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TITLE: Investigating Striatal Attentional Circuits to Understand and Mitigate Deficits in Cognitive Flexibility Due to Sleep Loss

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CONTRACTING ORGANIZATION: Washington State University

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14. ABSTRACT
Sleep loss compromises specific cognitive abilities that are both critical to real-world performance and dissociable from impairments in vigilant attention. Specifically, sleep loss impairs cognitive flexibility, which is the ability to adapt to changing events and environmental contingencies. We hypothesize that sleep loss-induced adenosinergic disruption of striatal dopaminergic circuits explains reduced attentional flexibility. We aim to identify dopaminergic and adenosinergic neural circuits responsible for sleep loss-induced deficits in cognitive flexibility using transgenic rats and optogenetic techniques, and performance measures that parallel task requirements for human cognitive flexibility. We seek to obtain converging evidence for the role of these circuits in humans by analyzing genotype differences in the effectiveness of wake-promoting agents during sleep deprivation.
Year 2 of this ongoing project has focused on development of the transgenic rat models and on animal and human subject data collection.

15. SUBJECT TERMS
Sleep deprivation, performance impairment, attentional control, cognitive flexibility, resilience, striatum, caffeine, modafinil, optogenetic stimulation

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1. INTRODUCTION

This project aims to investigate whether and how adenosinergic disruption of striatal dopaminergic circuits explains the reduced attentional flexibility caused by sleep deprivation. In animal studies, we optimize behavioral techniques that model the effects of sleep loss on cognitive flexibility observed in humans. Next, we use transgenic rats that express Cre and Flp recombinase-dependent viral DNA constructs in striatopallidal medium spiny neurons of the striatum that express both the Adora2a and DrD2 receptors. We use optogenetic methods to either activate these neurons, mimicking the effects of sleep deprivation on task performance in rats injected with flox/Frt -ChR2-GFP, or inactivate these neurons to recover normal task performance in sleep-deprived rats. For human subjects, we compare the effectiveness of standardized doses of modafinil and caffeine during total sleep deprivation in promoting cognitive flexibility based on dopaminergic and adenosinergic genotype. Beyond demonstrating that our animal model of attentional circuitry compromised by sleep loss generalizes to humans, these studies will shed light on the effectiveness of pharmacological agents countering the effects of sleep loss in settings that require the ability to rapidly adapt to changing circumstances. Thus, our research will have immediate real-world relevance for health and safety in industrial settings, emergency occupation, and people engaged in military operations.

2. KEYWORDS

Sleep deprivation, performance impairment, attentional control, cognitive flexibility, resilience, striatum, caffeine, modafinil, optogenetic stimulation

3. ACCOMPLISHMENTS

What were the major goals of the project?

Specific Aim 1: Develop behavioral model of sleep loss and cognitive flexibility in rodents.	**Timeline**	**Completed**
Study Preparations	Months	
Milestone(s) Achieved: Behavior techniques are validated and properties of transgenic rats have been verified for Aim 1.	14	100%
Data Analysis		
Milestone(s) Achieved: Aim 1 analyses completed.	14	100%
Specific Aim 2: Perform optogenetic experiments with transgenic rats.		
Data Collection		
Milestone(s) Achieved: Completion of data collection for Aim 2.	36	In progress
Data Analysis		
Milestone(s) Achieved: Aim 2 analyses completed.	36	n/a
Specific Aim 3: Demonstrate genotype differences in wake-promoting agents' effect on cognitive flexibility during sleep deprivation.		
Study Preparations	Months	
Milestone(s) Achieved: Procedures documented and IRB/HRPO approvals obtained.	6 (delays incurred due to contracting delays at NMRU-D; no major impact on study)	WSU IRB approval: 4 Jan 2019 HRPO approval: 2 Apr 2019 NMRU-D IRB approval: 6 Dec 2019
Data Collection		
Milestone(s) Achieved: Aim 3 data collection completed from 90 subjects (3 groups of 30 subjects in sleep deprivation condition with caffeine, modafinil, placebo).	36	In progress
Data Analysis		
Milestone(s) Achieved: Aim 3 analyses completed.	36	n/a
Final Report Preparation		
Compilation of analyses from aims 1–3 and drafting of report and briefing	30-36	n/a
Presentation of study results to the DoD	36	n/a
Milestone(s) Achieved: study completed	**36**	**In progress**

What was accomplished under these goals?

During Year 2, the major activities to be completed to achieve the goal milestones were:

- Optimize the dose and localization of viral injections for Aim 2 – in progress
- Determine the most effective virus promoter and serotype – in progress
- Aim 3 implementation review meeting with DoD consultant, Senior Research Psychologist Dr. Lynn Caldwell – completed
- Data collection for Aim 3 – in progress

During Year 2, the specific objectives were to:

- Optimize expression and injection coordinates, volumes and concentration of viral constructs in transgenic rats.
- Collect behavioral data from monotransgenic and bitransgenic rats for Aim 2.
- Collect data in human research participants for Aim 3.

During Year 2, key outcomes included the following:

- The Aim 1 (completed) and Aim 2 rodent behavioral tasks were selected and found to be responsive to sleep deprivation protocols.
- 13 human subjects completed the laboratory study of Aim 3.

Aim 1: During Year 2, significant progress was made optimizing the selected behavioral tasks. We implemented a version of the pairwise discrimination (PD) reversal task. In this version after learning criteria were met in the PD training phase, contingencies were reversed at the 30-minute mark in each 60-minute session (Fig. 1).

Male Long-Evans rats (n=7) were started on food restriction at six weeks of age. After one week, rats were introduced to the operant chambers and underwent habituation and pretraining trials consisting of five 1-hour training sessions per week, in which approaching the touch screen or nose pokes of the area of the touchscreen associated with a random image resulted in a sugar pellet reward. After pretraining, rats began pairwise discrimination where an

image of a fan or an image of marbles was rewarded with a sugar pellet, also five sessions per week. The images were randomized among rats; 3 rats had the marbles image and 4 rats had the fan image rewarded during PD. The PD training sessions continued until criterion for pairwise discrimination was achieved: 80 trials with at least 80% correct responses for two sessions, where correct responses were defined as a nose poke of the sucrose pellet-rewarded image.

After PD criteria were met the rewarded image was reversed during the second half of each session, so that the previously unrewarded image was now rewarded, and vice versa. However, unlike previous versions of the task where the reversal of the PD contingency remained for the entire session, here the original PD contingency was presented during the first half (30 minutes) of the reversal session fort each of the 15 sessions in the reversal phase. The rats had difficulty acquiring this within-session contingency reversal paradigm, as seen in poor responding (Fig. 2; white circles). Furthermore, as the reversal sessions progressed, rats also reduced responding in the first half of the session (Fig. 2; black circles). Thus, the design was too difficult for the rats to learn and produced extinction in the PD contingency portion of the reversal phase.

