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

PRINCIPAL INVESTIGATOR: Hans P.A. Van Dongen, PhD
Professor and Director
Sleep and Performance Research Center

CONTRACTING ORGANIZATION: Washington State University

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6. AUTHOR(S)
Hans P.A. Van Dongen, Christopher J. Davis

E-Mail: hvd@wsu.edu

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Washington State University
p/a Daniel Nordquist
240 French Admin Bldg,
Pullman, WA 99164-1001

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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.

While the human sleep laboratory was closed due to the COVID-19 pandemic, Year 3 of this ongoing project focused on further development of the transgenic rat models and on animal 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.

In Year 3 of the project, the human sleep laboratory was closed due to the COVID-19 pandemic, and effort on the project focused primarily on further development of the transgenic rat models and on animal data collection. A one-no-cost extension was requested and granted, extending the timeline of the project by 12 months.

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.	48	In progress
Data Analysis		
Milestone(s) Achieved: Aim 2 analyses completed.	48	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 during NAMRU-D contracting but no major impact on study)	WSU IRB approval: 4 Jan 2019 HRPO approval: 2 Apr 2019 NAMRU-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).	48	In progress
Data Analysis		
Milestone(s) Achieved: Aim 3 analyses completed.	48	n/a
Final Report Preparation		
Compilation of analyses from aims 1–3 and drafting of report and briefing	42-48	n/a
Presentation of study results to the DoD	48	n/a
Milestone(s) Achieved: study completed	**48**	**In progress**

What was accomplished under these goals?

During Year 3, the major activities to be completed to achieve the goal milestones were:

- Optimize the dose and localization of viral injections for Aim 2.
- Determine the extent to which behavioral and vigilance states are affected by optogenetic stimulation.
- Prepare manuscripts detailing our rodent work.
- Aim 3 implementation review meeting with DoD consultant, Senior Research Psychologist Dr. Lynn Caldwell.
- Data collection for Aim 3 (on hold due to the COVID-19 pandemic).

During Year 3, the specific objectives were to:

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

During Year 3, key outcomes included the following:

- The inhibitory opsin (halorhodopsin) transfection dose and localization was verified in DrD2-Cre rats.
- A significant experimental milestone was achieved in that the Aim 2 pairwise discrimination reversal task performance and post task EEG were disrupted by striatopallidal activation via optogenetic stimulation in DrD2-Cre rats, compared to control virus transfected rats.
- One manuscript detailing the rodent work was submitted for publication, and another is in preparation.
- Extensive preparations made to resume human subjects data collection in the laboratory study of Aim 3 once approval to reopen is received (expected in summer 2021).

Aim 2: During Year 3, work on Aim 2 centered on validating the DrD2-Cre+ rats with circuit activating (representative sample in Fig. 1A-C) and inhibiting (representative sample in Fig. 1D-F) opsins (n=3/construct). Histology showed robust expression of DrD2 positive cells in the dorsal striatum for both constructs. *These results laid the groundwork for the execution of activating the striatal indirect pathway in the context of cognitive flexibility described next, and also for the rescue experiments slated for the near future.*

