AN INTERNATIONAL ORGANIZATION DEDICATED TO PROTECTING THE RIGHTS OF ALL ANIMALS

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Via e-mail: Betty.J.Goldentyer@usda.gov

Dear Dr. Goldentyer,

I hope this correspondence finds you well. I am writing on behalf of People for the Ethical Treatment of Animals (PETA) and our more than 6.5 million members and supporters to respectfully request that the U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) investigate possible violations of the Animal Welfare Act (AWA) related to the use and treatment of monkeys in a laboratory at a National Institute of Mental Health (NIMH) Intramural Research Program (IRP) within the National Institutes of Health (NIH; Certificate No. 51-F-0016) in Bethesda, Maryland.

In response to several Freedom of Information Act requests, PETA received 43 hours of video footage and dozens of pages of documents from NIMH related to experiments carried out by Principal Investigator Elisabeth A. Murray on rhesus macaques. A review of these documents—including the detailed procedures described in Murray's protocol (Animal Study Protocol [ASP] # LN-20), "The Neural Substrates of Sensory Memory, Reward, and Emotion"—reveals treatment of animals that we believe constitutes violations of Animal Welfare Regulations (AWRs), including:

1. Failure on the part of NIMH's Animal Care and Use Committee (ACUC) to ensure that animals would not be used in more than one major operative surgery from which they were allowed to recover [9 C.F.R. §2.31(d)(1)(x)];
2. Failure to report the use of animals in the appropriate USDA category for pain and distress [9 C.F.R. §2.36];
3. Failure on the part of NIMH's ACUC to ensure that Murray had considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animals [9 C.F.R. §2.31(d)(1)(ii)];
4. Failure to ensure provision of adequate veterinary care to animals [9 C.F.R. §2.33(a)];
5. Failure to ensure that the attending veterinarian has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use [9 C.F.R. §2.33(a)(3)]; and
6. Failure to adequately address social grouping for nonhuman primates in an effort to promote their psychological well-being [9 C.F.R. §3.81(a)].

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I. Failure to ensure that animals would not be used in more than one major operative survival surgery

Section 2.31(d)(1)(x) of the AWRs stipulates that in its review of "proposed activities related to the care and use of animals," the Institutional Animal Care and Use Committee (IACUC) must ensure that "no animal will be use in more than one major operative procedure from which [he or she] is allowed to recover."

However, according to ASP # LN-20, the rhesus macaques used in PI Murray's experiments are subjected to three or more craniotomies, where the skin and muscles of the head are cut into, a portion of the skull is removed, and incisions are made into the dura, or the connective tissue that surrounds the brain beneath the skull. Some monkeys also have head posts surgically affixed to the tops of their skulls; and some monkeys have chambers cut into their skulls. These multiple major operative procedures are described below:

1. Excitotoxic lesion surgeries: The monkeys are subjected to two or more craniotomies and are then given intracranial injections of excitotoxins to cause permanent brain damage to a region of interest. The injection of excitotoxins can cause tachycardia (rapid heart rate) or respiratory arrest which may take 30 minutes to five hours to resolve. These conditions place severe stress on the body's immune system, internal organs, and normal physiological function. Hippocampal lesions require two craniotomies, and monkeys included in the laboratories "disconnection" experiments undergo two or three separate invasive surgeries to lesion different parts of their brain in stages. Additional surgeries are sometimes required to repair misplaced or incomplete lesions.
2. Head post placement surgeries: Some of the already-lesioned monkeys are subjected to an additional major operative surgery in which head posts are surgically affixed to the tops of their skulls. It takes up to four weeks for the monkeys to heal just from this surgery alone, and some of them end up living with these posts attached to their skulls for years. The dental acrylic used to affix these posts make the monkeys extremely vulnerable to discomfort, infection and inflammation, as well as bone and skin degradation.
3. Chamber placement surgeries: After recovering from head post surgeries, many of these monkeys undergo yet another major operative surgery, in which holes are cut and chambers are placed into their skulls to allow experimenters to inject pharmaceutical compounds directly into their brains. For these procedures, the experimenter uses a number of non-pharmaceutical grade drugs. The doses used in the systemic injections may be toxic and may cause the animals distress. In some instances, if acceptable medical treatments and/or procedures are not effective, the monkeys will be euthanized. During some of these surgeries, experimenters accidentally hit a blood vessel resulting in cerebral hemorrhage, infarctions and raised intracranial pressure. Additional surgeries are sometimes required to remove bone that has grown into the chambers.
4. Tracer studies: Prior to euthanasia, monkeys are subjected to one or more additional craniotomies to allow for tracer injections.