We will attempt another version of the task wherein the reversal phase will maintain the same image contingencies throughout the phase, but to better parallel the repeated contingency switching in the human cognitive tasks used in this project, a second reversal back to the original PD will be carried out after 10 sessions.

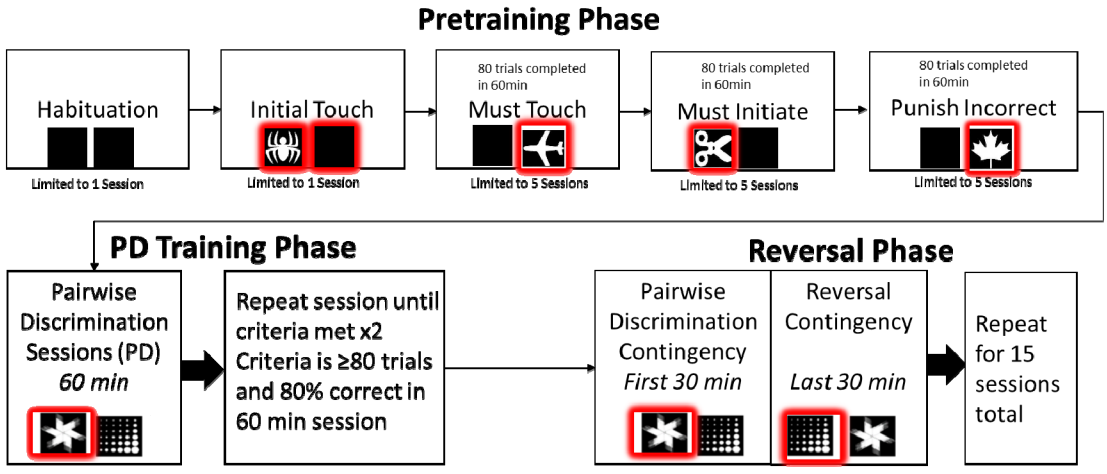


Figure 1. The three phases of the pairwise discrimination (PD) operant task. Red encircled images denote sugar pellet rewarded images in a subset of the rats (note that rewarded images were not cued by a red surround in the actual operant chambers). The opposite contingency for PD training and reversal phases was used in the other subset.

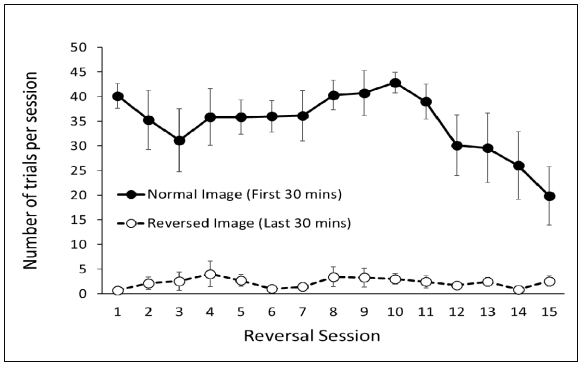


Figure 2. Image reversal occurring 30 minutes into the session during the reversal phase was not adequately learned (white circles). In addition, this implementation of the PD paradigm also extinguished performance during the first 30 minutes of the session (black circles).

In a separate experiment, however, we demonstrated successful implementation of the PD task and showed that 10 hours of sleep deprivation was sufficient to delay acquisition of PD task reversal (described below) as compared to rats with undisrupted sleep (Fig. 3). Food-restricted male Long Evans rats (n=6) underwent pretraining and PD training to criterion. As above, the fan and marble images were used, and the images were counterbalanced among rats (3 rats had the marbles image and 3 rats had the fan image rewarded during PD). After PD criteria were met, the rewarded image was reversed, so that the previously unrewarded image was now rewarded and vice versa. In this version the reversal contingency remained constant for the entire 60 min session and throughout the 10 sessions of the reversal phase.

Prior to the first reversal session rats were subjected to 10 hours of mechanical sleep deprivation (rotating bar) with direct researcher supervision. While the majority of rats stay awake for the 10-hour sleep deprivation with rotary bar, prior data showed that some rats have learned to sleep during the session by riding the bar. Therefore to ensure wakefulness direct observation and, if needed, manual perturbation were instituted for the last 5 hours of the sleep deprivation session. Rats were undisturbed before all subsequent reversal sessions and inter-session intervals, and the number of correct trials per session was compared to two cohorts (n=12) of rats that were treated similarly except they were allowed normal sleep prior to the first reversal session. Sleep deprivation impaired performance as measured by increased intertrial intervals (likely due to a decrement in sustained attention), while the number of correct trials over the first 5 sessions of the reversal phase was reduced; see Fig. 3. *This finding is particularly important to the project because with the sleep deprivation-induced performance*

deficits we are now poised to implement the optogenetic inquiry of the striatal circuitry to rescue or mimic these deficits (Aim 2).

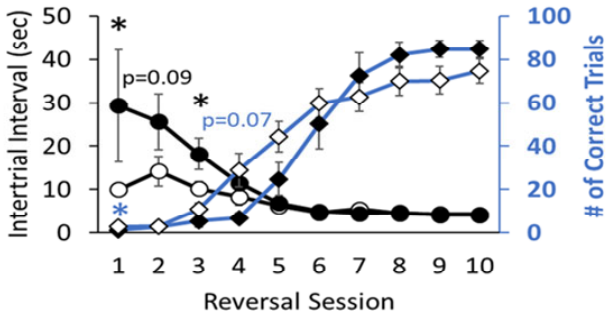


*Figure 3. Pairwise discrimination reversal task performance is disrupted by 10 hours of sleep deprivation (closed symbols) compared with controls (open symbols) as demonstrated by increased intertrial intervals (circles) and fewer correct trials (diamonds; *p<0.05) after cue reversal.*

We also completed the initial studies on our second reversal paradigm and demonstrated its susceptibility to sleep deprivation. The Light Actuating Search Task (LAST) is a circular open field maze, which requires rats to find an unmarked, quasi-randomly positioned target using visual feedback: light intensity based on the rodent's proximity from a target point. Floor vibrations are used to motivate ambulatory behavior, and when the rat arrives at the target, the lights and vibrations cease. In the Bright to Dim (Fig. 4; left panels) condition as the rats approach the target the light intensity increases (Fig. 4; yellow bars, sessions 1–6) and in the reversal phase as the rats approach the target light intensity decreases (Fig. 4; black bars, sessions 7–10). Alternatively, in the Dim to Bright condition (Fig. 4; right panels) as the rats approach the target the light intensity decreases (Fig. 4; black bars, sessions 1–6) and in the reversal phase as the rats approach the target light intensity increases (Fig. 4; yellow bars, sessions 7–10).