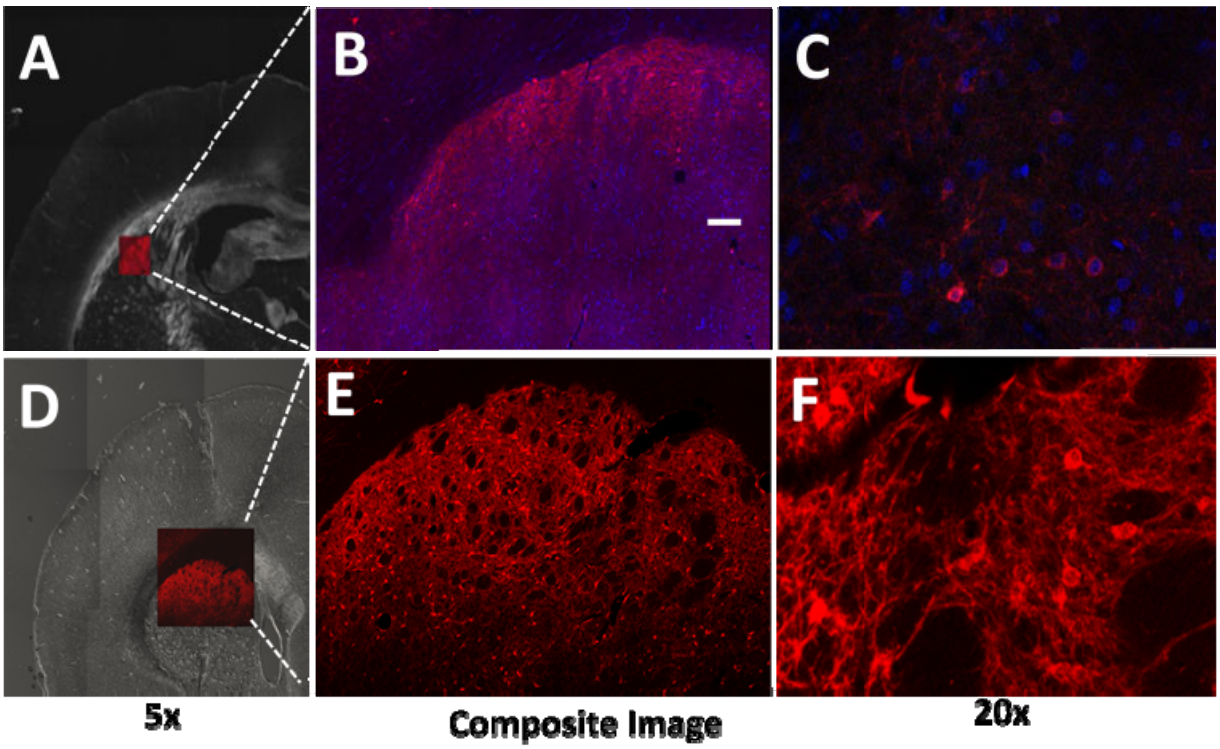


Figure 1. Upper panels: DrD2-Cre+ rats (8–9 weeks) transfected with 3 µl pAAV-EF1a-cDIO-hChR2(H134R)-mCherry Serotype 5, three weeks prior, at stereotaxic coordinates D/V 3.5 mm; A/P-0.6; M/L ±3.0. (A) Image of section showing placement of fluorescent inset of image B. (B) Image showing distribution of target protein in rat dorsal striatum (scale bar on bottom right). (C) Cellular localization of protein, blue is Dapi, red is mCherry. Lower panels: DrD2-Cre+ rats (6 weeks) surgically infused with 3 µl pAAV-EF1a-DIO-eNpHR3.0-mCherry-WPRE, Serotype 5, six weeks prior, with the above coordinates. (D) Image of section showing placement of fluorescent inset of image E. (E) Image showing the distribution of target protein in dorsal striatum. (F) Cellular localization of protein, red is enhanced mCherry.

Significant progress on Aim 2 was also made conducting the pairwise discrimination reversal task in conjunction with optogenetic stimulation. Male DrD2-Cre rats (LE-Tg(Drd2-icre)1Ottc)

were procured from the Rat Research and Resource Center (RRRC; strain #0768) and bred in house to Long Evans wild-type females (Envigo). Offspring were weaned and genotyped at 21 days and group housed until undergoing surgery. Genotyping was performed by Transnetyx (Cordova, TN) using sequences provided by the RRRC. Only DrD2-Cre positive male rats were used in the experiment.

Six-week-old rats (n=20) were anesthetized with a ketamine (29 mg/kg, IM) and xylazine (4.33 mg/kg, IM) injection and then maintained with 1% isoflurane for virus infusion (Fig. 2). The rats were prepared for standard stereotaxic surgery, given 0.5% lidocaine subcutaneous on the skull apex and craniotomized. The dorsal skull surface was cleaned with 3% hydrogen peroxide followed by 0.09% saline. The lambda and bregma reference points were leveled. Rats received 3 µl per hemisphere of pAAV-EF1a-cDIO-hChR2(H134R)-mCherry, serotype 5 (n=10; ChR2 group) or pAAV-EF1a-cDIO-mCherry, Serotype 5 (n=10; Control group) (UNC Vector Core, Raleigh, NC). Viral vectors were infused at A/P 0.6, M/L ±3.0, D/V -3.5 mm at a rate of 200 nl/min with needle equilibration holds of 5 min before and after the infusion. After the craniotomy was sutured, rats received a single injection of Buprenorphine SR Lab (3 mg/ml, 1 mg/kg, SC; ZooPharm) and then administered carprofen daily (5 mg/kg, SC) for 3 days.

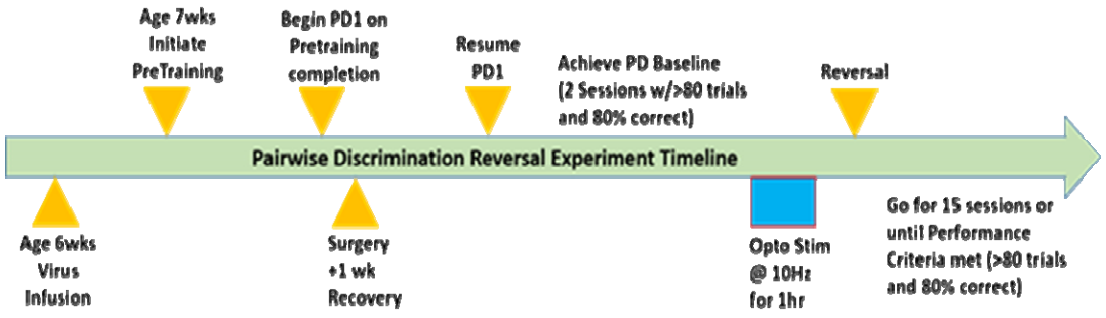


Figure 2. Experimental timeline of pairwise discrimination reversal with optogenetic stimulation.

