic receptor blocker mecamylamine (5 mg/kg, i.p), or scopolamine (0.5 or 1 mg/kg, s.c.) either immediately or up to 7 hrs after CFC. Drugs will be USP grade from Sigma, dissolved in saline, pH 7.2-7.4, such that the injection volumes will be 1 ml/kg. Rats are likely to experience a momentary pain from the injection itself. It is known that at these doses neither drug produces adverse side effects, i.e. exploratory locomotion is unaffected (Harris et al., 1996; Barak and Weiner, 2006).Drugs will be administered once either individually or in combination. To evaluate the specificity of drug action, some rats will receive first an antagonist (mecamylamine), then an agonist (nicotine). All drugs will be pharmaceutical grade, usually purchased from Sigma.  **Blood collection through a tail draw:** In a subset of rats we will evaluate both the baseline and the stress-induced levels of corticosterone to further validate our model and demonstrate to scientific reviewers that both the simulated cat and the CFC training elevate the levels of corticosterone above baseline. Additionally, we will compare, within the same animals, the levels induced by the two stressful events. Rats will be anesthetized with Isoflurane until areflexive and blood will be drawn through a 22 Ga needle from a tail vein or the tail artery. Only ~500 ul of blood will be collected at a time. As the average weight of the rats will be ~300 gr, 500 ul of blood represents 0.00017% of their body weight. There will be no more than 4 tail draws per animal (baseline, after ‘cat’ exposure, baseline, after CFC) and there will be at least 2 days between draws.  **Experimental endpoints:** Rats will be anesthetized with Isoflurane and decapitated at different points after CFC training or after extinction of CFC.  Barak, S. and I. Weiner (2006). "Scopolamine Induces Disruption of Latent Inhibition which is Prevented by Antipsychotic Drugs and an Acetylcholinesterase Inhibitor." Neuropsychopharmacology **32**(5): 989-999.  Blanchard, D. C. and R. J. Blanchard (1972). "Innate and conditioned reactions to threat in rats with amygdaloid lesions." J Comp Physiol Psychol **81**(2): 281-90.  File, S. E. (1996). "Recent developments in anxiety, stress, and depression." Pharmacol Biochem Behav **54**(1): 3-12.  Harris, G., M. Hedaya, W. Pan and P. Kalivas (1996). "Beta-adrenergic antagonism alters the behavioral and neurochemical responses to cocaine." Neuropsychopharmacology **14**(3): 195-204.  Vazdarjanova, A., L. Cahill and J. McGaugh (2001). "Disrupting basolateral amygdala function impairs unconditioned freezing and avoidance in rats." European Journal of Neuroscience **14**(4): 709-718.  Zangrossi, H., Jr. and S. E. File (1994). "Habituation and generalization of phobic responses to cat odor." Brain Res Bull **33**(2): 189-94. |

A. Anticipated adverse affects – Check all that apply in the first column and indicate how often animals will be checked in the 2nd column.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | | |  | Not Applicable |
| X | | | weekly | Weight loss |
|  | | |  | Infection |
|  | | |  | Dehydration |
|  | | |  | Loss of appetite |
|  | | |  | Paralysis |
|  | | |  | Behavioral Changes e.g. hunched up, difficulty breathing, not grooming, diarrhea |
| **Explain here:**  Some PTSD-prone rats may show weight loss due to heightened anxiety. | | | |
|  | Other | | | |
| **Explain here:** | | | |

B. Methods to be used for monitoring adverse affects - Check all that apply in the first column and indicate how often animals will be checked in the 2nd column.

|  |  |  |
| --- | --- | --- |
|  |  | Not Applicable |
| X |  | Observing for changes in behavior, activity, or posture |
|  |  | Observing for evidence of pain or discomfort in localized area |
|  |  | Observing procedure area for redness, swelling, discharge or dehiscence |
|  |  | Observing for decreased ability to move |
|  |  | Measuring daily food and/or water consumption |
|  |  | Paralysis |
| X | weekly | Weighing animals. Need to include the frequency below. |
| **Explain here:** | |
|  | | Other |
| **Explain here:** | |