Twenty-seven male Long Evans rats habituated to the maze and handling for 1 week were tested with 9 trials per day on one of two paradigms for 6 days, either Bright to Dim or Dim to Bright, then either were subjected to 10 hours of sleep deprivation by gentle handling, or remained in their home cage for spontaneous sleep. Next, rats proceeded to receive the opposite paradigm for the remaining 4 days. Our results showed that sleep deprivation (Fig. 4; striped bars) significantly impedes rats' ability to learn a reversal paradigm as indicated by decreased path distance, increased time to target, and increased failure rate in session 7,

compared to the spontaneous sleep group (Fig. 4; solid bars). Rats also showed reduced overall learning when cued to approach a bright light in both the initial training or in the reversal, likely due to the rat's photophobia limiting their motivation to navigate toward bright light, as required to succeed.

Although the deficits incurred by sleep loss are clear using the LAST, we will test a version of the LAST that uses different light wavelengths (green vs. ultraviolet) to resolve the darkness preference disparity in the light vs. dark reversal reported here. If successful, the LAST reversal approach has an advantage over the pairwise discrimination reversal in carrying out Aim 2, because SD only affects session 7, so the application of optogenetic stimulation at a single time point in the LAST is possible.

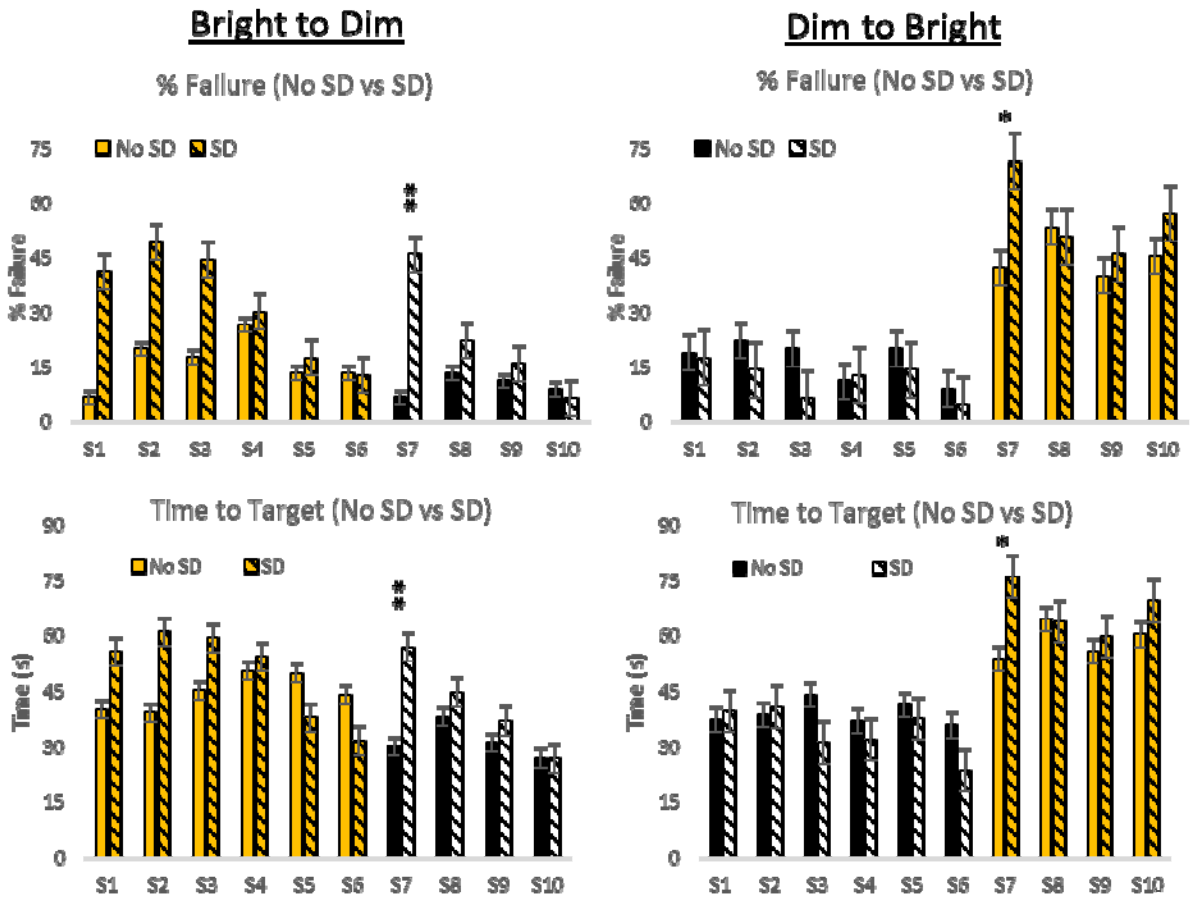


*Figure 4. Rat performance in the VAST is compromised by sleep deprivation for 10 hours, regardless of light intensity condition. This impairment resolves by the second day of reversal (session 8; *p<0.05, **p<0.01).*

Aim 2: During Year 2, work on Aim 2 centered on validating the ADORA2a and DRD2 monotransgenic and bitransgenic rats. First, three LE-Tg(Drd2-iCre)1ottc positive rats (8–9 weeks old) were unilaterally transfected with 1.5 or 3 µl pAAV-EF1a-cDIO-hChR2(H134R)-mCherry-serotype 5 at stereotaxic coordinates D/V 3.5 mm; A/P-0.6; M/L ±3.0. Histology performed three weeks later on the DRD2 monotransgenic rats showed better expression in the dorsal striatum with 3 µl injection compared to 1.5 µl injection (representative sample in Fig. 5).

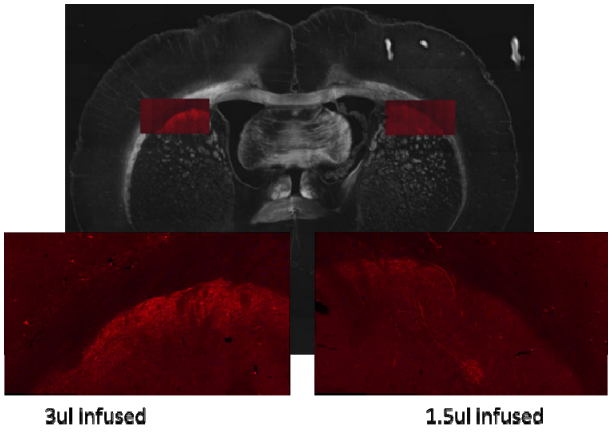


Figure 5. Strong expression of channel rhodopsin in DRD2-Cre monotransgenic rats at the 3 µl dose. This monotransgenic strain can be used to carry out the studies in Aim 2.