Following surgery, rats were housed in pairs within microisolator cages (Techniplast) containing a custom-made cage divider that allowed for smelling and nose touching their cage mate but not rough play or food sharing. The divider protected surgical recovery and individual feeding regimens as well as limited socialization. All handling and training sessions were initiated at ZT 10. Food restriction began 5 days following surgery. Rats were weighed and given 90% of the predicted food (0.7 g/100 g body weight) based on male Long Evans development curves

reported by Envigo. Thereafter, rats were weighed each week day and kept at 83–87% of the predicted body weight with feeding following session completion. Two days before the rats were scheduled to begin training they were given 10–15 sugar pellets to reduce palate neophobia. The rats were also handled four times before the beginning of training to acclimate transport in and out of the operant chambers. The training was composed of three phases: pretraining, PD training and reversal (Fig. 3).

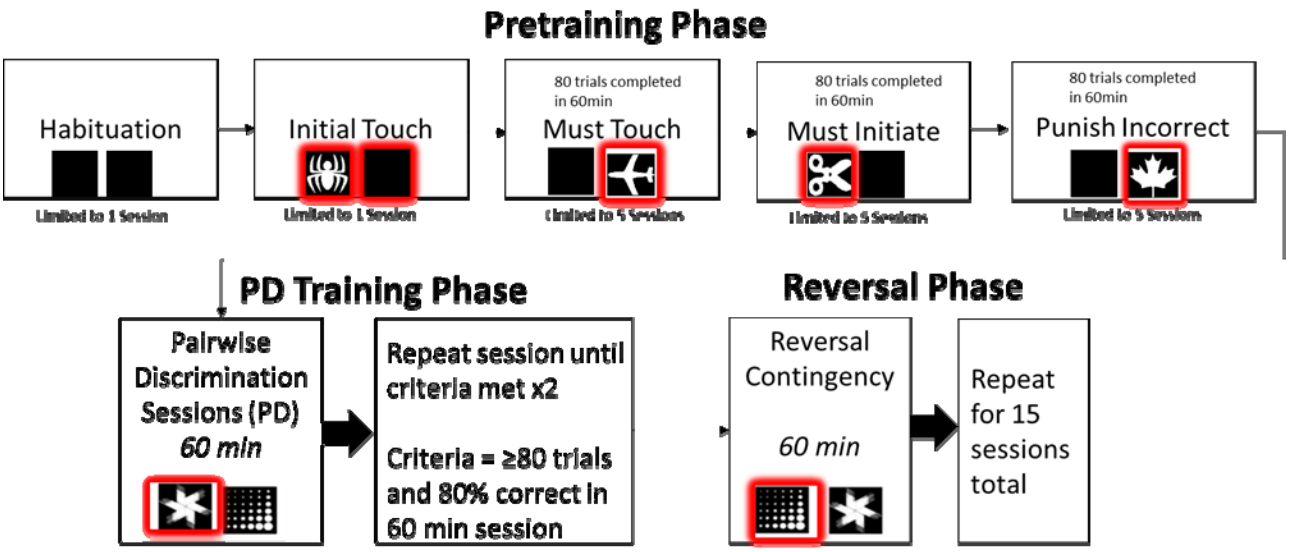


Figure 3. The three phases of the pairwise discrimination (PD) operant task. Red encircled images denote sugar pellet rewarded images in a subset of the rats (note that rewarded images were not cued by a red surround in the actual operant chambers). The opposite contingency for PD training and reversal phases was used in the other subset.

Pretraining Phase: Training began with a single Habituation session of 30 min in the operant chamber where the rats were given 15 sugar pellets (45 mg each) and allowed to explore. The remaining sessions were each 60 min long and had a maximum of 100 trials. The second session was Initial Touch. This session presented an image on either the left or right side in a pseudo random fashion so that a single image did not appear on a side more than three times in a row. If the rats touched the screen, they would receive three pellets, otherwise they received 1 pellet per image. This taught the rats to associate the images on the screen with a food reward. There was a possibility of receiving up to 300 pellets during this session (100 trials x 3 pellets/correct image) and was therefore limited to a single instance of this session. The rats advanced to Must Touch, where they had to complete at least 80 of 100 trials to move to the

next training step. Within Must Touch the rats learned to touch the screen to receive the food reward. Must Initiate required the rats to start each trial by turning off a light within the food hopper before being presented with a randomly presented image. They had to complete at least 80 trials to move to the next stage. Punish Incorrect was the final stage of training, wherein the rats had to incorporate previous response contingencies in addition to distinguishing the blank square from the randomly presented image. When the rats touched the blank square the chamber light came on and a tone was given. If an incorrect response was made the rats entered a correction trial. For the correction trial the stimulus was presented on the same side again before continuing to the next actual trial. If the rats correctly touched the image, they received a sucrose pellet. After completing at least 80 trials with 80% correct the rats moved to the PD training phase.

