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OMB APPROVED  
0579-0036

This report is required by law (7 U.S.C. 2143). Failure to report according to the regulations can result in an order to cease and desist and to be subject to penalties as provided for in Section 2150.

Interagency Report Control  
No. 0180-DOA-AN

Fiscal Year: 2009

UNITED STATES DEPARTMENT OF AGRICULTURE  
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

REGISTRATION NUMBER: 31-R-0027

Customer Number: 233

2. HEADQUARTERS RESEARCH FACILITY (Name and Address, as registered with USDA, include ZIP Code)

University Of Cincinnati Off Of Rsch  
51 Goodman Drive  
Po Box 210572  
Cincinnati, OH 45221  
Telephone: (513) 558 3024

NOV 30 2009

ANNUAL REPORT OF RESEARCH FACILITY  
(TYPE OR PRINT)

3. REPORTING FACILITY (List all locations where animals were housed or used in actual research, testing, teaching, or experimentation, or held for these purposes. Attach additional sheets if necessary.)

FACILITY LOCATIONS (Sites) See Attached Listing

See Attached Listing

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY (Attach additional sheets if necessary or use APHIS FORM 7023A.)

A.  Animals Covered By The Animal Welfare Regulations	B.  Number of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes.	C.  Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.	D.  Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.	E.  Number of animals upon which teaching, experiments, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. (An explanation of the procedures producing pain or distress on these animals and the reasons such drugs were not used must be attached to this report.)	F.  TOTAL NUMBER OF ANIMALS  (Cols. C + D + E)
4. Dogs		8	25		33
5. Cats					0
6. Guinea Pigs		14			14
7. Hamsters		8	148	83	239
8. Rabbits			100		100
9. Non-human Primates					0
10. Sheep			2		2
11. Pigs			90		90
12. Other Farm Animals					0
Goats			24		24
13. Other Animals					
Wild Mice		34			34

ASSURANCE STATEMENTS

- Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anesthetic, analgesic, and tranquilizing drugs, prior to, during, and following actual research, teaching, testing, surgery, or experimentation were followed by this research facility.
- Each principal investigator has considered alternatives to painful procedures.
- This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and approved by the Institutional Animal Care and Use Committee (IACUC). A summary of all such exceptions is attached to this annual report. In addition to identifying the IACUC approved exceptions, this summary includes a brief explanation of the exceptions, as well as the species and number of animals affected.
- The attending veterinarian for this research facility has appropriate authority to ensure the provisions of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL  
(Chief Executive Officer (C.E.O.) or Legally Responsible Institutional Official (L.O.))  
I certify that the above is true, correct, and complete (7 U.S.C. Section 2143).

DATE SIGNED

11/25/09

(b)(6), (b)(7)c

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**1. 83 hamsters used in the study.**

**Explanation of procedures:**

The research focuses on the behavioral, endocrine, and neurochemical changes produced by acute and chronic psychosocial stressors and nonsocial stressors. To study the effects of social stress on behavioral, endocrine and neuroendocrine parameters, the resident intruder paradigm will be utilized. On occasion, we will also determine the impact of nonsocial stressors on these same parameters as well (i.e., restraint, cold exposure and swimming).

Restraint: Animals are individually confined to a well-ventilated restraint cage for up to 120 min and then returned to their cages. For studies involving chronic stress animals may be exposed to restraint stress up to 120 min daily for a maximum period not extending beyond 4 weeks.

Cold Exposure: Animals will be placed in individual cages in a cold room (4 degrees C) for up to 2 hours daily for a maximum period not extending beyond 1 week.

Swim: Animals will be placed in a tank filled with tepid water (26-33 degrees C) for up to 15 min. The tank is filled to a depth not exceeding 9.5 in (to ensure that animals cannot support themselves without swimming or floating).

