Attachment to 2013-14 APHIS FORM 7023, Annual Report of Research Facility, Nathan Kline Institute for Psychiatric Research, Orangeburg, NY 10962

Certificate # 21-R-0084 Customer # 448

For the reporting period of 10/01/13 – 09/30/14: With regard to the NKI-IACUC SOP for Social Housing of Primates as well as other regulations and guidelines noted below, the following species and number of animals along with a description of their use are listed:

a. **Five (5) rhesus monkeys (sp. Macaca mulatta) were used in controlled water access procedures and were at times single-housed in relation to those procedures as part of IACUC-approved studies.**
b. **Two (2) rhesus monkeys were single-housed as a result of social incompatibility.**
c. **Two (2) capuchin monkeys (sp. Cebus apella) were at times single-housed in relation to procedures specified in an IACUC-approved study.**
d. **Two (2) capuchin monkeys were single-housed as a result of social incompatibility.**

All other monkeys of both species were either pair-housed or small-colony-housed. The research animals cited in a. and c. above were also socially housed when not in the phase of their protocols that required temporary isolation.

Guidelines and regulations:

I. Animal Welfare Regulations, 2002 9CFR Ch.1. Subpart C. Research Facilities, 2.38 Miscellaneous, (f) Handling (2)(ii) Deprivation of food or water shall not be used to train , work or otherwise handle animals; Provided, however, That the short-term withholding of food or water from animals, when specified in an IACUC-approved activity that includes a description of monitoring procedures is allowed by these regulations.

II. Animal Welfare Regulations, 2002 9CFR Ch.1. Subpart D. Nonhuman Primates, 3.83 Watering. Potable water must be provided in sufficient quantity to every nonhuman primate housed at the facility. If potable water is not continually available to the nonhuman primates, it must be offered to them as often as necessary to ensure their health and wellbeing, but no less than twice daily for at least 1 hour each time, unless otherwise required by the attending veterinarian, or as required by the research proposal approved by the Committee at research facilities.

III. Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Research, Commission on Life Sciences, National Research Council, 8th edition, 2011.

Food and Fluid Regulation, pg 30: Regulation of food or fluid intake may be required for the conduct of some physiological, neuroscience, and behavioral research protocols. The regulation process may entail *scheduled access* to food or fluid sources, so an animal consumes as much as desired at regular intervals, or *restriction*, in which the total volume of food or fluid consumed is strictly monitored and controlled (NRC 2003b). The objective when these studies are being planned and executed should be to use the

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least restriction necessary to achieve the scientific objective while maintaining animal well-being.

The development of animal protocols that involve the use of food or fluid regulation requires the evaluation of three factors: the necessary level of regulation, potential adverse consequences of regulation, and methods for assessing the health and well-being of the animals (NRC 2003b). In addition, the following factors influence the amount of food or fluid restriction that can be safely used in a specific protocol: the species, strain, or stock, gender, and age of the animals; thermoregulatory demand; type of housing; time of feeding, nutritive value, and fiber content of the diet (Heiderstadt et al. 2000; Rowland 2007); and prior experimental manipulation. The degree of food or fluid restriction necessary for consistent behavioral performance is influenced by the difficulty of the task, the individual animal, the motivation required of the animal, and the effectiveness of animal training for a specific protocol-related task.

The animals should be closely monitored to ensure that food and fluid intake meets their nutritional needs (Toth and Gardiner 2000). Body weights should be recorded at least weekly and more often for animals requiring greater restrictions (NRC 2003b). Written records should be maintained for each animal to document daily food and fluid consumption, hydration status, and any behavioral and clinical changes used as criteria for temporary or permanent removal of an animal from a protocol (Morton 2000; NRC 2003b). In the case of conditioned-response research protocols, use of a highly preferred food or fluid as positive reinforcement, instead of restriction, is recommended. Caloric restriction, as a husbandry technique and means of weight control.

