AN INTERNATIONAL ORGANIZATION DEDICATED TO PROTECTING THE RIGHTS OF ALL ANIMALS

PETA

PEOPLE FOR
THE ETHICAL
TREATMENT
OF ANIMALS

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February 21, 2023

Wanda Jones, Dr.P.H.
Acting Director
Office of Research Integrity

Alexander Runko, Ph.D.
Director
Office of Research Integrity
Division of Investigative Oversight

Via e-mail: Wanda.Jones@hhs.gov; Alexander.Runko@hhs.gov

Dear Drs. Jones and Runko:

On behalf of People for the Ethical Treatment of Animals (PETA), I'm writing to ask the Office of Research Integrity (ORI) to investigate whether misleading information was knowingly included in an actively funded grant application submitted to the National Institutes of Health (NIH) by Principal Investigator (PI) Agnès Lacreuse of the University of Massachusetts–Amherst.

Through a Freedom of Information Act (FOIA) request, PETA received copies of grant application documents for Project Number 5R21AG074251, titled "Experimental Sleep Fragmentation and Cognition in Aged Marmosets."[1] The project plans to simulate sleep difficulties experienced by patients with Alzheimer's disease (AD) in marmoset monkeys, who will then be tested for evidence of cognitive decline, and their cerebral spinal fluid will be assessed for AD-related biomarkers. The current budget for this two-year project is $199,375. We are deeply concerned that deliberately misleading information, which is reviewed below, was included in the "Innovation," "Significance," and "Approach and Institutional Environment" sections of the grant application for this project. According to the NIH Grants Policy Statement, these statements are all key factors for the Scientific Review Group (SRG) to consider when calculating the overall impact of the project. If your office determines that the applicantknowingly misled the SRG, we urge you to take appropriate action.

Misrepresentation of Innovation and Significance

Throughout the application,[1] as in the following examples, the PI indicates that this project with marmosets is necessary and significant because such procedures can't be performed with human volunteers:

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

[1]Exhibit 1. Lacreuse A. Experimental Sleep Fragmentation and Cognition in Aged Marmosets. Application funded by the National Institute of Aging as Project AG-074251, August 2021.

- The "**Summary**" portion of the application states that "human studies are unable to determine whether sleep disturbances precede or follow the development of AD pathology" (p. 8 of attached document).
- Under "**Specific Aims**," the applicant states that "human studies are unable to determine whether sleep fragmentation precedes or follows the development of AD pathology" and that "Studies in animal models are necessary to investigate this issue." (p. 31).
- The "**Significance**" section of the application states that "human studies cannot determine whether sleep disruptions precede or follow the development of AD neuropathology. Characterizing the effects of sleep disruptions on neuropathology and cognition in animal models are [*sic*] key to uncover novel preventative or therapeutic targets for AD" (p. 32).
- "**Specific Aim 1**" of the application states that "human studies cannot determine whether sleep disturbances are a cause or consequence of neuropathology" (p. 33).
- The "**Justification**" portion of the "**Vertebrate Animals**" section of the application states, "There is no alternative or *in vitro* models available for this purpose" (p. 39).

However, the claim that there is no human data available on the causative role of sleep fragmentation on AD pathology, made *repeatedly* in the application, is inaccurate. Several published studies using data acquired from healthy human volunteers have investigated the causal role of sleep deprivation on neuropathology. The following list of publications represents only a sample of such studies, many of which were funded by NIH:

- Barthélemy NR, Liu H, Lu W, Kotzbauer PT, et. al. Sleep Deprivation Affects Tau Phosphorylation in Human Cerebrospinal Fluid. *Ann Neurol*. 2020 May;87(5):700–709.
- 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.
- 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.
- Ju YS, Ooms SJ, Sutphen C, et al. Slow Wave Sleep Disruption Increases Cerebrospinal Fluid Amyloid-β Levels. *Brain*. 2017 August;140(8):2104–2111.
- Kam K, Parekh A, Sharma RA, et al. Sleep Oscillation–Specific Associations With Alzheimer's Disease CSF Biomarkers: Novel Roles for Sleep Spindles and Tau. *Mol Neurodegener*. 2019;14(1):10.
- Olsson M, Ärlig J, Hedner J, et al. Sleep Deprivation and Cerebrospinal Fluid Biomarkers for Alzheimer's Disease. *Sleep*. 2018 May;41(5):10.
- Mander BA, Dave A, Lui KK, et al. Inflammation, Tau Pathology, and Synaptic Integrity Associated With Sleep Spindles and Memory Prior to β-amyloid Positivity. *Sleep*. 2022 September;45(9):zsac135.
- Thomas J, Overeem S, Dresler M, et al. Shift-Work-Related Sleep Disruption and the Risk of Decline in Cognitive Function: The CRUISE Study. *J Sleep Res*. 2021 April;30(2):e13068.

Given the PI's expertise in sleep disruption, aging, and cognitive decline, it seems unlikely that she was unaware of the numerous publications, which appear in a simple literature review of the topic, that document the effects she purports to study. It seems more plausible and is far more concerning that the investigator *may have knowingly overstated the import and novelty of this study in order to secure federal funding and justify her use of nonhuman primates*.

Misrepresentation of the Institutional Environment
Documents indicate that the PI misrepresented the *resources* available at the intended recipient institution. Under "**Facilities and Other Resources**," the applicant indicates that all marmosets used in this project will be housed at the Lacreuse laboratory at the University of Massachusetts–Amherst (UMass), noting the following:

> The monkeys for the proposed project will be housed in two rooms that are shielded from outside noise and have restricted access when the behavioral experiments are in progress. Animals are fed a balanced diet including fruits, vegetables, eggs and mealworms. Marmosets are housed in male-female pairs in stainless steel cages that are approximately 80″x48″x36″ and maximize vertical space. The cages are equipped with tree branches, shelves, a nest box, a hammock, PVC tubes and enrichment devices to promote species-typical behaviors (see *Vertebrate Animals).* Right across from the laboratory is a necropsy room available for euthanasia and brain extraction. The UMass veterinary staff is available for medical consultation and assistance and supervised technicians are responsible for animal care at UMass. A surgery suite for veterinary care and a set of rooms for animal quarantine are available across the hall from the marmoset laboratory. (p. 9)

The PI also lists numerous additional UMass-specific resources, including, but not limited to, the following:

- "[N]umerous core facilities, such as animal imaging, flow cytometry, genomics including deep sequencing, electron microscopy, and a new, staffed Nikon Center of Excellence optical microscopy core which provides access to light sheet (for CLARITY), multiphoton, confocal and super resolution imaging. They also have access to the Massachusetts Green High-Performance Computing Center and other local computing clusters that will allow large-scale research computing as necessary." (p. 10)
- "[T]he **Center for Neuroendocrine Studies**, which consists of the research groups of seventeen faculty members who share an interest in understanding the relationships among hormones, the brain, physiology, and behavior. The Center fosters neuroendocrine research by sponsoring scientific meetings, facilitating the flow of information among laboratories, and coordinating efforts to obtain funding for training, equipment, and research." (p. 10)
- "**Marmoset caging.** The Lacreuse lab has 10 large stainless steel cages (80″x48″x36″) that maximize vertical space and that are appropriate for marmoset housing." (p. 11)
- "**Cognitive testing.** One touchscreen CANTAB computer system (Cambridge Neuropsychological Testing Automated Battery, Model 80951A). Touchscreens will be purchased for this project." (p. 11)
- "**Activity monitors.** Ten Actiwatch-Mini devices (CamNTech, Cambridge, Mass.) are available for use in this project." (p. 11)
- "**Heart rate monitors.** The Lacreuse lab has a telemetry-based system available for recording heart rate data." (p. 11)
- "**Thermal imaging.** FLIR A325sc camera (FUR Systems, NH)." (p. 11)

The grant application associated with this project clearly indicates UMass as the proposed location of the study and that it is aptly suited to carry out the proposed experiments. However, shortly after the Notice of Award was given by the National Institute of Aging (NIA) in August

2021,[2] the PI notified NIH Grants Management Specialist Eva Lawson-Lipton that a subcontract would be needed because the recipient facility *did not* have an adequate number of marmosets to perform the studies.[3]

According to the March 15, 2022, Research Performance Progress Report (RPPR) for this project, "[T]he subcontract with WNPRC has not yet been implemented."[4] This RPPR also states, "The WNPRC has all the infrastructure and resources necessary to conduct the work as planned." However, as of February 1, 2023, the WNPRC has been unable to commence collecting data,[5] at least in part because it doesn't have the necessary equipment.[6]

To the best of our knowledge, NIH chose to fund a project that has yet to be initiated, even though its stated start date is August 15, 2021, and the end date is April 30, 2023.

Altered Approach

In a December 23, 2021, letter to Grants Management Specialist Elizabeth Yeomans at NIH[7] regarding the need for a subcontract, the PI wrote the following:

> Due to the national shortage of marmosets, it has become clear that UMass Amherst will not be able to acquire the animals needed for this project within the budget and timeframe of the awarded grant. To address this challenge, we propose to conduct the animal work at the Wisconsin National Primate Research Center (WNPRC) of the University of Wisconsin-Madison. Dr. Ricki Colman, Associate Professor and Senior Scientist in the Cell and Regenerative Biology department, has made marmosets available for the project, and has agreed to serve as subcontractor to set-up and conduct the experiment on site, with the input and expertise of Dr. Karatsoreos and myself at UMass Amherst.
>
> Impact on project scope. 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.

The statement above that the hormonal status of the monkeys is irrelevant and will have no impact on the project's specific aims is **in direct contrast with information in the grant application and the PI's entire body of work**. The grant application explicitly states that "females will be ovariectomized to model low sex hormone levels milieu of menopausal women" and that the PI expects "females to be more affected than males" by the project's procedures. In fact, the majority of this PI's NIH-funded work has focused on the effect of sex hormones on sleep, cognition, and age-related decline.

The PI's **personal statement** in the grant application notes the following:

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[2]Exhibit 2. Notice of Award for R21AG074251
[3]Exhibit 3. September 14, 2022. E-mail from Agnès Lacreuse to Eva Lawson Lipton requesting a sub contract.
[4]Exhibit 4. RPPR_2022-03-15.
[5]Exhibit 5. January 30, 2023. Response to PRR request to UW-Madison.
[6]Exhibit 6. May 2022. Correspondence between UW-Madison and UMass-Amherst regarding the subcontract.
[7]December 2021 Letter to NIH from Agnès Lacreuse.

> The Lacreuse laboratory investigates neurocognitive function across the lifespan in nonhuman primate models of human aging, with a particular focus on sex differences and menopause.

In the "**Contribution to Science**" section, the PI states the following:

> We are also developing the marmoset as a new model for studying **menopausal symptoms**, including **sleep disturbances**, thermoregulation and cognitive deficits. Our latest research demonstrates **a crucial role of neuroestrogens** in these processes. [*Emphasis added.*]

The change from ovariectomized to intact females likely will alter the scope of the original grant application as it was originally submitted, and it will certainly limit the ability of this PI to integrate these data with all of her previous studies that used ovariectomized females.

Public Accountability Demands That the Misrepresentation in This Grant Application Be Investigated

The misinformation included about the necessity of primate use, the available resources, and the existence of similar data in the grant application for Project 5R21AG074251likely misled members of the SRG about the overall impact of the project. According to the NIH Grants Policy Statement, reviewers in the assigned SRG assess the overall impact as follows:

> [O]verall impact in the determination of scientific and technical merit … is defined differently for different types of applications. When considering applications for research grants and cooperative agreements, reviewers will provide an overall impact score to reflect their assessment of the likelihood for the project to exert a sustained, powerful influence on the research field(s) involved, taking into account, among other pertinent factors: Significance, Investigator(s), Innovation, Approach, and Environment.

We believe that the misinformation supplied by Lacreuse in the grant application for this project may have misled the SRG tasked with scoring the application on the significance and innovation of the project as well as the environment in which it would be taking place. The change in environment, which required the use of non-ovariectomized females, also changes the approach.

Given the limited resources NIH has available to fund research, it's critical that accurate information be included in grant applications for reviewers to consider when assessing the overall impact of each project.

I hope your office will look into this important matter. I would be happy to provide any additional information you may need or answer any questions you may have.

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

Exhibit 1

PI: **Lacreuse, Agnes**	Title: Experimental sleep fragmentation and cognition in aged marmosets	
Received: 11/09/2020	FOA: PA20-195	Council: 05/2021
Competition ID: FORMS-F	FOA Title: NIH Exploratory/Developmental Research Grant Program (Parent R21 Clinical Trial Not Allowed)	
1 R21 AG074251-01	Dual:	Accession Number: 4517380
IPF: 850904	Organization: UNIVERSITY OF MASSACHUSETTS AMHERST	
Former Number:	Department: Dept: Psychology and Brain Sci	
IRG/SRG: ZRG1 IFCN-U (02)M	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 150,000 Year 2: 125,000	Animals: Y Humans: N Clinical Trial: N Current HS Code: Evaluative Info HESC: N HFT: N	New Investigator: Early Stage Investigator:
Senior/Key Personnel:	*Organization:*	*Role Category:*
Agnes Lacreuse	University of Massachusetts Amherst	PD/PI
Redacted by agreement	University of Massachusetts Amherst	Co-Investigator

OMB Number: 4040-0010
Expiration Date: 12/31/2022

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	**State Application Identifier** MA

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number

c. Previous Grants.gov Tracking Number

2. DATE SUBMITTED 2020-11-09

Application Identifier 3187

5. APPLICANT INFORMATION **Organizational DUNS*:** 153926712

Legal Name*: University of Massachusetts Amherst
Department: Dept: Psychology and Brain Sci
Division: Coll: Natural Sciences
Street1*: Office of Research and Engagement
Street2: Venture Way Center, 100 Venture Way, Suite 201
City*: Hadley
County:
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 01035-9450

Person to be contacted on matters involving this application
Prefix: First Name*: Carol Middle Name: Last Name*: Sprague Suffix:
Position/Title: Director, Grants & Contracts
Street1*: Mass Ventures Building, Rm 201
Street2:
City*: Amherst
County:
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 01003-0000
Phone Number*: 413-545-0698 Fax Number: Email: sprague@research.umass.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 1043167352B5

7. TYPE OF APPLICANT* H: Public/State Controlled Institution of Higher Education

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

● New ❍ Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
NIH-NATIONAL INSTITUTES OF HEALTH

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Experimental sleep fragmentation and cognition in aged marmosets

12. PROPOSED PROJECT
Start Date* 07/01/2021
Ending Date* 06/30/2023

13. CONGRESSIONAL DISTRICTS OF APPLICANT
MA-002

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: Agnes Middle Name: C Last Name*: Lacreuse Suffix:
Position/Title: Professor U of M
Organization Name*: University of Massachusetts Amherst
Department: Dept: Psychology and Brain Sci
Division: Coll: Natural Sciences
Street1*: Tobin Hall, Rm 531
Street2:
City*: Amherst
County:
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 01003-0000
Phone Number*: 413-545-2183 Fax Number: Email*: alacreuse@psych.umass.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$438,625.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$438,625.00
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
DATE:

b. NO ○ PROGRAM IS NOT COVERED BY E.O. 12372; OR
● PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: Sarah Middle Name: M Last Name*: Vega-Liros Suffix:
Position/Title*: Grant & Budget Admin II
Organization Name*: University of Massachusetts Amherst
Department:
Division:
Street1*: Dickinson Hall, Rm 104A
Street2:
City*: Amherst
County:
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 01003-0000
Phone Number*: 413-545-7559 Fax Number: Email*: sarahv@research.umass.edu

Signature of Authorized Representative*
Sarah M Vega-Liros

Date Signed*
11/09/2020

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:RRSF424_Cover_Letter.pdf

424 R&R and PHS-398 Specific
Table Of Contents

OMB Number: 4040-0010
Expiration Date: 12/31/2022

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	University of Massachusetts Amherst
Duns Number:	153926712
Street1*:	Office of Research and Engagement
Street2:	Venture Way Center, 100 Venture Way, Suite 201
City*:	Hadley
County:	
State*:	MA: Massachusetts
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	01035-9450
Project/Performance Site Congressional District*:	MA-002

Additional Location(s) File Name:

Funding Opportunity Number: PA-20-195. Received Date: 2020-11-09T16:05:24.000-05:00

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ○ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ○ Yes ○ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6 _ 7 _ 8

If NO, is the IRB review Pending? ○ **Yes** ○ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ● **Yes** ○ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ● Yes ○ No

IACUC Approval Date:

Animal Welfare Assurance Number D16-00337

3. Is proprietary/privileged information included in the application?* ○ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ○ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ○ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	ProjectSummary.pdf
8. Project Narrative*	Narrative.pdf
9. Bibliography & References Cited	Bibliography.pdf
10.Facilities & Other Resources	Facilities.pdf
11.Equipment	Equipment.pdf

Funding Opportunity Number: PA-20-195. Received Date: 2020-11-09T16:05:24.000-05:00

SUMMARY

Elderly people frequently experience sleep disturbances, which contribute to age-related cognitive decline and are thought to be a core component of Alzheimer's disease (AD) and its pathophysiology. However, whether sleep disturbances *cause* cognitive impairment and neuropathology in AD remain unclear. Indeed human studies are unable to determine whether sleep disturbances precede or follow the development of AD pathology. Identifying the precise sequence of events linking sleep fragmentation and disease progression is a crucial step in better understanding the etiology of AD and identifying new therapeutic targets. Studies in animal models are needed to investigate this issue. Rodents are useful to identify basic mechanisms underlying the relationships between sleep and brain function, but also have limitations due to substantial differences from humans in sleep, cognitive and brain aging phenotypes. Using a more translational animal model with regards to sleep, cognitive function and neuropathology would likely provide critical new insights into the role of sleep disturbances in driving AD. The common marmoset (*Callithrix jacchus)* is ideally suited as such a model. This diurnal nonhuman primate exhibits monophasic sleep, shows age-related decline in several cognitive domains, and possesses two hallmarks of AD neuropathology, amyloid-β deposition and accumulation of hyperphosphorylated tau proteins. In addition, its short lifespan of about 10-12 years is ideal for longitudinal studies. Marmosets will be fitted with an actigraphy device to monitor sleep/wake patterns. The monkeys will be trained on a battery of cognitive tasks administered on touchscreens in their home cage. After baseline recording of sleep and cognitive performance, they will be randomly assigned to a sleep fragmentation (SF) or undisturbed sleep (control) group. The SF group will be chronically exposed to periods of disrupted sleep designed to mimic fragmented sleep in AD patients, whereas the control group will be kept undisturbed. Changes in physiology and cognitive function will be assessed throughout the experiment. The proposed study should validate the marmoset as a translational preclinical model for future studies focusing on the role of SF on AD neuropathology. The availability of such a model will be crucial for identifying preventative or therapeutic strategies that are clinically relevant.

