



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institute of Health  
National Institute of Mental Health  
Intramural Research Program  
10 Center Drive MSC 1381  
Bldg. 10, Room Room #  
Bethesda, MD 20892-1381

**Date:** October 3, 2014

*MS*

**Through:** Constantine Stratakis, M.D., D.Sc.  
Scientific Director, NICHD

**To:** Michael M. Gottesman, M.D.  
Deputy Director for Intramural Research, NIH

**From:** Karl Pfeifer, Ph.D. *KP*  
Chair, NICHDH Animal Care & Use Committee

**Subject:** Animal Welfare Investigation – Animal Welfare Assurance A4149-01 [Case 9Y]

The following comments are provided in response to the NIH Office of Laboratory Animal Welfare's questions outlined in their September 9, 2014 memorandum relating to Animal Welfare Investigation-Case 9Y. The behavioral tests in question were conducted by the late Dr. James T. Winslow, Director, Nonhuman Primate Core, National Institute of Mental Health (NIMH), in collaboration with Dr. Stephen Suomi, National Institute of Child Health and Human Development (NICHD). Other procedures referred to in the news report or shown on the video footage that are performed by NICHD personnel include maternal-infant separation and subsequent nursery rearing, neonatal assessment (handedness test), and CSF taps. This NICHD response will primarily address these procedures where our program has oversight of the Dr. Suomi's studies, the LCE nursery and the care of the infant monkeys.

All research procedures in this study were approved by the NIMH ACUC; the animals used belonged to the NICHD lab of Dr. Suomi, the Lab of Comparative Ethology (LCE). Dr. Suomi informed his ACUC of all work conducted, including the collaborative work. His approved ASP included a description of all procedures conducted including those conducted under collaboration. Many of the questions raised center around three (3) tests conducted by the NIMH Nonhuman Primate Core that were designed to evaluate differences in the behavior and temperament of infant macaques. These studies included: a) Human "Intruder" Paradigm (HIP), b) Human "Intruder" Startle Test (HIS), and c) a Novel Objects Test. These specific tests are addressed fully in the NIMH response.

- 1) *Provide an explanation of how discomfort, distress and pain was avoided or minimized, consistent with sound scientific practices and research design. As noted in the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training, "Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals."*

The procedures in question were not designed to induce discomfort, distress, or pain; but rather to develop the research paradigm or assess the individual animal. How the potential for discomfort, distress, and pain were avoided or minimized each of these procedures is detailed below.

a. Maternal-infant separation and subsequent nursery rearing:

- i. Mother and infant are separated as soon after birth as possible, always 72 hours.
- ii. Infants that are hand reared in the nursery receive human contact multiple times each day, including being held in a species appropriate manner. In addition they receive indirect human interaction during routine husbandry duties
- iii. Surrogate design mimics normal nursing posture and clinging behavior of rhesus infants. Fleece surrogate allow the infant to cling onto the substrate as they would with a live mother. The infant clings on upright and nurses from the attached bottle in the same position as if they were nursing from a mother in the animal runs. Surrogates are made from commercially manufactured ferret sacks which are hung from the side creating a pocket. This gives infants the choice to climb inside or cling to the outside of the sack.
- iv. Socialization occurs in both nursery groups. Peer reared infants are housed with other infants in the same cage. Surrogate reared infants have the ability to touch cohorts in adjacent cages at all times and are given time in a play cage with other cohort members for approximately two hours a day. All infants have constant visual, olfactory, auditory and tactile contact with other infants in the nursery.
- v. All infant cages have an enriched environment where novel items and manipulanda are rotated regularly, providing opportunities for exploratory behavior and stimulation. The additional stimulation allows for normal cognitive development and decreases stress. Natural foraging opportunities are provided when the youngest infant in a rearing group reaches 2 months of age. Foraging items rotate daily between grains, cracked corn, millet, sunflower seeds and trail mix. Afternoon snacks are provided which include peanuts, sweet feed, apples, bananas, popcorn, primatreats, oranges, grapes. On "Fun Snack Friday" infants are introduced to novel fruits and other

healthy food items. The goal of the enrichment plan is to provide novel, interesting and rotating feed items to developing infants.

vi. Behavioral assessments are performed on all non-human primates including nursery infants. These assessments continue through juvenile and adult. Nursery reared infants exhibit different behaviors but do not appear distressed; they are capable of adapting to normal daily stressors later in life.

b. Neonatal assessments:

These are adapted from human infant assessments including handedness test, imitation tests and the Brazelton battery of tests, depending on the age of the infant. They are performed on awake human newborns to assess whether the infant has normal responses and no neural deficits. They are not considered to cause pain or distress in human infants. These assessments are performed at different ages, as detailed in the ASP, from within one day of birth to post weaning age. The infant is swaddled in an absorbent pad and held by a human caregiver during this procedure. "Swaddling" involves holding and wrapping the infant as is done with human infants using a blanket and is comforting to the infant. The infant is returned to the nursery or its mother as appropriate to its cohort group as soon as possible after the assessment. When infants reach 2 months of age they will often receive a special food treat after completing cognitive testing.