C. How frequently will you/your staff observe possible health changes (Check one)?

|  |  |
| --- | --- |
|  | Not Applicable |
| X | Daily |
|  | Twice a day |
|  | Once a week |
|  | Twice a week |
|  | Other (explain below) |
| **Explain here:** | |

D. Criteria for removing animals from the study (Check all that apply):

|  |  |
| --- | --- |
|  | Not Applicable |
| X | Veterinary recommendation |
| X | Weight loss more than 15% of body weight |
| X | Inability to ambulate properly |
| X | Inability to eat or drink adequately |
|  | Reduced response to stimuli |
|  | Other |
| **Explain here:** | |

##### 7. RESTRAINT - Check one

|  |  |  |
| --- | --- | --- |
|  | | Routine Restraint - confined manually i.e. restraint used for injections or in a standard species specific  restraint device for < than 15 min.) |
|  | | Non-routine i.e. Primate housing, rodent restraint in inhalation chambers, restraint device > than 15 min. |
| **Restraint device employed and justification: Explain here:** A chamber with saturated Isoflurane vapors to induce anesthesia.Rats will be placed in the chamber until areflexive. | | |
| **Length of time animals are restrained (Minutes/Hours) Please explain here:** Our previous experience shows that confined in our inhalation chamber rats become areflexive in <30s. | | |
| **During this time will animals be given food and water? (Depriving animals of food or water requires scientific justification whether for short or long periods of time.) Please explain here: N/A** | | |

#### 

A. Movement possible while in the device (check all that apply)

|  |  |
| --- | --- |
|  | Not applicable **(if this box is checked proceed to question 8).** |
| X | Restricted ambulation |
|  | Normal postural changes |
|  | Limited postural changes |
|  | No movement is allowed |

B. Describe adverse effects of restraint on behavior, health or well being of the restrained animal:

|  |
| --- |
| **Explain here: None** |

###### C. Describe procedure for acclimating animals to non-routine restraint:

|  |
| --- |
| **Explain here:** |

D. How often are animals monitored during non-routine restraint?

|  |
| --- |
| **Explain here:** |

8. Surgery (check all that apply):

|  |  |
| --- | --- |
| X | No Surgery Involved **(If you checked this box proceed to question 12)** |
|  | Non-Survival – Animals euthanized before recovery from anesthesia. |
|  | Survival |
|  | Multiplesurvival surgeries **(must be discussed with attending veterinarian** |
|  | **Multiple major** survival surgeries **(must be discussed with attending veterinarian)** |

A. If more than one surgical procedure is being performed on a single animal, provide a

scientific justification for using more than one procedure. (Economic justifications are not

permitted by federal regulations).

|  |
| --- |
| **Explain here:** |

9. Describe each surgical procedure for each species. Include number to be used, preoperative procedures (i.e. shaving, preanesthesia, fasting etc.), monitoring, describe aseptic technique and supportive care during surgery. Surgeries must be discussed with Drs. Rodriguez or Charlton before commencing.

|  |
| --- |
| **Explain here:** |

A. Type of suture material to be used and method of skin or wound closure (if sutures are to be used

include type of suture material to be used and state when the sutures will be removed if applicable).

|  |
| --- |
| **Ex Explain here:** |

B. Who will perform surgery and what are their qualifications and/or experience.

|  |
| --- |
| **Ex Explain here:** |

C. Location where surgery will be done (**Building Code and Room Number**)

|  |
| --- |
| **Ex Explain here:** |

**If you answered nonsurvival surgery on question 8 proceed to question 10.**

D. Anticipated/potential adverse affects – **Check all that apply in the 1st column and in the 2nd**

**column indicate how often animals will be checked.**

|  |  |  |
| --- | --- | --- |
|  |  | Weight loss |
|  |  | Infection |
|  |  | Dehydration |
|  |  | Loss of appetite |
|  |  | Paralysis |
|  |  | Behavioral changes e.g. hunched up, difficulty breathing, not grooming, diarrhea |
|  |  | Other |
| **Explain here:** |