NARRATIVE

This proposal designs a sleep fragmentation procedure for marmosets that mimics the sleep fragmentation observed in patients with Alzheimer's disease (AD) to determine whether fragmented sleep causes cognitive impairment and physiological changes. The findings should validate the marmoset as a translational model to study the mechanisms by which sleep fragmentation drives the development of AD in the aged primate brain.

Facilities and Other Resources

Lacreuse laboratory (UMass Amherst)

Marmoset Laboratory Redacted by agreement marmosets. The marmosets are housed Redacted by agreement Redacted by agreement Redacted containing a work station for data entry and processing. The monkeys for the proposed project will be housed in two rooms that are shielded from outside noise and have restricted access when the behavioral experiments are in progress. Animals are fed a balanced diet including fruits, vegetables, eggs and mealworms. Marmosets are housed in male-female pairs in stainless steel cages that are approximately 48"W x 36"D x 80"H and maximize vertical space. The cages are equipped with tree branches, shelves, a nest box, a hammock, PVC tubes and enrichment devices to promote species-typical behaviors (see *Vertebrate Animals*). Right across from the laboratory is a necropsy room available for euthanasia and brain extraction. The UMass veterinary staff is available for medical consultation and assistance and supervised technicians are responsible for animal care at UMass. A surgery suite for veterinary care and a set of rooms for animal quarantine are available across the hall from the marmoset laboratory.

Office space and computers Dr. Lacreuse recently moved to a new office with her neuroscience colleagues, including Dr. Karatsoreos, in the renovated Neuroscience Wing of Morrill IV North Science center. The building is approximately 10 min walk from the building housing the marmosets. Dr. Lacreuse has two laptop computers and one desktop computer that provide the appropriate software for writing, data analysis and internet access. The Lacreuse laboratory is also equipped with 3 PC desktop computers, a laptop computer and a laser printer. The University has strong IT resources which can provide all the support necessary in case of computer or wireless failure. Dr. Lacreuse has assigned office space for graduate students, postdoctoral and research staff.

Karatsoreos laboratory (UMass Amherst)

Wet Laboratory: The Karatsoreos lab is housed in the newly completely renovated Morrill IV North Science center. This space has been custom designed to accommodate a collaborative research environment with 6 other members of the Neuroscience and Behavior program. This facility is directly adjacent to the recently constructed Life Sciences building, providing direct and easy access to collaborators and research core in the Institute for Applied Life Sciences (IALS), of which Dr. Karatsoreos is a member. Specifically, the Karatsoreos laboratory has approximately 1000 sqft of dedicated wet lab space. Shared space includes tissue culture rooms, multiple fume hoods, neurotechnology workshop, tissue processing/staining room, a large cold room, and space for freezers and other large equipment.

Sleep and Circadian Rhythms Laboratory: Redacted by agreement Redacted by agreement Redacted by Specifically dedicated to the Karatsoreos lab are three rooms (~150 sqft each) are used as housing for mice in circadian boxes allowing for numerous computer controlled light-cycles to be used in tandem. These rooms each have a capacity of 90 cages (or 450 mice, group housed). In addition a large breeding vivarium is used in the adjacent and connected building where all breeding takes place.

Metabolic Screening Laboratory: The Karatsoreos laboratory has established a mouse metabolic screening laboratory within its assigned animal space. This lab includes a dedicated 16-channel TSE Phenomaster system for behavioral and metabolic phenotyping within the home cage (see Equipment) as well as a dedicated EchoMRI-100 (EchoMRI Corp) Whole Body Composition analyzer (see Equipment). The lab also has access additional shared animal imaging equipment in Redacted by agreement

Common Use Laboratory Space: The research space in Morrill IVN has shared perfusion rooms, surgical suites, tissue culture facilities, centrifuges (refrigerated, standard, and ultra), freezer storage (-80°C and -20°C), and general lab maintenance facilities (autoclaves, glass washers, ice machines, water polishers) on each floor.

Office: Dr. Karatosoreos has a 150 sqft private office, adjacent to the lab, access to a conference rooms and has have assigned office space for graduate students, postdocs and research staff.

Institutional Environment:

The University of Massachusetts, Amherst (UMass Amherst) is a large university with many excellent training graduate training programs. The university has a current enrollment of 23,515 undergraduates, 7,078 graduate students and 1,300 full-time instructional faculty members. In FY2017, UMass supported research activities that totaled $210 million dollars. UMass is situated within the five-college region, a coalition of universities including Hampshire College, Smith College, Mount Holyoke College and Amherst College. Many research collaborations exist between the faculty and students from each school have the opportunity to take classes on each campus and also to work in research labs outside of their home campus. It is an exciting time for Neuroscience at UMass. The development of the UMass Neuroscience Initiative and the UMass Institute for Applied Life Sciences has promoted the recruitment of many new neuroscientists. This growing neuroscience community spans across Biology, Psychological and Brain Sciences, Engineering and Computing sciences and has led to multiple seminars, symposia, and meetings that stimulate collaborative work.

The Department of Psychological and Brain Sciences has a dedicated machine shop for custom fabrication needs and a full staff dedicated to budget and financial administration, and assists in the day-to-day management of all laboratory finances, and a team of dedicated staff offers support for any administrative or secretarial needs. The College of Natural Sciences provides dedicated grant and budget prepartion support, along with a host of other support services for investigators.

As members of the **Institute for Applied Life Sciences (IALS)**, Drs. Lacreuse and Karatsoreos have access to numerous core facilities, such as animal imaging, flow cytometry, genomics including deep sequencing, electron microscopy, and a new, staffed Nikon Center of Excellence optical microscopy core which provides access to light sheet (for CLARITY), multiphoton, confocal and super resolution imaging. They also have access to the Massachusetts Green High-Performance Computing Center and other local computing clusters which will allow large-scale research computing as necessary.

Drs. Lacreuse and Karatsoreos are also members of the **Center for Neuroendocrine Studies**, which consists of the research groups of seventeen faculty members who share an interest in understanding the relationships among hormones, the brain, physiology, and behavior. The Center fosters neuroendocrine research by sponsoring scientific meetings, facilitating the flow of information among laboratories, and coordinating efforts to obtain funding for training, equipment, and research. In addition to monthly research meetings, the CNS offers team-taught seminars, hands-on technical workshops, and an Annual Symposium on Neuroendocrinology. The Neuroscience and Behavior graduate program, of which both Dr. Lacreuse and Karatsoreos are members, offers mutliple opportunities for interactions with faculty, postdoctoral fellows and students across multiple departments.

Equipment

Lacreuse laboratory

Marmoset caging. The Lacreuse lab has 10 large stainless steel cages (48"W x 36"D x 80"H) that maximize vertical space and that are appropriate for marmoset housing.

Cognitive testing. One touchscreen CANTAB computer system (Cambridge Neuropsychological Testing Automated Battery, Model 80951A). Touchscreens will be purchased for this project.

Activity monitors. Ten Actiwatch-Mini devices (CamNTech, Cambridge, MA) are available for use in this project.

Heart rate monitors. The Lacreuse lab has a telemetry-based system available for recording heart rate data (Data Sciences International). This includes two receivers, a matrix and a dedicated PC computer for data acquisition and four external telemetry-based heart rate monitors (CA-EXT) and marmoset jackets (Lomir Biomedical).

Thermal imaging. FLIR A325sc camera (FLIR Systems, NH).

Computers: The Lacreuse laboratory is equipped with 3 PC desktop computers, a laptop computer and a laser printer. All computers are able to access the campus intranet as well as the Internet. Most computers have Microsoft Office suites. Several computers include software packages for Statistical analysis (SPSS) and MATLAB.

Karatsoreos Laboratory

Rodent Surgery: Dedicated mouse surgery suite in the vivarium one floor below the lab, with three surgical work stations, bead sterilizer, tabletop autoclave, digital stereotaxic system (Stoetling) and VetEquip mobile gas anesthesia machine.

Non-Tethered Circadian Rhythms Recording System: Mini-Mitter batteryless animal telemetry system; VitalView software; Light-tight animal housing boxes, with individual ventilation and light control (8 boxes, 10 group cages/box; 320 mice).

Cell and Molecular Biology Wet Laboratory: MagPix Instrument (Luminex) for fluorescent magnetic bead multiplex analysis, Guava MUSE benchtop cell analysis instrument; BioRad GelDoc gel imaging system; dissecting scopes; cryostat; freeszing microtome; vibratome; -80'C and -20'C freezers, PCR systems, refrigerated centrifuge, microfuges, incubators, power supplies, gel boxes, balances, etc.

Biosensor for real-time recording of CNS Glucose and Lactate concentrations: Biosensors that are capable of providing real-time changes in glucose or lactate concentration in the brains of rodents. This is integrated into the sleep recording system, but can be used independently. Both lactate and gluctamate biosensors reject all common electroactive interferents including ascorbate. The lactate biosensor has an average linear range of at least 2mM, works in vivo, and can provide continuous monitoring of brain lactate concentration changes for at least 96 hours. The glucose biosensor has an average linear range of at least 4mM, works in vivo, and can provide continuous monitoring of brain lactate concentration changes for at least 96 hours. Currently, the system includes 4 simultaneous channels, but will be increased to 8 by February 2021.

Electroencephalographic/Electromyographic Recordings*:* For tethered recordings, a Pinnacle system features four configurable channels for EEG, EMG, and biosensor data acquisition. Each channel features independent, adjustable gain and filter settings, and adjustable bias settings when configured as a biosensor channel. The system supports sampling rates from 200 - 2000 Hz with 14 bit resolution on each channel.

Behavioral Phenotyping and Metabolic Screening Equipment: The Karatsoreos lab has a dedicated TSE Phenomaster indirect calorimetry system for 16 cages. Phenomaster works within a *home cage* environment, with the capacity for habitat enrichment. The gas analyzers (oxygen and CO_2) have a resolution of one part per

million (0.0001%) or better, and water vapor analyzers with a resoluton of 0.001kPA. Fod and water uptake sensors are mounted on the roof of the cage, and have a resolution of 0.003g, or better, and can detect changes in uptake mass (resolvable difference before and after an event) of 0.005g or less. Body mass is measured automatically through an enrichment habitat suspended from a mass sensor. Infrared technology monitors animal activity in the X, Y, and Z planes. Importantly, the system is capabale of acquirng readings from all gas analyzers and other sensors at a maximum frequency of not less than 1Hz, and all raw data from all sensors is retained for later analysis. This system is housed within light-tight boxes, allowing for multiple light-dark cycles to be run at the same time in the system, providing increase throughput and the ability to run studies in parallel not just sequentially.

The Karatsoreos lab also has a dedicated EchoMRI-100 (EchoMRI Corp) Whole-body composition analyzer for mice and rats up to 100g. This system allows for the rapid (2min-4min) assessment of Lean-Mass, Fat-Mass, Total-Water, and Total-Body-Water.

Computers: Numerous computers for data collection including both PC and Macintosh systems. All computers are able to access the campus intranet as well as the Internet. Most computers have Microsoft Office suites. Several computers include software packages for Gene expression analysis, Statistical analysis (GraphPad), Circadian rhythms analysis (VitalView and Clocklab), and Behavioral anlysis (Noldus).

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: Agnes Middle Name C Last Name*: Lacreuse Suffix:

Position/Title*: Professor U of M
Organization Name*: University of Massachusetts Amherst
Department: Dept: Psychology and Brain Sci
Division: Coll: Natural Sciences
Street1*: Tobin Hall, Rm 531
Street2:
City*: Amherst
County:
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 01003-0000

Phone Number*: 413-545-2183 Fax Number:

E-Mail*: alacreuse@psych.umass.edu

Credential, e.g., agency login: eRA Commons User Name

Project Role*: PD/PI Other Project Role Category:

Degree Type: PhD Degree Year: 1994

Attach Biographical Sketch*: File Name: Agnes_C_Lacreuse_Biosketch.pdf

Attach Current & Pending Support: File Name:

Tracking Number: GRANT13241346

Funding Opportunity Number: PA-20-195 . Received Date: 2020-11-09T16:05:24.000-05:00

PROFILE - Senior/Key Person

Prefix: First Name*: Ilia Middle Name Last Name*: Karatsoreos Suffix:

Position/Title*: Assoc Professor U of M
Organization Name*: University of Massachusetts Amherst
Department: Dept: Psychology and Brain Sci
Division: Coll: Natural Sciences
Street1*: Morrill Science Center IV, Rm N216
Street2:
City*: Amherst
County:
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 01003-0000

Phone Number*: 413-577-9203 Fax Number:

E-Mail*: ikaratsoreos@umass.edu

Credential, e.g., agency login:

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PhD Degree Year:

Attach Biographical Sketch*: File Name: Ilia_Karatsoreos_Biosketch.pdf

Attach Current & Pending Support: File Name:

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Lacreuse, Agnès

eRA COMMONS USER NAME (credential, e.g., agency login): eRA Commons User Name

POSITION TITLE: Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University Paul Sabatier, Toulouse, France	DEA (MS)	1991	Behav Neurosci
University Paul Sabatier, Toulouse, France	Ph.D.	1994	Behav Neurosci
University of Georgia, Athens, GA	Postdoc	1995	Behav Neurosci
Emory University, Atlanta, GA	Postdoc	1997	Behav Neurosci

A. Personal Statement

The Lacreuse laboratory investigates neurocognitive function across the lifespan in nonhuman primate models of human aging, with a particular focus on sex differences and menopause. In an ongoing project supported by an NIH R01, we assess the role of sex differences in cognitive and brain aging, including Alzheimer's disease (AD)-like pathology, in a primate with a short lifespan, the marmoset, ideally suited for longitudinal studies. This project has revealed age- and sex-related differences in cognitive function in marmosets, with females exhibiting greater decline than males. In addition, sleep quality was worse in females than males. Furthermore, large individual differences in cognitive decline suggest that some marmosets exhibit pathological, rather than normal aging. Ongoing analyses of brain tissues of cognitively characterized monkeys provide preliminary support for an association between worsening cognitive performance and increased neuropathology. Based on these findings, the aim of our future studies is to address whether sleep fragmentation drives neuropathological processes and cognitive impairment in aging marmosets. ***As a first step towards this goal, the current R21 proposal designs a sleep fragmentation procedure for aged marmosets and examines its impact on cognitive function and measures of inflammation and metabolic function.*** The application combines expertise in primate cognitive aging (Lacreuse, PI) and circadian rhythms and metabolic function (Karatsoreos, Co-I). The findings should form the basis for a larger project to determine whether sleep fragmentation drives AD-like neuropathology in a nonhuman primate that naturally develops two hallmarks of AD.

