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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. The animal study with behavioral and brain-based data collection in transgenic rats has been completed, and a manuscript describing the results is nearly finished. After extended closure of the human sleep laboratory due to the COVID-19 pandemic and subsequent delays in subject recruitment associated with the post-pandemic worker shortage affecting availability of the population we draw from, the double-blind human study continued in Year 5 and will require one additional year to be completed.					
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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. 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 in order to mimic the effects of sleep deprivation on task performance in rats injected with ChR2-mCherry, or inactivate these neurons in order 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.

After extended closure of the human sleep laboratory due to the COVID-19 pandemic, the double-blind human study was resumed in Year 4 and continued in Year 5. The parallel animal study involving transgenic rat models and behavioral and brain-based data collection has been completed. Another 1-year no-cost extension was granted, extending the timeline of the project by 12 additional 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.	60	100%
Data Analysis		
Milestone(s) Achieved: Aim 2 analyses completed.	60	100%
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).	72	In progress
Data Analysis		
Milestone(s) Achieved: Aim 3 analyses completed.	72	n/a
Final Report Preparation		
Compilation of analyses from aims 1–3 and drafting of report and briefing	69-72	n/a
Presentation of study results to the DoD	72	n/a
Milestone(s) Achieved: study completed	72	In progress

What was accomplished under these goals?

During Year 5, the major activities to be completed to achieve the goal milestones were:

- Perform data analyses for aim 2 to determine the extent to which behavioral and vigilance states are affected by optogenetic stimulation.
- Prepare manuscripts detailing our rodent work (Aims 1 and 2).
- Continue data collection for the human study of Aim 3.

During Year 5, the specific objectives were to:

- Perform analyses of the data collected for Aim 2 – completed.
- Collect data in double-blind study of human research participants for Aim 3 – ongoing.

During Year 5, key outcomes included the following:

- A second and third manuscript detailing the rodent work were accepted for publication (Foakes et al., 2022; Strobel et al., 2022).
- A manuscript describing the results of the animal experiments of Aim 2, showing that 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.
- Human subjects data collection for Aim 3 progressed, albeit at a slower rate than hoped for due to the post-pandemic worker shortage, which adversely affected the availability of the population we draw from.

The human study of Aim 3 remains double-blind to stimulant condition and genotype and can therefore not yet be analyzed and reported on, and the preparatory work for the animal experiments of Aim 1 were completed and reported on previously. That said, novel and significant scientific progress can be reported for Year 5 based on the full set of results obtained in Aim 2. These results have been reported in recent quarterly reports; they are included here again in a reworked format that will be turned into a comprehensive journal publication as the main product resulting from Aim 2.

1. Introduction

Cognitive flexibility, the ability to alter decision-making strategies in the face of negative feedback during real-time tasks, is known to be impaired after sleep deprivation (SD). Cognitive flexibility is regulated by the dorsal striatum [Bonnavion et al., 2019; Qiao et al., 2017; Sala-Bayo et al., 2020]. GABAergic dorsal striatal medium spiny neurons (MSNs) of the indirect pathway are known to support habitual behavior [i.e., cognitive *inflexibility*/rigidity; Kehagia et al., 2010; Wei et al., 2011; Yu et al., 2009], and their activation by A2a receptors under conditions of SD may underlie cognitive rigidity in this state. Moreover, both dopamine type 2 receptors (DrD2) [Muck et al., 2020; Tomasi et al., 2016; Volkow et al., 2012] and A2a receptors [Bodenmann et al., 2012; Rétey et al., 2007], for which these cells are enriched, have been implicated through genetic and pharmacological approaches in humans to be involved in the cognitive deficits resulting from SD. To better understand the cellular mechanisms underlying the negative effects of sleep loss on cognitive flexibility, we used an optogenetic approach to interrogate the role of the dorsal striatal DrD2-positive population in the cognitive effects of SD.

DrD2 and A2a receptors interact antagonistically at the biochemical level in dorsal striatal indirect pathway MSNs. At the circuit and receptor level, the striatum has been examined in the contexts of its functional effects on arousal state [Eban-Rothschild et al., 2017; Qui et al., 2010; 2019; Yuan et al., 2017; Zhang et al., 2013] and its role in learning and behavior [Agnoli et al., 2013; Garr & Delamater, 2020; Nonomura et al., 2018; Radke et al., 2018]. Herein, our aim was to query the intersection of these two functions in the dorsal striatum specifically, to gain a mechanistic understanding of its contribution to the cognitive utility of adaptive response efficiency following SD. We hypothesized that striatal indirect pathway MSNs are necessary and sufficient to confer the negative impacts of SD on cognitive flexibility. More specifically, we posited that activating these cells would mimic the effect of SD and induce performance deficits and EEG signatures of sleep loss.