Section 2.31(d)(1)(x) of the AWRs identifies a number of exceptions to the prohibition on multiple major survival surgeries, including justification based on "scientific reasons by the principal investigator." However, the "justification" provided by Murray in ASP # LN-20 for these experiments is specific to the "disconnection lesion surgeries" and simply states that it is "widely accepted that this is the only way to determine whether a given function, in this case, a specific kind

of sensory memory, is dependent upon the integrity of the anatomical connections between certain specified brain structures." However, as described in the attached report, this is inaccurate—there are numerous tools available to study the import of individual brain regions and/or their anatomical connections in sensory memory behaviors. Additionally, no scientific justification was provided in ASP # LN-20 for subjecting individual monkeys to excitotoxic lesions, head posts, and chambers.

Using monkeys in more than one major survival surgery, even with "justification," isn't only a question of whether Murray and NIMH's ACUC complied with federal regulation. The cumulative harms inflicted by Murray on individual monkeys, as described in Murray's own protocol, are so extreme that it's quite likely that monkeys are experiencing significant morbidity and mortality during the surgical procedures and post-surgically. This would mean that *by design* and with the approval of NIMH's ACUC, "discomfort, distress, and pain to the animals" was not minimized, as is required by Section 2.31(d)(1)(i) of the AWRs.

II. Failure to report animal use in the appropriate USDA category for pain and distress

Section 2.36 of the AWRs stipulates that research facilities must submit an annual report to the USDA, stating "the common names and the numbers of animals upon which experiments, teaching, research, surgery, or tests were conducted" and classifying the USDA pain and distress category for the procedures in which the animals were used.

The rhesus macaques used in Murray's protocol were reported in NIH's Annual Report under Category D, that is, "procedures which would involve more than slight or momentary accompanying pain or distress, and for which appropriate anesthetic, analgesic, or tranquilizing drugs, were used." However, a veterinary assessment of this protocol suggests that the manipulations to which the macaques are subjected in Murray's protocol would produce significant unrelieved pain and distress.

The multiple invasive surgeries described in the earlier section cause physical and psychological stress, immune system suppression, and may impair spatial memory and cause cognitive decline. Brain surgery causes high levels of both acute and chronic pain. The skin and muscles of the head and scalp are extensively enervated with pain-transmitting nerves, as is the dura. Tissue injury and nerve entrapment, compression, transection, or other damage in the scalp, cranial muscles, and dura lead to extensive pain following the surgeries. Additionally, the permanent brain damage inflicted in these animals causes myriad negative behavioral outcomes, including impaired emotional responsivity, aberrant social interactions, altered response to fearful and threatening stimuli, and impaired reward processing.

Monkeys in this laboratory are subjected to multiple painful intramuscular (IM) injections that can cause bruising, swelling, and impaired movement. Some monkeys will receive painful IM injections of neurotransmitter receptor agonists and antagonists, which can cause dyskinesia (uncontrolled muscle twitching), sedation, and agitation.

For training and behavioral testing, the monkeys in this laboratory are fitted with a metal or hard plastic collar and strapped into a restraint chair that keeps their head, arms, and/or legs immobilized. In some behavioral experiments, monkeys' autonomic responses (pupil responses, heart rate, blood pressure) are recorded. This requires the monkeys' arms to be tied behind their backs and their heads completely immobilized via the implanted head post. Monkeys are subjected to this type of restraint for hours at a time, as many as five days a week.