PD Training Phase: At the beginning of discrimination training the rats were randomly assigned to either “fan” or “marbles” as the correct image. The rats proceeded with only these two images until they reached at least 80 trials with 80% correct. After rats completed the first session of the training phase (PD1) they underwent a second surgery to implant electrodes and guide cannulas to record cortical biopotentials and optogenetically stimulate. Rats were anesthetized and craniotomized as before. Phosphoric acid etchant was applied to the skull for 10 seconds and irrigated with saline. The skull was leveled, and EEG screws (invovo1) placed bilaterally above the prefrontal cortex (approximately A/P 3.0, M/L ±2.0 mm) and one in each hemisphere behind bregma (approximately A/P-4.0, M/L ±3.0 mm) for differential recording. Guide cannulas were placed at the site of viral infusion (A/P 0.6, M/L ±3.0, D/V -3.5 mm) and fixed with UV cure dental cement. Two EMG wires with metal paddles (invivo1) were inserted bilaterally under the nuchal muscles. The electrodes were connected to a 6-pin connector (Plastics One) and the connector and wires were encased in dental cement. Rats received post-surgical analgesics as before and received *ad lib* food a week before reinstating food restriction and reentering PD training. During subsequent PD and reversal sessions the rats were tethered to a dummy commutator. The total number of trials completed, the number of trials correctly answered, and the number of correction trials completed within each session were recorded. When the rats met criterion (at least 80 trials with 80% correct) twice, these two sessions were averaged to generate baseline performance values.

Reversal Phase: Following the second baseline session, the rats were removed from the touch-screen operant chamber, the dummy tether was removed, and a headstage instrumented with

preamplifiers and right and left rat friction fit LED (445 nm) optogenetic fiber probes was attached. Bilateral LED optrodes were inserted into the guide cannula and the rat placed into a recording chamber where baseline sleep was recorded. Recordings were conducted at 500 Hz using Sirenia software (Pinnacle Technology Inc.). At ZT 9 the following day the rats received optogenetic stimulation. Stimulation parameters were 300 mA, 10 Hz, 20 ms PW, 20% duty cycle for 1 h using a Pinnacle 8487 2 Opto, 2 EEG, 1 EMG recording system. At ZT 10, the rats were removed from the recording chamber, transported to the touch-screen operant chamber, and connected to the dummy commutator, and underwent the first reversal session. Following the 1 h reversal session, the rats were returned to the Pinnacle chamber to record recovery sleep. The rats were removed at ZT 5 the following day and returned to their home cage until the next reversal session. Reversal sessions continued until performance criterion was met (at least 80 trials with 80% correct) or for a maximum of 15 reversal sessions. Rats that did not meet performance criterion at any phase or lost a headcap were omitted from the study. This included five rats from the ChR2 group and five rats from the Control group. For the ten remaining rats (n=5/group), PD reversal data were analyzed and EEG records were scored.

At the end of the study, rats were anesthetized with sodium pentobarbital (150 mg/kg, IP) and transcardially perfused with cold PBS followed by 10% formalin. Brains were extracted and stored in 10% formalin for 24 h at 4 ºC before being transferred to 30% sucrose in PBS and stored at 4 ºC. Brains were blocked A/P ±10.0 mm from the guide cannula imprint and sectioned in the coronal plane at 40 µm using a cryostat (ThermoFisher) and mounted to slides. A confocal microscope (Leica DMi8, TCS SPE) was used to verify AAV expression and cannula/ optrode placement.

Preliminary behavioral data suggest that activation of the striatal indirect pathway, immediately before the initial reversal session, disrupts the ability of rats to adapt to changes in contingencies (Fig. 4). This result is similar to performance decrements observed in rat cognitive flexibility when preceded with acute sleep deprivation. *These anticipated findings are critical to the project because they support our hypothesis that activation of the DrD2+ medium spiny neurons in the dorsal striatum induces a state of high sleep propensity which in turn compromises adaptive decision making.*