B. Positions and Honors

Positions and Employment

1991-1994 Ph.D. student (Toulouse III), Center for Research in Cognitive Neuroscience, Marseille, France
1995-1997 Postdoctoral Fellow, Department of Psychology, University of Georgia, Athens, GA
1997-2002 Research Associate, Yerkes Natl Primate Res Ctr, Emory University, Atlanta, GA
2002-2006 Assistant Research Professor, Yerkes Natl Primate Res Ctr, Emory University, Atlanta, GA
2006-2012 Assistant Professor, Department of Psychology, University of Massachusetts, Amherst, MA
2012-2020 Associate Professor, Psychology and Brain Sci, University of Massachusetts, Amherst, MA
2020- Professor, Psychology and Brain Sci, University of Massachusetts, Amherst, MA

Other Experience and Professional Memberships

Member of professional societies

1999- Society for Neuroscience
2000- American Society of Primatologists
2001- International Primatological Society
2004- Society for Behavioral Neuroendocrinology
2009- Organization for the Studies of Sex Differences

Editorial boards

2011-2018 Review editor, *Frontiers in Neurodegeneration*
2012-2015 Editorial board member, *Journal of Primatology*
2016-2018 Editorial board member, *Scientific Reports*
2015-2020 Review editor, *Frontiers in Aging Neuroscience*
2015- Editorial board member, *Hormones and Behavior*
2018- Editorial board member, *American Journal of Primatology*
2020- Associate editor, *Frontiers in Aging Neuroscience*

NIH study sections

2012 Biobehavioral Regulation, Learning and Ethology, BRLE (*ad hoc*)
2013 Special Emphasis Panel ZRG1 ETTN-L (03) M (*ad hoc*)
2014 Special Emphasis Panel, ZRG1-IFCN-Q (*ad hoc*)
2014 Cognition and Perception (*ad hoc*)
2015 BRLE (*ad hoc*)
2015 Special Emphasis Panel, ZRG1 IFCN-Q (55) (*ad hoc*)
2015 Neuroendocrinology, Neuroimmunology, Rhythms and Sleep, NNRS (*ad hoc*)
2016 Special Emphasis Panel, BBBP-Y (45) R (*ad hoc*)
2016 NNRS (*ad hoc*)
2016 BRLE (*ad hoc*)
2020 2020 NIH, BRAIN Initiative Marmoset Colonies and Coordination Centers ZMH1 ERB-G (03) R
2017-2023 Permanent member of the NNRS study section

Honors

1991-1994 Doctoral Fellowship from the French Ministry of Research
1995-1996 Post-doctoral fellowship, Fondation Fyssen
1996-1997 Post-doctoral fellowship, Fondation pour la Recherche Médicale
2001 Travel award, American Aging Association, Madison, WI
2008 UMass College of Social and Behavioral Science Grant Award
2010 Travel award, UMass Center for Teaching
2011 Mellon mentoring award
2013-2014 Center for Research on Families Research Scholar

C. Contributions to Science

1. Estrogens and cognitive aging in nonhuman primates: My early work in collaboration with Redacted by agreement Redacted by agreement at Emory University focused on the effects of estradiol replacement on cognitive function in female nonhuman primates. In a series of studies in the rhesus monkey for which I received NIH funding, we demonstrated that selective domains of cognitive function are sensitive to ovariectomy and estrogen replacement in aged females. In contrast, young adult females show minimal effects of estrogen replacement, with the exception of tasks that involve the processing of social cues. These age differences in the sensitivity to estrogens have been confirmed by other research groups and remain to be explained at a mechanistic level. My most recent work proposed a primate model with a short lifespan, the common marmoset, as a complementary primate model for cognitive aging. We conducted the first study on the effect of estrogens on cognition in this species. We are also developing the marmoset as a new model for studying menopausal symptoms, including sleep disturbances, thermoregulation and cognitive deficits. Our latest research demonstrates a crucial role of neuroestrogens in these processes.

a. **Lacreuse, A.**, Chang, J., Metevier, C., LaClair, M., Meyer, J.S. & Ferris, C. (2014) Oestradiol modulation of cognition in adult female marmosets (*Callithrix jacchus*). *Journal of Neuroendocrinology,* 26, 296-309. PMCID: PMC4040528.
b. **Lacreuse, A.**, Mong, J.A. & Hara, Y. (2015) Neurocognitive effects of estrogens across the adult lifespan in nonhuman primates: State of knowledge and new perspectives. *Hormones and Behavior,* 74,157-66.
c. Gervais, N. J., Mong, J. A. & **Lacreuse, A.** (2017). Ovarian hormones, sleep and cognition across the adult female lifespan: An integrated perspective. *Frontiers in Neuroendocrinology*, 47, 134-153.
d. Gervais, N.J., Remage-Healey, L., Starrett, J.R., Pollak, D. J., Mong, J.A. & **Lacreuse, A.** (2019) Adverse effects of aromatase inhibition on the brain and behavior in a nonhuman primate. *Journal of Neuroscience*, 39, 918-928. PMCID: PMC6382974.

2. Androgens and cognition in male nonhuman primates: My lab was the first to investigate the effects of androgens on cognition in adult nonhuman primates. Our studies in young and older male macaques showed that testosterone has no or minimal effect on cognitive function, but enhances selective aspects of emotional reactivity.

a. **Lacreuse, A.**, Chiavetta, M.R., Shirai, A.A., Meyer, J.S., Grow, D.R., 2009. Effects of testosterone on cognition in young adult male rhesus monkeys. *Physiology & Behavior* 98, 524-531.
b. **Lacreuse, A.**, King, H.M., Kurdziel, L.B., Partan, S.R., Caldwell, K.M., Chiavetta, M.R., Millette, M.M., Meyer, J.S., Grow, D.R., 2010. Testosterone may increase selective attention to threat in young male macaques. *Hormones and Behavior* 58, 854-863.
c. King, H.M., Kurdziel, L.B., Meyer, J.S., **Lacreuse, A.**, 2012. Effects of testosterone on attention and memory for emotional stimuli in male rhesus monkeys. *Psychoneuroendocrinology* 37, 396-409.
d. Kelly, B., Maguire-Herring, V., Rose, C.M., Gore, H.E., Ferrigno, S., Novak, M.A., **Lacreuse, A.**, 2014. Short-term testosterone manipulations do not affect cognition or motor function but differentially modulate emotions in young and older male rhesus monkeys. *Hormones and Behavior* 66, 731-742. PMCID: PMC4262694.

3. Sex differences in cognitive aging in nonhuman primates: Additional contributions demonstrated that biological sex modulates cognitive aging in nonhuman primates. My collaborators and I showed that young male rhesus monkeys outperform females in a task of spatial working memory, but that older males lose this advantage, suggesting greater age-related decline in males than in females. In addition, we demonstrated that males show greater age-related decline than females in fine motor function. These papers remain to date the only studies on sex differences in cognition and motor function in aged nonhuman primates. Based on these data, my lab is currently investigating longitudinal change in cognition, stress reactivity, motor function and brain function (resting state brain networks) in male and female as they age to middle-age to old age. Recent data from this project indicate sex differences in executive function that remain stable across two years, with a male advantage related to glutamate availability in the prefrontal cortex, and patterns of resting state connectivity.

a. **Lacreuse, A.,** Moore, C. M., LaClair, M., Payne, L., & King, J. A. (2018). Glutamine/Glutamate (Glx) concentration in prefrontal cortex predicts reversal learning performance in the marmoset. *Behavioural Brain Research,* 346, 11-15. PMCID: PMC5860997
b. Workman, K. P., Healey, B., Carlotto, A. & **Lacreuse, A.** (2018). One-year change in cognitive flexibility and fine motor function in middle-aged male and female marmosets (*Callithrix jacchus*). *American Journal of Primatology*, 81, e22924. PMCID: PMC6816503.
c. LaClair, M.G, Febo, M., Nephew, B., Gervais, N.J, Poirier, G., Workman, K.P., Chumachenko, S., Payne, L., Moore, M.C., King, J.A. & **Lacreuse, A.** (2019). Sex differences in cognitive flexibility and resting brain networks in middle-aged marmosets. *eNEURO, 6 (4)*. PMCID: PMC6658914.
d. Nephew, B.C., Febo, M., Cali, R., Workman, K.P., Payne, L., Moore, M.C., King, J.A. & **Lacreuse, A.** (2020). Robustness of sex differences in functional connectivity over time in middle-aged marmosets. *Scientific Reports* **10,** 16647. PMCID: PMC7538565.

4. Comparative cognitive aging Our species is characterized by a uniquely long post-reproductive longevity among primates. Important questions in the field of aging are to understand whether the extension of longevity past the fertile years is associated with reduced age-related cognitive decline in humans and, in contrast, whether Alzheimer's disease is linked to extreme longevity and unique to our species. Investigating cognitive aging and menopause in our closest relative, the chimpanzee (*Pan troglodytes*), as well as other primates is key to answering these questions. In a series of studies in collaboration with James Herndon (Emory University), we provided evidence that menopause is a rare phenomenon in female chimpanzees and described for the first time patterns of cognitive and motor aging in our closest relative. In a recent review, we find that executive function is a hallmark of cognitive aging across primate orders.

a. **Lacreuse, A.**, Chennareddi, L., Gould, K.G., Hawkes, K., Wijayawardana, S.R., Chen, J., Easley, K.A., Herndon, J.G. (2008). Menstrual cycles continue into advanced old age in the common chimpanzee (*Pan troglodytes*). *Biology of Reproduction* 79, 407-412. PMCID: PMC2547989
b. **Lacreuse, A.**, Russell, J.L., Hopkins, W.D., Herndon, J.G. (2014). Cognitive and motor aging in female chimpanzees. *Neurobiology of Aging* 35, 623-632. PMCID: PMC3864620.
c. **Lacreuse, A.**, Parr, L., Chennareddi, L., & Herndon, J.G. (2018). Age-related decline in cognitive flexibility in female chimpanzees. *Neurobiology of Aging*, 72, 83-88. PMCID: PMC6215734
d. **Lacreuse, A.**, Raz, N., Schmidtke, D., Hopkins, W.D & Herndon, J.G. (2020). Age-related decline in executive function as a hallmark of cognitive ageing in primates: an overview of cognitive and neurobiological studies. *Philosophical Transactions of the Royal Society B.* PMCID: PMC7540957.

Complete list of Published Work in MyBibliography
https://www.ncbi.nlm.nih.gov/myncbi/1D3hxxac-bL/bibliography/public/

D. Research Support

Ongoing Research Support

1R01AG046266-01A1 (Lacreuse, PI) 9/1/14-5/31/21
Sex differences in cognitive aging: a primate model NCE
The goal of this project is to use the common marmoset to examine sex differences in neurocognitive aging
Role: PI

R21 grant AG053841 (Lacreuse and Mong, MPI) 7/15/17-5/31/21
Sleep, hot flashes and cognition: a nonhuman primate model for menopausal symptoms NCE
The goal of this project is to develop a marmoset model for menopausal symptoms, including sleep disturbances, cognitive dysfunction and hot flashes
Role: PI

F32 NRSA AG064925 (Rothwell, PI) 12/1/19-11/30/22
"Behavioral and Neural Indicators of Prodromal Alzheimer's Disease"
The goal of this fellowship is to identify behavioral predictors of brain pathology in aged marmosets
Role: Sponsor

Completed Research Support (past 3 years)

R01 AG046266-5S1 9/1/18-5/31/19
Alzheimer's disease-like pathology in marmosets (Lacreuse, PI)
Administrative supplement to the grant "Sex differences in cognitive aging: a primate model"
Role: PI

R01 grant AG046266-04S1 (Lacreuse, PI) 7/1/17-5/31/18
Administrative supplement to the grant "Sex differences in cognitive aging: a primate model"
Role: PI

R21 AG 053841-2S1 9/15/18-5/31/19
Administrative supplement to the grant "Sleep, hot flashes and cognition: a nonhuman primate model for menopausal Symptoms". The goal of this project is to examine whether the prodrug DHED has the same effect as estradiol on sleep disturbances, cognitive dysfunction and hot flashes
Role: PI

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Ilia N. Karatsoreos

eRA COMMONS USER NAME (credential, e.g., agency login): eRA Commons User Name

POSITION TITLE: Associate Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date	FIELD OF STUDY
University of Toronto, Toronto, Ontario, Canada	Hon.BSc.	06/2001	Psychology/Neuroscience
Columbia University, New York, NY, USA	PhD	02/2008	Psychology/Neuroscience
The Rockefeller University, New York, NY, USA	Postdoctoral	12/2011	Neuroendocrinology

A. Personal Statement:

I am an Associate Professor in Psychological and Brain Sciences at the University of Massachusetts Amherst. The focus of my laboratory is on understanding how homeostatic systems are integrated and regulated, and the consequences of disrupting these systems on health. Specifically, we are interested in how altered circadian rhythms and sleep impact brain-body interactions in health and disease. I have characterized a form of circadian disruption that results in obesity and metabolic dysregulation, and neurobehavioral deficits (Karatsoreos et al., 2011). My laboratory has built upon this work, showing that our model of circadian disruption leads to significant reduction in sleep quality, without sleep deprivation (Phillips, et al., 2015), altered neurometabolic processes (Wallace et al., 2020) and changes in central, peripheral, and behavioral responses to immune challenge (Pearson et al., 2020; Phillips et al., 2015). In addition to my work in circadian rhythms, I also probe the effects of altered glucocorticoid signaling on metabolic function (Karatsoreos et al., 2010; Bowles et al., 2015), and have been exploring how endocannabinoids (eCB) can modulate these effects (Bowles et al., 2015). Currently, the lab has undertaken a detailed characterization of the effects of circadian desynchronization on hepatic function, including physiological and gross morphological assessments, as well as effects on the liver transcriptome (e.g. clock genes, metabolic genes, eCB genes) both *in vivo* and *ex vivo*.

A key principle of work in my lab is understanding the underlying mechanisms by disrupted homeostatic systems contribute to negative mental and physical health outcomes. My lab employs an integrative strategy, from cell and molecular (e.g. gene and protein expression) to whole animal (e.g. *in vivo* imaging, detailed metabolic and behavior analyses), approaches, with a keen eye on brain-periphery interactions. I have significant expertise in studying metabolism and metabolic function. We also undertake extensive work understanding the interactions between circadian rhythms/sleep and stress on immune function, using high-throughput multiplexed arrays along with more standard assessments of inflammatory measures in brain and periphery. For this project, my group will bring this expertise to bear in support of Dr. Lacreuse by using high-throughput multiplex approaches to help understand changes in metabolic and immune related biomarkers over time in different bodily tissues of marmoset subjects, and provide guidance on the interpretation of data collected by these methods.

B. Positions and Honors:

Positions

2001-2008	Graduate Fellow, Columbia University (Rae Silver)
2008-2011	Postdoctoral Fellow, The Rockefeller University (Bruce McEwen)
2012-2017	Assistant Professor, Washington State University, Pullman
2016-2020	Associate Director of Graduate Studies, IPN, Washington State University, Pullman
2017-2020	Associate Professor, Washington State University, Pullman
2020-Present	Associate Professor, University of Massachusetts Amherst

Honors and Awards

W.M. Keck Foundation Grantee (2020-2023)
National Science Foundation CAREER Award (2016-2021).
Dean's Award for Outstanding Faculty Research, Washington State University (2016)
Postdoctoral Fellowship, Canadian Institutes of Health Research (2008-2011)
Young Investigator Award, Society for Behavioral Neuroendocrinology (2006)
Predoctoral Fellowship, Natural Sciences and Engineering Research Council of Canada (2002-2006).

C. Contributions to Science

- **Helped to identify commonalities in structure and function of the mammalian brain clock.**

Central Findings: My work revealed that the mouse SCN was nearly identical to the structure of the rat SCN, and using functional assays determined that the functional organization of the SCN was also conserved. Specifically, I showed that the light-inducible region of the mouse SCN was delineated by a population of gastrin-releasing peptide (GRP) containing neurons in the central and lateral SCN, that were distinct from the highly rhythmic arginine vasopressin (AVP) neurons of the more medial SCN (Karatsoreos et al., 2004). Moreover, I showed that GRP receptors had unique distribution within the SCN (Karatsoreos et al., 2006) that helped explain how GRP communicates photic information from the eye to the brain clock.

Influence of Findings: This work had several impacts on the field. First, it helped clarify that the structure of the SCN was responsible for its unique function, given that this organization was highly conserved between species (Yan et al., 2008). This contributed to the development of mathematical models to integrate cellular, molecular, and genetic data that have been collected over the past decades. The second impact of this work was that it helped to explain the pharmacological effects of GRP demonstrated over the previous 20yrs. While it was known the SCN had many different cell types, our work helped solidify a theoretical model for how SCN cells create a coherent output, and how light could impact the function of the clock.

Karatsoreos, I.N., Yan, L., LeSauter, J., Silver, R. (2004) "Phenotype Matters: Identification of Light Response Cells in Mouse SCN". *Journal of Neuroscience*, 24 (1): 68-75.

Karatsoreos, I.N., Romeo, R.D., McEwen, B.S., Silver, R. (2006) "Diurnal Regulation of the Gastrin-releasing Peptide Receptor in Mouse Suprachiasmatic Nucleus" *European Journal of Neuroscience*, 23(4):1047–1053.

Yan, L., **Karatsoreos, I.**, LeSauter J., Welsh D.K., Kay, S., Foley, D., Silver R. (2008) "Exploring Spatiotemporal Organization of SCN Circuits." *Cold Spring Harbor Symp. on Quant. Biol.* Jan 1; 72:527-541.

- **Clarified a role for gonadal hormones in the regulation of circadian rhythms and behavior.**

Central Finding: I determined that androgen receptors were localized to the mouse SCN, and specifically that they were localized to the GRP subregion that I had previously identified in my earlier published work (Karatsoreos et al., 2007). Moreover, I demonstrated in a series of papers that androgens had significant impact on the functional output of the SCN, specifically identifying how it androgens alter the response to light (Karatsoreos et al., 2011). In addition, I provided a potential framework for how some sex differences in circadian function may be related to androgen receptor expression within the SCN (Iwahana et al., 2008).

Influence of Findings: These findings helped identify a novel role for steroid hormone modulation of neurobehavioral function. This resulted in several "News and views" articles in the journal *Endocrinology*, which is one of the flagship journals in the field. In addition, I was invited to provide a review to *Endocrinology* highlighting how our findings related to existing findings. This work contributed to my "Young Investigators Award" from the Society for Behavioral Neuroendocrinology.

Karatsoreos I.N., Wang, A., Sasanian, J., Silver, R. (2007). "A Role for Androgens in Regulating Circadian Behavior and the Suprachiasmatic Nucleus." *Endocrinology*, 148(11):5487-95.

Karatsoreos, I.N. and Silver, R. (2007) "The neuroendocrinology of the suprachiasmatic nucleus as a conductor of circadian time in mammals" *Endocrinology*. Dec; 148 (12):5640-7

Karatsoreos, I.N., Butler, M.P., LeSauter, J., Silver, R. (2011) "Androgens modulate structure and function of the suprachiasmatic nucleus brain clock." *Endocrinology* 152: 1970-1978.

