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PETA

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Monica M. Bertagnolli, M.D.
Director
National Institutes of Health

Via e-mail: monica.bertagnolli@nih.gov; NIHDirectorInvitations@nih.gov

Dear Dr. Bertagnolli:

On behalf of People for the Ethical Treatment of Animals (PETA), I'd like to congratulate you on your appointment as director of the National Institutes of Health (NIH). We look forward to the exciting new directions you will take the agency and biomedical research in the U.S. Today, however, I'm writing to share grave concerns about a project currently being funded by the National Institute on Aging (NIA). Specifically, I'm writing about Project Number 5R21AG074251-02, titled "Experimental Sleep Fragmentation and Cognition in Aged Marmosets."[1] This NIA-funded project is led by Principal Investigator (PI) Agnès Lacreuse of the University of Massachusetts–Amherst and is being conducted at the Wisconsin National Primate Research Center at the University of Wisconsin–Madison. As outlined below, the proposed experiments involve causing nonhuman primates irreversible harm for experiments that offer little to no new scientifically valuable knowledge or human benefit.

Irreversible Harm

As part of the proposed project, aging marmoset monkeys would be subjected to procedures that may cause long-lasting harm, including chronic sleep deprivation, restraint, and fluid restriction. For these experiments, marmosets would be exposed to audio stimuli at 60 to 90 decibels lasting for six minutes every 15 minutes throughout the night. By comparison, the noise that an average vacuum cleaner makes is about 70 decibels. The marmosets would be awakened as often as 46 times per night for a total of 276 minutes, three consecutive nights a week. There is no existing literature on the negative effects of sleep deprivation in nonhuman primates. However, the literature suggests that in humans, sleep deprivation is associated with various aversive effects, ranging from impaired cognition to altered gut microbiota and increased risk for anxiety, depression, and immunological impairments. To ensure cooperation on the cognitive tests that the laboratory subjects them to, the marmosets are deprived of one of their most basic necessities—water—for hours on end. Water restriction can cause "decreased skin turgor, dry mucous membranes, increased plasma osmolality, and behavior suggestive of extreme thirst or hunger. Distressed primates might also show behavioral changes such as lethargy, agitation, or altered patterns of aggression,"[2] and smaller species of primates "may be especially susceptible to dehydration." The procedures proposed for this project are severe enough that the

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Institutional Animal Care and Use Committee (IACUC) at UW-Madison classified them as category E because the animals would be "subjected to painful or stressful procedures" that induce permanent physical and psychological harm and that wouldn't be "alleviated through the use of anesthetics, analgesics, or tranquilizers."[3]

Moreover, marmosets in laboratories are at high risk of developing a condition referred to as "marmoset wasting syndrome" (also called "chronic lymphocytic enteritis"), a systemic inflammatory disorder that leads to weight loss, diarrhea, anemia, alopecia, weakness, intestinal inflammation, osteoporosis, paralysis, and death.[4,5] Efforts to curb the profound and deadly weight loss associated with improper nutrition have only caused additional health concerns. If they don't waste away, marmosets in laboratories become obese and suffer from health complications associated with that condition, including altered glucose metabolism, reduced insulin sensitivity, increased risk of heart disease and diabetes, and various metabolic dysfunctions.[6] Captive marmosets are also prone to metabolic bone disease,[7] which results in bone lesions and fractures and may be the cause of the oral disease, including tooth decay, frequently affecting these animals. Experimenters currently believe this issue may be related to the high vitamin D requirements of marmosets, differences in vitamin D metabolism in marmosets, or vitamin D deficiency caused by complete deprivation of sunlight in laboratory cages.[8] Marmosets in laboratories are also likely to suffer from secondary systemic amyloidosis[9] and insulin resistance.[10] Use of them in laboratories should be highly selective, if not forbidden altogether.

Limited Scientific Value or Merit

The purpose of the experiments is to determine whether sleep deprivation precedes or succeeds cognitive decline and related Alzheimer's disease (AD) biomarkers in these animals, assuming that this would provide insight into the role of sleep disruption and neurodegenerative disease in humans. PI Lacreuse repeatedly asserts in her grant application that these invasive experiments are necessary and significant because "human studies are unable to determine whether sleep disturbances precede or follow the development of AD pathology."[11] However, this claim is untrue and misleading. A multitude of *already published* studies conducted using human volunteers answer this question.[12,13,14,15,16,17,18,19,20,21,22,23] These experiments are wholly unnecessary for understanding the relationship between sleep disruption, cognitive impairment, and the risk for neurodegenerative disease.