1. Methods to be used for monitoring adverse effects (Check all that apply in the 1st column and indicate

how often animals will be checked in the 2nd column).

|  |  |  |
| --- | --- | --- |
|  |  | Observing for changes in behavior, activity, or posture |
|  |  | Observing for evidence of pain or discomfort in localized area |
|  |  | Observing procedure area for redness, swelling, discharge or dehiscence |
|  |  | Observing for decreased ability to move |
|  |  | Measuring daily food and/or water consumption |
|  |  | Paralysis |
|  |  | Weighing animals. Need to include the frequency below. |
|  |  | Other |
| **Explain here:** |

F. How frequently will you/your staff observe possible health changes (Check one)?

|  |  |
| --- | --- |
|  | Daily |
|  | Twice a day |
|  | Once a week |
|  | Twice a week |
|  | Other (explain below) |
| **Explain here:** | |

G. Criteria for removing animals from the study (Check all that apply):

|  |  |
| --- | --- |
|  | Veterinary recommendation |
|  | Animals will be weighed and removed if weight loss is >15% of their body weight |
|  | Inability to ambulate properly |
|  | Inability to eat or drink adequately |
|  | Reduced response to stimuli |
|  | Other |
| **Explain here:** | |

10. Which of the following aseptic techniques will be used? (Aseptic technique must be used on all survival surgeries including rodents). Check all that apply.

|  |  |  |
| --- | --- | --- |
|  | Surgery (rodents) will be performed in an uncluttered area using sterile instruments | |
|  | Removal of hair from surgery site will be performed in an area separate from where the surgery is to  be conducted | |
|  | Nonsurvival – remove hair from surgical area, non sterile gloves and clean instruments | |
|  | Surgical gloves | |
|  | Face Mask | |
|  | Shoe covers | |
|  | Gown | |
|  | Sterilized instruments | |
|  | Surgical prep of surgery site. Indicate antiseptics used (i.e. alcohol, betadine) below. | |
|  | Other | |
| **Explain here:** |

11. Post-operative procedures (check all that apply)

|  |  |
| --- | --- |
|  | Animal moved to a warm, dry area and monitored during recovery |
|  | Returned to cage after fully conscious |
|  | Observed continuously until fully conscious |
|  | Body temperature properly maintained until fully conscious |
|  | Food and water withheld until fully conscious |
|  | Body temperature recorded |
|  | Surgical record kept |
|  | Monitor incisions for swelling, pain or dehiscence |
|  | When will wound clips or sutures be removed? |
| **Explain here:** | |

A. Identify the person from your lab who will be responsible for postoperative care and pain relief.

|  |
| --- |
| **Explain here:** |

B. How often will the animal be observed?

|  |  |
| --- | --- |
|  | Twice a day |
|  | Once a day |
|  | Every other day |
|  | Other. Explain below |
| **Explain here:** | |

12. Anesthesia (Must be Pharmaceutical Grade) – Please provide drug, dose, volume, and route.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Drug | Dose (mg/kg) | Route  IV, IM, IP, SQ | Volume Volume | Frequency of Administration | Duration of Drug Treatment | Species |
| Isoflurane | 2 ml/ 1000cm3 | inhalation |  | continuous | <5 min | Rats |
|  |  |  |  |  |  |  |

A. If you are using inhalant anesthetics are you protecting personnel by using:

|  |  |  |
| --- | --- | --- |
|  | Fume Hood | |
|  | Vacuum Trap | |
| X | Other |
| **Explain here: The experimenters have minimal exposure to Isoflurane. The anesthesia chamber is closed at all times except when putting in or removing a rat. Furthermore, as Isoflurane is more than 6 times heavier than air (185 vs. 29 gr/mol, respectively), it tends to sink to the floor after evaporating and thus minimally affects the experimenter who is at least 50 cm above and to the side of the anesthesia chamber while placing or taking an animal out.** | | |