Iwahana E.*, **Karatsoreos, I.N.***, Shibata, S., Silver, R. (2008) "Gonadectomy reveals sex differences in circadian rhythms and SCN androgen receptors in mice." *Hormones and Behavior*. 53 (3):422-430.

- **Contributed to our understanding of how stress mediators affect metabolism and brain function**

Central Finding: My work has helped clarify how mediators of environmental and psychological stress affect both short- and long-term function, particularly on the processes of adaptation to stress. Specifically, we showed disrupting normal corticosterone levels (and rhythms) causes profound metabolic dysregulation. Current work in my lab shows disrupting dynamic glucocorticoid responses causes a "mismatch" of behavioral, hormonal, and neural stress responses, with ramifications for long term function (Kinlein, 2015; 2018). In addition, we have investigated how excess glucocorticoids drive changes in metabolic function.

Influence of Findings: Our work builds on the concept of allostatic load, or the cumulative "wear and tear" on brain and body systems. Specifically, my work has helped to define circadian rhythms (and circadian desynchronization) as an important regulatory system in allostatic responses. I have been invited to contribute several review papers that outline how our proposed model plays a role in the broader context of mental and physical health. I have also organized, chaired, and participated in many international scientific meetings presenting our published and new findings, including talks at both national and international conferences. Importantly, these contributions span a variety of areas of research, including metabolism/obesity, circadian rhythms/sleep, and stress research, highlighting their interdisciplinary applicability and appeal.

Kinlein, S.A., Wilson, C.D., Savenkova, M., **Karatsoreos, I.N.** (2015) "Disruption of HPA axis leads to divergent neurobehavioral responses to stress." *Frontiers in Psychiatry.* 6:31. PMID: 25821436.

McEwen, B.S., Bowles, N.P., Gray, J.D., Hill, M.N., Hunter, R.G., **Karatsoreos, I.N.,** Nasca, C. (2015) "Mechanisms of stress in the brain." *Nature Neuroscience*. 18; 1353-1363. PMID: 26404710.

Kinlein, S.A., Shahanoor, J., Romeo, R.D., **Karatsoreos, I.N.** (2017) "Chronic corticosterone treatment during adolescence has significant effects on metabolic measures and skeletal development in male C57BL6/N mice". *Endocrinology*. 158:7; 2239-2254. PMID: 28510653

Kinlein, S.A., Phillips, D.J., Keller, C., **Karatsoreos, I.N.** (2019) "Role of corticosterone in altered neurobehavioral responses to acute stress in a model of compromised hypothalamic-pituitary-adrenal axis function." *Psychoneuroendocrinology*. 102; 248-255. PMID: 30594817

- **Investigating how disrupted circadian clocks impact health and disease in brain and periphery.**

Central Finding: I initially determined that mice housed in LD10:10 showed disrupted locomotor and body temperature rhythms, increased weight gain, elevated leptin and insulin levels, and increased triglycerides. In the brain, I found that Layer II/III pyramidal neurons in the medial prefrontal cortex (mPFC) atrophied, and that several mPFC related behaviors were significantly impacted, including impulsive-like behaviors and reduced cognitive flexibility. This was followed by our work demonstrating our model does not result in sleep deprivation, but instead reduced sleep quality (Phillips et al., 2015). We have extended this to demonstrate significant changes in PFC neurometabolic function (Wallace et al., 2020), as well as altered immune and behavioral responses to immune challenge (Pearson et al., 2020; Phillips et al., 2015).

Influence of Findings: Our work is at the forefront of exploring how environmental disruption leads body-wide effects, garnering attention from many fields because of its cross-cutting interdisciplinary nature. I have been invited to present these data at several national and international conferences, and the Phillips (2015) paper is was selected as one of the "most significant findings in sleep research for 2015" at the international SLEEP meeting. Current funding (NIH, R01 and NSF CAREER) supports this ongoing work.

Karatsoreos, I.N., Bhagat, S.M., Bloss, E.B., Morrison, J.H., McEwen, B.S. (2011) "Disruption of circadian clocks has ramifications for metabolism, brain and behavior." *PNAS;* 108(4):1657-62.

Pearson, G.L., Savenkova, M., Barnwell, J.J., **Karatsoreos, I.N.** (2020) "Circadian desynchronization alters metabolic and immune responses following lipopolysaccharide inoculation in male mice." *Brain Behav Immun. 88:220-229. Epub 2020 May 12.* PMID: 32413558

Wallace, N.K., Pollard, F., Savenkova, M., **Karatsoreos, I.N.** (2020) "Effect of Aging on Daily Rhythms of Lactate Metabolism in the mPFC of Male Mice." *Neuroscience. (20)30475-9.* PMID: 32717298.

Phillips, D.P., Savenkova, M., **Karatsoreos, I.N.** (2015) "Environmental disruption of the circadian clock leads to altered sleep and immune responses in mouse." *Brain, Behav, Immunity.* Jul*;47:14-23.* PMID: 25542734.

Publications URL: **If link does not open, please copy and paste into your browser**
http://www.ncbi.nlm.nih.gov/sites/myncbi/ilia.karatsoreos.1/bibliography/41567840/public/?sort=date&direction=descending

D. Research Support.

Ongoing: *No projects will have significant overlap should this proposal be funded.*

Private Source — Karatsoreos (Co-PI) — 01/01/2020 – 12/31/2023
"A Brain Self Defense Mechanism: Keeping Time to Guard from the "threat" Within.
Determine the functional consequences of bacterial cell wall components and signaling molecules in the brain on sleep and circadian rhythms.
Role: Co-PI

1R01 DK119811-01A1 — Karatsoreos (PI) — 12/15/2018 – 11/30/2023
National Institute of Diabetes and Digestive and Kidney Diseases
"The role of endocannabinoids in circadian disruption induced metabolic dysregulation"
Determine the role of endocannabinoids in modulating the central vs. peripheral mechanisms of circadian disruption induced metabolic dysregulations.
***Role:* PI**

NSF CAREER Award — Karatsoreos (PI) — 03/01/2016 – 02/28/2021
National Science Foundation.
"Biological Timing and Brain Circuits: Circadian influences on Prefrontal Cortex function".
Determine the role of circadian rhythms in regulation of PFC structure and function.
***Role*: PI**

Completed (3yrs):

1R21DA043722-01A1 — McLaughlin (PI) — 09/01/2017 – 08/30/2019
National Institute of Drug Abuse
"Effects of developmental cannabis exposure on prefrontocortical structure and function."
Investigate the effects of early life exposure to cannabis vapor on the structure and function of the prefrontal cortex.
***Role:* Co-I**

1R21AG050054-01A1 — Karatsoreos (PI) — 08/01/2015 – 03/30/2018
National Institute of Aging
"Environmentally driven metabolic dysregulation as a model of accelerated aging"
Determine if circadian disruption causes aging-like changes in brain and periphery
***Role:* PI**

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 02/28/2023

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ○ Yes ● No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

○ Yes ○ No

If "No" to AVMA guidelines, describe method and provide scientific justification

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period *Anticipated Amount ($) *Source(s)

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ○ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Human Fetal Tissue Section

*Does the proposed project involve human fetal tissue obtained from elective abortions? ○ Yes ● No

If "yes" then provide the HFT Compliance Assurance

If "yes" then provide the HFT Sample IRB Consent Form

5. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ○ Yes ○ No

If the answer is "Yes" then please answer the following:

*Previously Reported: ○ Yes ○ No

6. Change of Investigator/Change of Institution Section

❑ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

Funding Opportunity Number: PA-20-195 . Received Date: 2020-11-09T16:05:24.000-05:00

PHS 398 Modular Budget

OMB Number: 0925-0001
Expiration Date: 02/28/2023

Budget Period: 1

Start Date: 07/01/2021 End Date: 06/30/2022

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium Indirect (F&A)*	150,000.00
Consortium Indirect (F&A)	0.00
Total Direct Costs*	150,000.00

B. Indirect (F&A) Costs

	Indirect (F&A) Type	Indirect (F&A) Rate (%)	Indirect (F&A) Base ($)	Funds Requested ($)
1.	Standard Federal MTDC	59.50	150,000.00	89,250.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS,Michael Stanco (212) 264-0920

Indirect (F&A) Rate Agreement Date

Total Indirect (F&A) Costs: 89,250.00

C. Total Direct and Indirect (F&A) Costs (A + B)

Funds Requested ($): 239,250.00

Funding Opportunity Number: PA-20-195. Received Date: 2020-11-09T16:05:24.000-05:00

PHS 398 Modular Budget

Budget Period: 2

Start Date: 07/01/2022 End Date: 06/30/2023

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium Indirect (F&A)*	125,000.00
Consortium Indirect (F&A)	0.00
Total Direct Costs*	125,000.00

B. Indirect (F&A) Costs

	Indirect (F&A) Type	Indirect (F&A) Rate (%)	Indirect (F&A) Base ($)	Funds Requested ($)
1.	Standard Federal MTDC	59.50	125,000.00	74,375.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS,Michael Stanco (212) 264-0920

Indirect (F&A) Rate Agreement Date

Total Indirect (F&A) Costs: 74,375.00

C. Total Direct and Indirect (F&A) Costs (A + B)

Funds Requested ($): 199,375.00

Funding Opportunity Number: PA-20-195. Received Date: 2020-11-09T16:05:24.000-05:00

PHS 398 Modular Budget

Cumulative Budget Information

1. Total Costs, Entire Project Period

Section A, Total Direct Cost less Consortium Indirect (F&A) for Entire Project Period ($)	275,000.00
Section A, Total Consortium Indirect (F&A) for Entire Project Period ($)	0.00
Section A, Total Direct Costs for Entire Project Period ($)	275,000.00
Section B, Total Indirect (F&A) Costs for Entire Project Period ($)	163,625.00
Section C, Total Direct and Indirect (F&A) Costs (A+B) for Entire Project Period ($)	438,625.00

2. Budget Justifications

Personnel Justification	PHS_ModBud_PersonJustif.pdf
Consortium Justification	
Additional Narrative Justification	

Tracking Number: GRANT13241346
Funding Opportunity Number: PA-20-195. Received Date: 2020-11-09T16:05:24.000-05:00

Personnel Justification: University of Massachusetts Amherst

Senior Personnel:

Agnès Lacreuse, Ph.D. Principal Investigator (EFFORT each year). Dr. Lacreuse is a professor in the department of Psychological and Brain Sciences at UMass Amherst. She will be responsible for maintaining the marmoset colony and will direct all aspects of the Specific Aims 1-2 at UMass Amherst, in close collaboration with co-I Ilia Karatsoreos. She will supervise Ms. Edward, graduate student and will be responsible for data publication.

Ilia Karatsoreos, **Ph.D.** Co-Investigator EFFORT each year) is an Associate professor in the Department of Psychological and Brain Sciences at the UMass Amherst. Dr. Karatsoreos has expertise is circadian biology, metabolism, and stress physiology, and he will be responsible for the design of the biomarker assay experiments, and data analysis and manuscript preparation associated with this aspect of the project. He will be supervising the research associate and provide necessary training in both experimental techniques and experimental protocols to Ms Edwards.

Other Personnel:

Redacted by agreement in the UMass Neuroscience and Behavior program EFFORT EFFORT each year) will participate in the design of the marmoset experiment, run the physiological assays with the research associate and Dr. Karatsoreos' assistance and participate in data analysis and manuscript preparation.

Redacted by agreement in the Lacreuse lab (EFFORT each year) will maintain the marmoset colony and run all procedures involving marmosets.

TBN Research associate in the Karatsoreos lab (1.0 calendar month each year) will directly undertake the biomarker studies in the project, working under the supervision of Dr. Karatsoreos. S/he will be responsible for data collection, quality control and data analysis.

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 02/28/2023

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	
Research Plan Section	
2. Specific Aims	PHS_ResearchPlan_SpecificAims.pdf
3. Research Strategy*	PHS_ResearchPlan_ResearchStrategy.pdf
4. Progress Report Publication List	
Other Research Plan Section	
5. Vertebrate Animals	PHS_ResearchPlan_VertebrateAnimals.pdf
6. Select Agent Research	
7. Multiple PD/PI Leadership Plan	
8. Consortium/Contractual Arrangements	
9. Letters of Support	
10. Resource Sharing Plan(s)	PHS_ResearchPlan_ResourceSharingPlans.pdf
11. Authentication of Key Biological and/or Chemical Resources	
Appendix	
12. Appendix	

Funding Opportunity Number: PA-20-195. Received Date: 2020-11-09T16:05:24.000-05:00

Specific Aims

Sleep plays a crucial role in brain function. Sleep disturbances impair learning and memory, attention, executive function and emotional regulation. In the brain, sleep disruptions have pro-inflammatory effects and increase the levels of amyloid β (Aβ) and tau proteins. Importantly, sleep disturbances, specifically sleep fragmentation, are frequent in the elderly, contribute to age-related cognitive decline and are thought to be a core component of Alzheimer's disease (AD) pathophysiology. ***However, whether sleep fragmentation causes cognitive impairment and neuropathology remains unclear.*** Indeed, human studies are unable to determine whether sleep fragmentation precedes or follows the development of AD pathology.

Studies in animal models are necessary to investigate this issue. Cognitive deficits following sleep disturbances can be successfully modeled in rodents. However, unlike humans, mice and rats are nocturnal, with polyphasic sleep (multiple sleep-wake cycles across a 24 h period), and differ substantially from humans in their cognitive and brain aging phenotypes. Using a more translational animal model with regards to sleep, cognitive function and neuropathology would likely provide new insights into the role of sleep disturbances in driving AD-like neuropathology, with great potential for uncovering new preventative or therapeutic targets for AD. The common marmoset (*Callithrix jacchus*) is ideally suited as such a model. This small diurnal nonhuman primate exhibits monophasic sleep, is able to perform a range of cognitive tasks, shows age-related decline in several cognitive domains and possesses two hallmarks of AD neuropathology, Aβ deposition and accumulation of hyperphosphorylated tau in the brain. In addition, its relatively short lifespan of about 10-12 years is ideal for longitudinal studies. ***As a first step towards the validation of this model for future studies focusing on the relationships between chronic sleep fragmentation, AD-neuropathology and cognitive impairment, this proposal designs an experimental sleep fragmentation procedure adapted to marmosets that mimics sleep fragmentation observed in AD.***

Middle-aged marmoset will be fitted with an actigraphy device to monitor sleep/wake patterns. The monkeys will be trained on a battery of cognitive tasks administered on touchscreens in their home cage. After baseline recording of sleep and cognitive performance, they will be randomly assigned to a sleep fragmentation (SF) or undisturbed (control) sleep group. The SF group will be progressively exposed to longer periods of disrupted sleep (40% of baseline sleep) whereas the control group will be kept undisturbed. Changes in cognitive performance and physiological measures of inflammation and metabolic function will be assessed throughout. ***The overarching hypothesis that chronic SF drives cognitive impairment, dysregulates metabolism and increases inflammatory status in a nonhuman primate will be tested via the following aims:***

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 levels of orexin, Aβ and tau levels. We hypothesize that SF will increase inflammatory processes and impair metabolism and that these changes will correlate with cognitive impairment.

The findings should validate the aged marmoset as a translational animal model to study the mechanisms by which SF drives AD-like neuropathology and cognitive impairment. The availability of this preclinical model will be crucial for identifying new preventative or therapeutic targets for AD.

A. SIGNIFICANCE

Alzheimer's disease (AD) is one of the most pressing challenges of our time. The aged population in the United States is estimated to reach 88 million by 2050, with 13.8 million developing AD[1,2]. Despite these alarming projections and many decades of research, treatments to slow, prevent or cure the disease are still elusive. AD is characterized by increasingly severe cognitive impairment that eventually interferes with everyday life activities and two neuropathological hallmarks: the progressive accumulation in the brain of extracellular plaques made of amyloid β protein (Aβ), and of intracellular neurofibrillary tangles made of hyperphosphorylated tau (p-tau) protein. Associated changes include widespread inflammation and severe brain atrophy. ***Importantly, neuropathological changes can be observed 15-20 years before any cognitive symptoms.*** This preclinical period is key to identify predictors of AD and design interventions to slow disease progression. In recent years, animal and clinical studies have highlighted sleep disruptions as a core component of AD[3,4]. Evidence is rapidly mounting for a bidirectional link between sleep disturbances, cognitive impairment and Aβ and tau deposition in the brain. ***However, human studies cannot determine whether sleep disruptions precede or follow the development of AD neuropathology. Characterizing the effects of sleep disruptions on neuropathology and cognition in animal models are key to uncover novel preventative or therapeutic targets for AD.***

For the past 50 years, rodents have been the models of choice to study the neurobiology of sleep and its impact on cognition[5]. This body of research has led to major advances in our understanding of the effect of sleep on brain function. Landmark studies in AD transgenic mice (Tg2576) documented that Aβ levels in the brain interstitial fluid were 25% higher during the waking phase than during the inactive phase[6]. Following sleep disruption (4h for 21 days), Aβ plaques increased by two-fold, an effect attenuated by the administration of an orexin receptor antagonist[6]. Later studies revealed that Aβ proteins and tau oligomers were transported from the interstitial fluid space out of the brain via the glymphatic system[7] and that slow wave sleep enhanced glymphatic Aβ clearance from the brain when compared to wakefulness[8]. Aging of the glymphatic system is now regarded as a key factor in AD and other neurological diseases[9]. Interestingly, many of the human data support the rodent findings: Aβ levels in cerebrospinal fluid (CSF) show diurnal variation, with higher levels found during the day than during the night[10] and sleep disturbances affect both Aβ and tau levels: a single night of sleep deprivation is sufficient to increase Aβ accumulation in the hippocampus (HPC) and thalamus[11] as well as $A\beta_{42}$ in CSF[12], while 36h of sleep deprivation leads to a 25-30% overnight increase in CSF Aβ[13]. Furthermore, poor sleep quality is associated with increased CSF tau[14], while sleep deprivation increases CSF tau and tau pathology spreading[15,16].