In addition to being redundant, there are several critical limitations to the scientific value of this study:

- The PI is planning on titrating the level of sleep disturbance to determine what amount of sleep disruption is necessary to manufacture the level of cognitive impairment she wants to obtain. This necessarily removes the investigators' ability to determine any causative relationship between sleep disruption, cognitive impairment, and AD-related biomarkers they choose to measure in these animals (the purported purpose), as the experiment's design has already predetermined this relationship for the marmosets.
- The sleep disruption associated with cognitive impairments and AD in humans isn't caused by unpredictable (and potentially frightening and stressful) loud noises—nor are these periods of wakefulness likely to occur at set, discrete increments over the course of a night. Instead, most humans who experience sleep disruption in old age report poor quality of sleep throughout the night (due to dampening circadian rhythms, increased time in non-REM sleep, changes in daytime work, eating and exercise habits, increased daytime napping, and/or side effects from medications).[24,25,26] Similarly, aging humans report difficulty returning to sleep after waking, not due to increased heart rates and alertness associated with being suddenly awakened by loud,

startling sounds but rather due to racing thoughts, physical discomfort, or not feeling tired.[27] The sleep deprivation this protocol is attempting to create is unlike that experienced by aging humans. Any disruption in cognition or increase in AD pathology would be difficult to compare to that seen in humans.

- The experimenters are attempting to disrupt the sleep of the marmosets to the point where they show impairments on an over-trained task *but do not* demonstrate "sleepiness," as measured by reduced overall physical activity. Unfortunately, this approach also makes the experiments a poor "model" of what humans who endure sleep disruption actually experience. Most humans who report cognitive difficulty associated with poor sleep attribute it to fatigue sleepiness (which is the most common complaint associated with sleep loss in aging humans and is associated with cognitive difficulties).[28] The titrations that the investigators are proposing would further interfere with the translatability of the data.

In addition, marmosets are an exceptionally inappropriate species to study human sleep disruption and its relationship to age-related cognitive and neurological change. Inherent differences in sex hormone function, aging, and gene expression between marmosets and humans; critical differences in endocrine and neurological processes between the two species; and the profound impact of captivity on marmosets' physiological systems render data from these experiments irrelevant to humans.

Please consider the following information:

- There are fundamental differences in gene expression and protein function in the brains of marmosets compared to those of humans.[29] There are also differences in neurodevelopment[30,31] and neuroanatomy,[32,33] including in the timing, rate, and patterns of gray- and white-matter development across a lifetime.[34,35,36,37,38,39] In marmosets, tau—a protein that makes up a major component of the neurofibrillary tangles in Alzheimer's disease—is actually much more similar to the protein found in rodents' brains than that found in humans'.[40] While marmosets exhibit some evidence of cognitive decline with age, they don't develop human-like Alzheimer's disease, a condition unique to humans that has never been successfully recapitulated in another species.[41,42,43,44,45]
- Marmosets' accelerated development makes them an inappropriate choice for studying the much more protracted age-related changes in human cognitive behavior, sleeping patterns, or age-related changes in the human brain. In a recent review (2019), biological anthropologist and experimenter at Yerkes National Primate Research Center (now Emory National Primate Research Center) Todd Preuss wrote:

> The very small size of the marmoset brain makes it very likely that the functions of its cortical systems differ in important ways from those of larger-brained primates, if only because of the much more limited amount of neural machinery marmosets and other callitrichines have to work with. … Given the small size and rapid development of marmosets, it is tempting to view marmoset life history as a condensed version of that of longer-lived primates. Yet there is evidence primates vary in patterns of postnatal growth and development. Bogin (2007) indicates that cercopithecoid and hominoid development includes an extended period of slow growth, defining a juvenile stage that has no counterpart in marmosets. This difference, and the specializations of human development recognized by Bogin—namely, the addition of childhood and adolescent stages—imply differences in the hormonal control of development.[46]

Lack of Competence
After a series of public records requests submitted by PETA to both NIH and UW-Madison, we now have new information that, despite having had more than two years to initiate the project, data collection has not yet commenced on it. As noted in our earlier letter, documents received via public records requests indicate there have been a host of difficulties getting this project started at UW-Madison, including delays in purchasing equipment and/or shipping it from UMass-Amherst to UW-Madison, training personnel at UW-Madison, staff absences and changes, and investigators who have been too busy with conferences, study sections, and/or teaching responsibilities to get the project up and running. In June 2023 correspondence between the two institutions, investigators at UMass-Amherst requested that their counterparts at UW-Madison collect unplanned baseline blood values from marmosets in order to have tangible measurements to demonstrate "progress" on the project. In short, it has been more than two years since the Notice of Award was sent to Lacreuse, but no data has been collected for this project and design details of the experiments haven't been finalized.

We have attempted to contact leadership at NIA and the UW-Madison IACUC on multiple occasions but have received no response. It's deeply concerning that taxpayer funds are being used to support a project that is not only unnecessary and harmful to animals but also was so poorly planned that the investigators are *still* unclear about procedural details, the effects of the procedures on the animals, and the equipment needed to conduct the experiments. Please consider discontinuing funding for these extremely invasive experiments so that those resources can be directed toward research that could actually help our ever-growing aging population.

Thanks for your consideration. I look forward to hearing from you.

Sincerely,

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