We employed a pairwise discrimination (PD) reversal learning paradigm to model SD-induced cognitive rigidity (Strobel et al., 2022) in transgenic rats that express Cre recombinase-dependent viral DNA constructs in dorsal striatal DrD2-positive neurons. We used optogenetic techniques to activate these MSNs in rats transfected with channelrhodopsin-2 (**ChR2**). We compared them to rats injected with a control viral construct (*sans* ChR2) that were either sleep deprived for 9 h (**9 SD**) or left undisturbed (**0 SD**) prior to task reversal. We also recorded cortical EEG to determine the extent to which optogenetic activation of the dorsal striatum DrD2-

positive neurons artificially induced characteristic SD recovery phenotypes including sleep rebound and elevated delta power from fast-Fourier transforms (FFT) of the biopotentials.

2. Methods

2.1 Subjects

Male breeder DrD2-Cre rats (LE-Tg(Drd2-icre)1Ottc) procured from the Rat Research and Resource Center (RRRC, University of Missouri, USA; strain #0768) were paired in-house to Long Evans wild-type females (Envigo). Offspring were weaned and genotyped at 21 days old. Genotyping was performed by Transnetyx (Cordova, TN) using sequences provided by the RRRC. Only DrD2-Cre-positive male rats (DrD2+) were used in these experiments. Prior to surgeries, all rats were provided cage enrichment materials and maintained on a 12/12 h light-dark cycle at 24 ± 2 °C with access to food and water. After the completion of the experiments, rats were anesthetized and perfused, and subjected to histological verification of transfection efficiency and optrode tip placement.

2.2 Virus Injections

At six weeks of age, male DrD2+ rats (n=25; Fig. 1) 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 injection. The rats were prepared for standard stereotaxic surgery, given 0.5% lidocaine subcutaneously 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 μ l per hemisphere of pAAV-EF1a-cDIO-hChR2(H134R)-mCherry, serotype 5 (ChR2 group n=9) or pAAV-EF1a-cDIO-mCherry, Serotype 5 (0 SD group n=10 and 9 SD group n=6; UNC Vector Core). Viral vectors ($3.4\text{--}5.2 \times 10^{12}$ particles/ml) 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 incision was closed, rats received a single injection of Buprenorphine SR (3 mg/ml, 1 mg/kg, SC; ZooPharm) and then were administered carprofen daily (5 mg/kg, PO) for 3 days.

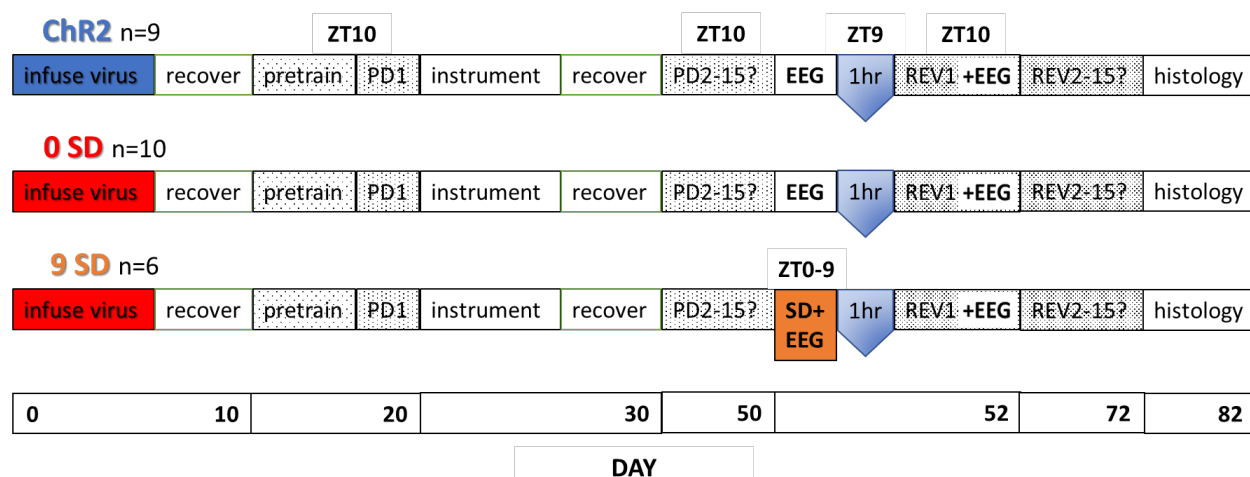


Figure 1. Experimental time course and design. The data collection protocol for each of the three treatment groups (ChR2 (top row), 0 SD (second row), and 9 SD (third row)) are shown across protocol days (bottom row). At 6 weeks of age, rats entered protocol DAY 0. The blue-shaded pentagon indicates optogenetic stimulation. (SD=sleep deprivation, ZT#=zeitgeber time, PD#=pairwise discrimination session, EEG=electroencephalogram, REV#=reversal session).