It is now critical to determine whether sleep disruptions <u>drive</u> neuropathological processes in the primate brain and identify underlying mechanisms. Such studies should be carried out in an animal model that closely recapitulates the human phenotype in terms of sleep architecture, cognitive function and AD neuropathology. The common marmoset (Callithrix jacchus), is ideally suited as such a model. Unlike rodents, marmosets are diurnal, with monophasic sleep and their sleep patterns are remarkably similar to those of humans[17-21]. Marmosets have a well-developed brain and are able to perform a range of cognitive tasks[22,23]; in addition, their short life expectancy of about 10 years[24] is ideal for longitudinal studies. Marmosets show signs of aging by the age of 8, an age at which declines in working memory[25] and executive function (see preliminary data) are observed. Importantly, marmosets exhibit two hallmarks of AD neuropathology, cortical Aβ deposition[26] and p-tau[27], a major component of neurofibrillary tangles. In one study[27], the accumulation of p-tau progressed from the entorhinal cortex to the HPC region with increasing age, consistent with the human pattern. ***Although sleep is well characterized in marmosets[18,20,28-30], the effects of sleep disruptions on cognitive function and neuropathology have not been studied in this species. As a first step towards the validation of this model for future studies focusing on neuropathological processes, this proposal tests the hypothesis that chronic experimental sleep fragmentation (<u>SF</u>) causes cognitive impairment and increases inflammatory and metabolic dysfunction in the aged marmoset.***

B. INNOVATION

Several aspects of this project are highly innovative (1) this study will be the first to investigate how chronic SF that mimics fragmented sleep in AD affects cognitive performance in a nonhuman primate; (2) .we develop a novel procedure for chronic SF studies in nonhuman primates; (3) we develop a wireless homecage testing for longitudinal tracking of cognitive performance; (4) we use cutting-edge technology for assessing peripheral and central markers of inflammation and metabolism; (5) we include sex as a variable, a variable often overlooked in nonhuman primate studies.

C. APPROACH

Overview: 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[31]. 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 experience[32,33], we 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 3 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, Aβ and tau levels will be measured (Aim 2).

Choice of Animal model: The common marmoset (*Callithrix jacchus*) is an NHP becoming increasingly attractive for neuroscience[34-36], due to its small body size (300-500 g), a short lifespan (10-12 years), and the breadth of its behavioral repertoire[37]. The marmoset has a brain with a well conserved structural and functional organization when compared to the macaque or human brain[38-41]. The marmoset is ideal for this project, as it shows high degrees of construct and face validity. The marmoset exhibits sleep patterns very similar to those of humans[18,20], has sophisticated cognitive abilities[22,42,43], and is characterized by high levels of prosocial behavior[37]. Marmosets show age-related cognitive decline in a number of domains[44,45] and an age-related increase in Aβ cortical deposition and p-tau that resembles the human pattern[27]. Due to their short lifespan, they are ideally suited for longitudinal studies. ***Based on these characteristics, the aged marmoset is expected to display a high degree of predictive validity as a model of preclinical AD, with potential to help identify therapeutic strategies that are clinically relevant.***

SPECIFIC AIM 1: TO DEVELOP A CHRONIC SF PROCEDURE AND CHARACTERIZE ITS EFFECTS ON COGNITIVE FUNCTION AND BEHAVIOR IN AGING MALE AND FEMALE MARMOSETS

Rationale: Sleep disturbances are a core feature of AD. However, human studies cannot determine whether sleep disturbances are a cause or consequence of neuropathology. In this aim, we develop an SF procedure for aged marmosets that mimics SF observed in AD, in order to test the hypothesis that chronic SF induces cognitive impairment in this NHP model of preclinical AD.

Development of an experimental sleep fragmentation (SF) procedure for the marmoset: **Background:** Age-related changes in sleep have been thoroughly described in humans. Older adults tend to have advanced sleep timing, longer sleep-onset latency, shorter overall sleep duration, increased SF, more fragile sleep, reduced amount of slow wave sleep (SWS), increased time spent in lighter stages N1 and N2, shorter and fewer non rapid eye movement (NREM)-REM sleep cycles, and increased time spent awake throughout the night[46]. However, human studies show that AD risk, Aβ pathology and accumulation of tau aggregates are more closely related to SF[47,48] rather than overall shorter sleep duration. ***To our knowledge, studies manipulating sleep in marmosets are not available and the few studies employing experimental SF in other NHPs are unlike human SF. In this aim, we will implement an SF procedure for marmosets that is designed to mimic SF observed in AD patients, rather than restricting or depriving sleep, as used in most animal studies[5]***.

Experimental Design: The data reported by Vitiello and Prinz[49] show an average of 10 to 25 awakenings per night in AD patients, which add up to nearly 40% of bed time spent awake. Proprietary Info Proprietary Info The authors used auditory stimuli (60-90 dB) delivered every 15 min throughout the night (~ 8 hours) and repeated every 10 sec to induce 2 min of wake or a total of 1 hour of total wake. The SF was administered for a total of 3 consecutive nights. As aged marmosets in our colony sleep for an average of 11.5 hours (690 min)[20], a disruption of 40 % of that time amounts to about 4.6 hours (276 min) of wake time. We will distribute the fragmentation throughout the night, by playing the audio stimuli every 15 min for 6 min each (n = 46 audio stimuli), inducing 276 min of wake per night. Initially, will administer the SF with these parameters for 1 SF night only and assess its effect on behavior (including naps), cognitive performance during the following days and sleep patterns during the recovery night (Phase I, **Fig. 1**). If excessive naps, lack of participation in cognitive tasks, or concerns with animals' health is noted, the SF parameters will be adjusted to reduce wake time (i.e., 30 % of wake time). At least a week of rest will be granted between SF trials. Once the targeted parameters are set, 3 consecutive nights of SF per week will be administered (Phase II; **Fig. 1**). Cognitive performance and behavior will be monitored throughout to ensure the well-being of the animals. The SF disruption will be adjusted, as justified by changes in eating habits,

Proprietary Info

Sleep/wake assessments: **Preliminary Data** The Lacreuse laboratory uses wireless telemetric systems (Data Science International)[20] as well as wearable activity monitors to measure sleep and activity in aged marmosets. Polysomnography (PSG) as recorded by telemetry is a useful technique that allows to analyze sleep stages EEG waveforms[18,20,28], but also involves a challenging surgery and a long recovery period[51] that would be problematic for this project. Here we propose to use actigraphy, a validated method to measure sleep/wake activity in both humans[47] and marmosets[31]. We will use a device called the Actiwatch Mini (CamNTech, UK), which marmosets wear on a collar custom-adjusted to their size (**Fig. 2**). The device records motor activity and can be used to evaluate sleep[31]. Using this system, we recorded nighttime activity from male and female marmosets **(Fig. 3**). We found that males had a shorter latency to sleep and shorter wake bouts than females throughout the night. **Experimental Design** Marmosets will be fitted with a custom-sized collar provided with an Actiwatch Mini. The system can record up to 90 days of sleep/wake activity patterns, with easy battery replacement. To gain a full picture of SF effects on sleep, we will record 5 nights each week consisting of (1) a night of undisturbed sleep; (2) the SF nights and (3) the recovery night, with the same nights recorded for the control group. Importantly, activity during the day will also be recorded, in order to uncover changes in activity levels and potential naps. Access to the nest boxes will be removed during the day in both groups to minimize SF-induced sleep recovery during the day. **Expected outcomes** Significant change in sleep patterns are expected in the SF relative to the control group, such as a compensatory increase in sleep depth and duration[52,53] as well as increases in napping frequency. We also expect sex differences in the ability to recover from SF, with females showing less recovery than males[54,55]. As suggested by our preliminary data, sex differences in sleep patterns may also be observed in the control group **(Fig. 3)**.

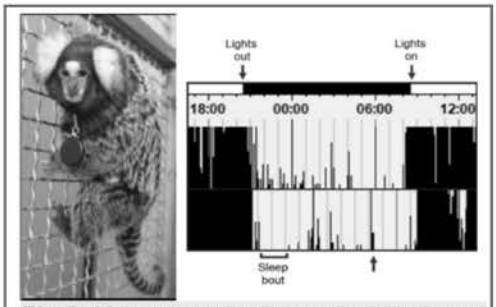

Fig 2. Marmoset wearing an Actiwatch and actogram showing reduced activity during nighttime.

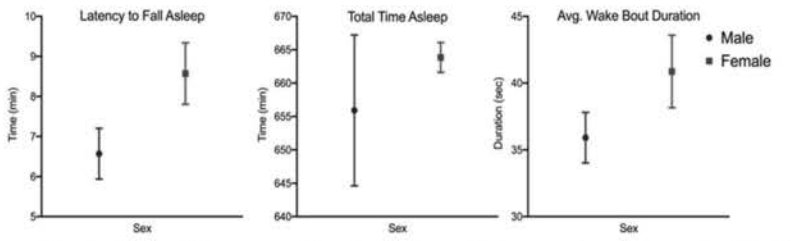

Fig 3. Sex differences in sleep in middle-aged marmosets. Data represent 84 nights from n=10 males and 66 nights from n=10 females.

Cognitive assessments: **Background:** sleep disturbances affect cognitive function in humans, including in old age, but it is unclear whether SF observed in AD patients is a cause or consequence of AD. Rodent studies have

established that SF impairs cognitive function and increases neuropathology, in particular increases accumulation of Aβ and tau in the brain but more translational animal models are clearly needed to characterize these processes in the primate brain and provide predictive validity. NHP data provide a strong rationale to posit that sleep disturbances adversely affect cognitive function. For example, age-related changes in sleep patterns[56] were associated with spatial learning and memory impairments in the rhesus monkey[57]. Sleep deprivation (8h) impaired memory retrieval in grey mouse lemurs[58]. In a recent study in 5 species of lemurs[59], spatial memory and selective behaviors were sensitive to sleep disruption (4h for 1 week or 10h audio clips for 2 days). However, to our knowledge no NHP study has attempted to mimic SF that occurs in AD and to measure associated changes in cognition. We propose to use the marmoset, an NHP which presents many similarities with humans in sleep patterns, cognitive function and brain organization to identify these links for the first time. **Preliminary data** The Lacreuse laboratory routinely tests marmosets on cognitive tasks. We have used the CANTAB touchscreen system (Cambridge Neuropsychological Test Automated Battery), developed for standardized cognitive testing of humans[60] and NHPs[61,62] to train marmosets on reversal learning and attentional set-shifting[33]. We found that reversal learning performance declined with age in marmosets that were followed from middle-age (4-5 years) to old age (8-9 years), with deficits starting around age 8 (years 3 to 4 in **Fig. 4**). Monkeys were presented with a series of 3 pairs of stimuli/year for which they had to perform a discrimination (select the rewarded stimulus, 90% learning criterion), followed by a reversal (select the previously incorrect stimulus). Females took longer than males to acquire the reversals and the trajectory of decline was worse in females than in males. In addition, the large individual variation suggested that some animals may experience pathological, rather than healthy aging. These data demonstrate our ability to test marmosets longitudinally on touchscreen tasks, and highlight age- and sex-related changes in cognitive function in this species. **Experimental Design** To minimize drawbacks associated with our previous set-up (physical separation from partner, low number of trials performed and need for an experimenter), monkeys will perform the cognitive tasks in their home cage, at their own pace, and in presence of their partner, maximizing participation and eliminating anxiety associated with partner separation and/or confinement in a transport cage. The homecage will be fitted with touchscreen units controlled by the Radio Frequency Identification (RFID) of the subjects. This wireless system will initiate subject-specific tasks and provide automatic data logging and dispensing of food reward (banana milkshake), using the NIMH MonkeyLogic software[63,64]. Task parameters will adapt to each monkey's performance to facilitate learning. Once the monkeys acquire the tasks (80% correct), they will be given access to the touchscreens to perform the cognitive battery with new stimuli. To increase motivation, access will be restricted to specific intervals of time during the day (i.e, 8-10 h and 14-16h), where they will have the opportunity to complete all tasks at will (e.g, 60 trials of DNMS, 50 trials of WCST, 100 trials of PR). All correct trials will be rewarded with banana milkshake.

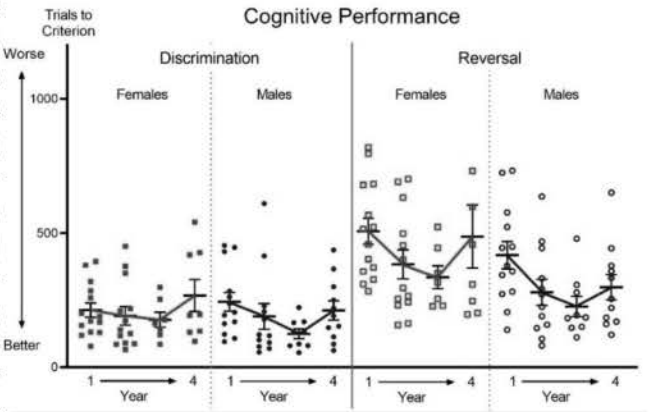

Fig. 4. Age-related decline in reversal learning in marmosets. Trials to criterion in the discrimination and reversal of 3 pairs of stimuli /year. Females show impaired reversal performance compared to males and decline more than males

The tasks, written in MATLAB, assess cognitive domains known to be affected by age, sleep and AD (**Fig. 5**). Given the intensity and duration of the proposed SF, we focus on 3 tasks in order to maximize participation 1) Delayed Non-Matching-to-Sample (DNMS) The DNMS is a task dependent on the HPC[65] and PFC[66] that assesses *visual recognition memory*. Visual recognition memory declines with age in rhesus monkeys[67], is impaired in AD [68], and is sensitive to sleep deprivation in both humans[69] and rhesus monkeys[70]. Marmosets can be successfully trained on the DNMS[71] but the effects of sleep disruptions on DNMS performance have not been assessed; in the DNMS, monkeys are presented with a sample stimulus, and after a specific delay (1, 3, 6 and 10s)[71] to be rewarded. Monkeys will be given the opportunity to perform as many trials as they wish during touchscreen times. Delays will be randomly distributed between trials and images will randomly be drawn from a pool of 1000

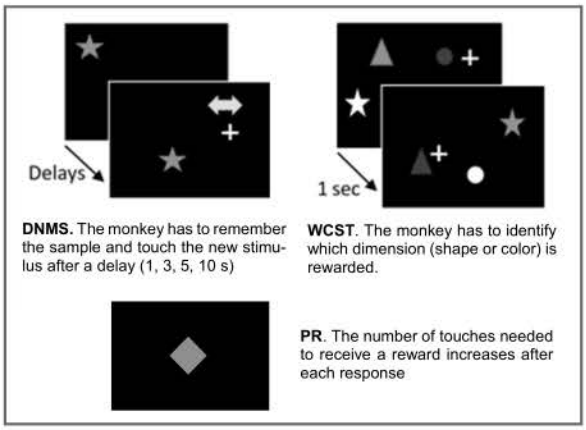

Fig. 5. Schematic of the cognitive tasks

stimuli. The number of trials performed, as well as the accuracy and response times will be recorded, are represented with the same stimulus along with a new stimulus. They have to touch the new stimulus (2) Wisconsin Card Sort Test (WCST) The WCST assesses *set-shifting*, the ability to switch from one dimension (e.g., shape) to another (e.g., color). WCST performance declines with age in humans and NHPs[72], is impaired in AD[73] and with sleep deprivation in humans[74]. To our knowledge, marmoset data on the WCST are not available, but we have successfully tested marmosets on another task of set-shifting, the Intradimensional/Extradimensional task[33]. Therefore, we do not foresee any major difficulty in testing marmosets on the WCST. In this task, the monkey is presented with 3 shapes of 3 different colors at random locations on the screen. They have to determine which one of the dimensions is rewarded, for example the color red. Once they produce 8 consecutive correct responses, the correct the dimension switches to a new one (for example the shape triangle) and they have to adapt their responses to the new set. 5 switches will be given; the total number of trials performed as well as the number of errors and response times are recorded (3) Progressive ratio (PR) The PR provides a measure of *motivation*, which is crucial to the interpretation of cognitive deficits[75]. The task measures the number of responses the monkey is willing to make for obtaining a single reward. The number of responses required for a reward increases at each trial according to a specific schedule. Marmosets have been tested on PR[22], including aged marmosets in the Lacreuse laboratory. **Expected outcomes** We expect SF marmosets to show cognitive impairment in both cognitive tasks compared to the controls, and females to be more affected than males. We predict that cognitive impairment will increase with longer periods of SF and that executive function, which may capture early cognitive decline[72], will be particularly affected. We will also determine the extent to which decreasing motivation, as assessed by the PR, contributes to cognitive deficits. Comparison of am and pm data will also provide an indication of cognitive and motivational changes throughout the day.

Behavioral assessments: **Experimental Design** We will assess general behavior 5 days/week[33]. Monkeys will be observed using a modified frequency scoring system in which ~20 behaviors of interest are recorded for the focal animal at 15-s intervals for 5 min. Behaviors include measures of locomotion, sociality, and aggression, adapted from an extensive marmoset ethogram[76]. **Expected outcomes** We predict that SF monkeys will show decreased social interactions[77], likely accompanied by an increase in agonistic interactions[59,78] and decrease in locomotion[59], relative to control monkeys.