Continued examination of transfection efficacy in five LE-Tg(Adora2a-Flp) positive rats using 3 µl of pAAV-EF1a-fDIO-hChR2(H134R)-eYFP-serotype 5 virus at 3, 4- and 5-weeks post-injection showed little expression. See Fig. 6; in this representative rat, only one cell appeared to immunofluorescence (white arrow) in the 4-week condition (middle panel).

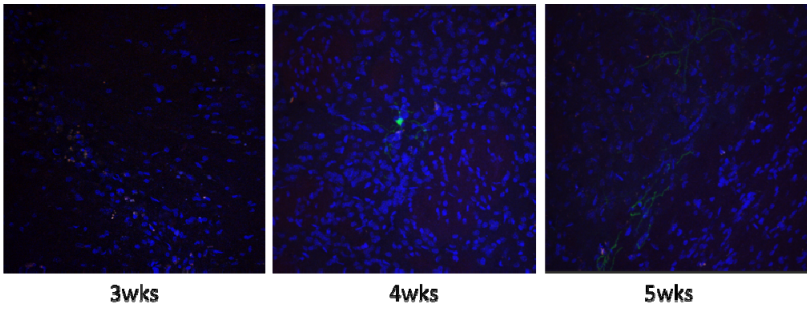


Figure 6. Extended transfection times in Adora2a-Flp monotransgenic rats did not demonstrate striatal expression in eYFP-tagged serotype 2 virus. The serotype 2 virus was suspect.

In discussions with collaborators in the Deisseroff lab (who famously developed the ChR2 optogenetic model) it was suggested that we try a newly available viral construct that combines three different serotypes. *We ordered these constructs to troubleshoot the poor ChR2 expression patterns in ADORA2a-Flp rats.*

Aim 3: We implemented the study design depicted in Fig. 7 for the human laboratory sleep deprivation study of Aim 3. A total of 13 out of 90 subjects completed the study before the COVID-19 pandemic forced the laboratory to suspend operations; see Fig. 8. This is a double-blind study; no analyses can be done until after unblinding.

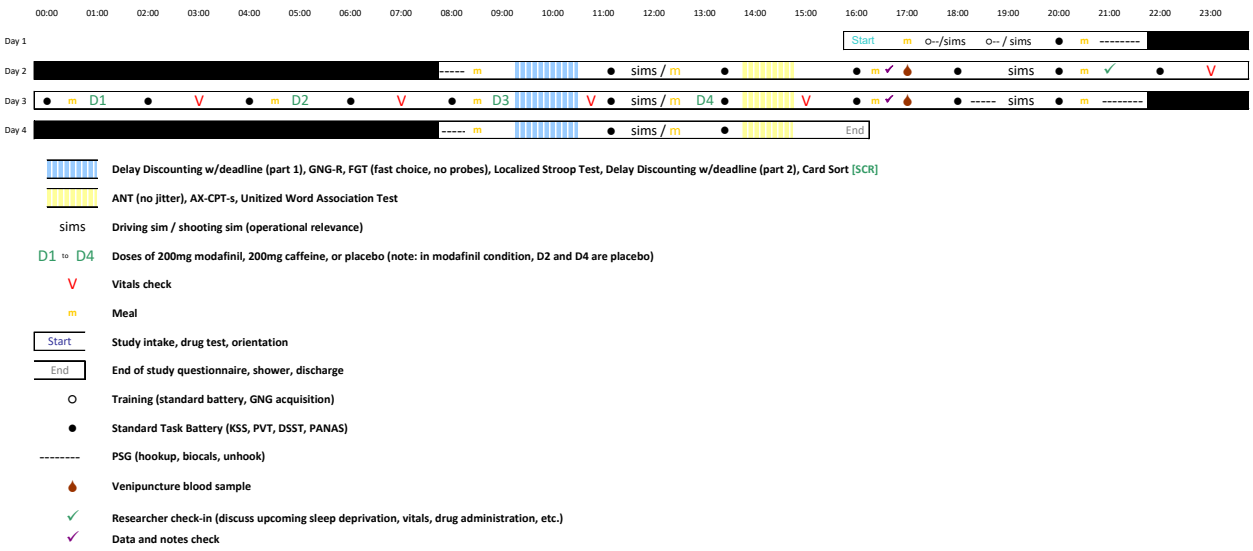


Figure 7. Schematic of the 4-day (3-night) human laboratory study of sleep deprivation to investigate cognitive flexibility and the effects of caffeine and modafinil thereon. GNG, GNG-R: go/no-go task (with reversal); FGT: framed gambling task; SCR: skin conductance response measurement; ANT: attention network test; AX-CPT-s: AX continuous performance task with switch; KSS: Karolinska Sleepiness Scale; PVT: psychomotor vigilance test; DSST: digit–symbol substitution task; PANAS: positive and negative affect schedule; PSG: polysomnography.

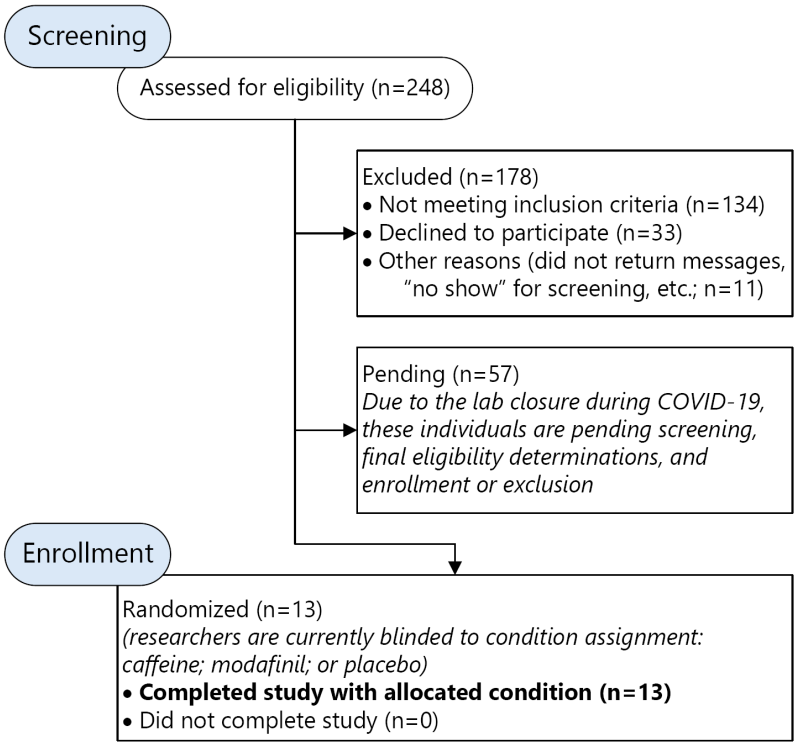


Figure 8: CONSORT diagram of subject recruitment and study completion.