For structural neuroimaging, monkeys in this laboratory are sedated several times a month, requiring repeated fasting and prolonged restraint, and resulting in post-anesthetic malaise. For functional neuroimaging experiments they are also required to lie awake, with their bodies and heads completely immobilized, inside a magnetic resonance imaging (MRI) scanner, in some cases for up to five hours at a time. Some monkeys receive injections of a substance containing iron for fMRI studies. In order to prevent toxicity due to high levels of iron, these monkeys also receive IM injections of iron chelators. Side effects include pain and swelling at injection site, itching, redness, hearing impairment, and blurred vision. They may receive these injections three to four times per week while undergoing fMRI studies.

To improve the monkeys' willingness to repeatedly perform behavioral tests, experimenters often restrict their food and water intake. In one behavioral paradigm, to get the monkeys to cooperate, the experimenters withhold food and water until they perform the required task, then provide them with the entire daily food ration at one time. The biscuits are presented in "mash" form to both increase the ease of consumption and to restrict access to water. This so-called "lunch box" procedure forces the monkeys to "earn" their entire daily allotment of fluid and food while "working" in the apparatus. This requires that the monkeys consume their full day's nourishment within a 15-minute window of time. In addition to the acute gastrointestinal dilation that would likely occur with this quick devouring of a large quantity of food, causing pain and discomfort in the monkeys, this practice would also cause psychological distress in the monkeys since they would access to food for only 15 minutes during a 24-hour period.

Given the extensive catalogue of invasive, painful, and distressing procedures carried out on the macaques in Murray's laboratory, the suggestion that the complete universe of pain and distress suffered by the monkeys—which is frankly, overwhelming and unimaginable—is preposterous. The rhesus macaques used in PI Murray's experiments should be reported as Category E experiments, reflecting their unrelieved pain and distress.

III. Failure to consider alternatives to painful procedures

Section 2.31(d)(1)(ii) of the AWRs stipulates that in its review of "proposed activities related to the care and use of animals," the IACUC must ensure that the principal investigator has "considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animals."

However, the animal study proposal for these experiments indicates that the experimenters failed to conduct an adequate search for alternative procedures. When searching on the PubMed database, the investigators used the term "primate" in each individual search, eliminating the possibility of discovering human-based research methodologies. In other searches, the investigators employed databases dedicated to primate experimentation. As described in the attached report, there are numerous non-animal alternatives available for these experimenters to investigate their research questions. Had the experimenters chosen proper search terms and databases these alternatives would have revealed themselves, and hundreds of monkeys could have been spared extensive suffering.

Also, it is unclear from Murray's protocol whether any consideration was given to alternatives to dental acrylic/cement to facilitate attachment of the head post. As noted earlier, these materials are more likely to fail and are known to cause irritation and infection for the monkeys. European neuroscientists and some experimenters at the University of Pennsylvania have done away with such materials for these reasons. Instead they are refining their techniques using 3D scans of the skulls to

fabricate precisely fitted attachments. These refinements also mean that the monkeys are less likely to undergo 'repair' surgeries. It is unclear whether Murray conducted a search for alternatives to the use of dental acrylic/cement to affix head posts in the monkeys, and it is unclear whether the NIMH ACUC requested that such a search be carried out. It is amply clear that by continuing to use an antiquated method of implanting head posts, Murray failed to minimize discomfort, distress, and pain to the animals—and the ACUC failed to ensure that her protocol complied with federal animal welfare regulations in this regard.

IV. Failure to establish and maintain a program of adequate veterinary care

Section 2.33(a) of the AWRs stipulates that "[e]ach research facility shall have an attending veterinarian who shall provide adequate veterinary care to its animals." Policy 3 of the USDA's *Animal Care Policies* expounds on this directive as it relates to the use of pharmaceutical-grade compounds in research. In particular, the policy states:

> Investigators are expected to use pharmaceutical-grade medications whenever they are available, even in acute procedures. Non-pharmaceutical-grade chemical compounds should only be used in regulated animals after specific review and approval by the IACUC, for reasons such as scientific necessity or non-availability of an acceptable veterinary or human pharmaceutical-grade product. Cost savings is not a justification for using non-pharmaceutical-grade compounds in regulated animals.