13. Anesthesia monitoring (check all that applies):

|  |  |
| --- | --- |
|  | Not applicable |
| X | Response to toe/skin pinch |
|  | Heart rate monitoring |
|  | Blood pressure monitoring |
|  | Palpebral/corneal reflex (not applicable to rodents) |
| X | Monitoring of physiological response |
| X | Other |
| **Explain here: Observing the animal for loss of muscle tone, then checking for response to toe pinch.** | |

14. Frequency of monitoring: Check one

|  |  |
| --- | --- |
| X | Every 5-10 min. |
|  | Every 10-15 min. |
|  | Every 15-30 min. |

15. Analgesics (Pharmaceutical Grade Only) – Please provide drug, dose, volume, and route**. LAS veterinary staff discourages the use of analgesics to the drinking water i.e. Acetaminophen, Tylenol**. Please contact veterinarians for alternative analgesics’.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Drug | Dose | Route | Volume Volume | Frequency of Administration | Duration of Drug Treatment | Species |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |

If pain or distress cannot be relieved by analgesics scientific justification must be provided.

|  |
| --- |
| **Explain here:** |

16. Method of euthanasia for all animals including pups (Check all that apply)

|  |  |  |
| --- | --- | --- |
|  | C02/thoracotomy (**Preferred by the IACUC for Rodents**) | |
| X | Decapitation under anesthesia or tranquilization. Identify which animals (species) and at what age,  who will be performing decapitation and how much experience they have with this procedure. | |
| **Explain here:** The PI, Dr. Bunting and Rebecca Nalloor are all proficient in performing the decapitations. Each one has performed over 80 such decapitations. |
|  | Decapitation **without** anesthesia or tranquilization. Justify the use and identify which animals (species)  and at what age, who will be performing decapitation and how much experience they have with  this procedure. | |
| **Explain here:** |
|  | Cervical dislocation under anesthesia or tranquilization. Identify what species this will be performed on & who will be performing this procedure and how much experience they have with this procedure . | |
| **Explain here:** |
|  | Cervical dislocation **without** anesthesia or tranquilization. Identify what species this will be performed on and justify the use. Also identify who will be performing this procedure and how much experience they have with this procedure. | |
| **Explain here:** |
|  | Anesthetic overdose | |
| **Species:**  **Drug Name:**  **Dose:**  **Route:** |
|  | Other | |
| **Explain here:** |
|  | Animals will not be euthanized. | |
| **Explain their disposition here:** |

17. **Item 17 requires a literature-search narrative for Class D and E procedures** that describes the methods and sources used to determine that there are no adequate alternatives to procedures that may cause "more than momentary or slight pain or stress to animals.” Animals must be claimed under the highest class involved at any point prior to euthanasia or release. Euthanasia does not constitute a Class D procedure in-and-of itself e.g. Antibody production, however, a terminal procedure, such as a terminal bleed on a rabbit, is considered a Class D procedure, even if the animal is anesthetized. Procedures involving more than momentary or slight pain or distress must be discussed with the attending veterinarian (Dr. Nancy Rodriguez or Dr. Patricia Charlton) in the planning of the research project. See [Pain or Distress Classification and Consideration of Alternatives](http://grants2.nih.gov/grants/olaw/sampledoc/oacu3040-2.htm#paindist)

|  |  |
| --- | --- |
|  | Class C – Animal Upon which teaching, research, experiments, or tests will be conducted involving no pain, distress, or use of pain-relieving drugs (If euthanasia is the only procedure to be performed on live animals (such as in antibody production). |
| X | Class D – Animals upon which experiments, teaching research, surgery, or tests will be conducted involving accompanying pain or distress to the animals and/or for which appropriate anesthetic, analgesic, or tranquilizing drugs will be used |
|  | Class E - Animals upon which experiments, teaching research, surgery, or tests will be conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs **will not be used**. |

1. If you checked Class E you must provide scientific justification, state how many animals this

applies to and include endpoint criteria.