During Year 2, other achievements included:

- Submission of four manuscripts for publication (under review).

What opportunities for training and professional development has the project provided?

The project provides significant opportunities for undergraduate and graduate education and professional development:

- A Ph.D. student in the Experimental Psychology program at Washington State University, Amanda Hudson, is involved in dissertation research integrated with the project. This includes programming of cognitive performance tasks in E-Prime software, data reduction and statistical analysis. The graduate student is involved in the project under the direct mentorship of the Co-PI (Honn) of the project. During Year 2 of the project, she began her dissertation project.

- Undergraduate interns Wei Chin and Jemima Onih, under the mentorship of Co-PI Chris Davis, constructed their first two research posters and presented them at laboratory meetings. Wei Chin was trained in surgeries and statistical analysis. Jemima Onih was trained in immunohistochemistry.

- Two post-baccalaureate research assistants, Julie Erwin and Myles Finlay, are undergoing training to become Registered Polysomnographic Technicians (RPSGT).

- Two additional post-baccalaureate research assistants, Kirsie Lundholm and Cecilia Moeller, are intensively involved in the project. Ms. Lundholm is also managing the response to COVID-19 in order to resume the human subjects laboratory study (Aim 3).

- More than a dozen undergraduate students have been involved in the project. They provided around-the-clock staffing and constant behavioral monitoring during the 24/7 laboratory experimentation of the project. Our records over the last 15 years show that the experience gained in the laboratory helps these students significantly with their applications for graduate and medical school.

How were the results disseminated to communities of interest?

The PI (Van Dongen) presented on the research at the DoD Sleep Workshop (Arlington, VA, Feb 2020) and at a Congressional Neuroscience Caucus Briefing (Washington, D.C., Sep 2019).

What do you plan to do during the next reporting period to accomplish the goals?

For the animal studies (Aim 2): During Year 3 of the project, we will pursue enhanced viral expression and determine the extent to which cortical biopotentials and vigilance states are affected by them. We will also submit two manuscripts detailing our work.

For the human study (Aim 3): During Year 3 of the project, we will continue to enroll human subjects until we meet our target of 90 healthy adults completed. We will also submit two additional manuscripts detailing our work.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?

The field of sleep and performance research will be enhanced by construction of an effective rodent model for cognitive flexibility and the optogenetic dissection of associated neural pathways that are susceptible to insufficient sleep.

What was the impact on other disciplines?

The construction of an effective rodent model for cognitive flexibility has broad implications on the fields of learning and memory, cognitive neuroscience and even the search for treatments for neurodegenerative diseases.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS

Changes in approach and reasons for change

- In the animal study (Aim 2), we are examining alternative viral constructs to elicit higher transfection in our ADORA2a rats. This is primarily due to our observed robust opsin expression in striatal dopaminergic cells (see Q8 quad chart in section 8 below), but only sparse expression in striatal adrenergic cells 3-4 weeks after viral injection in monotransgenic rat lines. We will develop constructs with alternative general neural promoters.

- In the human study (Aim 3), beginning in Year 3, we will omit the polysomnographic recording of sleep, as the hands-on procedure involved in electrode application greatly increases the risk of transmission of COVID-19. An IRB application to replace polysomnography with an additional obstructive sleep apnea risk questionnaire during screening and additional wrist actigraphy during the laboratory study, which requires no person-to-person physical contact and will suffice as a sleep measurement technology for addressing Aim 3, is pending for review. After IRB approval it will be submitted to the HRPO.

- We are making various other changes to the laboratory setting and the procedures of the human study (Aim 3) in order to mitigate the risk of COVID-19. These changes, which are minor and inconsequential for the scientific aspect of the human study, will be reviewed by the Washington State University IRB and then submitted to the HRPO.

Actual or anticipated problems or delays and actions or plans to resolve them

- We have incurred considerable delays in the animal and human studies of Aims 2 and 3, respectively, due to the COVID-19 pandemic, which forced us to shut down our laboratories in March 2020. As soon as we have permission to reopen the animal and human laboratories, we will intensify our research efforts to compensate for the delay. That said, because of the pandemic, it is anticipated that we will need to request a no-cost extension to be able to fully meet our objectives. Barring any unexpected delays in

reopening the laboratory before the end of the summer, we expect that a 1-year no-cost extension will be sufficient to complete the project.

Changes that had a significant impact on expenditures

Because of the laboratory shut-downs in response to the COVID-19 pandemic, our expenditure rate has slowed down over the last quarter. Because of the delays incurred due to the pandemic, we anticipate that we will need to request a no-cost extension and that we will spend out the full budget at that time. We do not expect to require any change in overall project funding.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report.

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS

Journal publications

Four manuscripts are under review at peer-reviewed journals.

Books or other non-periodical, one-time publications

Nothing to report.

Other publications, conference papers, and presentations

Van Dongen HPA (oral presentation): Sleep deprivation and loss of situational awareness: Mechanisms and mitigation strategies. SLEEP 2019 conference; San Antonio, TX. Jun 2019.

Van Dongen HPA (oral presentation): Resilience to vigilant attention deficits due to sleep deprivation: Non-additive interaction of TNFα genotype and caffeine. Military Health System Research Symposium; Orlando, FL. Aug 2019.

Van Dongen HPA (oral presentation): Investigating striatal attentional circuits to understand and mitigate deficits in cognitive flexibility due to sleep loss. DoD Sleep Workshop; Arlington, VA. Feb 2020.

Van Dongen HPA (invited lecture): DoD-supported research on sleep deprivation at Washington State University. Congressional Neuroscience Caucus Briefing; Washington, D.C. Sep 2019.

Van Dongen HPA (invited lecture): Fatigue management and prevention of accidents. “SLAAP2019” congress; Ermelo, The Netherlands. Nov 2019.

Van Dongen HPA (invited lecture): Sleep and vigilance. “SLAAP2019” congress; Ermelo, The Netherlands. Nov 2019.

Website(s) or other Internet site(s)

Nothing to report.

Technologies or techniques

Nothing to report.

Inventions, patent applications, and/or licenses

Nothing to report.