However, Murray reports the use of non-pharmaceutical-grade drugs on her study, including GDR 12909 and nomifensine. Murray admits that the doses used in the systemic injections of these non-pharmaceutical-grade compounds may be toxic. It is unclear from the protocol whether the ACUC considered key issues in allowing Murray to use non-pharmaceutical-grade drugs in her study, including the level of pain and distress suffered by the monkeys injected with the compounds; whether purity differences between pharmaceutical-grade and non-pharmaceutical-grade compounds would result in toxic and adverse effects, and possibly, an increase in pain and distress.

V. Failure to ensure that the attending veterinarian has appropriate authority

Section 2.33(a)(2) of the AWRs stipulates that the research facility must ensure "that the attending veterinarian has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use."

However, the animal study proposal for these experiments specifies that the veterinarian must consult "with the investigator" before "an animal [who] is experiencing distress that cannot be relieved by applying acceptable medical treatments and procedures" can be euthanized. This deference to the investigator undermines the authority of the veterinarian and opens the door to the possibility that the investigator's desire for experimental data will trump the imperative to prioritize the animal's welfare.

VI. Failure to promote psychological well-being of nonhuman primates

Section 3.81 of the AWRs stipulates that "research facilities must develop, document, and follow an appropriate plan for environment enhancement adequate to promote the psychological well-being of nonhuman primates." Section 3.81(a) of the AWRs addresses the issue of social grouping, stating:

"The environment enhancement plan must include specific provisions to address the social needs of nonhuman primates of species known to exist in social groups in nature."

Rhesus macaques in the wild live in multi-male, multi-female groups within a profoundly social environment. However, the brain lesions inflicted in the monkeys as part of Murray's protocol cause behavioral deficits that impair their ability to engage normally with conspecifics. These induced deficits are, and have been, used to justify the confinement of many monkeys in this laboratory in isolation.

To be clear, Murray has been inflicting brain lesions in rhesus macaques—and caging monkeys in isolation—for more than 30 years. While the importance of housing primates in social groupings was understood 30 years ago and certainly in 1989 when the USDA promulgated regulations aimed at promoting the psychological well-being of nonhuman primates, today the scientific literature is replete with overwhelming and irrefutable evidence that social isolation causes primates severe psychological and physiological harm. Caging monkeys alone frequently leads to the development of abnormal and self-injurious behaviors including hair plucking and pulling, biting, digit sucking, eye poking, and self-clasping, and other forms of self-mutilation that can lead to significant injury and morbidity. These very behaviors can be seen in the video footage produced by experimenters working in Murray's laboratory and obtained by PETA via a FOIA request; a small sampling of this footage may be viewed here.

Conclusion

For 30 years, Murray's protocols have necessitated caging monkeys in isolation; for 30 years, this egregious privation has caused extreme psychological suffering for rhesus macaques in Murray's laboratory; for 30 years, Murray has requested exemptions from social grouping requirements; and for 30 years, the NIMH ACUC has approved the exemptions. The ACUC has also approved the use of monkeys in multiple invasive survival surgeries and other harmful procedures that resulted in acute and chronic pain and distress for the animals. Murray's use of cruel and archaic experimental methods and the ACUC's rubberstamping of her protocols have violated the spirit and letter of the Animal Welfare Act and its implementing regulations. As a result, hundreds of rhesus macaques have been condemned to suffer lives marked by loneliness, depression, anxiety, and depression.

We urge you to investigate the concerns summarized in this letter and, if the claims are substantiated, to take swift and decisive action against NIMH. If you have any questions about these concerns, please contact me at KatherineR@peta.org. Thank you for your time and consideration.

Sincerely,

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Encl.: Brief Review of Neurological Experiments on Rhesus Macaques at the NIH