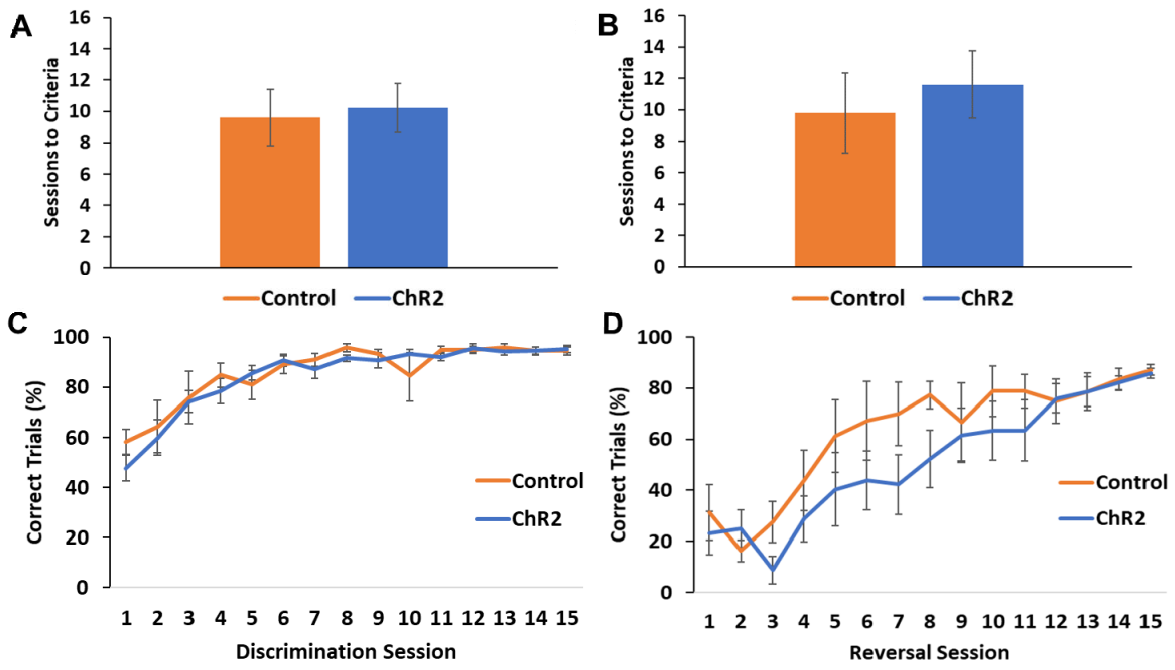


Figure 4. Optogenetic activation of the striatal indirect pathway disrupts cognitive flexibility in rats. Prior to stimulation of striatal DrD2+ medium spiny neurons, ChR2 virus infused rats (n=5; blue) had similar performance as determined by the number of sessions to criteria (A) and the percent correct trials (B) as control virus infused rats (n=5; orange). However, after optogenetic stimulation and image reversal, ChR2 rats took ~2 sessions longer to meet performance criteria (C) and exhibited less percent correct trials (D) for 10 sessions compared to control rats. Data are expressed as group mean ± SEM.

We also recorded EEG cortical arousal states prior to and after the initial reversal session that was preceded by optogenetic stimulation, with the expectation that sleep would be increased in ChR2 rats compared to controls. EEG data were scored in 10 s epochs as wake, NREM or REM. In ChR2 rats, optogenetic activation of the striatal indirect pathway led to a 48% increase in sleep rebound in the 3 h period following the 10 Hz stimulation and the initial reversal session compared to control virus rats that also received the 10 Hz stimulation (Fig. 5). *This preliminary result is encouraging because that amounts to a ~30 min sleep increase (REM and NREM combined) at a time when rats, as nocturnal animals. normally have a high propensity for wake. It supports the preliminary behavioral data and our hypothesis that the striatal indirect pathway is particularly susceptible to the effects of sleep loss.*