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 opportunities for socialization. To incentivize performance, rats were given 90% of their predicted food consumption (0.7 g/100 g body weight) based on male Long Evans development body weight curves reported by Envigo. Thereafter, rats were weighed each weekday and kept at 83–87% of the predicted body weight with feeding following session completion. Food restriction began 5 days after surgery. During training, pellets would constitute about 13% of the daily total caloric intake if a 300 g rat received 60 pellets in a session with restricted feeding.

2.3 Pairwise Discrimination Protocol

The Bussey-Saksida touchscreen operant platform (Lafayette) controlled by ABETII software (version 22.03.22-touch-release-0-g236135349 32-bit) was used to conduct the PD reversal task. For all three treatment groups, the handling and training sessions were initiated at ZT10.

Two days before the rats were scheduled to begin pretraining, they were provided 10–15 banana-flavored sucrose pellets (45 mg/16 kCal each; Bio-Serv) in their home cage to reduce palate neophobia.

The rats were also handled four times before the beginning of training to acclimate transport into and out of the operant chambers. The training was composed of three phases: pretraining, PD training, and reversal (Fig. 2).

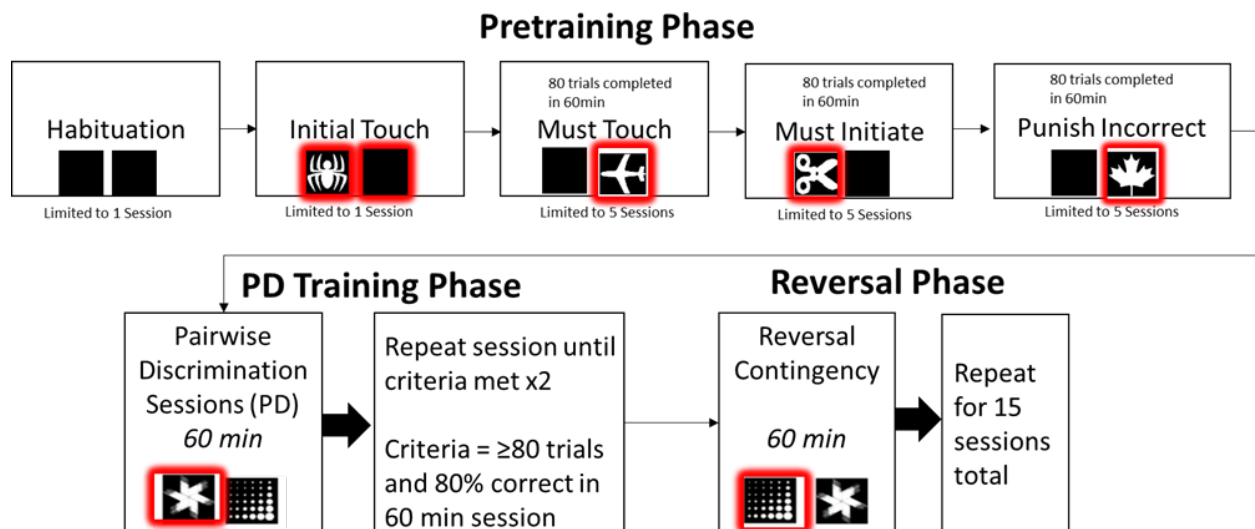


Figure 2. The three phases of the pairwise discrimination (PD) operant task. Red encircled images denote rewarded images in a subset of the rats (note that rewarded images were not cued by a red surround in the actual operant chambers). The opposite image was rewarded in PD training and reversal phases for the other subset of rats.

2.3.1 Pretraining Phase

Pretraining began with a 30 min *Habituation* session where the rats were allowed to explore the operant chamber with 15 pellets available in the food hopper. All other pretraining sessions were 60 min and had a maximum of 100 trials. The second *Initial Touch* session presented an image on either the left or right side of the touchscreen in a pseudo-random fashion so that an image did not appear on a particular side more than three consecutive times. If the rats touched the screen they received three pellets; if they did not, they received one pellet per image

presentation. In the *Must Touch* session, rats completed 80 trials of rewarded screen touches to advance to the next pretraining step or they were advanced after five sessions. The *Must Initiate* sessions required rats to trigger the trial start by turning off a light in the food hopper via beam break detection (i.e., non-specific locomotor activity) at which time rats were presented with a randomly presented image on the touchscreen. Again, if the rat achieved 80 trials in a 60 min session or completed five sessions, they advanced to the next stage. In the final stage of pretraining, *Punish Incorrect*, rats were trained to distinguish a blank square from random images from the image bank. If rats touched the blank square, an overhead chamber light came on and a tone was given signaling an **incorrect response**. If an incorrect response was made, the rats entered a correction trial. In a **correction trial**, the same image was presented on the same side again before continuing to the next actual trial. If the rats correctly touched the image, they received a reward and a 20 s intertrial interval was entered after which beam break initiated the next trial. After completing at least 80 trials with 80% **correct responses** in a session, the rats moved to the PD training phase.

