AN INTERNATIONAL ORGANIZATION DEDICATED TO PROTECTING THE RIGHTS OF ALL ANIMALS

PETA

PEOPLE FOR
THE ETHICAL
TREATMENT
OF ANIMALS

Washington
1536 16th St. N.W.
Washington, DC 20036
202-483-PETA

Los Angeles
2154 W. Sunset Blvd.
Los Angeles, CA 90026
323-644-PETA

Norfolk
501 Front St.
Norfolk, VA 23510
757-622-PETA

Info@peta.org
PETA.org

November 10, 2023

Kevin Shea
Administrator
USDA/APHIS

Patricia A. Brown, V.M.D., M.S.
Director, Office of Laboratory Animal Welfare
National Institutes of Health

Via e-mail: kevin.a.shea@usda.gov; brownp@od.nih.gov

Dear Mr. Shea and Dr. Brown:

People for the Ethical Treatment of Animals, Inc. (PETA) requests that the U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) and the National Institutes of Health's (NIH) Office of Laboratory Animal Welfare (OLAW) investigate possible violations of the federal Animal Welfare Act (AWA) and noncompliance with the Public Health Service Policy on Care and Use of Laboratory Animals (PHS Policy) related to the use and treatment of common marmoset monkeys (*Callithrix jacchus*) in sleep fragmentation experiments. The procedures in question are being led by NIH-funded principal investigator (PI) Ricki Colman and conducted at the University of Wisconsin–Madison (UW-Madison; USDA Certificate No. 35-R-0001, Animal Welfare Assurance D16-00239), and involve subjecting marmoset monkeys to sleep deprivation and other harmful procedures. We believe the approval of these procedures violates AWA regulations and PHS Policy.

In response to several Freedom of Information Act requests, PETA received hundreds of pages of documents related to experiments PI Colman is planning on conducting on common marmosets. A review of these documents, which include copies of the grant application for NIH funded project R21AG074251, "Experimental sleep fragmentation and cognition in aged marmosets," correspondence between PI Colman and investigators at the grant recipient institution and the University of Massachusetts-Amherst, and the approved protocol (#G006540), reveal proposed treatment of animals that will constitute violations of Animal Welfare Regulations (AWRs) and animal welfare guidelines associated with PHS Policy.

These violations include the following:

1. Failure on the part of UW-Madison's Institutional Animal Care and Use Committee (IACUC) to ensure to ensure that no member was allowed to participate in the IACUC review or approval of an activity in which that

Entities
- PETA Asia
- PETA India
- PETA France
- PETA Australia
- PETA Germany
- PETA Switzerland
- PETA Netherlands
- PETA Foundation (U.K.)

member has a conflicting interest [9 C.F.R. § 2.31(d)(2)); Public Health Service Policy on Humane Care and Use of Laboratory Animals, Section IV.C.2].
2. Failure on the part of the IACUC to ensure that the proposal to conduct these experiments contained a scientifically valid rationale for involving animals, and for the appropriateness of the species and numbers of animals to be used. [9 C.F.R. §2.31(e)(2)].

PETA believes that inadequate oversight by the UW-Madison IACUC and conduct in apparent violation of its conflict of interest policy are responsible for the improper approval and upcoming use of live animals in unnecessary experiments.

1. Failure to consider PI Colman's conflict of interest (COI)

The University of Madison's Conflict of Interest (COI) Policy states that an "ACUC member or consultant who has a COI may be present to provide information about the protocol requested by the ACUC, but will be requested to leave the room for further discussion or vote on the protocol."[1] Both the Animal Welfare Act (9 C.F.R. § 2.31(d)(2)) and PHS Policy on Humane Care and Use of Laboratory Animals (Section IV.C.2) states "No [IACUC] member may participate in the IACUC review or approval of a research project in which the member has a conflicting interest (e.g., is personally involved in the project) except to provide information requested by the IACUC; nor may a member who has a conflicting interest contribute to the constitution of a quorum." However, the PI for this project, Ricki Colman, was permitted to remain present during an August 28, 2023, IACUC vote to decide whether the protocol should be amended. In addition to being the PI for the protocol being reviewed, Dr. Colman also serves as the chair of the IACUC, and her presence has the potential to unduly influence the decisions of the other committee members.

