AN INTERNATIONAL ORGANIZATION DEDICATED TO PROTECTING THE RIGHTS OF ALL ANIMALS

PETA

PEOPLE FOR
THE ETHICAL
TREATMENT
OF ANIMALS

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Richard J. Hodes, M.D.
Director
National Institute on Aging

Noni Byrnes, Ph.D.
Director
Center for Scientific Review

Sheila Garrity, J.D., M.P.H., M.B.A.
Director
Office of Research Integrity

Via e-mail: hodesr@31.nia.nih.gov; byrnesn@mail.nih.gov; AskORI@hhs.gov

Dear Dr. Hodes, Dr. Byrnes, and Ms. Garrity:

Good morning. I'm writing on behalf of People for the Ethical Treatment of Animals (PETA) to ask that the National Institute of Aging (NIA), the Center for Scientific Review (CSR), and the Office of Research Integrity (ORI) investigate whether there was misuse of funding or research misconduct associated with Project R21AG074251, awarded to the University of Massachusetts–Amherst and led by principal investigator (PI) Agnès Lacreuse.

The overarching goal of Project R21AG074251, as described in the application submitted to NIH, was to "provide critical new insights into the role of sleep disturbances in driving AD [Alzheimer's disease]." The investigators proposed to subject marmoset monkeys to chronic sleep deprivation in order to measure its effects on their physiology and behavior. The scientific value of these experiments was questionable from the start, given the species gap and the already existing research documenting the causal relationship between sleep fragmentation (SF) and Alzheimer's disease pathology in humans. However, as detailed below, documents obtained by PETA via open records requests indicate that Lacreuse **failed to complete any of the goals or objectives** outlined in the original proposal and failed to collect the majority of the measurements or carry out the majority of the procedures funded for the project. She also omitted critical information regarding the integrity and usability of the partial data that *was* included in the Final Research Performance Progress Report (FRPPR).

We hope you will review the information provided below and take appropriate action, including requiring that UMass-Amherst reimburse NIH more of the funds spent on this poorly and incompletely executed project than it already has.

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

Failure to Accomplish Any Specified Aims or Goals
As mentioned above, the goal of this project was to assess the relationship between chronic sleep fragmentation and the onset of AD pathology by repeatedly disrupting the sleep of marmoset monkeys and collecting multiple behavioral and biological measurements from them. However, Lacreuse failed to achieve this, in part because she asked for (and received) a last-minute change of testing location to a facility that was unable to complete the project as planned.

In her application for funding, Lacreuse clearly indicated that all marmosets used in this project would be housed and tested in her laboratory at UMass-Amherst and mentions numerous UMass-specific resources, including particular computer equipment required for behavioral and cognitive testing.

After receiving notice that the project would be funded, Lacreuse informed NIH that she did not, in fact, have access to a suitable number of marmosets to complete it. She requested and received permission for a subcontract with the University of Wisconsin–Madison's Wisconsin National Primate Research Center (WNPRC), noting, "The WNPRC has all the infrastructure and resources necessary to conduct the work as planned" and that "Dr. Ricki Colman, Associate Professor and Senior Scientist in the Cell and Regenerative Biology department, has made marmosets available for the project." She also stated, "The scope of the grant is unchanged. One minimal change from the original grant will be the use of gonadally intact females instead of ovariectomized females. This should have no impact on the specific aims, as the purpose of this grant is to develop a method for fragmenting sleep, which does not depend on the hormonal status of the animals."

However, in her FRPPR, Lacreuse describes a very different impact of transferring this project to the WNPRC and acknowledged the following:

> This project encountered many challenges that prevented us from accomplishing all the objectives of the original application. First, as a result of the national shortage of marmosets, we had great difficulty locating suitable marmosets for the project and for this reason requested to transfer the project to the WNPRC. This transfer incurred a lengthy delay and was not established before May, 2022. Second, due to various experimental and practical constraints at WNPRC only 6 animals (3 heterosexual pairs), of suitable age (3 females mean age= 6.99, and 3 males mean age = 6.97) could be assigned to the project, instead of the 18 originally planned in the grant. Given this constraint, the PI and co-PIs decided to switch to an alternate design, with animals serving as their own control. Finally, despite the implementation of a touchscreen system and training protocol identical to those in place in the Lacreuse lab at UMass, the assigned animals failed to reliably engage with the system after several months of training. As a result, we had to abandon our plan to assess multiple domains of cognition via this computerized system and pivot to a task with faster acquisition times for this group of animals, the detour reaching task, which assesses executive function. Despite these setbacks the following aspects of the project were successfully accomplished.

In fact, Lacreuse (and Colman) failed to complete *any* of the reviewed project's aims and objectives.

The original grant application proposed to accomplish the specific aims below.

> Specific Aim 1: to design an experimental SF procedure that mimics the sleep fragmentation observed in AD and to characterize the effects of chronic SF on sleep, cognitive function and behavior. We hypothesize that SF will be associated with altered sleep patterns, impaired cognitive function and specific behavioral changes (i.e., social interactions).
>
> Specific Aim 2: to determine whether chronic SF induces changes in peripheral measures of inflammation and metabolic function and CSF [cerebrospinal fluid] levels of orexin, Al3 and tau levels. We hypothesize that SF will increase inflammatory processes and impair metabolism and that these changes will correlate with cognitive impairment.