IV. Guidelines for the care and use of mammals in neuroscience and behavioral research / Committee on Guidelines for the Use of Animals in Neuroscience and Behavioral Research. National Academy of Sciences. 2003.

FOOD AND FLUID REGULATION: Neuroscience-related protocols occasionally require the regulation of animals' food or fluid intake to achieve a specific experimental goal. The regulation process may entail *scheduling* of access to food or fluid sources so an animal consumes as much as desired at regular intervals, or *restriction*, in which the total volume of food or fluid consumed is strictly monitored and controlled. As stated in the *Guide*, "the least restriction that will achieve the scientific objective should be used" (p. 12). Regulation of food or fluid is commonly used as motivation in experiments that require animals to perform a behavioral task with a high degree of repeatability (Toth and Gardiner, 2000), but the food or fluid consumption is not the experimental variable. In those studies, food and fluid regulation is used to motivate the animals to perform a specific behavioral task for a food or fluid reward; regulation of food or fluid outside the experimental session ensures response reliability to the food and fluid reward in each session (NIH, 2002). That allows the investigator to elicit and monitor the same movement repeatedly, to present the sensory stimuli under highly controlled conditions, and to obtain physiologic discriminations from the animal. For example, water-regulated monkeys may be trained to press a button for a juice reward, while the investigator measures the effect on neuronal firing rates. In conditioned-response experiments, (for example, a monkey may be conditioned to associate a light with a fluid reward), consideration should be given to whether the use of highly preferred food or fluid as positive reinforcement can be used instead of restriction. Fluid reward is preferable to food reward in some types of experiments. For example, studies that monitor neuronal activity in the brain may require the minimization of jaw or head movement to avoid

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displacing a microelectrode from its position. Because fluid rewards can be delivered through a tube positioned near the animal's mouth and tongue, they offer a particular advantage: licking and swallowing a fluid reward are much less disruptive to the neuronal recordings than chewing or crunching movements of the teeth or jaws that accompany the consumption of food rewards (NIH, 2002). Fluids offer additional experimental advantages. They can be easily delivered in small quantities, maximizing the number of trials that can be executed before satiation of the animal. In contrast with food rewards that require chewing before swallowing, fluids are quickly consumed, reducing the inter trial interval an important advantage when an animal must perform a behavior hundreds or even thousands of times in an experimental sessionto allow for statistical analysis.

Determination of Minimum Fluid Consumption

Assessing the ad libitum fluid consumption for each fluid-regulated experimental animal might be an important step in ensuring the health and well-being of the animal. However, voluntary fluid-consumption levels in a laboratory setting might not be equivalent to the animal's minimal fluid-requirement levels. Limited availability of fluid is a common determinant of consumption in natural settings, and physiologic and behavioral mechanisms have evolved to enable animals to adapt to the limitation. For example, rats and monkeys quickly learn to consume much, if not all, of their daily fluid needs in a short, restricted period (reviewed by Evans, 1990). Species that drink from watering sites only once per day invoke homeostatic mechanisms to control urine output in relation to their hydration state (Toth and Gardiner, 2000). Mammals may also use torpor to adapt to the dry season in their natural habitat (Schmid and Speakman, 2000). Thus, it is difficult to designate specific minimum fluid needs, because requirements may vary with species, strain, environment, efficiency of fluid-saving mechanisms, and so on. IACUCs, veterinarians, and researchers should take into account the possibility that laboratory animals can be adequately physiologically sustained with less fluid than they would voluntarily consume.

At the start of a new research protocol involving restricted or altered access to fluid, the amount of fluid consumed, body weight, and a hydration assessment should be recorded daily for each animal, as individual animals may manifest physiologic and behavior differences. Those data will help in refining the protocol and evaluating the adequacy of access to fluid. In evaluating the adequacy of access to fluid, each animal should be evaluated individually to determine how it is adapting to the imposition of restricted or altered access. For example, if an animal attains and then maintains a new body weight, it could suggest successful adaptation even if the new weight is below the weight recorded during ad libitum access to fluid.