Other Products

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*Hans P.A. Van Dongen, Ph.D.*
Project Role:	*PI*
Researcher Identifier:	*ORCID ID: 0000-0002-4678-2971*
Nearest person month worked:	1
Contribution to Project:	*Dr. Van Dongen has provided general oversight, co-authored and submitted publications, and gave presentations on the study. He has coordinated between the animal and human studies.*
Funding Support:	

Name:	*Chris Davis, Ph.D.*
Project Role:	*Co-PI*
Researcher Identifier:	*ORCID ID: 0000-0002-9613-928X*
Nearest person month worked:	3
Contribution to Project:	*Dr. Davis directed operations and workforce for the rat behavioral research, wrote a COVID-19 research reduction contingency plan for the animal laboratory, took responsibility for the mutant rats, and optimized the behavioral paradigm (Aim 2).*
Funding Support:	

Name:	*Kimberly A. Honn, Ph.D.*
Project Role:	*Co-PI*
Researcher Identifier:	*ORCID ID: 0000-0001-8911-6277*
Nearest person month worked:	2
Contribution to Project:	*Dr. Honn led the human subjects laboratory study of Aim 3 and took responsibility for regulatory compliance.*
Funding Support:	

Name:	*John M. Hinson, Ph.D.*
Project Role:	*Co-PI*
Researcher Identifier:	*ORCID ID: 0000-0002-5012-5974*
Nearest person month worked:	1
Contribution to Project:	*Dr. Hinson contributed to the specification of the operant reversal paradigm for Aim 2 and the cognitive performance tasks used in Aim 3.*
Funding Support:	

Name:	*Paul Whitney, Ph.D.*
Project Role:	*Co-PI*
Researcher Identifier:	*ORCID ID: 0000-0003-1973-5261*
Nearest person month worked:	1
Contribution to Project:	*Dr. Whitney contributed to the cognitive performance tasks used in Aim 3.*
Funding Support:	

Name:	*Marcos Frank, Ph.D.*
Project Role:	*Co-PI*
Researcher Identifier:	*ORCID ID: 0000-0002-6233-516X*
Nearest person month worked:	1
Contribution to Project:	*Dr. Frank provided general oversight of resources and budget for Aim 2.*
Funding Support:	

Name:	*Jonathan Wisor, Ph.D.*
Project Role:	*Co-PI*
Researcher Identifier:	*ORCID ID: 0000-0003-4948-4379*
Nearest person month worked:	1
Contribution to Project:	*Dr. Wisor directed the optogenetic experimentation for Aim 2.*
Funding Support:	

Name:	*Brieann Satterfield, Ph.D.*
Project Role:	*Postdoctoral Researcher*
Researcher Identifier:	*ORCID ID: 0000-0002-8688-2416*
Nearest person month worked:	1
Contribution to Project:	*Dr. Satterfield managed the day-to-day operation of the human laboratory study of Aim 3.*
Funding Support:	

Name:	*Devon Hansen, Ph.D., LMHC*
Project Role:	*Assistant Research Professor*
Researcher Identifier:	*Washington State University ID: 10064965*
Nearest person month worked:	1
Contribution to Project:	*Dr. Hansen managed staffing for the human laboratory study of Aim 3.*
Funding Support:	

Name:	*Julie Erwin, M.S.*
Project Role:	*Research Associate*
Researcher Identifier:	*Washington State University ID: 11657249*
Nearest person month worked:	*2*
Contribution to Project:	*Ms. Erwin coordinated staffing and logistics for the study, and assisted with polysomnographic recordings for the human study of Aim 3.*
Funding Support:	

Name:	*Amanda Hudson*
Project Role:	*Ph.D. Student*
Researcher Identifier:	*Washington State University ID: 11624214*
Nearest person month worked:	*2*
Contribution to Project:	*Ms. Hudson managed performance testing for the human study of Aim 3.*
Funding Support:	

Name:	*Samantha Riedy, M.S.*
Project Role:	*Ph.D. Student*
Researcher Identifier:	*Washington State University ID: 11365976*
Nearest person month worked:	1
Contribution to Project:	*Ms. Riedy performed data analyses for the human subjects research of Aim 3.*
Funding Support:	

Name:	*Kirsie Lundholm*
Project Role:	*Postbaccalaureate Research Assistant*
Researcher Identifier:	*Washington State University ID: 11723261*
Nearest person month worked:	2
Contribution to Project:	*Ms. Lundholm conducted screening sessions, performed laboratory set-up for the experiment, and monitored subjects in the laboratory for the human study of Aim 3.*
Funding Support:	

Name:	*Matthew E. Layton, M.D., Ph.D.*
Project Role:	*Physician of Record*
Researcher Identifier:	*ORCID ID: 0000-0002-3287-9203*
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Layton implemented procedures for the pharmacogenetic interventions in the human subjects.*
Funding Support:	

Name:	*Samuel Joseph, D.O.*
Project Role:	*Medical Oversight*
Researcher Identifier:	*Washington State University ID: 11475855*
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Joseph performed medical exams during screening and oversaw subject health and well-being in the human subjects study of Aim 3.*
Funding Support:	

Name:	*Dawn DePriest, Ph.D., FNP-C*
Project Role:	*Medical Oversight*
Researcher Identifier:	*Washington State University ID: 11458230*
Nearest person month worked:	*1*
Contribution to Project:	*Together with Dr. Joseph, Dr. DePriest performed medical exams during screening and oversaw subject health and well-being in the human subjects study of Aim 3*
Funding Support:	

Name:	*Patti Grossman, MSN, FNP-C*
Project Role:	*Medical Oversight*
Researcher Identifier:	*Washington State University ID: 11573115*
Nearest person month worked:	*1*
Contribution to Project:	*Together with Dr. Joseph and Dr. DePriest, Ms. Grossman performed medical exams during screening and oversaw subject health and well-being in the human subjects study of Aim 3*
Funding Support:	

Name:	*Stephen James, Ph.D.*
Project Role:	*Researcher*
Researcher Identifier:	*ORCID ID: 0000-0003-4139-7967*
Nearest person month worked:	*1*
Contribution to Project:	*Dr. James implemented operationally relevant testing procedures in the simulators used in the human laboratory study of Aim 3.*
Funding Support:	

Name:	*Michelle Schmidt*
Project Role:	*Research Technician*
Researcher Identifier:	*Washington State University ID: 11126756*
Nearest person month worked:	3
Contribution to Project:	*Ms. Schmidt performed rat EEG surgery and managed the wild-type, DRD2::Cre and Adora2a::Flp rat colonies for Aim 2. She also did histology to determine viral transfection and optrode placement.*
Funding Support:	

Name:	*Daniel Harvey*
Project Role:	*Research Technician*
Researcher Identifier:	*Washington State University ID: 10245277*
Nearest person month worked:	3
Contribution to Project:	*Mr. Harvey implemented the behavioral paradigm for the rat experiments of Aim 2. He also maintained the equipment and wrote code for data analysis files.*
Funding Support:	