SPECIFIC AIM 2 TO IDENTIFY SF-DRIVEN CHANGES IN PERIPHERAL INFLAMMATION AND METABOLISM AND CSF LEVELS OF OREXIN, Aβ AND TAU.

Rationale: In this aim, we test the hypothesis that chronic SF induces substantial changes in peripheral markers of inflammation and dysregulated metabolism, as well as CSF levels of orexin, Aβ and tau levels.

Table 1. Analytes, media, assay method and predictions in SF vs. control marmosets

Analyte	Medium	Method	SF vs. controls
IL-6	Serum	Luminex	+
CRP	Serum	Luminex	+
Insulin	Serum	Luminex	+
Glucose	Serum	Luminex	+
Cortisol	Urine	ELISA	+
T	Urine	ELISA	-
Orexin	CSF	ELISA	+
Aβ	CSF	Luminex	+
Tau	CSF	Luminex	+

Metabolic and inflammatory assessments Inflammation is an important factor in the pathologies of aging, including AD. Central and peripheral inflammatory states not only seem to reflect wider deficits in cognition, mental and physical health, but also may be causal in driving some of these changes[79-82]. Both metabolism and inflammation are intimately linked with sleep, in a bidirectional manner[83-85]. In fact, many cytokines in the brain are directly shown to regulate sleep[86-89]. Increasing evidence also shows that sleep disorders affect metabolic function in humans. Various degrees of sleep loss have been shown to decrease insulin sensitivity and impair glucose metabolism[90-92]. Sleep deprivation is associated with increased levels of circulating inflammatory markers such as C-reactive protein (CRP)[93] and interleukin-6 (IL-6)[94,95]. In addition, increased CSF orexin levels are related to sleep deterioration and cognitive decline in AD[96], and orexin receptor antagonist treatment in AD-mouse models reduces Aβ levels, while intracerebral injections of orexin increase Aβ levels[6]. One of our goals is to determine the temporally dynamic changes in biomarkers that are directly related to metabolic status and inflammation. By tracking over time, we anticipate being able to construct biomarker panels that could be used to predict how an individual may respond to SF. To measure multiple analytes in the same sample, we will employ a custom MagPix Luminex fluorescent bead assay, in use in the Karatsoreos laboratory. These assays allow for the interrogation of over 20 analytes in the same small (10-30µl) sample collected into EDTA coated tubes. This enables us to probe the

relationships between these various biological factors, and to evaluate how these relationships may change with chronic SF. The Luminex system has been validated in marmosets and other NHPs[97,98]. **Experimental Design** Blood samples will be collected weekly from a femoral vein for quantification of cytokines and chemokines in serum using a validated Monkey Cytokine Magnetic 29-plex Panel (Luminex Platform Kit, Invitrogen). A marmoset-specific ELISA kit (Ucytech Bioscience) will be used for further validation. Quantification of insulin will be performed using a 9-plex ProcartaPlex Panel (Invitrogen) configured for NHPs. Concentrations of $A\beta_{42}$ and tau concentrations in CSF samples (collected during anesthesia) will be measured using the Aβ/tau Neurodegenerative Human Magnetic 3-plex panel (Invitrogen). CSF orexin levels and blood levels of fasting glucose will be assessed via ELISA assays. Daily non-invasive collection of urine samples, routinely in use in the Lacreuse laboratory[33], will be used to track daily changes in cortisol and testosterone (T) in males. Indeed, 1 week of sleep restriction to 5h/night resulted in a 10% to 15% decrease in testosterone levels in young healthy men[99]. Urinary cortisol and T levels will be assessed by ELISA. Because the females will be ovariectomized, levels of ovarian hormones should remain low throughout the experiment and will not be assessed. **Expected outcomes** Monkeys in the SF groups will show an increase in circulating inflammatory cytokines and CRP, consistent with inflammation, exhibit metabolic dysfunction, characterized by insulin resistance, elevated glucose and cortisol, and show lower testosterone levels. CSF levels should show a time-dependent increase in Aβ and tau levels and orexin.

Rigor, Reproducibility and Sex as a Biological Variable: This project is based on ***rigorous prior research***. Our published research and preliminary data demonstrate our ability to track cognitive changes over time in aging marmosets[33]. We include ***sex as a biological variable***. In humans, biological sex has been shown to affect sleep[55,100], AD risk and disease severity[101,102]. Inclusion of this variable is therefore a key component of this proposal; we previously found reliable sex differences in sleep, cognition and resting state networks in marmosets[33]. ***Experimental rigor*** will be achieved by having experimenters blind to experimental conditions during data analyses. In keeping with the animal welfare's "three Rs" (replacement, reduction, refinement), the number of animals is reduced to the minimum necessary for sound statistical results, which is achieved by our proposed sample of 16 monkeys. Analyses of variance with Condition (SF, control) and Sex as factors will be conducted on each dependent variable investigated under the Aims. The physiological data collected in Aim 2 will be integrated with the cognitive data collected in Aim 1 using multiple regressions. Power analysis for a repeated measures analysis of variance with a within-between interaction shows that with n=16 across 4 groups (condition x sex) and repeated measurement occasions (weeks), we have 95% power to detect a large effect (f=0.39) and 80% power to detect a medium effect (f=0.31). As our previous research found large effects of sex on reversal learning (d=0.76) and sleep efficiency (d=1), we are confident that both SF and Sex effects will be detected.

Result interpretation, potential pitfalls and alternative strategies: ***We expect monkeys in the SF group to develop a phenotype characterized by altered sleep patterns, impaired cognition, reduced social behaviors, dysregulated metabolism, increased inflammation and increased CSF AD biomarkers***. One potential problem with the cognitive assessments (Aim 1), based on the literature[71] and our own experience, is that not all monkeys acquire proficiency on touchscreen tasks. We believe that testing in the home cage will reduce drop-out rates. Attrition of the sample is also a potential concern. The Lacreuse laboratory has been successful in maintaining a colony of aged marmosets at UMass with minimal attrition. Another problem is the validation of the Luminex assays for marmosets (Aim 2). Although preliminary data in marmosets are lacking for this aim, we are confident that the instrument will successfully assay the analytes under consideration, based on the literature[97]. Overall, we anticipate no substantive pitfalls for the two aims of the proposal.

Overall Timeline and Future studies:

Table 2 shows the estimated timeline for the proposed research. The results should provide crucial data for validating the marmoset as a model to understand the relationships between sleep and AD. These data will form the foundation for proposing more substantial studies on the ***mechanisms by which SF drives neuropathology and cognitive impairment in aged marmosets***. Implementation of this research in an animal model with a phenotype recapitulating many features of AD will be essential to identify sleep therapies of clinical relevance.

Table 1: Timeline of the proposed research

Aims	Year 1	Year 2
1	• Purchase monkeys • Lab set-up (wireless homecage testing) • Cognitive training • Pilot the SF procedure (phases I and II)	• Chronic SF procedure (Phases III and IV) • Cognitive testing
2	• Sample collection • Pilot MagPix measurements with marmoset samples	• Sample collection and physiological assays • Data analysis and write-up for publication

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001

Expiration Date: 02/28/2023

Use of Human Specimens and/or Data

Does any of the proposed research in the application involve human specimens and/or data *

❍ Yes ● No

Provide an explanation for any use of human specimens and/or data not considered to be human subjects research.

Are Human Subjects Involved

❍ Yes ● No

Is the Project Exempt from Federal regulations?

❍ Yes ● No

Exemption Number

❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8

Other Requested Information

VERTEBRATE ANIMALS

Use of animals These studies will use 16 common marmoset monkeys (*Callithrix jacchus*), approximately 6 years of age (middle-aged), weighing between 350–550g, and consisting of 8 gonadally intact males and 8 gonadectomized females. The monkeys will be Redacted by agreement, with lights on from 0700 to 1900 h, ambient temperature at approximately 27 °C, and humidity at approximately 50%. Each male will be socially housed with a female to promote species typical behavior and psychological well-being. The females will be gonadectomized prior to the start of the experiment to model menopause and prevent breeding. The pairs will be housed in stainless steel mesh cages measuring 48"W x 36"D x 80"H. The cages are furnished with perches, platforms, nest boxes, hammocks and a variety of enrichment devices to promote species-typical behavior including foraging, scent-marking, and climbing. The marmoset diet consists of fresh fruits, vegetables, nuts and seeds, various breads and ZuPreem marmoset food. Marmosets are fed twice daily with fruits and nuts provided between 0800h and 0900h. Water is available *ad libitum*. The monkeys will be provided with daily enrichment, including foraging tubes and a variety of toys, in accordance with the federally mandated Environmental Enrichment Program. The animals are cared for in accordance with the guidelines published in the *Guide for the Care and Use of Laboratory Animals 8th Edition* (National Academies Press, Washington D.C, 2011).

- Ovariectomies Upon study entry, females will be ovariectomized to model low sex hormone levels mileu of menopausal women and to prevent breeding, as in our previous study[1].
- Proprietary Info

  Proprietary Info If at any point, monkeys show adverse effects that alter their eating habits, induces weight loss, aggression or excessive daytime sleepiness that prevents them from participating at cognitive tasks, the SF procedure will be adjusted to a reduced frequency/duration. The main purpose of this R21 is to design an SF procedure that mimics that observed in AD, without compromising the animals' health or ability to perform cognitive tasks.
- Behavior and Cognitive Testing At the beginning of the experiment, the monkeys will be trained on a battery of cognitive tasks administered on touchscreen units in their home cage. They will also be observed for behavior and trained for noninvasive urine collection. Urine collection will occur once a week from all animals.
- Activity Monitor Collars At the beginning of the experiment, animals will be acclimated to wearing the activity monitors (actiwatch mini, CamNTech) affixed to collars around their necks. A custom-made collar will be designed for each monkey to account for size differences. The monkey will be acclimated to wearing the collar by increasing durations of wear over successive sessions. Once animals are acclimated to the collar, they will wear the activity monitors in their home cages.
- Blood samples Blood (0.5-1 ml) will be collected from a femoral vein in awake monkeys manually restrained by an experimenter. Blood will be collected every bimonthly for assessment of inflammatory and metabolic biomarkers relevant to AD, including cytokines, glucose, insulin and HbA1c.
- CSF collection CSF collection will be performed by an experienced veterinarian in anesthetized monkeys, following a procedure described in details in Schultz-Darken and collaborators [2]. Briefly, after appropriate positioning of the animal, a 27-gauge needle will be carefully introduced vertically into the aseptically prepared site and CSF volume (50-150 ml) will be collected. The entire procedure will be completed within 5 min. After full recovery from anesthesia, monkeys will be released into their home cage. CSF collection will be performed at baseline and after 4 and 8 weeks of SF.

Justification Owing to their similarities to humans in neuroanatomy, physiology, brain function and behavior, nonhuman primates are indispensable to understand brain function. There is no alternative or *in vitro* models available for this purpose. The marmoset shows age-related cognitive decline and has sleep patterns remarkably similar to those of humans. Moreover, marmosets display neuropathology relevant to AD including β-amyloid deposition and hyperphosphorylated tau. Its short lifespan of about 10 years makes it ideal for longitudinal studies such as the one proposed here.

Sample Size We have designed the experiments so that they yield a maximum of information with the minimum of animals. We have determined that a number of 8 subjects per group is needed for valid interpretation of the data. Based on our preliminary data, a total sample size of 16 across four groups, we will have 95% power to detect large effect sizes (f=0.39) and greater than 80% power to detect medium effect sizes (f=0.31). Our previous research found large effects for Age on reversal learning in males (d=0.59) and females (d=0.92), and large effect of Sex on sleep efficiency (d= 1). Therefore, we should be sufficiently powered with n=16 for the proposed.

Veterinarian Care The UMass veterinarian staff monitors the animals daily. None of the procedures planned in the studies should harm the animals. However, if monkeys were to become ill or injured, we would rely on the attending veterinarian to diagnose the problem and treat the animal. All research activity would be suspended during the treatment.

Procedures to minimize discomfort, distress, pain and injury The main sources of pain in this experiment will be the ovariectomies and CSF sampling, performed by a UMass veterinarian. Monkeys will be anesthetized with isoflurane for both procedures and appropriate analgesics will be administered as needed. The veterinarian staff and lab personnel will monitor the animals daily for any sign of pain or distress. In the event that distress is observed, we will follow the recommended treatments. All research activity will be suspended during treatment. Mild stress may also be experienced for the procedures involving physical restraint (e.g., blood sampling). Blood sampling will be performed by experienced staff and will only require a few minutes. The monkeys will be acclimated to the procedures involving the wear of collars before the start of the experiments.

Euthanasia Euthanasia is not the endpoint of this study. However, should any animal develop irreversible clinical illness during the course of this study, they will be euthanized. They will be anesthetized with Ketamine and euthanized with an overdose of pentobarbital (100 mg/kg to effect administered iv) in order to spare animals unwanted discomfort. These procedures are in accordance with the recommendations of the Panel on Euthanasia of the American Veterinary Association, 2007.

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DATA SHARING PLAN

Our laboratories will not develop a model organism or other constructs that need formal sharing plans. However, the PI and co-PI embrace the need to have project data publicly accessible after publication. Should other researchers ask us for our expertise in cognitive tasks, sleep and physiological assessments as applied to the marmoset, we will gladly provide assistance. In addition, we will continue to disseminate our findings through publishing manuscripts as well as meeting presentations, as described below.

Data sharing among team members
The PI, co-PI and other project participants will communicate weekly, either by Zoom or email (or in person if pandemic situation allows) through laboratory meetings. This will ensure that all team members are informed of data progress, are able to discuss any change in the direction of the research project and encouraged to give input on data handling and analysis.

Data sharing with the wider scientific community
We will share our results by submitting abstracts to national/international scientific meetings (including, but not limited to, meetings of the American Society of Primatologists, the Marmoset Bioscience Symposium and the Society for Neuroscience) and through peer-reviewed scientific publications.

Peer-reviewed scientific publications
Any manuscript that has been accepted for publication in a peer-reviewed journal that describes scientific results originating from this project will be submitted to PubMed Central in accordance with NIH requirements.

Exhibit 2

Recipient Information

1. Recipient Name
UNIVERSITY OF MASSACHUSETTS
COMMONWEALTH AVE

AMHERST, MA 01003

2. Congressional District of Recipient
02

3. Payment System Identifier (ID)
104316735 2B5

4. Employer Identification Number (EIN)
043167352

5. Data Universal Numbering System (DUNS)
153926712

6. Recipient's Unique Entity Identifier

7. Project Director or Principal Investigator
Agnes Lacreuse, PHD
Associate Professor
alacreuse@psych.umass.edu
413-545-2183

8. Authorized Official
Redacted by agreement

Federal Agency Information

9. Awarding Agency Contact Information
Olusola Shoyelu
Grants Management Specialist
NATIONAL INSTITUTE ON AGING
shoyeluo@mail.nih.gov

10. Program Official Contact Information
MANUEL H MORO
Program Officer
NATIONAL INSTITUTE ON AGING
morom@mail.nih.gov
301-496-6402

Federal Award Information

11. Award Number
1R21AG074251-01

12. Unique Federal Award Identification Number (FAIN)
R21AG074251

13. Statutory Authority
42 USC 241 42 CFR 52

14. Federal Award Project Title
Experimental sleep fragmentation and cognition in aged marmosets

15. Assistance Listing Number
93.866

16. Assistance Listing Program Title
Aging Research

17. Award Action Type
New Competing

18. Is the Award R&D?
Yes

Summary Federal Award Financial Information

19. Budget Period Start Date 08/15/2021 **– End Date** 04/30/2022	
20. Total Amount of Federal Funds Obligated by this Action	$239,250
20 a. Direct Cost Amount	$150,000
20 b. Indirect Cost Amount	$89,250
21. Authorized Carryover	$0
22. Offset	$0
23. Total Amount of Federal Funds Obligated this budget period	$239,250
24. Total Approved Cost Sharing or Matching, where applicable	$0
25. Total Federal and Non-Federal Approved this Budget Period	$239,250
26. Project Period Start Date 08/15/2021 **– End Date** 04/30/2023	
27. Total Amount of the Federal Award including Approved Cost Sharing or Matching this Project Period	$239,250

28. Authorized Treatment of Program Income
Additional Costs

29. Grants Management Officer - Signature
Jessica Perez

30. Remarks

Acceptance of this award, including the "Terms and Conditions," is acknowledged by the recipient when funds are drawn down or otherwise requested from the grant payment system.

EXPLORATORY/DEVELOPMENT GRANT
Department of Health and Human Services
National Institutes of Health

NATIONAL INSTITUTE ON AGING

SECTION I – AWARD DATA – 1R21AG074251-01

Principal Investigator(s):
Agnes Lacreuse, PHD

Award e-mailed to: opam@umass.edu

Dear Authorized Official:

The National Institutes of Health hereby awards a grant in the amount of $239,250 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF MASSACHUSETTS AMHERST in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award, including the "Terms and Conditions," is acknowledged by the recipient when funds are drawn down or otherwise requested from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute On Aging of the National Institutes of Health under Award Number R21AG074251. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please direct questions to the Federal Agency contacts.