Fluid-Regulation Design

When fluid regulation is selected as a behavioral motivator, access to fluid outside the experimental setting has to be regulated to motivate performance of the rewarded behavior (NIH, 2002). Generally, fluid regulation is patterned after one of two designs. In "fluid restriction," animals are given access to a metered volume of fluid per day and may consume that volume over any length of time. In "fluid scheduling," the experimenter determines the time of day during which the animal has access to fluid, but the duration of drinking and the volume consumed are determined by the behavior of the animal. For example, in many behavioral protocols, animals are given continual access to fluid for as

long as they continue to perform a task. Because food and fluid generally are not freely available in the wild and some effort (foraging) is required to obtain them (NIH, 2002), such scheduling designs may model the effort expenditure necessary to obtain food and fluid in the wild For both types of fluid regulation, animals generally should be given free access to fluid for some period on days when experimental sessions are not scheduled, unless scientifically justifiable reasons preclude such fluid supplementation (NIH, 2002).

When developing a restriction design to motivate an animal to perform a task, the main consideration is determining what level of restriction is necessary to achieve the desired performance. Generally, the more complicated the task, the more stringent the restriction protocol needs to be. For example, in a study of water-restricted rats, where the rats were required to bar press to obtain their daily allotment of water (Collier and Levitsky, 1967), mild restriction (rats receive 75% of their average ad libitum intake) resulted in poor performance while a more stringent restriction (rats receive 32% of their average ad libitum intake) resulted in maximal performance. This is why fluid restriction levels used in one study may not provide adequate motivation for learning or performing other more demanding tasks (Toth and Gardiner, 2000). However, the most severe restriction is not always necessary for achieving maximal performance. In this same study (Collier and Levitsky, 1967), bar-press rates were similar when water was restricted to 32%, 42%, or 56% of average ad libitum intake.

Species- and Strain-Specific Considerations

Other Influences on Fluid Homeostasis In some situations, fluid reinforcers (such as fruit juice) are used because they may maintain behavioral performance when access to fluid is restricted; for example, some monkeys prefer fruit juice when performing long behavioral sessionsin which many reinforcements are delivered (NIH, 2002). Investigators, veterinary personnel, and IACUCs should consider and monitor for any potential physiologic ramifications of total substitution of solute-containing fluids for water in a fluid-restricted protocol. Sweetened milk or juices may be unfavorable choices for use in a long-term study in which an animal will participate for many months or years, because of the potential for dental caries (NIH, 2002). Provision of treats, such as fruits or vegetables, is recommended when appropriate to provide variety and nutritional balance to an experimental animal's diet (NRC, 1996). The water content of these dietary supplements can be difficult to estimate, so their potential contribution to hydration should not be considered in determining the minimal ration of fluids to be given to the animal (see Pennington et al., 1998, for data on water content of fruit and vegetable supplements). However, investigators, veterinary personnel, and caretakers should be aware of the potential need for restriction or substitution of supplemental food items in fluid-regulated animals.

NKI IACUC SOP SOCIAL HOUSING OF PRIMATES update 11/6/12.

The default husbandry environment for primates at NKI is paired or group housing. However there are specific cases when primates need to be single housed. The following are examples of such cases:

1. During training when water consumption required to maintain health is quantified and during testing when a specific amount is allocated for a specific individual.
2. During post-operative and post-procedure periods or for veterinary care when the presence of another individual(s) may impair recovery or health and well being.

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3. Individuals found to be incompatible or aggressive toward other individuals such that injuries may result from co-habitation and this may result in the necessity of removing animals from an ongoing study undermining the integrity of the study.

4. Placement of opposite sexes in co-habitation that may result in pregnancy and neccessitate the removal of animals from an ongoing study undermining the integrity of the study.

5. During the course of the study for administration of experimental substances, sampling, or observations that are required to aquire necessary experimental data.