Social defeat protocol: Social defeat stress will involve the use of the resident/intruder paradigm. This paradigm has been used to investigate the effects of social stress on a number of behavioral and physiological parameters. This is common practice using this social defeat paradigm and avoids requirement of a large number of stimulus animals for experiments. For the social defeat stressor, an intruder hamster will be removed from its home cage and placed into the home cage of the resident hamster on four separate occasions (every third day). Defeat is defined as the display of 4 seconds of supine posture of the intruder and occurs within 5 minutes of introduction of the intruder into the resident cage. Each resident male will be used repeatedly to defeat different intruder hamsters. Repeated use of resident male hamsters is a common practice that is well established in the literature using this social defeat protocol and reduces unnecessary use of additional animals. In some instances, immediately after each defeat, the intruder hamster will be placed within a protective cage with wire mesh walls inside the larger resident cage and will be exposed to the resident's threats for up to 30 minutes. The protective cage employed will prevent injurious attacks, but will allow auditory, visual, and olfactory contact.

Decapitation without Anesthesia: Animals will be euthanized by decapitation so that blood & brain tissue may be rapidly removed and frozen for mRNA measurements and without influencing levels of stress-related hormones.

**Justification of the procedures:**

Restraint, Cold Exposure, Swim and Social Defeat Protocol: The current work aims to delineate the critical interaction between peripheral hormones and CNS systems involved in an animal's response to chronic psychosocial stress and its effects on food intake and body mass regulation. Additionally, changes in neuronal morphology, neurogenesis and/or neurodegeneration may contribute to changes in any or all of these systems. The execution of these experiments is critical to further our understanding of the CNS and endocrine systems involved in both the acute and long-term stress situations and in understanding how dysregulation of these systems might underlie certain psychiatric disorders such as depression, overeating and stress-induced obesity. In addition, these studies will further characterize the differences between the dominant and subordinate animals and document the plasticity of the animal's neuroendocrine and neurochemical systems as a consequence of chronic social stress. Further understanding of these systems is necessary before effective treatments for instances of dysregulation of these systems can be devised. Exploring such interactions in the control of behavior is simply not possible to do in isolated cell culture, tissue culture, or computer simulation systems. The need to make detailed measurements of in vivo alterations in CNS function and the need for carefully controlled genetic backgrounds make this work impractical in humans.

## Column E Explanation

Decapitation without anesthesia: It is necessary to collect fresh blood and brain tissue for some of the assays. Animals will be euthanized by decapitation so that blood & brain tissue may be rapidly removed and frozen for mRNA measurements and without influencing levels of stress-related hormones. There is a wealth of data that indicates that the stress axis responds with secretion of corticotropin releasing factor and ACTH within seconds following a stressor (some example references: (Cook et al., 1973; Engeland et al., 1980; Wilkinson et al., 1981; Walker et al., 1991). Changes in brain, which are the ultimate endpoint of our studies, precede the observed endocrine changes. Furthermore, anesthesia itself is a potent stressor (e.g., inhalants are potent activators of the stress axis) (Engeland et al., 1980; Wilkinson et al., 1981), as is hypercapnia (CO<sub>2</sub>) inhalation (e.g., (Marotta et al., 1976; Raff and Roarty, 1988)). Injectable anesthetics (e.g., barbiturates) take too long to achieve suitable depth of anesthesia; by the time animals are anesthetized, the animal has had ample opportunity to mount stress axis responses to handling and injection. Overall, anesthesia introduces a powerful confounding variable into our experimental analyses. Thus, while we understand the controversy regarding decapitation, we have no viable alternative for this euthanasia procedure.

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## Summary of Exceptions Approved by the IACUC

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### **1. Audiology Clinic**

#### Exception to sanitizable surface (AWA—9CFR3.11)

The satellite location is a carpeted room. This is absolutely critical to the service since the room acts as a sound booth similar to what humans are tested in. The enclosure must be as quiet and vibration free as possible to allow the equipment to give results. The equipment essentially produces an EEG based on the brain's and ear's response to auditory stimuli. The response of the brain and ear can be confounded by muscle movement and noise. The equipment is designed to average muscle movement to a large degree but not ambient noise. Reverberation and abnormal room noise confounds results or does not allow results to be given at all. This room is outfitted to provide the required quiet conditions that will not confound the tests.

We have special rubber mats that are placed over the carpet under and around the table that the dog is tested on. These mats and the table are disinfected and cleaned after each test and at the end of each day to preclude allergens. In addition, the carpets are cleaned nightly by janitorial staff and can be steam cleaned monthly.

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