The test shown in the news report where the swaddled infant is being held by a lab staff member is the handedness test, this test is to determine if the animal appears to be left handed or right handed.

c. CSF taps:

CSF taps are performed in humans as an awake procedure. On this study the rhesus are sedated with Ketamine for the safety of the human researchers and the animals themselves. The animals are given a dose of ketoprofen as CSF taps in humans are reported to occasionally cause a headache in the patient. Animals are monitored during recovery and assessed after for any sign of complications.

2) *Provide information on any procedures or circumstances that may result in more than momentary discomfort, distress, pain or injury and describe the methods used to alleviate this.*

None of the procedures in question were designed to induce discomfort, distress, or pain; but rather to develop the research paradigm or assess the individual animal's development. None of the procedures in question induce more than momentary discomfort, distress or pain. As currently approved none of the research procedures on the NICHD ASP are considered to be more than

momentarily painful or distressful. In response to PETA's allegations, the NICHD ACUC is reopening this question and will provide an additional response after their October meeting.

Procedures specified in the news report:

The restraint cage was used in NIMH studies and is addressed in their response. In NICHD studies, sedation of the mother is required so that the researchers can safely remove the infants for the behavioral studies described in the ASP. This occurs at days 14 and 30 (Brazelton battery with CSF and blood draws), at 2 months (blood draw), and around 4 months (behavioral bioassessment). After sedation with ketamine, the infant remains with the mother for the minimum time required for human safety which is typically around 5-10 minutes until the mother is fully sedated.

- 3) *Provide information on the steps which were taken to ensure that the use of stressors was the minimum to obtain valid results. Provide information on the timelines, habituation, mitigation or supportive actions taken to reduce stress to the minimum. Specifically address the length of the stress/fear inducing procedures involving the small restraint cage and the length of time the baby was on the sedated mother.*

The procedures discussed in this report are not considered to be more than brief, transient stressors. The two procedures specified in the directive above are addressed in the NIMH response

- 4) *Provide justification as to why alternatives to animals could not be used and indicate the potential benefits and knowledge to be gained*

The studies in question were designed to investigate the influence of the mother-infant bond on the behavior, temperament, and social competence of the infant. The numerous physiological, biochemical, genetic, psychopathological, neurological, social, and environmental variables related to behavior necessitates the use of an animal model. In addition, the requirements to control, minimize, or evaluate various nondependent variables (i.e. experiential, genetic, environmental, etc.) makes controlled human studies unfeasible. These scientific justifications are provided by the Principal Investigator and certified by his supervisor who confirms that the study has undergone peer review as part of the quadrennial review of the LCE research program organized by the NICHD Board of Scientific Counselors.

- 5) *Indicate the steps taken to replace, reduce, or refine the use of animal.*

There are no suitable alternatives to the use of animals that would meet the experimental goals outlined for this study. The number of animals used was reduced to the minimum required to obtain statistical significance. In addition, by working in collaboration with the NIMH to share animals and develop methodologies which would support the mission of both institutes, the number of animals required was further reduced.

Current practices regarding nursery and mother reared infants – breeding and birthing season are synchronized via use of non-invasive birth control methods. Infants are born during a limited window of time to ensure that they are raised as a cohort group. This allows the statistically necessary number of infants to be available during a specific time window, without having excess animals born throughout the year.

Additionally infants who are abandoned or neglected by their mothers in the mother-reared setting are brought into the nursery and raised by the human care providers. On the rare occasions when this occurs it saves the life of that infant and allows for one less human induced separation. In the wild, infants are often abandoned or neglected, especially by primiparous mothers, the infants in fully natural settings do not survive.