Sincerely yours,

Jessica Perez
Grants Management Officer
NATIONAL INSTITUTE ON AGING

Additional information follows

Cumulative Award Calculations for this Budget Period (U.S. Dollars)

Federal Direct Costs	$150,000
Federal F&A Costs	$89,250
Approved Budget	$239,250
Total Amount of Federal Funds Authorized (Federal Share)	$239,250
TOTAL FEDERAL AWARD AMOUNT	$239,250
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$239,250

SUMMARY TOTALS FOR ALL YEARS (for this Document Number)		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
1	$239,250	$239,250
2	$199,375	$199,375

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

Payment System Identifier:	1043167352B5
Document Number:	RAG074251A
PMS Account Type:	P (Subaccount)
Fiscal Year:	2021

IC	CAN	2021	2022
AG	8033155	$239,250	$199,375

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: 1CAMPMO / **OC**: 41021 / **Released**: Perez, Jessica 08/05/2021
Award Processed: 08/13/2021 12:21:53 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 1R21AG074251-01

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – STANDARD TERMS AND CONDITIONS – 1R21AG074251-01

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2**.** As such, auditees should identify NIH

awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R21AG074251. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:

Additional Costs

SECTION IV – AG SPECIFIC AWARD CONDITIONS – 1R21AG074251-01

Clinical Trial Indicator: No

This award does not support any NIH-defined Clinical Trials. See the NIH Grants Policy Statement Section 1.2 for NIH definition of Clinical Trial.

RESTRICTION: Funds included in this award for research involving live vertebrate animals are restricted and may not be used for any other purpose without NIA approval. Under governing PHS Policy no funds may be drawn down from the payment system and no obligations may be made against federal funds for research involving live vertebrate animals prior to submission of

valid Institutional Animal Care and Use Committee (IACUC) approval in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals. The present award is made without currently valid verification of IACUC approval for this project. Only activities that do not involve live vertebrate animals may be conducted pending NIA's acceptance of verification of IACUC approval. The verification of IACUC approval must be submitted to the Grants Management contact identified on the Notice of Award. Failure to submit the verification of IACUC approval or to otherwise comply with the above requirements can result in suspension and/or termination of this award, withholding of support, audit disallowances, and/or other appropriate action.

Funding for this award has been provided by Alzheimer's Disease Initiative funds.

This is a Modular Grant Award without direct cost categorical breakdowns issued in accordance with the guidelines published in the NIH Grants Policy Statement. See: http://grants.nih.gov/grants/policy/nihgps/HTML5/section_13/13_modular_applications_and_awards.htm# . Recipients are required to allocate and account for costs related to this award by category within their institutional accounting system in accordance with applicable cost principles.

None of the funds in this award shall be used to pay the salary of an individual at a rate in excess of the current salary cap. Current salary cap levels can be found at the following URL: http://grants.nih.gov/grants/policy/salcap_summary.htm

In accordance with the Notice: NOT-OD-02-017 entitled, "GRADUATE STUDENT COMPENSATION" published on December 10, 2001, in the NIH Guide for Grants and Contracts, total direct costs (salary, fringe benefits and tuition remission) for graduate students are provided at a level not to exceed the NIH maximum allowable amount (zero level of the Ruth L. Kirschstein National Research Service Award stipend in effect at the time of the competing award). Support recommended for future years has been adjusted accordingly, if applicable. The full guide Notice describing the level of compensation allowed for a graduate student can be found at: http://grants.nih.gov/grants/guide/notice-files/NOT-OD-02-017.html

This award includes funds for twelve months of support. The competing budget period is awarded for less than 12 months. Continuation awards will cycle each year on May 1. The Research Performance Progress Report (RPPR) is due 45 days prior to this date for SNAP awards or 60 days prior for non-SNAP awards.

SPREADSHEET SUMMARY
AWARD NUMBER: 1R21AG074251-01

INSTITUTION: UNIVERSITY OF MASSACHUSETTS AMHERST

Budget	Year 1	Year 2
TOTAL FEDERAL DC	$150,000	$125,000
TOTAL FEDERAL F&A	$89,250	$74,375
TOTAL COST	$239,250	$199,375

Facilities and Administrative Costs	Year 1	Year 2
F&A Cost Rate 1	59.5%	59.5%
F&A Cost Base 1	$150,000	$125,000
F&A Costs 1	$89,250	$74,375

Exhibit 3

Subject: subcontract needed for R21 AG074251
From: Agnès Lacreuse <lacreuse@umass.edu>
Date: 9/14/2021, 9:01 PM
To: "Lawson-Lipchin, Eva (NIH/NIA/ERP) [E]" <eva.lawson-lipchin@nih.gov>

Dear Ms. Lawson-Lipchin,

I have been trying to reach Dr. Moro with regards to R21 AG074251, for which I need a subcontract that requires his approval.
Should I proceed with the subcontract paperwork/new budget in the era commons?
If this would be better discussed over the phone, please let me know

Thank you very much for any advice you may provide

Sincerely,

Agnès Lacreuse

--
Agnès Lacreuse, PhD.
Professor
Psychological and Brain Sciences
University of Massachusetts
Morrill IV North, 204N
639 North Pleasant st
Amherst MA 01003
Email: lacreuse@umass.edu
https://www.lacreuselab.com/

Exhibit 4

A. COVER PAGE

Project Title: Experimental sleep fragmentation and cognition in aged marmosets	
Grant Number: 5R21AG074251-02	**Project/Grant Period:** 08/15/2021 - 04/30/2023
Reporting Period: 08/15/2021 - 04/30/2022	**Requested Budget Period:** 05/01/2022 - 04/30/2023
Report Term Frequency: Annual	**Date Submitted:** 03/15/2022
Program Director/Principal Investigator Information: AGNES LACREUSE , PHD **Phone Number:** 413-545-2183 **Email:** alacreuse@psych.umass.edu	**Recipient Organization:** UNIVERSITY OF MASSACHUSETTS AMHERST Office of Post-Award Management 100 Venture Way Suite 201 HADLEY, MA 010359450 **DUNS:** 153926712 **EIN:** 1043167352B5 **RECIPIENT ID:**
Change of Contact PD/PI: No	
Administrative Official: JAMES WILLIAM WARREN Office of Pre-Award Services 100 Venture Way Hadley, MA 010359450 **Phone number:** 4138833902 **Email:** jameswarren@umass.edu	**Signing Official:** JAMES WILLIAM WARREN Office of Pre-Award Services 100 Venture Way Hadley, MA 010359450 **Phone number:** 4138833902 **Email:** jameswarren@umass.edu
Human Subjects: No	**Vertebrate Animals:** Yes
hESC: No	**Inventions/Patents:** No

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Specific Aim 1 to design an experimental SF procedure that mimics the sleep fragmentation observed in early AD/MCI and to characterize the effects of chronic SF on sleep, cognitive function and behavior.

Specific Aim 2 to determine whether chronic SF induces changes in peripheral measures of inflammation and metabolic function and CSF levels of orexin, Aβ and tau levels.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File Uploaded : Accomplishments.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Once the subcontract is in place, we will initiate the experiments at the WNPRC. The protocol has already been approved. The animals will be selected and the lab set-up with the touchscreen computerized system. The animals will be divided into an experimental and a control group. They will be trained on touchscreen cognitive testing. Once they reach criterion on the tasks, the sleep fragmentation (SF) procedure will be implemented (phases I and II). The SF procedure will target MCI/early AD patterns of sleep disturbance, [redacted] Cognitive performance and behavior will be monitored throughout to ensure the well-being of the animals.

B.2 (Accomplishments.pdf)

Given the shortage of age-appropriate marmosets available for purchase in the country, we have requested to conduct the experiment at the Wisconsin National Primate Research Center (WNPRC), which houses suitable animals. NIH has approved this change, but the subcontract with WNPRC has not yet been implemented.
As a result, the grant has not yet been started and there is nothing to report.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

No

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

NOTHING TO REPORT

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? No

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization? No

C.5 OTHER PRODUCTS AND RESOURCE SHARING

NOTHING TO REPORT

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
ALACREUSE	Y	Lacreuse, Agnes	PHD	PD/PI	0.0	0.0	0.1			NA

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

Yes

File Uploaded: Colman Bio.3.7.22.pdf

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

No

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

NA

OMB No. 0925-0001 and 0925-0002 (Rev. 12/2020 Approved Through 02/28/2023)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Colman, Ricki Jean

eRA COMMONS USER NAME (credential, e.g., agency login): rcolman

POSITION TITLE: Associate Professor of Cell and Regenerative Biology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Washington University, St. Louis, MO	B.A.	05/1991	Biology, Anthropology
Washington University, St. Louis, MO	M.A.	05/1993	Biological Anthropology
University of Wisconsin, Madison, WI	M.S.	05/1998	Biological Anthropology
University of Wisconsin, Madison, WI	Ph.D.	12/1998	Biological Anthropology
WI National Primate Research Center, Madison, WI	Postdoc	01/2001	Primate Aging

A. Personal Statement

I am an Associate Professor of Cell and Regenerative Biology at the University of Wisconsin School of Medicine and Public Health and a Senior Scientist at the Wisconsin National Primate Research Center where I chair the Energy Metabolism and Chronic Diseases Working Group and serve on the Executive Committee. My research is focused on the impact of nutrition and social/behavioral factors on health outcomes across the lifespan. My background in biological anthropology informs my evolutionary perspective on the aging process and my over 25 years of experience with nonhuman primate models provides the expertise to explore relevant questions in humans' closest relatives. I have an extensive body of work focused on the effects of long-term, adult-onset caloric restriction in rhesus macaques and have successfully translated many of the techniques used in that work to the smaller, shorter-lived marmoset model. I have worked with captive common marmosets since 1998 and have extensive experience evaluating their behavior, cognition, health, and aging trajectories. This experience is highlighted by numerous successful NIH grant applications and publications in peer-reviewed journals.

Ongoing and recently completed projects that I would like to highlight include:

NIH U24MH123422 **Co-Investigator** 07/01/2020 – 06/30/2025
Collaborative expansion of marmoset colonies for neuroscience research
This collaborative grant will support the expansion of our marmoset breeding colony specifically for use by neuroscience researchers. As part of this grant, we will explore ways to improve marmoset reproductive success. Drs. Colman and Ross will be responsible for this part of the project and Dr. Phillips will assist in interpretation. Dr. Colman will additionally participate in decision making regarding animal breeding and allocation.

NIH U24MH123696 **Co-Investigator** 07/01/2020 – 06/30/2025
Coordinating center for collaborative marmoset research
The goal of this collaborative mechanism is to work with NIH and the marmoset neuroscience colonies to direct breeding decisions and allocation of resources. Dr. Colman will serve on the management committee for this project and participate in decision making regarding directed animal breeding and allocation.

NIH U34AG068466 **Principal Investigator** 07/01/2020 – 06/30/2023
Enhancing the marmoset aging model through biomarker development
The goal of this study is to improve the utility of the common marmoset as a model for aging research by developing and validating necessary biomarkers of energy regulation, inflammation, and frailty. Dr. Colman has responsibility for oversight and performance of all portions of this project.

NIH R01HD086057 **Principal Investigator** 7/01/2016 – 6/30/2022

Dietary fat ratio's influence on adolescent depression: A nonhuman primate model.
This grant examines the role of balanced fatty acids in promoting healthy brain development for cognition, the reward system, and social behaviors from childhood through to adulthood in the common marmoset. Dr. Colman has full responsibility for the design, oversight, and performance of this study.

NIH R24OD020347 **Principal Investigator** 4/01/2016 – 3/31/2022
Research to improve and standardize marmoset nutrition and dietary husbandry.
This study will identify critical features of a standardized basic diet for captive common marmosets; determine links between diet, gut microbiome, and disease; and establish standards for healthy weights, body condition, and biomarkers of metabolic function. Dr. Colman along with Drs. Ross and Power has responsibility for the design, oversight, and performance of this study.

NIH R21AG061635 **Principal Investigator** 12/01/2019 – 5/31/2022
Translational analysis of a novel intervention for diet-induced obesity
This project seeks to determine if a diet low in branched chain amino acids can reverse diet-induced obesity in a primate model and to understand the molecular mechanisms behind such actions. Dr. Colman has full responsibility for the design, oversight and performance of the marmoset monkey portions of this study.

NIH R01HD083001 **Co-Investigator** 1/01/2016 – 12/31/2022
Hypothermia to prevent neurotoxic side effects of pediatric drugs.
This study is designed to determine whether hypothermia can protect the nonhuman primate brain from histological, behavioral, and neurocognitive toxicity of common drugs used for anesthesia, prolonged sedation or antiepileptic therapy in human neonates and infants. Dr. Colman has responsibility for cognitive testing in infant through subadult macaques and will work with the PI on data analysis and reporting.

NIH P51OD011106 **Principal Investigator, EMCD** 5/01/2017– 4/30/2022
Primate Research Center support.
This base-operating grant for the Primate Center includes funds for an energy metabolism and chronic diseases working group led by Dr. Colman.

NIH R61NS115102 **Co-Investigator** 12/01/2019 – 11/30/2024
Modeling frontotemporal dementia in rhesus macaques
This project aims to create a resource to study frontotemporal dementia by taking advantage of a family of rhesus monkeys carrying an exact replicate of a frontotemporal dementia-related mutation. Dr. Colman has full responsibility for the cognitive testing portions of this study and will work with the PI on data analysis and reporting.

NIH R01AG040178 **Principal Investigator** 6/01/2016 – 5/31/2021
Caloric restriction and aging in rhesus monkeys.
This project explores the possibility that long-term dietary restriction retards aging processes in a nonhuman primate species. Dr. Colman has full responsibility for the design, oversight, and performance of this study.

Citations:

1. Power ML, Adams J, Solonika K, Colman R, Ross CN, Tardif SD. Diet, digestion and energy intake in captive common marmosets (*Callithrix jacchus*): research and management implications. *Sci Reports*. 9(1):12134, 2019. doi:10.1038/s41598-019-48643-x [Epub ahead of print]. PMCID:PMC6702194.
2. Ash H, Ziegler T, Colman RJ. Early learning in the common marmoset (*Callithrix jacchus*): effect of sex and family interactions on cognitive development. *Am J Primatol*. 2020 Jun 9:e23159. doi:10.1002/ajp.23159. PMCID:PMC7440670.
3. Colman RJ, Capuano S, Bakker J, Keeley J, Nakamura K, Ross C. Marmosets: welfare, ethical use, and IACUC/regulatory considerations. *ILARJ*. 2021 Feb 23;ilab003. doi:10.1093/ilar/ilab003. PMCID in progress.
4. Ross CN, Colman RJ, Power M, Tardif S. Marmoset metabolism, nutrition and obesity. *ILARJ*. 2021 May 10;ilab014. doi:10.1093/ilar/ilab014. PMCID in progress.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments:

2021-present Associate Professor, Cell & Regenerative Biology, University of WI, Madison
2021-present Faculty Trainer, Graduate Program in Nutritional Sciences, University of WI, Madison

2021-present	Member, Stem Cell & Regenerative Medicine Center, University of WI, Madison
2021-present	Chair, Research Professor Promotions Committee, University of WI, Madison
2020-present	Affiliated Faculty, Psychology, University of WI, Madison
2019-present	Standing Member, Aging Systems and Geriatrics Study Section, NIH
2017-present	Faculty Trainer, Endocrinology & Reproductive Physiology, University of WI, Madison
2017-2021	Assistant Professor, Cell & Regenerative Biology, University of WI, Madison
2016-present	Co-Chair, LSVC Animal Care and Use Committee, University of WI, Madison
2015-present	Chair, Energy Metabolism & Chronic Dis., Wisconsin National Primate Research Center
2014	Co-Chair, Energy Metabolism & Chronic Dis., Wisconsin National Primate Research Center
2013-2015	Vice-Chair, All Campus Animal Planning & Advisory Committee, University of WI, Madison
2012-2015	Chair, Graduate School Animal Care and Use Committee, University of WI, Madison
2010-present	Senior Scientist, Wisconsin National Primate Research Center
2006-2010	Associate Scientist, Wisconsin National Primate Research Center
2002-2013	Core Leader, Aging and Metabolism, Wisconsin National Primate Research Center
2006-2011	Advisor, Behavioral Management Unit, Wisconsin National Primate Research Center
2002-present	Unit Leader, Aged Rhesus Monkey Resource, Wisconsin National Primate Research Center
2002-present	Affiliate Member, Institute on Aging, University of WI, Madison
2001-2005	Assistant Scientist, Wisconsin National Primate Research Center
1998-2001	Research Associate, Wisconsin National Primate Research Center
1994-1998	Research Assistant, Wisconsin National Primate Research Center
1992-1993	Teaching Assistant, Anthropology, Washington University

Honors:

2018	Vilas Faculty Early Career Investigator Award, University of WI, Madison
2016	Fellowship Status Award, Gerontological Society of America
2015	Permanent Principal Investigator Status Award, University of WI, Madison