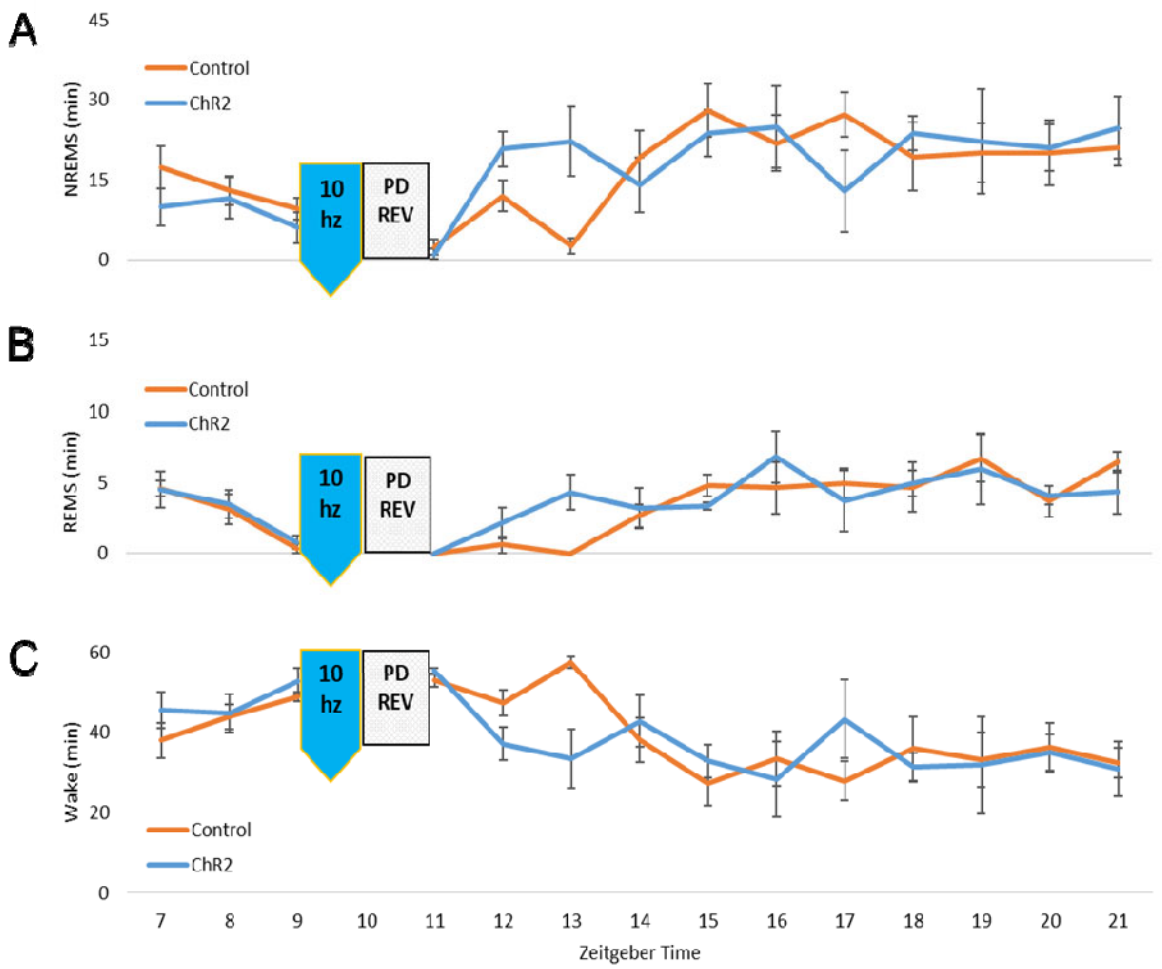


Figure 5. *Optogenetic activation of the striatal indirect pathway produces a sleep rebound. In the 3 h preceding stimulation of striatal DrD2+ medium spiny neurons, ChR2 virus infused rats (blue lines; n=5) and control virus infused rats (n=5; orange lines) had similar EEG arousal states. However, in the 3 h following optogenetic stimulation (blue arrow) and the image reversal task (PD REV), ChR2 rats manifested increased NREM sleep (A) and REM sleep (B), and decreased wakefulness (C) compared to controls. Data are expressed as group mean ± SEM.*

Aim 3: Due to the COVID-19 pandemic, the human sleep laboratory was closed throughout Year 3, and no experimentation could be conducted. The laboratory is expected to reopen in the summer of 2021. Activity pertaining to Aim 3 was limited to a review of the laboratory procedures implemented in Year 2, with our DoD (NAMRU-D) consultant, Senior Research Psychologist Dr. Lynn Caldwell. The review was done via videoconference and embedded in a symposium review of all ongoing science in our group (human and animal studies) related to the

project to help place the work in a larger context. Additionally, data management and reduction was done for data collected in Year 2 (but no data analyses as we are blinded to condition), and procedures were developed and implemented for seamless resumption of the human laboratory study once the laboratory has reopened. To date, a total of 13 out of 90 subjects completed the study; the remaining subjects will be studied during a no-cost extension that has been granted to complete the work.

During Year 3, other achievements included:

Two manuscripts published on results obtained during preparatory work for Aim 3 (PDF copies enclosed).

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 Neuroscience program at Washington State University, Darian Lawrence-Sidebottom, was involved in dissertation research integrated with the project. She was first author on one of the publications resulting from the project in Year 3. She graduated and earned her Ph.D. degree in December 2020.

- 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 has been involved in the project under the direct mentorship of the Co-PI (Honn) of the project, and she published a paper related to the project in Year 2 which formed a basis for her dissertation project. During Year 3 of the project, she continued her dissertation research.

- A mini-symposium was organized in Year 3, and oral presentations regarding progress on the animal and human work were presented by trainees Daniel Harvey, Amanda Hudson, and Rachael Muck.

- Two post-baccalaureate research assistants, Julie Erwin and Myles Finlay, completed their board certification to become Registered Polysomnographic Technologists (RPSGT).

How were the results disseminated to communities of interest?

In preparation of upcoming in-person conferences (e.g., Military Health System Research Symposium 2021), a mini-symposium was held on 2021 March 24 via videoconference, with participation from the different research groups involved in the project as well as NAMRU-D consultant Dr. Lynn Caldwell. Following an introduction by the PI, Dr. Hans Van Dongen, the following presentations were included:

- Michelle Schmidt: *Optimizing Optogenetics: Targeting the Striatal Indirect Pathway*
- Daniel Harvey: *Developing the Bichromatic Light Actuating Search Task (BLAST): An Open Field Rodent Task Assessing Cognitive Flexibility*
- Christopher Davis, PhD: *Progress with Rat Operant Tasks of Cognitive Flexibility*
- Ashley Ingiosi, PhD: *Defining a Role for Non-Neuronal Cells in Sleep and Sleep Homeostasis*
- Lynn Caldwell, PhD: *Naval Medical Research Unit Dayton Fatigue Assessment and Countermeasures Research*
- Kimberly Honn, PhD: *Workload and Time-on-Task in the PVT*
- Brieann Satterfield, PhD: *ARC Genotype Modulates Slow Wave Sleep Following Total Sleep Deprivation*
- Courtney Kurinec, PhD: *Can Unitization Promote Associative Memory during Sleep Deprivation?*
- Amanda Hudson, PhD: *Speed/Accuracy Trade-Off in the Effects of Acute Total Sleep Deprivation on a Sustained Attention and Response Inhibition Task*
- Rachael Muck: *DRD2 C957T Genotype Modulates the Time-on-Task Effect during Total Sleep Deprivation*

Additionally, the PI (Van Dongen) presented on the mechanisms underlying alertness at the Advances in Sleep & Circadian Science Series of the Sleep Research Society, on 2021 March 8, via videoconference; and at the Brigham and Women's Hospital / Harvard Medical School

Division of Sleep Medicine Analytic and Modeling Unit journal club, 2021 May 5, via videoconference.

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

For the animal studies (Aim 2): During Year 4 of the project (under no-cost extension), we will complete the mimicry and rescue studies in order to finish the project. In addition to the manuscript currently under review, we will submit two additional manuscripts detailing our work.

For the human study (Aim 3): During Year 4 of the project (under no-cost extension), 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 is 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 moved to monotransgenic DrD2 rats to execute the mimetic and rescue studies. This will benefit the project because the number of rats omitted due to technical issues with the headcap can be accommodated by exploiting a single transgenic line to finish the mimicry and rescue experiments.

- In the human study (Aim 3), as previously reported, we will omit the polysomnographic recording of sleep, as the hands-on procedure involved in electrode application 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, has been approved, and will be submitted to HRPO along with notification of resumption of the human study when the laboratory reopens (expected summer 2021).

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. While the animal research was allowed to resume a few months later, the human sleep laboratory remains closed. As soon as we have permission to reopen the human laboratory (expected for summer 2021), we will resume and intensify our human research efforts. A 1-year no-cost extension has been granted 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 slowed down over Year 3. The remaining funds will be needed to complete the study during

the 1-year no-cost extension that was granted to finish the project. 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

Lawrence-Sidebottom D, Hinson JM, Whitney P, Van Dongen HPA, Honn KA. Reversal learning deficits during sleep deprivation: Investigating the role of information acquisition failures. *Chronobiology International*, 2020; 37: 1445–1451.

Muck RA, Van Dongen HPA, Schmidt MA, Wisor JP, Layton ME, DePriest DM, Honn KA, Satterfield BC. *DRD2* C957T genotype modulates the time-on-task effect during total sleep deprivation. *Chronobiology International*, 2020; 37: 1457–1460.

Books or other non-periodical, one-time publications

Nothing to report.

Other publications, conference papers, and presentations

Van Dongen HPA (oral presentation): Beyond the two-process model of sleep regulation: Long-term dynamics of alertness. Advances in Sleep & Circadian Science Series, Sleep Research Society; via videoconference, Mar 2021.

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 provided general oversight, co-authored and submitted publications, and gave presentations on the research. He 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, implemented a COVID-19 research reduction contingency plan for the animal laboratory, took responsibility for the transgenic 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 developed a COVID-19 research reduction contingency plan for the human 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 and implementation of the operant reversal paradigm for Aim 2.*
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 oversaw gene analyses and paper writing.*
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 research of Aim 3.*
Funding Support:	

Name:	*Lillian Skeiky*
Project Role:	*Ph.D. Student*
Researcher Identifier:	*Washington State University ID: 11656455*
Nearest person month worked:	1
Contribution to Project:	*Ms. Skeiky prepared an article on the role of genes in human subjects performance during sleep deprivation.*
Funding Support:	

Name:	*Amanda Hudson*
Project Role:	*Ph.D. Student*
Researcher Identifier:	*Washington State University ID: 11624214*
Nearest person month worked:	1
Contribution to Project:	*Ms. Hudson implemented performance testing for the human study of Aim 3.*
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 contributed to COVID-19 protocols for the resumption of human laboratory research.*
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 maintained simulator equipment and software.*
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 and DRD2::Cre rat colonies for Aim 2. She also did histology to determine viral transfection for halorhodopsin constructs.*
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 equipment and wrote code for data analysis.*
Funding Support:	

Name:	*Lynn Caldwell, PhD / NAMRU-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:

Congressionally Directed Medical Research Programs, "Resilience to Sleep Loss and Stress: A Framework for Investigation and Intervention," September 2020–September 2023 (PI: Van Dongen HPA). This grant provides funding for the development of a conceptual framework that explains the effects of both sleep loss and stress on cognitive functioning.