|  |
| --- |
| **Explain here:** |

B.The Animal Welfare Act regulations require principal investigators to consider alternatives to procedures that may cause more than momentary or slight pain or distress to the animal (**Class D and Class E)** [pdf/alternativesSearchTips.pdf](http://www.mcg.edu/research/animal/pdf/alternativesSearchTips.pdf). Your search must include at least **2 databases** used.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Check** | Database | **Keywords and/or search strategy used when searching a database i.e. alternative, in vitro (You MUST include animal alternative as a keyword)** | **Years Covered e.g.**  **1980-1995** | **Date Search Conducted** |
| X | Medline/PubMed <http://medline.cos.com/> | Animal alternative AND psychological distress AND footshock AND predator scent AND rats | 1955-2006 | Aug 15, 2008 |
|  | Agricola - <http://www.nal.usda.gov/ag98/ag98.html> |  |  |  |
| X | Altweb <http://altweb.jhsph.edu/> | Animal alternative AND psychological distress AND footshock AND predator scent AND rats | 1985-2006 | Aug 15, 2008 |
|  | CAB Abstracts <http://www.isinet.com/isi/products/specialized/cababstracts/> |  |  |  |
|  | TOXNET  <http://toxnet.nlm.ni> |  |  |  |

**Other Sources**

1. Consultation with Experts: (Names and dates)
2. Scientific Meetings: Specify
3. Other Databases: Specify

|  |
| --- |
|  |

|  |
| --- |
| Results of the research. Provide a written narrative and include adequate information for the IACUC to assess that a reasonable and good faith effort was made to determine the availability of alternatives or alternative methods including refinement, reductions, and replacements. [(Literature search summary example)](http://www.mcg.edu/research/animal/documents/examplesummaryliteraturesearch.pdf) **Narrative:** **The PI did not find alternative methods for inducing psychological distress in rats that is as well-controlled as a defined level of footshock or a defined volume of a predator hair.** |

18. List all personnel (include Pi if applicable) who will be working with animals under this protocol. **(If you are using a core facility please i.e. behavior core, telemetry etc include the PI’s name of the core)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Name | Is he/she enrolled in MCG’s Occupational Health and Safety Program | Techniques/  Procedures to be performed | Species | Level of Experience:  Approx. number of times you have performed the procedure in this species | | |
| None  (<5) | Limited  (e.g. 5-20) | High  (e.g. >20) |
| Almira Smith | Yes | Analgesia administration, behavioral training, decapitation | Rats |  |  | >400 |
| Kristopher Smith | Yes | Analgesia administration, behavioral training, decapitation | Rats |  |  | >200 |
| Rebecca Smith | Yes | Analgesia administration, behavioral training, decapitation | Rats |  |  | >80 |

A. For individuals that have none or limited experience or training, explain who will provide the training and the experience they have with the procedure(s).

|  |
| --- |
| **Explain here:** |

B. What will be done for training? Check one

|  |  |
| --- | --- |
|  | Personnel will be trained under the direct supervision of the Principal Investigator until the personnel  listed can competently and professionally perform ALL procedures in which they will be  involved as described in this Animal Care and Use Protocol without supervision |
|  | Personnel will need LAS to provide training and will contact Dr. Tambrallo, Dr. Rodriguez or Dr. Charlton |
|  | Personnel listed had previously trained at another institution or with another PI with procedures described  in this protocol. |
| **Explain here:** | | |
|  | Other training plan |
| **Explain here (provide specifics):** | | |

19. Please provide your Chemical Safety Committee PI Authorization Number (Mandatory for all PI ‘s)

|  |
| --- |
| **Enter number here: 228** |

(If you have not obtained your PI Authorization Number please contact the MCG Chemical Safety Officer at 1-2591)

A. Are you using any chemicals from the MCG List of High Hazard Chemicals? The MCG List of High Hazard Chemicals can be found on their website.

|  |  |
| --- | --- |
|  | Yes (explain below) |
| X | No |
| **Explain here:** | |