2. Failure to ensure that a proposal to conduct an activity involving animals contains a scientifically valid rationale for involving animals

C.F.R. Title 9, Section 2.31(e)(2) of the AWRs requires that a proposal to conduct an activity involving animals must contain a "rationale for involving animals, and for the appropriateness of the species and numbers of animals to be used." Under approved protocol G006540[2], "Sleep fragmentation and cognition," marmosets will be "chronically exposed to periods of disrupted sleep" through exposure to loud noises. More specifically, marmosets will 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 at about 70 decibels. The marmosets are awoken as often as 46 times per night, for a total of 276 minutes a night, three consecutive nights a week. The justification for subjecting marmosets to this extensive sleep deprivation is to allow the experimenters to identify "…the sequence of events linking sleep fragmentation and disease progression."

However, there are several critical limitations to the scientific rationale for the proposed species:
- 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 they want to obtain. This necessarily removes the investigators' ability to determine any causative relationship between sleep disruption, cognitive impairment, and Alzheimer's disease (AD) related biomarkers they choose to measure in these animals (the purported purpose), as the marmosets have had this relationship predetermined by the experimental design.

- The sleep disruption associated with cognitive impairments and AD in humans is not 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 the night. In fact, 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).[3,4,5] Similarly, aging humans report difficulty returning to sleep after waking not due to increased heart rates and alertness associated with being suddenly awoken from loud startling sounds, but rather due to racing thoughts, physical discomfort, or not feeling tired.[6] 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 will 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 also makes this 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).[7] The titrations the investigators are proposing will further interfere with the translatability of the data.

Additionally, marmosets are not an appropriate species to study human sleep disruption and its relationship to age-related cognitive and neurological change. Inherent differences in age-related changes in hormone function, brain structure and function, and gene expression between marmosets and humans 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 humans.[8] There are differences in neurodevelopment[9,10] and neuroanatomy,[11,12] including in the timing, rate, and patterns of gray- and white-matter development across the animal's lifespan.[13,14,15,16,17,18] In marmosets, tau—a protein that makes up a major component of the neurofibrillary tangles in Alzheimer's disease (AD) —is actually much more similar to the protein found in rodents' brains than that found in humans.[19] While marmosets exhibit some evidence of cognitive decline with age, they do not develop human-like Alzheimer's disease (AD), a condition unique to humans that has never been successfully recapitulated in another species.[20,21,22,23,24]
- 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 (2019) review, biological anthropologist, and experimenter at Yerkes National Primate Research Center (now Emory National Primate Research Center), Todd Preuss writes:

> 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.[25]

- 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.[26,27] Efforts to curb the profound and deadly weight loss associated with improper nutrition have only caused additional health concerns. If they are not wasting away, marmosets in laboratories are becoming obese and suffering 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.[28] Captive marmosets are also prone to metabolic bone disease,[29] 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 higher 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.[30] Marmosets in laboratories are also likely to suffer from secondary systemic amyloidosis[31] and insulin resistance.[32] All of these physiological effects of captivity on marmosets interferes with the validity of the data PI Colman is planning to collect.

Moreover, these experiments are not necessary. In her protocol application, Colman writes "…human studies are unable to determine whether sleep disturbances precede or follow the development of Alzheimer's disease." This is inaccurate and misleading. A review of the literature identifies several existing and ongoing projects use data acquired from healthy human volunteers to investigate the causal role of sleep deprivation on AD related neuropathology, including collecting CSF data to test for Alzheimer's disease–related biomarkers.[33,34,35,36,37,38] These published studies have already successfully linked sleep deprivation to increased risk of age-related cognitive decline and AD in humans.

The IACUC failed to ensure that approved protocols contained a "rationale for involving animals, and for the appropriateness of the species and numbers of animals to be used."

Conclusion

As detailed above, violation of the AWRs and noncompliance with the PHS Policy appear evident:

1. The PI was present during the UW-Madison IACUC voting on August 28, 2023, when they were deciding whether the protocol needed to be amended to classify the experiments as USDA Pain Category E, disregarding AWA, PHS, and UW-Madison's own COI policies.
2. The UW-Madison IACUC gave approval for Colman to perform sleep deprivation experiments on marmosets, even though a scientifically valid rationale for conducting these experiments is absent and marmosets are not an appropriate species for the research project.

PETA requests that APHIS and OLAW investigate this matter and order corrective action and appropriate penalties.

Thank you for your attention to this important matter.