National Institutes of Health, "Resilience, Cultural Alignment, and Social Support in Brain Aging: Data from the Strong Heart Study," September 2020–May 2025 (PI: Suchy-Dicey A). This grant uses previously collected data to examine factors associated with resilience in brain aging in underserved populations.

National Institutes of Health, "Astroglial Mechanisms in Sleep Homeostasis," April 2021–June 2025 (PI: Frank MG). This grant focuses on the role of astrocytes in regulating sleep.

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:

National Institutes of Health, "Exploratory Models of Cortical Consolidation," July 2017–June 2020 (PI: Frank MG). This exploratory grant involved animal research to lay a foundation for a larger investigation of how experience and sleep shape the developing brain.

National Institutes of Health, "Mutual Effects of Sleep and Pain in Veterans with Chronic Pain: A Supplemental Grant," July 2016–June 2020 (PI: Jensen MP). This grant involved testing of behavioral intervention to mitigate pain in military veterans, with a specific focus on the mediating role of sleep.

National Institutes of Health, “Farnesol Analogues as Novel Treatment for Succinic Semialdehyde Dehydrogenase (SSADH) Deficiency,” July 2018–July 2020 (PI: Roullet JB). This grant examined a possible new treatment for a rare neurological disorder.

National Institutes of Health, “Neurotrophin Regulation of Sleep *in Vivo*,” September 2017–July 2020 (PI: Frank MG). This grant investigated the function of neurotrophin as a sleep regulatory substance.

National Institutes of Health, “Innovations in Research and Practice Improving Shiftworker Health and Safety,” August 2019–July 2020 (PI: Van Dongen HPA). This grant provided funding for organizing the 24th International Shiftwork and Working Time Symposium.

National Institutes of Health, "Circadian Clock Disruption as a Risk Factor for Environmental Carcinogenesis," November 2019–October 2020 (PI: Gaddameedhi S). This project provided a mechanistic understanding of how the circadian clock regulates DNA damage response signaling and how circadian disruption affects genomic stability

Congressionally Directed Medical Research Programs, “The Role of Sleep in Mediating Post-Traumatic Stress Disorder,” August 2018–January 2021 (PI: Vanderheyden WM). This animal study investigated the effects of sleep and sleep loss on the development of PTSD symptomology.

Transport Canada, “Fatigue Science Advisor,” May 2019–March 2021 (PI: Honn KA). This contract involved review of proposed railroad rule sets in Canada and advice on fatigue risk management.

National Institutes of Health, “Rapalog Therapy in Heritable and Vigabatrin-Induced GABA Metabolic Disorders,” May 2017–April 2021 (PI: Gibson M). This grant investigated a possible therapy for a class of rare metabolic disorders.

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

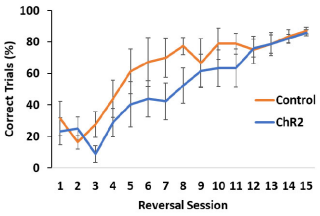
Study/Product Aims

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.

Mimicking sleep loss via striatopallidal circuit activation 60-min before pairwise discrimination reversal decreases % of correct trials (mean ± SEM) for over a week. This time course closely resembles the cognitive flexibility performance decrement occurring after ten hours of physical sleep deprivation. Control rats (n=5) have the same viral construct as the ChR2 rats (n=5) but lack the activating rhodopsin.



Accomplishments: Preliminary (as yet to be completed) data set analyzed from DrD2+ rats with optogenetic striatal stimulation prior to pairwise reversal demonstrated EEG and cognitive deficits consistent with sleep loss mimicry compared with control virus injected rats; inhibitory opsin transfection efficiency was verified (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 restrictions are lifted (projected for mid to late summer).

Timeline and Cost

Activities CY	18	19	20	21	22
1. Development of rodent model	completed				
2. Optogenetic experiments					
3. Human genotype differences					
Estimated Budget ($K)	**$445K**	**$910K**	**$955K**	**$488K**	**no-cost**

Updated: 2021 June 7

Goals/Milestones

CY21 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 once COVID-19 permission to reopen has been obtained.

Comments/Challenges/Issues/Concerns

- Mandatory human laboratory closure due to COVID-19 pandemic disrupted human subjects research activities which continue to be suspended.

Budget Expenditure to Date

Projected Expenditure: $2,798K.

Actual Expenditure: $2,400K (lagging due to COVID-19 pandemic; expenditures will catch up during approved 1-year no-cost extension).

9. APPENDICES

Lawrence-Sidebottom D, Hinson JM, Whitney P, Van Dongen HPA, Honn KA. Reversal learning deficits during sleep deprivation: Investigating the role of information acquisition failures. *Chronobiology International*, 2020; 37: 1445–1451.

Muck RA, Van Dongen HPA, Schmidt MA, Wisor JP, Layton ME, DePriest DM, Honn KA, Satterfield BC. *DRD2* C957T genotype modulates the time-on-task effect during total sleep deprivation. *Chronobiology International*, 2020; 37: 1457–1460.