Name:	*Wei Chin*
Project Role:	*Research Assistant*
Researcher Identifier:	*Washington State University ID: 11650093*
Nearest person month worked:	3
Contribution to Project:	*Mr. Chin collected data for the rat experiments of Aim 2.*
Funding Support:	

Name:	*Jemima Onih*
Project Role:	*Research Assistant*
Researcher Identifier:	*Washington State University ID: 11650094*
Nearest person month worked:	3
Contribution to Project:	*Ms. Onih collected data for the rat experiments of Aim 2.*
Funding Support:	

Name:	*Kelli Singer*
Project Role:	*Research Assistant*
Researcher Identifier:	*Washington State University ID: 11584759*
Nearest person month worked:	1
Contribution to Project:	*Ms. Singer was part of the group of research assistants providing around-the-clock staffing and carrying out the research protocol for the human study of Aim 3.*
Funding Support:	

Name:	*Jonah Scott*
Project Role:	*Research Assistant*
Researcher Identifier:	*Washington State University ID: 11698308*
Nearest person month worked:	1
Contribution to Project:	*Mr. Scott was part of the group of research assistants providing around-the-clock staffing and carrying out the research protocol for the human study of Aim 3.*
Funding Support:	

Name:	*Mary Peterson*
Project Role:	*Research Assistant*
Researcher Identifier:	*Washington State University ID: 11695706*
Nearest person month worked:	1
Contribution to Project:	*Ms. Peterson was part of the group of research assistants providing around-the-clock staffing and carrying out the research protocol for the human study of Aim 3.*
Funding Support:	

Name:	*Dustin Parmiter*
Project Role:	*Research Assistant*
Researcher Identifier:	*Washington State University ID: 11698327*
Nearest person month worked:	1
Contribution to Project:	*Mr. Parmiter was part of the group of research assistants providing around-the-clock staffing and carrying out the research protocol for the human study of Aim 3.*
Funding Support:	

Name:	*Deena Oubari*
Project Role:	*Research Assistant*
Researcher Identifier:	*Washington State University ID: 11698224*
Nearest person month worked:	1
Contribution to Project:	*Ms. Oubari was part of the group of research assistants providing around-the-clock staffing and carrying out the research protocol for the human study of Aim 3.*
Funding Support:	

Name:	*Cecilia Moeller*
Project Role:	*Research Assistant*
Researcher Identifier:	*Washington State University ID: 11582073*
Nearest person month worked:	1
Contribution to Project:	*Ms. Moeller was part of the group of research assistants providing around-the-clock staffing and carrying out the research protocol for the human study of Aim 3. She also assisted with participant screening.*
Funding Support:	

Name:	*Mari Metter*
Project Role:	*Research Assistant*
Researcher Identifier:	*Washington State University ID: 11509648*
Nearest person month worked:	1
Contribution to Project:	*Ms. Metter was part of the group of research assistants providing around-the-clock staffing and carrying out the research protocol for the human study of Aim 3.*
Funding Support:	

Name:	*Madeline McDougal*
Project Role:	*Research Assistant*
Researcher Identifier:	*Washington State University ID: 11643204*
Nearest person month worked:	1
Contribution to Project:	*Ms. McDougal was part of the group of research assistants providing around-the-clock staffing and carrying out the research protocol for the human study of Aim 3.*
Funding Support:	

Name:	*Emily Mathis*
Project Role:	*Research Assistant*
Researcher Identifier:	*Washington State University ID: 11698242*
Nearest person month worked:	1
Contribution to Project:	*Ms. Mathis was part of the group of research assistants providing around-the-clock staffing and carrying out the research protocol for the human study of Aim 3.*
Funding Support:	

Name:	*Amanda Kiefer*
Project Role:	*Research Assistant*
Researcher Identifier:	*Washington State University ID: 11698233*
Nearest person month worked:	1
Contribution to Project:	*Ms. Kiefer was part of the group of research assistants providing around-the-clock staffing and carrying out the research protocol for the human study of Aim 3.*
Funding Support:	

Name:	*Cynthia Hernandez*
Project Role:	*Research Assistant*
Researcher Identifier:	*Washington State University ID: 11465731*
Nearest person month worked:	1
Contribution to Project:	*Ms. Hernandez was part of the group of research assistants providing around-the-clock staffing and carrying out the research protocol for the human study of Aim 3.*
Funding Support:	

Name:	*Anna Franzella*
Project Role:	*Research Assistant*
Researcher Identifier:	*Washington State University ID: 11656937*
Nearest person month worked:	1
Contribution to Project:	*Ms. Franzella was part of the group of research assistants providing around-the-clock staffing and carrying out the research protocol for the human study of Aim 3.*
Funding Support:	

Name:	*Katie Christanson*
Project Role:	*Research Assistant*
Researcher Identifier:	*Washington State University ID: 11583062*
Nearest person month worked:	1
Contribution to Project:	*Ms. Christanson was part of the group of research assistants providing around-the-clock staffing and carrying out the research protocol for the human study of Aim 3.*
Funding Support:	

Name:	*Lynn Caldwell, PhD / NMRU-D (on subcontract)*
Project Role:	*DoD Consultant*
Researcher Identifier:	*ORCID ID: 0000-0002-6461-4023*
Nearest person month worked:	1
Contribution to Project:	*Dr. Caldwell contributed expertise regarding the caffeine and modafinil administration protocols for Aim 3.*
Funding Support:	

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Grants started:

National Institutes of Health, “Innovations in Research and Practice Improving Shiftworker Health and Safety,” August 2019–present (PI: Van Dongen HPA). This grant provided funding for organizing the 24th International Shiftwork and Working Time Symposium.

Transport Canada, “Fatigue Science Advisor,” May 2019–present (PI: Honn KA). This contract involves review of proposed railroad rule sets in Canada and advice on fatigue risk management.

National Institutes of Health, “AMERICAN INDIAN CHronic disEase RIsk and Sleep Health (AI-CHERISH),” May 2019–present (PI: Nelson LA). This project investigates the relationship between sleep health and chronic disease risk in American Indians.

National Institutes of Health, “Glycemic Variability and Fluctuations in Cognitive Status in Adults with Type 1 Diabetes,” April 2019–present (PIs: Germine L, Chaytor NS). This study examines the relationship between glycemic control and cognition in patients with type 1 diabetes.

National Institutes of Health, “Characterizing Evolutionarily Conserved Mechanisms Underlying Sleep, Clocks, and Memory,” July 2019–present (PI: Gerstner JR). This project will test phylogenetically diverse species that integrate the circadian rhythm of rest-activity cycles with changes in sleep need and identify evolutionarily conserved cellular and molecular mechanisms underlying activity-dependent changes in synaptic activity that are sensitive to sleep and are critical for cognitive function.