Sincerely,

Katherine V. Roe Ph.D.
Chief, Science Advancement and Outreach
Laboratory Investigations Department
People for the Ethical Treatment of Animals
501 Front Street Norfolk, VA 23510
KatherineR@peta.org
240-893-7292

---

[1] Policy UW-4124. Animal Program: Identifying and Managing Conflicts of Interest. University of Wisconsin Research Animal Resources and Compliance Office.
[2] Colman R. Sleep fragmentation and cognition. Animal study protocol #G006540. University of Wisconsin-Madison.
[3] Gilley RR. The Role of Sleep in Cognitive Function: The Value of a Good Night's Rest. *Clin EEG Neurosci*. 2023;54(1):12-20.
[4] Carlson EJ, Wilckens KA, Wheeler ME. The Interactive Role of Sleep and Circadian Rhythms in Episodic Memory in Older Adults. *J Gerontol A Biol Sci Med Sci*. 2023;78(10):1844-1852.
[5] Taillard J, Gronfier C, Bioulac S, Philip P, Sagaspe P. Sleep in Normal Aging, Homeostatic and Circadian Regulation and Vulnerability to Sleep Deprivation. *Brain Sci*. 2021;11(8):1003.
[6] Neikrug AB, Ancoli-Israel S. Sleep disorders in the older adult - a mini-review. *Gerontology*. 2010;56(2):181-189.
[7] Carvalho DZ, St Louis EK, Boeve BF, et al. Excessive daytime sleepiness and fatigue may indicate accelerated brain aging in cognitively normal late middle-aged and older adults. *Sleep Med*. 2017;32:236-243.
[8]Bailey J. Monkey-based research on human disease: the implications of genetic differences. *Altern Lab Anim*. 2014;42(5):287-317. doi:10.1177/026119291404200504
[9]Charvet CJ, Finlay BL. Comparing adult hippocampal neurogenesis across species: translating time to predict the tempo in humans. *Front Neurosci*. 2018;12:706. doi:10.3389/fnins.2018.00706
[10]Sakai T, Komaki Y, Hata J, et al. Elucidation of developmental patterns of marmoset corpus callosum through a comparative MRI in marmosets, chimpanzees, and humans. *Neurosci Res*. 2017;122:25-34. doi:10.1016/j.neures.2017.04.001
[11]Fukushima M, Ichinohe N, Okano H. Neuroanatomy of the marmoset. In: Marini RP, Wachtman LM, Tardif SD, Mansfield K, Fox JG, eds. *The Common Marmoset in Captivity and Biomedical Research*. Academic Press; 2019:43-62. doi:10.1016/b978-0-12-811829-0.00003-0
[12]Charvet CJ, Palani A, Kabaria P, Takahashi E. Evolution of brain connections: integrating diffusion MR tractography with gene expression highlights increased corticocortical projections in primates. *Cereb Cortex*. 2019;29(12):5150-5165. doi:10.1093/cercor/bhz054
[13]Seki F, Hikishima K, Komaki Y, et al. Developmental trajectories of macroanatomical structures in common marmoset brain. *Neuroscience*. 2017;364:143-156. doi:10.1016/j.neuroscience.2017.09.021
[14]Walker LC, Jucker M. The exceptional vulnerability of humans to Alzheimer's disease. *Trends Mol Med*. 2017;23(6):534-545. doi:10.1016/j.molmed.2017.04.001
[15]Drummond E, Wisniewski T. Alzheimer's disease: experimental models and reality. *Acta Neuropathol*. 2017;133(2):155-175. doi:10.1007/s00401-016-1662-x
[16]Duyckaerts C, Potier MC, Delatour B. Alzheimer disease models and human neuropathology: similarities and differences. *Acta Neuropathol*. 2008;115(1):5-38. doi:10.1007/s00401-007-0312-8
[17]Neha, Sodhi RK, Jaggi AS, Singh N. Animal models of dementia and cognitive dysfunction. *Life Sci*. 2014;109(2):73-