- 6) *Provide the rationale for the age and choice of species used. The rationale should indicate the advantages of the species chosen and why alternative species are not appropriate. If less highly evolved or simpler animal models are available, provide the justification for using more advanced species.*

Although numerous mammalian and non-mammalian species display clear-cut individual differences in response to environmental challenges, nonhuman primates provide the most compelling models of human phenomena in terms of (a) magnitude of genetic overlap, (b) homology of relevant physiological systems, (c) similarity of behavioral responses to both social and nonsocial environmental stressors, and (d) highly parallel patterns and sequences of social and emotional developmental processes. Among the nonhuman primates, rhesus monkeys (*Macaca mulatta*) are arguably the most preferred species because there already exists an extensive empirical background regarding biobehavioral responses to environmental challenges across a wide range of laboratory and field settings. The complete genome for rhesus monkeys has been sequenced, facilitating studies of specific gene interactions. No comparable data base exists for any other nonhuman primate species. Moreover, the LCE possesses a unique colony of rhesus monkeys, including appropriate breeders, for whom genetic pedigrees (including specific genetic polymorphisms) and characteristic responses to environmental challenge have already been established. It would take years, if not decades, to establish a comparable pool of subjects in another nonhuman primate species.

- 7) *Provide the description of the living conditions of the young nonhuman primates which are appropriate for their species and contribute to their health and comfort.*

All NHPs assigned to either the NICHD ASP in question, whether mother reared in group-housed runs or nursery reared are housed within standards of the Guide to the Care and Use of Laboratory Animals as per temperature range, light cycle, humidity and space requirements.

All infants in the Lab of Comparative Ethology (LCE) nursery receive daily care and handling by nursery staff. Peer reared infants are co-housed together in groups of four infants. Singly reared infants are in the same room and have constant visual, olfactory, tactile and auditory contact with other infants. Singly reared infants also receive about two hours a day in a group play cage with other singly reared cohorts.

All infants in the LCE nursery receive a variety of enrichment toys and manipulanda which are frequently changed in order to provide a more stimulating environment. Ample cage space allows the infants to climb around freely.

The cage environment includes a plush fleece “surrogate” to which they can hold onto, which mimics the way they would hold onto their mother. Infants can also climb into the surrogate pocket should they choose to do so. This surrogate and placement of a self-feeding bottle allows normal species positioning during nursing and resting. Mother reared infants are raised in group runs typically of one male, several females and their offspring. They have constant interaction with their peers and the older monkeys. The groups in these runs have a well-developed enrichment program, a view of the outdoors, and the ability to come and go from indoor to outdoor runs.

All NHPs in the NICHD program have semiannual behavioral assessments at a minimum to detect distress and behavioral abnormalities. If these are noted, the facility veterinarian, the research staff and the enrichment technician work together to address specific issues and needs of that animal.

- 8) *Provide a brief synopsis of the qualifications and training of the individuals directly involved in the conduct of procedures and handling of the primates.*

The primate nursery manager has over seven years of experience working with non-human primates. All nursery staff receive training specific to infant care, including appropriate handling, daily feeding and weighing, behavioral assessment and emergency/critical of neonatal rhesus before they are allow to work unsupervised in the nursery.

Prior to working with animals, all personnel conducting research under an animal study protocol are required to complete the NIH training courses entitled “Using animals in Intramural Research: Guidelines for Animal Users” and “Working Safely with Nonhuman Primates”. These courses include information on the legal requirements of all personnel working with animals in research,

recognition of nonhuman primate behaviors, and procedures for avoiding and treating bites, scratches and exposures to nonhuman primate body fluids. Personnel were further trained by the principal investigator and veterinary staff on: a) the experimental and behavioral procedures to be conducted; b) the humane and safe handling of nonhuman primates; c) the identification of species specific signs of pain and/or distress; and d) methodologies to avoid or minimize distress.

Before performing CSF taps, a staff member would undergo a lengthy process of observing the procedure and then performing the task under supervision before being certified by the lab supervisor as proficient. The Facility Veterinarian can also perform CSF taps if needed.

The Facility Veterinarian has oversight of the care, health and welfare of all the NHP at the NICHD Shared Facility, including the nursery. The Facility Veterinarian has over 23 of NHP veterinary experience and has worked with non-human primates for over thirty years.

- 9) *Provide any additional salient information regarding measures taken to ensure the humane treatment of the baby primates used in the conduct of these studies.*

Specific to the maternally separated infants raised in the Lab of Comparative Ethology (LCE) nursery:

Infants are separated as soon as possible after birth. This practice is done to prevent development of mother-infant bonding and thus decreases the amount of stress at separation, particularly of the mother. The maternal-infant bond is thought to develop due to oxytocin release secondary to the infant nursing on the mother and is strengthened with time.

The facility enrichment program and the research enrichment program pay particular attention to animals which meet the criteria as requiring “special considerations”. As outlined in the Animal Welfare Act section 3.81 “Environmental enhancement to promote psychological well-being” infants and juveniles should be provided with special attention and an enhanced environment to promote their psychological well-being. Infants are provided with a complex variable rotating manipulanda experience, are provided with daily social contact with conspecifics, receive positive human interactions and species specific foraging opportunities.