National Institutes of Health, "Circadian Clock Disruption as a Risk Factor for Environmental Carcinogenesis," November 2019–present (PI: Gaddameedhi S). The project will provide a mechanistic understanding of how the circadian clock regulates DNA damage response signaling and how circadian disruption affects genomic stability and environmental carcinogenesis

There is no overlap among the new projects and the current project, and effort on the new project has been offset by reduced effort on completed and closed grants (see below).

The new projects did not stem from results obtained in the present project. However, we expect that follow-up funding may come in the future based on results obtained in Years 2 and 3.

Grants closed:

Washington Research Foundation, “Clinical Sleep Research Facility,” September 2018–August 2019 (PI: Van Dongen HPA). This grant provided construction funding for a new clinical lab in the Sleep and Performance Research Center at Washington State University.

National Institutes of Health, "Non-Neuronal Regulators of Sleep," June 2014–July 2019 (PI: Frank MG). This project examined the contributions of glial cells in sleep regulation.

Mars Wrigley Confectionery US, “Effect of Mastication on Sustained Attention,” December 2018–November 2019 (PI: Hansen DA). This contract sought to test the efficacy of mastication as a countermeasure for fatigue.

National Science Foundation, “Threat-Assessment Tools for Management-Coupled Cyber- and Physical- Infrastructure,” September 2015–August 2019 (PI: Roy S). This project analyzed physical, technological and fatigue threats to air traffic control and other systems.

Congressionally Directed Medical Research Programs, “Sleep Deprivation Effects on Cognitive Flexibility in Dynamic Decision-Making Environments,” July 2016–July 2019 (PI: Van Dongen HPA). This project developed cognitive flexibility training to mitigate decision making error due to sleep loss.

National Institutes of Health, “Understanding Hormonal Mechanisms of Sleep Restriction,” August 2014–December 2019 (PI: Liu PY). This laboratory study investigated hormonal effects on health and performance during partial sleep restriction.

Neuro Detective International, “No Gavage Control Replication of Stress-Induced Insomnia in Rats,” October 2019–March 2020 (PI: Davis CJ). This project investigated the extent to which procedural stress of gavage-associated restraint with an EEG headcap affects male cage exchange stress induced insomnia.

Congressionally Directed Medical Research Programs, “The Role of Sleep in Mediating Post-Traumatic Stress Disorder,” July 2018–February 2020 (PI: Vanderheyden WM) .The goals of this study were to develop a PTSD model in rats, quantify recovery sleep, and employ an aversive conditioning paradigm to confirm results.

National Institutes of Health, "Multimodal Imaging of Neuronal and Glial Contributions to Sleep Homeostasis *in Vivo*," September 2017–August 2019 (PI: Hayworth CR). This project used cutting-edge imaging techniques to examine the involvement of astrocytes in vigilance states.

What other organizations were involved as partners?

- Organization Name: Naval Medical Research Unit Dayton
- Location of Organization: Dayton, OH
- Partner's contribution to the project: Dr. Lynn Caldwell is subcontracted as a DoD consultant for the project.

8. SPECIAL REPORTING REQUIREMENTS

Quad Chart

Investigating Striatal Attentional Circuits to Understand and Mitigate Deficits in Cognitive Flexibility Due to Sleep Loss

BA170226 / W81XWH1810100

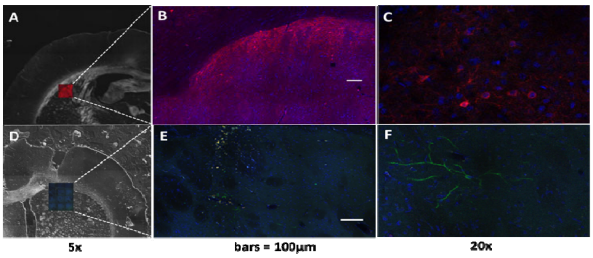
PI: Hans P.A. Van Dongen **Org:** Washington State University **Award Amount:** $2,797,841

Study/Product Aims

1. Develop behavioral model of sleep loss and cognitive flexibility in rodents.
2. Perform optogenetic experiments of sleep loss and cognitive flexibility with transgenic rats.
3. Demonstrate genotype differences in wake-promoting agents' effect on cognitive flexibility during sleep deprivation in humans.

Approach

In animal studies, we will optimize behavioral techniques that model the effects of sleep loss on cognitive flexibility observed in humans. Next, we will use transgenic rats that express Cre and Flp recombinase-dependent viral DNA constructs in striatopallidal medium spiny neurons of the striatum that express both the Adora2a and DrD2 receptors. We will use optogenetic methods to either activate these neurons, mimicking the effects of sleep deprivation on task performance in rats injected with flox/Frt -ChR2-GFP, or inactivate these neurons to recover normal task performance in sleep-deprived rats. For human subjects, we will compare the effectiveness of standardized doses of modafinil and caffeine during total sleep deprivation in promoting cognitive flexibility based on dopamine and adenosine genotype.



Accomplishments: Robust opsin expression in striatal dopaminergic cells (see panels A,B, and C), but only sparse expression in striatal adrenergic cells (see panels D,E, and F), after 3-4 weeks post viral injection in monotransgenic rat lines (Aim 2). For the human studies, 13 subjects completed the laboratory experiment (Aim 3), with another 10 subjects ready to be studied as soon as COVID-19 pandemic restrictions are lifted and the laboratory reopens.

Timeline and Cost

Activities CY	18	19	20	21
1.Development of rodent model	completed			
2.Optogenetic experiments				
3.Human genotype differences				
Estimated Budget ($K)	**$445K**	**$910K**	**$955K**	**$488K**

Updated: 2020 June 12

Goals/Milestones

CY20 Goals – Develop model of sleep loss and cognitive flexibility in transgenic rodents that responds to optogenetic manipulation in brain; continue human subject laboratory sleep deprivation experiments.

Comments/Challenges/Issues/Concerns

- Mandatory reduced effort on project due to COVID-19 pandemic disrupted animal and human research activities.
- Low opsin expression in ADORA2a cells, even after switch from serotype-5 virus to DJ (serotypes 2,5 and 8) virus. Will try to optimize transfection with Synapsin-1 promoter instead of Elongation Factor-1 promoter.

Budget Expenditure to Date

Projected Expenditure: approx. $1,832K.
Actual Expenditure: $1,781K (lagging due to COVID-19 pandemic).

9. APPENDICES

Nothing to report.

No manuscripts or presentation abstracts were published or in press during Year 2. Manuscripts submitted during Year 2 will be reported in Year 3 when published or accepted for publication.