C. Contribution to Science

1. My early publications validated methods for assessment of body composition in rhesus monkeys. At the time, dual-energy x-ray absorptiometry (DXA) was newly developed and generally only used clinically for evaluation of bone. We adapted this technology, working closely with the manufacturer, to measure total and regional body composition in rhesus monkeys. The development of these noninvasive techniques allowed for relatively easy and cost-effective methods for longitudinal assessment of total body and regional body composition, including both soft tissue and bone mass. Following this developmental phase, we evaluated the effects of aging and long-term, adult-onset caloric restriction (CR) on body composition and bone mass in rhesus monkeys. We showed that rhesus macaques accurately model human age-related changes in body composition and that CR prevented or delayed the expected age-related changes. Importantly, we were able to show that CR did not negatively impact skeletal health. We additionally utilized our large collection of information on rhesus monkeys to establish reference body composition for this species, important information that was lacking in the literature. I was fully responsible for all work associated with these publications from study design to data collection and interpretation to manuscript development.
    a. Colman RJ, Roecker EB, Ramsey JJ, Kemnitz JW. The effect of dietary restriction on body composition in adult male and female rhesus macaques. *Aging* 10:83-92, 1998.
    b. Colman RJ, Ramsey JJ, Roecker EB, Havighurst T, Hudson JC, Kemnitz JW. Body fat distribution with long-term dietary restriction in adult male rhesus macaques. *J. Gerontol.* 54A:B283-290, 1999.
    c. Raman A, Colman RJ, Cheng Y, Kemnitz JW, Baum ST, Weindruch R, Schoeller DA. Reference body composition in adult rhesus monkeys: glucoregulatory and anthropometric indices. *J. Gerontol.* 60A(12):1518-1524, 2005.
    d. Colman RJ, Beasley TM, Allison DB, Weindruch R. Skeletal effects of long-term caloric restriction in rhesus monkeys. *AGE*. 34(5):1133-1143, 2012. PMCID: PMC3448987.
2. Sarcopenia, the loss of muscle mass with advancing age, is a serious clinical concern leading to increased morbidity and mortality. People begin losing muscle mass at relatively young ages, but the mechanism behind this loss and ways to prevent sarcopenia are not well developed. The lack of an appropriate rodent model of sarcopenia greatly hinders this work. Therefore, we established a rhesus monkey model of sarcopenia establishing first that rhesus monkeys, like humans, experience sarcopenia and then determining the age at which this begins. We then showed that CR was able to delay the onset of sarcopenia in rhesus monkeys. More recently we have focused on the mechanisms behind the beneficial effect of CR on muscle

mass centering on a potential mechanism related to alterations in energy metabolism. Our most recent work incorporates measures of sarcopenia in the development of the rhesus monkey as a frailty model and shows the ability of CR to delay or prevent frailty. I designed and performed the initial experiments developing the techniques and validating the model and showing the effects of CR and the development and testing of the frailty model. The mechanism work is performed in collaboration with cellular biology colleagues with my full input and participation in study design, data collection and interpretation and manuscript preparation.

a. Colman RJ, McKiernan SH, Aiken JM, Weindruch R. Muscle mass loss in rhesus monkeys: age of onset. *Exp. Gerontol.* 40(7):573-581, 2005.
b. Colman RJ, Beasley TM, Allison DB, Weindruch R. Attenuation of sarcopenia by dietary restriction in rhesus monkeys. *J. Gerontol. A Biol. Sci. Med. Sci.* 63(6):556-559, 2008. PMCID: PMC2812805.
c. Pugh TD, Conklin MW, Evans TD, Polewski MA, Barbian HJ, Pass R, Anderson BD, Colman RJ, Eliceiri KW, Keely PJ, Weindruch R, Beasley MT, Anderson RM. A shift in energy metabolism anticipates the onset of sarcopenia in rhesus monkeys. *Aging Cell* 12(4):672-681, 2013. PMCID: PMC3714309.
d. Yamada Y, Kemnitz JW, Weindruch R, Anderson RM, Schoeller DA, Colman RJ. Caloric restriction and healthy lifespan: frail phenotype of non-human primates in the Wisconsin National Primate Research Center caloric restriction study. *J Gerontol Biol Sci*. 73(3):273-278, 2018. PMCID: PMC5861888.

3. Since I began my PhD work my research has focused on the use of nonhuman primate models to explore the impact of nutrition and metabolism on health across the aging continuum. A large focus of this research has been on the ability of CR, the long-term moderate reduction in food intake without malnutrition, to modulate the aging process. CR offers a powerful way to explore mechanisms of aging because it is the only environmental intervention that repeatedly and strongly increases maximum life span and delays aging in a diverse array of experimental organisms. In a landmark 2009 paper in *Science* and two follow-up papers in *Nature Communications* we showed that CR also improves health and survival in rhesus monkeys. The success of this paradigm then allowed us to begin to address the mechanisms behind this beneficial effect in primates. The inverse linear relationship between caloric intake and lifespan extension in rodents suggests a role for factors involved in the regulation of energy metabolism in the mechanisms of CR. Alterations in energy metabolism are observed in multiple species on CR, including humans. Therefore, over the past several years my CR research has focused largely on exploring the hypothesis that CR induces an altered state of energy metabolism that promotes health and longevity. As PI of the grant that funded this long-term work, I had complete responsibility for this work. We are one of the only groups that has had the opportunity to perform a long-term study of the effects of CR in a nonhuman primate model making these papers important contributions to the literature in this field. In addition to making information available through these publications, I make a concerted effort to make both data and biological samples from our study available to interested investigators to further advance the field.
    a. Colman RJ, Anderson RM, Johnson SC, Kastman EK, Kosmatka KJ, Beasley TM, Allison DB, Cruzen C, Simmons HA, Kemnitz JW, Weindruch R. Caloric restriction delays disease onset and mortality in rhesus monkeys. *Science*. 325:201-204, 2009. PMCID: PMC2812811.
    b. Colman RJ, Beasley TM, Kemnitz JW, Johnson SC, Weindruch R, Anderson RM. Caloric restriction reduces age-related and all-cause mortality in rhesus monkeys. *Nature Commun*. 5:3557, 2014. doi:10.1038/ncomms4557. PMCID: PMC3988801.
    c. Mattison JA*, Colman RJ*, Beasley TM, Allison DB, Kemnitz JW, Roth GS, Ingram DK, Weindruch R, deCabo R, Anderson RM. Caloric restriction improves health and survival of rhesus monkeys. *Nature Commun.* 8:14063, 2017. doi:10.1038/ncomms14063. PMCID: PMC5247583. *Joint first authors
    d. Maegawa S, Lu Y, Tahara T, Lee JT, Madzo J, Liang S, Jelinek JJ, Colman RJ, Issa J-PJ. Caloric restriction delays age-related methylation drift. *Nature Commun.* 8(1):539, 2017. doi:10.1038/s41467-017-00607-3. PMCID: PMC5599616.
4. Working with neuroscience collaborators, we began to investigate the important question of the effects of CR on brain structure and function. This work was designed to address a crucial question regarding any effects, either positive or negative, of long-term, adult-onset CR in a primate model. This work involved noninvasive imaging in addition to evaluations of cognitive function, motor function, physical activity and glucoregulatory function along with other covariates of interest. *Ex vivo* determinations were also performed. I had full responsibility for all live animal portions of this work including study design and data collection and interpretation for cognitive and motor function, physical activity and glucoregulatory function along with all sample collection, and was fully involved in all design, data interpretation and manuscript preparation.

a. Sridharan A, Willette AA, Bendlin BB, Alexander AL, Coe CL, Voytko ML, Colman RJ, Kemnitz JW, Weindruch RH, Johnson SC. Brain volumetric and microstructural correlates of executive and motor performance in aged rhesus monkeys. *Front. Aging Neurosci*. 4(Article 31):1-19, 2012. PMCID: PMC3492760.
b. Sridharan A, Bendlin BB, Gallagher C, Oh JM, Willette AA, Alexander AL, Kemnitz JW, Colman RJ, Weindruch RH, Johnson SC. Effect of age and calorie restriction on corpus callosal integrity in rhesus macaques: A fiber tractography study. *Neurosci Lett*. 569:38-42, 2014. PMCID: PMC4105191
c. Martin SA, DeMuth TM, Miller KN, Pugh TD, Polewski MA, Colman RJ, Eliceiri KW, Beasley TM, Johnson SC, Anderson RM. Regional and metabolic heterogeneity of the hippocampus is non-uniformly impacted by age and caloric restriction. *Aging Cell* 15(1):100-110, 2016. doi.10.1111/acel.12418. PMCID: PMC4717265.
d. Souder DC, Dreischmeier IA, Smith AB, Wright S, Martin SA, Sagar MAK, Eliceiri KW, Salamat SM, Bendlin BB, Colman RJ, Beasley TM, Puglielli L, Anderson RM. Rhesus monkeys as a translational model for late-onset Alzheimer's disease. *Aging Cell*. 2021;00:e13374. doi:10.1111/acel.13374. PMCID: PMC8208787.

5. There has long been a desire within the research community for a small, relatively short-lived nonhuman primate model of metabolism, aging, and age-related diseases. The common marmoset has generated much interest due, in large part, to their unique social structure and their rapid life history. However, development of a common marmoset model of aging and metabolism was severely limited by the lack of validated assays and assessment tools. We were able to begin to overcome this roadblock when we succeeded in validating an insulin assay along with other metabolic function biomarkers several years ago. This advance led the way to increased utility of this relatively short-lived nonhuman primate for many areas of biomedical research including early life influences on later life outcomes, early learning and cognition, aging and metabolism. In particular, ballooning interest in the marmoset model by the neuroscience community has led to a need to increase the number of available marmosets for research and to better understand and characterize these animals. This need was realized by NIH leading to several recent grant announcements focused on the use of the marmoset model in aging and neuroscience research. I have been fully involved in development of this model including refining husbandry and handling practices, developing tools specifically for marmoset research and understanding their development and aging processes. I was fully involved in all aspects of the following work including study design, data interpretation and manuscript preparation.
    a. Ziegler TE, Colman RJ, Tardiff SD, Sosa ME, Wegner FH, Wittwer DJ, Shrestha H. Development of metabolic function biomarkers in the common marmoset, *Callithrix jacchus*. *Am. J. Primatol*. 75(5):500-508, 2013. PMCID: PMC3771328.
    b. Saltzman W, Abbott DH, Binkley N, Colman RJ. Maintenance of bone mass despite estrogen depletion in female common marmoset monkeys (Callithrix jacchus). Am J Primatol. 2018 Aug 14 14:e22905. doi:10.1002/ajp.22905. PMCID: PMC7336524.
    c. Kraynak M*, Colman RJ*, Flowers MT, Abbott DH, Levine JE. Ovarian estradiol supports sexual behavior but not energy homeostasis in female marmoset monkeys. *Int J Obesity*. 43(5):1034-1045, 2019. doi:10.1038/s41366-018-0156-4. PMCID: PMC30022054. *Joint first authors.
    d. Goodroe A, Wachtman L, Allen-Worthington K, Bakker J, Burns M, Diaz LL, Dick E, Dickerson M, Eliades SJ, Gonzalez O, Graf D, Haroush K, Inoue T, Izzi J, Laudano A, Layne-Colon D, Leblanc M, Ludwig B, Mejia A, Miller C, Sarfaty A, Sosa M, Vallender E, Brown C, Forney L, Schultz-Darken N, Colman R, Power M, Capuano S, Ross C, Tardif S. Current practices in nutrition management and disease incidence of common marmosets (*Callithrix jacchus*). *J Med Primatol*. 50:164-175, 2021. doi.org/1111/jmp.12525. PMCID: PMC8422998.

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/ricki.colman.1/bibliography/public/

E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

NOTHING TO REPORT

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

The initiation of this grant has been significantly delayed due to the request to conduct the experiment at the WNPRC instead of UMass Amherst.
Approval of the change from NIH was obtained on 2/2/22. Implementation of the subcontract with WNPRC is still pending.

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subject

No Change

F.3.b Vertebrate Animals

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F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

F.3.b (animals.pdf)

The animals to be used will be housed at the WNPRC instead of UMass Amherst

G. SPECIAL REPORTING REQUIREMENTS SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

Yes

G.8 PROJECT/PERFORMANCE SITES

Organization Name	DUNS	Congressional District	Address
Primary: University of Massachusetts-Amherst	153926712	MA-002	Office of Post-Award Management 100 Venture Way Suite 201 HADLEY, MA 010359450

Wisconsin National Primate Research Center	103198813	WI-002	1220 Capital Court Madison, WI 53715

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

Yes

Estimated unobligated balance: $239,250

G.10.b Provide an explanation for unobligated balance:
Project has not yet started. In the absence of suitable animals for purchase, the animal work has been transferred to the WNPRC. Subcontract arrangements are in the process of being finalized.

G.10.c If authorized to carryover the balance, provide a general description of how it is anticipated that the funds will be spent
The funds have been rebudgeted so that the WNPRC receives the majority of the funds across the 2 years, namely 120 K in Y1 and 100 K in Y2 (for animal fee, per diem and personnel needed to run the experiment). Drs. Lacreuse and Karatsoreos will oversee the experiment and perform the assays.

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period? No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

Yes

In the absence of suitable animals for purchase, transferring the animal work to the WNPRC is the only alternative to implement the work described in the R21 and has been added as a subcontractor. The WNPRC has all the infrastructure and resources necessary to conduct the work as planned. Dr. Lacreuse has budgeted travel to WNPRC to help set-up the project. Bi-weekly virtual meetings are also planned to ensure satisfactory progress. Budgetary Impact Direct Costs: $51,191.22 UMass Amherst + $220,000 WNPRC = $271,191.22 Indirect Costs: $45,333.80 UMass Amherst (59.5% MTDC) + $122,100 WNPRC (55.5% MTDC) = $167,433.80 Total Project Cost: $438,625.02 The funds have been redistributed so that the subcontractor receives the majority of the funds across the 2 years, namely 120 K in Y1 and 100 K in Y2 (for animal fee, per diem and personnel needed to run the experiment).

Exhibit 5

Amy Meyer

From: Kaylie Flaugher
Sent: Tuesday, January 31, 2023 7:34 AM
To: Dr. Alka Chandna; Amy Meyer; Dr. Katherine Roe; Kathy Guillermo
Subject: UW Madison still says no records re marmoset sleep frag

Resubmitted our request yesterday with new dates and UW Madison says, "There are no records responsive to your request."

From: UW - Madison Public Records <universityofwisconsin@mycusthelp.net>
Sent: Tuesday, January 31, 2023 9:19 AM
To: Kaylie Flaugher <kaylief@peta.org>
Cc: lisa.hull@wisc.edu <lisa.hull@wisc.edu>
Subject: Kaylie Flaugher People for the Ethical Treatment of Animals (PETA) Public Records Request :: P002651-013023

--- Please respond above this line ---

WISCONSIN
UNIVERSITY OF WISCONSIN–MADISON

Public Records Request: P002651-013023

RE: PUBLIC RECORDS REQUEST of January 30, 2023.

Reference # P002651-013023.

Dear Requester,

I am writing in response to your public records request of January 30, 2023, in which you requested the following:

> Records associated with University of Wisconsin-Madison Protocol titled “Sleep fragmentation and cognition” with Protocol ID # G006540, approved by the College of Letters and Science and Vice Chancellor for Research and Graduate Education Centers Institutional Animal Care and Use

Committee.For the period from September 27, 2022 to the date of fulfillment, for all common marmosets assigned to Protocol ID # G006540, we request:1. All daily care logs, and2. All clinical/veterinary/surgical records, and3. All reports of adverse events, unintended instances, or other reportable instances, and4. All necropsy reports, and5. All photos and/or videos

There are no records responsive to your request.

The university has responded to your request and it is now closed.

Sincerely,

Elizabeth Wilkerson
Assistant Public Records Custodian
Office of Compliance
University of Wisconsin-Madison

Access the Public Records Center to View the Request

Exhibit 6

University of Massachusetts Amherst

College of Natural Sciences
Psychological and Brain Sciences
639 N Pleasant Street,
Morrill IV North, Room N204
Amherst, MA 01003

Elizabeth Yeomans
Grants Management Specialist
Grants and Contracts Management Branch (GCMB)
Re: award # R21AG074251-01 (UMass Amherst Kuali #5254)

December 23, 2021

Dear Liz Yeomans,

I am writing to request a revision of grant R21AG074251-01 "Sleep fragmentation and cognition in aged marmosets". Due to the national shortage of marmosets, it has become clear that UMass Amherst will not be able to acquire the animals needed for this project within the budget and timeframe of the awarded grant. To address this challenge, we propose to conduct the animal work at the Wisconsin National Primate Research Center (WNPRC) of the University of Wisconsin-Madison. Dr. Ricki Colman, Associate Professor and Senior Scientist in the Cell and Regenerative Biology department, has made marmosets available for the project, and has agreed to serve as subcontractor to set-up and conduct the experiment on site, with the input and expertise of Dr. Karatsoreos and myself at UMass Amherst.

Impact on project scope

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. The IACUC protocol has not yet been submitted as it will be initiated by WNPRC, pending approval of this request.

Benefits

In the absence of suitable animals for purchase, transferring the animal work to the WNPRC is the only alternative to implement the work described in the R21. The WNPRC has all the infrastructure and resources necessary to conduct the work as planned. Dr. Lacreuse has budgeted travel to WNPRC to help set-up the project. Bi-weekly virtual meetings are also planned to ensure satisfactory progress.

Budgetary Impact

Direct Costs: $51,191.22 UMass Amherst + $220,000 WNPRC = $271,191.22
Indirect Costs: $45,333.80 UMass Amherst (59.5% MTDC) + $122,100 WNPRC (55.5% MTDC) = $167,433.80
Total Project Cost: $438,625.02
The funds have been redistributed so that the subcontractor receives the majority of the funds across the 2 years, namely 120 K in Y1 and 100 K in Y2 (for animal fee, per diem and personnel needed to run the experiment). Drs. Lacreuse and Karatsoreos will oversee the experiment and perform the assays. Consequently, Dr. Lacreuse requests approval for a reduction of effort to 0.5. A revised budget and supporting documentation are attached.

Thank you for considering this request. Should it not be approved, we would not be able to perform the specific aims at UMass Amherst and would return the totality of the awarded funds to NIH.

Sincerely,

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