B. List of all Hazardous chemicals, including carcinogens (Carcinogen) being used:

|  |
| --- |
| **List here: None will be used on live animals.** |
| 1) Specify the containment methods to be followed in protecting other research animals and personnel from any of the agents listed above. Describe the procedures required for the safe handling and disposal of contaminated animals, caging, bedding, food and materials associated with this study. Describe methods for removal of radioactive waste and monitoring of radioactivity, if applicable. |
| **Explain here:** |
| 2) Have all chemicals and carcinogens been approved by the Chemical Safety Committee? What is the actual approval date (month/year). |
| **Explain here:** |
| 3) Describe the expected physical or physiological consequences to the animals due to administration of pathogens, hazardous chemicals, carcinogens, transgenes, or mutation(s). |
| **Explain here:** |
| 4) Describe any special care or monitoring that the animals will require. |
| Explain here: |
| 5) How long will the animals be maintained after introduction of the agent? |
| **Explain here:** |
| 6) Describe the criteria for interventional euthanasia. *(For example, signs of extreme distress in rodents include hunched posture, disheveled coat, reduced food consumption, emaciation, inactivity, difficulty in ambulation, respiratory problems, and solid tumor growth.)* |
| **Explain here:** |
|  |

20. Potential hazards: PI must submit an application to the appropriate Committee below and receive written approval for the use of potential hazards to animals or humans. The AUP cannot receive full approval until the safety review is completed and approved.

Potential hazards to be used. (Check all that apply)

|  |  |  |
| --- | --- | --- |
| X | | None |
|  | | Infectious pathogenic organisms (human or animal) |
| **Please identify and include the source:** | | |
|  | | Recombinant DNA. |
| **Please identify and include the source: None will be used on live animals.** | | |
|  | | Oncogenic viruses. |
| **Please identify and include the source:** | | |
|  | | Cell/Tissue cultures. |
| **Please identify and include the source. Will these cells/tissues be injected into rodents?** | | |
| **If yes, have these cells/tissue cultures been screened for rodent pathogens?** | | |
|  | | Yes. Provide proof of screening to Jenny Whitlock (IACUC) |
|  | | No. Please contact LAS Vet Tech to schedule screening |
|  | | Exempt. Source of cells/tissues are from routinely screened animals housed within  LAS animal facilities |

|  |  |  |
| --- | --- | --- |
|  | | Radioactive materials or radiation producing devices. |
| **Please identify and include the source:** | | |

"Will you be intercrossing one or more strains of genetically modified animals with another strain (i.e. with a different genetic background) such that the resulting offspring could be a new genetic strain?"

|  |  |
| --- | --- |
|  | Yes. |
| X | No |

A. If you checked any of the boxes (hazards) provide the date of your approval letter(s) include your Biosafety Protocol (BSP) number Radiation Safety approval number.

|  |
| --- |
| **Explain here:** |

21. Exemptions from environmental enrichment for Nonhuman primates, dogs, rabbits or singly housed rodents. Are you seeking an exemption for scientific reasons from the institution’s plan for environmental enrichment?

|  |  |
| --- | --- |
| X | Yes. |
|  | No |

If yes, provide the basis of the request below.

|  |
| --- |
| **Explain here:**  Single cage housing is necessary for the integrity of the scientific data. Expression of learning-related genes is very time sensitive (<3 min), therefore to properly control the timing of the different procedures which take place in different rooms, it is necessary to have one rat/cage. Although rats will be housed in single cages, they will be extensively handled and will be given multiple opportunities to learn/ be stimulated. |

22. Will animals be removed from the LAS animal housing facility:

|  |  |
| --- | --- |
| X | Yes. |
|  | No |

**If you answered Yes. Please answer the following:**

(1) Animals will be taken to (room number and building):

|  |
| --- |
| **Animals will be housed in CB3710C which is an LAS-approved housing room. Most behavior will be done in CB 3705B. Startle will be tested in the Small Animal Behavioral Core (BF 241).** |

1. Provide rationale for the need to remove animals from dedicated animal facilities and justify why such

work cannot be performed in dedicated, approved animal facility space

|  |
| --- |
| **Behavioral training and testing is done is a specific apparatus that is not available at the animal facility.** |