The "Approach" outlined in the application was described as follows:

> A *total of 16 marmosets* (~ 6 years old, 8 females, 8 males) will be studied in this project. Monkeys will be habituated to wearing an actigraphy device that records motor activity via an accelerometer and can accurately measure sleep in marmosets. The monkeys will be trained on a *battery of cognitive tasks assessing motivation, working memory and executive function*, administered on touchscreen in their homecage. Based on our estimate that the first 6–8 months of the project will be needed for colony and laboratory set-up and acquisition of the cognitive battery. After acquisition of baseline cognitive performance, monkeys will be assigned to a SF group or an undisturbed sleep group (8 marmosets/group). The SF procedure will be developed progressively to ensure the well-being of the animals but is designed to expose the marmosets *to consecutive nights of disrupted sleep/week for 2 months*. Changes in cognitive function (Aim 1), peripheral markers of inflammation and metabolism and CSF measures of *orexin, AB and tau* levels will be measured (Aim 2). [*Emphasis added*.]

However, despite being awarded $438,625, being allowed to change testing sites after assuring NIH officials that the transfer would not affect the study, *and* receiving a 12-month no-cost extension, the following took place:

- **The investigators failed to procure enough marmosets to have the necessary number of animals needed for their experimental group** and **failed to have *any* control group** as originally proposed. **Only six marmosets** in total were included in this study, and they served as their own controls.
- **The investigators failed to study *chronic* sleep deprivation.** The six marmosets they did manage to procure were only exposed to **one night of sleep deprivation** before the extended end-of-project date, which was not the chronic sleep deprivation the project was intended to assess. There are currently no theories of AD indicating that *one night* of poor sleep is part of its disease etiology.
- **The investigators failed to assess the role of sleep fragmentation on AD pathology.** The original project proposed to measure AD pathology using CSF samples from the marmosets. Urine cortisol levels were collected instead but not over the course of chronic sleep deprivation and only once before and once after a single night of fragmented sleep. **The experimenters did not assess whether chronic sleep disruption increased levels of tau and Aβ, which was a primary goal of the project.**

- The investigators **failed to assess *multiple* domains of cognition and behavior as described above**. Rather, due to an inability to train the marmosets on the necessary battery of tasks, only their ability to perform the detour-reaching task before and after one night of fragmented sleep was assessed. The detour-reaching tasks are a measure of the animals' inhibitory control and irrelevant to the sort of cognitive decline associated with AD. **No measurements of working memory, motivation, or social interactions were obtained as planned.**

Despite completing only a small fraction of the work outlined in the project, Lacreuse managed to spend 78% of the taxpayer funds allotted to it. This is concerning.

Selective Omissions in the Final FRPPR

Unfortunately, even the meager accomplishments outlined above are problematic. Several important health issues the marmosets were experiencing at the time of testing were omitted from the FRPPR.

During the months, weeks, and days leading up to the sleep fragmentation procedure, **the six marmosets used in these experiments were all experiencing chronic diarrhea**. At the time of testing and data collection, there appears to have been a colony outbreak of diarrhea, and all six marmosets were observed to have diarrhea in the cages for four to seven consecutive days at the time of testing, and some were observed to have swollen anuses or feces caked on their bodies.

One marmoset (CJ2122) had lost 3% of her bodyweight right before the sleep fragmentation procedure was administered. This same marmoset had been treated with Estrumate (cloprostenol) *nine times* to terminate unwanted pregnancies during the training phase of these experiments, including one administration given less than two weeks before the sleep fragmentation procedure and related urine collections took place.

Two marmosets (CJ2129 and CJ2093) had diarrhea so severe that they were treated with budesonide and required a special diet for weeks **before, during, and after testing was performed**. Budesonide is a steroid and likely altered any inflammatory markers measured in these animals' urine and sleep behavior at the time of testing. CJ2129 was also treated with cloprostenol for unwanted pregnancies while on this protocol.

Despite these ongoing health issues and the likelihood that they would impact the integrity of the data being collected, the experimenters chose to forge ahead and collect a tiny portion of the data outlined in their grant application. We can only speculate here, but it seems this was done to have *some* data to report in the closeout documents for the project. The decision to collect data from animals with ongoing health issues introduces various confounds into the small amount of data these experimenters managed to collect.

In short, the experimenters spent three years and $339,964 of taxpayers' money to study the effect of one night of poor sleep on the ability of six diarrhea-ridden marmosets to reach around an object to grab a piece of food. This doesn't seem like a responsible use of federal research resources, and it certainly doesn't contribute anything to our understanding of the relationship between chronic sleep fragmentation and AD pathology in humans. It also does nothing to validate the use of marmosets for this type of research.

Conclusions
PETA previously contacted leadership at NIH and the NIA expressing our concerns about these experiments' lack of scientific utility as well as the misrepresentation of resources in the original application. We also expressed our concerns regarding the impact the change of location for these experiments might have on the project in addition to its failure to progress. All these concerns were ignored. Now hundreds of thousands of dollars have been wasted on cruel experiments that resulted in no meaningful data and no benefits to the public or the scientific community.

We hope you will take the following actions:

1. Conduct an audit or other systematic review of Project R21AG074251
2. Require that UMass-Amherst reimburse NIH for a larger percentage of R21AG074251 funding
3. Bar Agnès Lacreuse from receiving additional NIH funding, as she is unable to shepherd such funds properly or responsibly

PETA has hundreds of pages of documents, which are available upon request, that we can provide to assist you with investigating this matter. I look forward to your prompt response.

Sincerely,

Katherine Roe, Ph.D.
Chief Scientist
Laboratory Investigations Department