86. doi:10.1016/j.lfs.2014.05.017
[18]Heuer E, Rosen RF, Cintron A, Walker LC. Nonhuman primate models of Alzheimer-like cerebral proteopathy. *Curr Pharm Des*. 2012;18(8):1159-1169. doi:10.2174/138161212799315885
[19]Sharma G, Huo A, Kimura T, et al. Tau isoform expression and phosphorylation in marmoset brains. *J Biol Chem*. 2019;294(30):11433-11444. doi:10.1074/jbc.RA119.008415
[20]Walker LC, Jucker M. The exceptional vulnerability of humans to Alzheimer's disease. *Trends Mol Med*. 2017;23(6):534-545. doi:10.1016/j.molmed.2017.04.001
[21]Drummond E, Wisniewski T. Alzheimer's disease: experimental models and reality. *Acta Neuropathol*. 2017;133(2):155-175. doi:10.1007/s00401-016-1662-x
[22]Duyckaerts C, Potier MC, Delatour B. Alzheimer disease models and human neuropathology: similarities and differences. *Acta Neuropathol*. 2008;115(1):5-38. doi:10.1007/s00401-007-0312-8
[23]Neha, Sodhi RK, Jaggi AS, Singh N. Animal models of dementia and cognitive dysfunction. *Life Sci*. 2014;109(2):73-86. doi:10.1016/j.lfs.2014.05.017
[24]Heuer E, Rosen RF, Cintron A, Walker LC. Nonhuman primate models of Alzheimer-like cerebral proteopathy. *Curr Pharm Des*. 2012;18(8):1159-1169. doi:10.2174/138161212799315885
[25]Preuss TM. Critique of pure marmoset. *Brain Behav Evol*. 2019;93(2-3):92-107. doi:10.1159/000500500
[26]Otovic P, Smith S, Hutchinson E. The use of glucocorticoids in marmoset wasting syndrome. *J Med Primatol*. 2015;44(2):53-59. doi:10.1111/jmp.12159
[27]Cabana F, Maguire R, Hsu CD, Plowman A. Identification of possible nutritional and stress risk factors in the development of marmoset wasting syndrome. *Zoo Biol*. 2018;37(2):98-106. doi:10.1002/zoo.21398
[28]Ross CN, Colman R, Power M, Tardif S. Marmoset metabolism, nutrition, and obesity. *ILAR J*. 2021;ilab014. doi:10.1093/ilar/ilab014
[29]Olson EJ, Shaw GC, Hutchinson EK, et al. Bone disease in the common marmoset: radiographic and histological findings. *Vet Pathol*. 2015;52(5):883-893. doi:10.1177/0300985815589354
[30]Goodroe A, Wachtman L, Benedict W, et al. Current practices in nutrition management and disease incidence of common marmosets (*Callithrix jacchus*). *J Med Primatol*. 2021;50(3):164-175. doi:10.1111/jmp.12525
[31]Ludlage E, Murphy CL, Davern SM, et al. Systemic AA amyloidosis in the common marmoset. *Vet Pathol*. 2005;42(2):117-124. doi:10.1354/vp.42-2-117
[32] Perez-Cruz C, Rodriguez-Callejas JD. The common marmoset as a model of neurodegeneration. *Trends Neurosci*. 2023;46(5):394-409.
[33]Anderson EL, Richmond RC, Jones SE, et al. Is disrupted sleep a risk factor for Alzheimer's disease? Evidence from a two-sample Mendelian randomization analysis. *Int J Epidemiol*. 2021;50(3):817-828. doi:10.1093/ije/dyaa183
[34]Olsson M, Ärlig J, Hedner J, Blennow K, Zetterberg H. Sleep deprivation and cerebrospinal fluid biomarkers for Alzheimer's disease. *Sleep*. 2018;41(5):10. doi:10.1093/sleep/zsy025
[35]Chen DW, Wang J, Zhang LL, Wang YJ, Gao CY. Cerebrospinal fluid amyloid-β levels are increased in patients with insomnia. *J Alzheimers Dis*. 2018;61(2):645-651. doi:10.3233/JAD-170032
[36]Thomas J, Overeem S, Dresler M, Kessels RPC, Claassen JAHR. Shift-work-related sleep disruption and the risk of decline in cognitive function: the CRUISE Study. *J Sleep Res*. 2021;30(2):e13068. doi:10.1111/jsr.13068
[37]Blattner MS, Panigrahi SK, Toedebusch CD, et al. Increased cerebrospinal fluid amyloid-β during sleep deprivation in healthy middle-aged adults is not due to stress or circadian disruption. *J Alzheimers Dis*. 2020;75(2):471-482. doi:10.3233/JAD-191122
[38]Barthélemy NR, Liu H, Lu W, Kotzbauer PT, Bateman RJ, Lucey BP. Sleep deprivation affects tau phosphorylation in human cerebrospinal fluid. *Ann Neurol*. 2020;87(5):700-709. doi:10.1002/ana.25702