(3) Estimated total time period live animals will be kept in the laboratory:

|  |
| --- |
| **8 hrs** |

(4) Will these animals be returned to the LAS animal facility:

|  |  |
| --- | --- |
|  | Yes. |
| X | No |

|  |
| --- |
|  |

23. Animal Husbandry: special housing, conditioning, diet or other conditions:

|  |  |  |
| --- | --- | --- |
| X | | None |
|  | | Increased frequency of cage changing |
|  | | BSL2 Housing |
|  | | Medicated water (explain what medication will be used and how you will monitor) |
|  | | Medicated feed (explain what medication will be used and how you will monitor) |
|  | | Housing in non-standard cage (i.e. Metabolic cages) |
|  | | Water restriction for longer than 12 hours (explain below) |
|  | | Food restriction for longer than 24 hours explain below) |
|  | | Prolonged exposure to high or low temperatures (explain below) |
|  | | Other. |
| **Explain here:** | | |

24. Is the project a non-clinical laboratory study evaluating the toxicity and/or the safety of a product, agent or device to be monitored by the Quality Assurance Unit in compliance with the FDA’s Good Laboratory Practice Regulations?

|  |  |
| --- | --- |
|  | Yes |
| X | No |

A. If yes, have you consulted a LAS representative?

|  |
| --- |
| **Explain here:** |

1. Will you be using controlled substances e.g. Ketamine, Pentobarbital, Buprenorphine etc. (**LAS Does Not Provide/Order Controlled Substances**)

|  |  |
| --- | --- |
|  | Yes. |
| X | No |

A. If yes, list the drugs being used and provide building and room number where drugs will be stored.

|  |
| --- |
| **Explain here:** |

26. The Animal Welfare Act requires that the principal investigator provide written assurance that any proposed research using animals is not unnecessarily duplicative of other research. Based on your professional knowledge of the literature, does this project unnecessarily duplicate previous research?

|  |  |
| --- | --- |
|  | Yes. |
| X | No |

A. If yes, provide justification for the need to duplicate previous work.

|  |
| --- |
| **Explain here:** |

27. Principal Investigator/Faculty Assurance

The information contained herein is accurate to the best of my knowledge. Procedures involving

animals will be carried out humanely and have been designed to assure that discomfort and pain to animals would be limited to that which is unavoidable. Project personnel have, or will receive, instruction and guidance on the proper handling and care of animals, aseptic surgical procedures, euthanasia, and the proper pre- and post- procedural use of anesthesia and analgesia used in this project. Check all that apply.

|  |  |
| --- | --- |
| X | I am experienced in conducting all the procedures on living animals covered in this protocol. |
|  | I am experienced in conducting some of the procedures on living animals covered in this protocol. |
|  | I am not experienced in conducting the procedures on living animals in this protocol. |

A. Describe the measures taken to gain experience on procedure(s) that you lack in experience and date of anticipated completion.

|  |
| --- |
| **Explain here:** |

28. Principal Investigator: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Signature Date

Note: Any modification to animal use procedures as described in this protocol cannot be implemented until IACUC approval is obtained.

**All word processors come with spell checkers. Use them!!!**

**FOR Veterinarian USE ONLY:** Check all that apply:

|  |  |
| --- | --- |
|  | Survival Surgery |
|  | Major Multiple Survival Surgery |
|  | Multiple Survival Surgery |
|  | Food/fluid restriction |
|  | Metabolic cages |
|  | Prolonged restraint |
|  | Ascites |
|  | Medication in feed |
|  | Use of Freund’s Complete Adjuvant |
|  | Medication in drinking water |
|  | Unavoidable pain/distress |
|  | BSL2 |
|  | Animals in PI labs >12 hours |
|  | IBC Approval |
|  | Chemical Safety Approval |
|  | Radiation Safety |
|  | Controlled Substances |

**USDA Pain/Distress Category: \_\_\_\_\_\_\_\_\_\_\_\_\_**

Veterinarian signature \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Date: \_\_\_\_\_\_\_\_\_

IACUC Official: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Date: \_\_\_\_\_\_\_\_\_\_

**Rev 6/2008**