

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

1. CERTIFICATE NUMBER: 14-R-0009
CUSTOMER NUMBER: 105

FORM APPROVED
OMB NO. 0579-0036

ANNUAL REPORT OF RESEARCH FACILITY
(TYPE OR PRINT)

Boston University Medical School
Director Laboratory Animal Science Center
Laboratory Animal Science
700 Albany Street, Rm W707
Boston, MA 02118

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

FACILITY LOCATIONS (Sites) - 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 animal 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 in these animals and the reasons such drugs were not used must be attached to this report)	F. TOTAL NUMBER OF ANIMALS (COLUMNS C + D + E)
4. Dogs					0
5. Cats			23		23
6. Guinea Pigs					0
7. Hamsters					0
8. Rabbits		15	11		26
9. Non-human Primates			40	7	47
10. Sheep					0
11. Pigs			38		38
12. Other Farm Animals					0
13. Other Animals					
Chinchillas			189		189

ASSURANCE STATEMENTS

- 1) 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.
- 2) Each principal investigator has considered alternatives to painful procedures.
- 3) 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.
- 4) The attending veterinarian for this research facility has appropriate authority to ensure the provision 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 or Legally Responsible Institutional Official)

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

DATE SIGNED

11/24/08

Column E Explanation

1. Registration Number: 14-R-0009

2. Number of animals used under Column E conditions in this study. Seven (7)

3. Species (common name) of animals used in this study. Rhesus monkeys

4. Explain the procedure producing pain and/or distress.

The proposed housing conditions in one protocol were determined by the IACUC to require the animals enrolled in this project be classified as USDA Pain Category E. Three exemptions from accepted standards of care for nonhuman primates, as described in the Guide for the Care and Use of Laboratory Animals (ILAR/NRC 1996) and the Animal Welfare Regulations, were approved as scientifically justified. The three points of exemption related to: illumination, socialization and environmental enrichment. These conditions were considered to be distressful to the animals and could not be relieved by the use of appropriate anesthetic, analgesic, or tranquilizing drugs. The IACUC approved these exemptions because it determined the conditions to be scientifically justified and the use of drugs would adversely affect the procedures, results, or interpretation of the research, experiments, surgery, or tests.

Exemptions:

- *Lighting.* This project involves housing animals in constant dim light (20 lux at cage top level) during constant condition parts of the study for a maximum of three separate periods which range from 6 to 30 weeks. During the remainder of the experimental period the animals are in environmentally controlled housing on a 12:12 hr light:dark cycle.
- *Socialization.* Throughout the entire protocol, the animals are housed in specially designed individual sleep/circadian chambers that contain an over-sized steel primate cage. The chambers provide controlled levels and timing of environmental illumination, low noise level and limited intra- and inter-species interactions. Limiting inter-specific and intra-specific interactions for periods up to 179 weeks, as well as exposure to facility noise to randomly scheduled times, ensures that each animal's behavior reflects endogenous processes rather than responsiveness to the events in their environment.
- *Environmental enrichment.* Isolation of the animals from each other is a critical part of the experimental design when studying circadian rhythms. Sources of enrichment that partially compensate for lack of social interactions that are used in the protocol include toys and mirrors in each cage and access to computerized performance exercises.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

The protocol is devoted to studying individual intrinsic circadian rhythms of behavior and physiology that is characteristic of a particular animal and differs from that of another one. Circadian body rhythms are defined as rhythms that persist in constant conditions with a cycle length of close-to-24 hours. The intrinsic circadian rhythms, defined by an animal's biological clock, located in its brain (suprachiasmatic nucleus of the hypothalamus, SCN), typically have a period of activity either shorter or longer than 24 hours. Thus, if not disturbed, they "free-run", i.e., display an intrinsic period, governing animal's physiology and behavior. These rhythms, however, can be altered by multiple environmental interferences, such as light or social environment. If a disrupting signal occurs with a period of 24 hours, the entire circadian system will adjust (entrain) to 24-hour day. This typically occurs in the majority of animals and humans, due to a 24-hour period of environmental light-dark cycle on our planet. If an environmental signal is administered randomly, it will increase day-to-day variability of circadian rhythm, requiring a longer evaluation of its true period. If the animals will be exposed to regular stimuli the data collected would reflect the circadian rhythm of those who interfere with the experiment, rather than those who are subject of that experiment. Any random signal will not entrain the animals. Each episode interfering with data collection would cut out a large piece of data from each day's recording, a piece much larger than the duration of the event itself. The only way to at least partially compensate for the data loss is to increase the number of observations. A paper describing the need for specialized housing conditions in studies of circadian rhythms is referenced (L.P. Morin (chair), Animal Issues Statement of the Society for Research on Biological Rhythms, *Journal of Biological Rhythms* 8: 97-106, 1993).

The light-dark cycle is the primary environmental stimulus for entrainment of circadian rhythms. Considering diurnal animals' well-being, it is very difficult to keep them in complete darkness. Constant dim light is typically used for both animal and human studies, allowing individuals to be fully oriented in their environment, while preserving intrinsic circadian body rhythm. In contrast, brighter light intensities can alter the circadian clock function (lengthening period) and at sufficiently high light intensities disrupt circadian rhythmicity, sleep and psychological well-being. Thus, exposing the animals to constant dim light and limiting their social interactions is necessary if one intends to study circadian rhythms.

Another important factor affecting the circadian studies is that animals' behaviors and physiological functions are significantly affected by other animals' or humans' behaviors, thus requiring social isolation. While studied under "entrained conditions", animals are exposed to some of the regular time cues, e.g., light: dark cycle. The protocol is addressing how each animal structures its behavior and physiologic functions throughout a 24-hour period and how these 24-h patterns change with age and in response to alterations in the circadian system. However, in order to reveal an individual pattern of specific physiological or behavioral function under entrained conditions, it is necessary to assure that whatever a specific animal is doing is not influenced by another animal. If one animal is eating, another animal would be likely to initiate food intake as well. If one animal is awake and running around, another animal would have a hard time falling asleep, even if it would like to. Since the protocol is specifically studying what this individual animal would do, and when, in contrast to another animal, it is necessary to minimize the effect of one animal's behavior on another. For this reason, in a study of circadian rhythms, social isolation of the animals from each other for significant periods of time is required.

Therefore, nonstandard lighting and socialization can not be relieved, nor can standard methods of environmental enrichment be provided, because of the requirement for constant dim lighting and isolation in circadian chambers which allow each animal to express and maintain individualized circadian pattern of activity, sleep and feeding, without interference from the facility noise or other monkeys. On each given day, animals eat or sleep, or do cognitive tests at different hours. Thus, experimental results can be based on the statistical analysis of a large data set that may reveal common patterns between many consecutive days of uninterrupted and undisturbed recordings in a statistically significant number of animals. These animals will be studied in both standard light-dark cycle and constant dim light conditions while being socially isolated and deprived of traditional environmental enrichment.

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