2.3.2 PD Training Phase

At the beginning of PD training, the rats were randomly assigned to either “fan” or “marbles” as the correct (rewarded) image. All trials in the PD training and reversal phases were restricted to the simultaneous presentation of the fan and marble images (Fig. 3). After rats completed the first session of the training phase (PD1) they underwent surgery to implant EEG electrodes and cannulas for optrode insertion (section 2.4). Following surgical recovery, rats reentered the PD training phase at PD2, and sessions persisted until they reached the criteria of at least 80 trials with 80% correct in two sessions or for 15 days, at which point they were eliminated from the subject pool if criteria had not yet been reached.

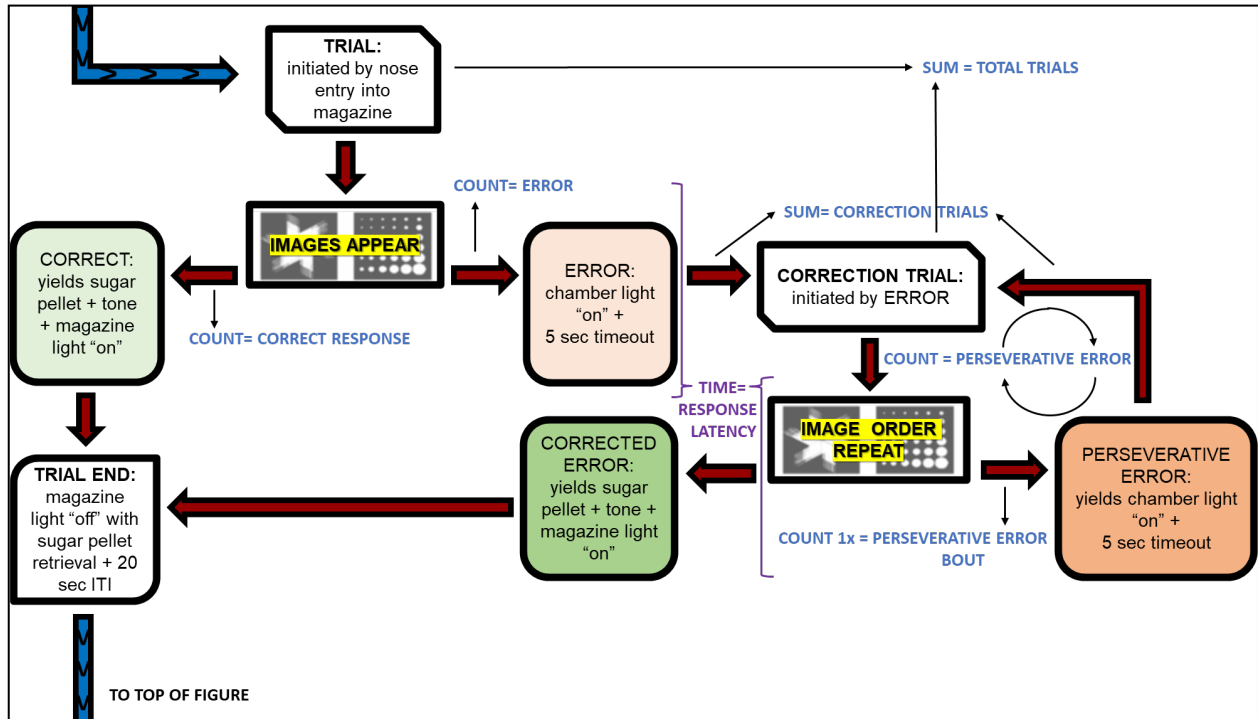


Figure 3. PD Trial Progression and Dependent Variable Extractions. This figure depicts the execution of PD training and reversal phase trials. The blue and purple fonts depict the primary dependent variables and how they were extracted for analyses (described in section 2.8 Data Collection and Processing).

2.3.3 Reversal Phase

When rats met PD training criteria, EEG baseline recording and LED optogenetic stimulation occurred (see section 2.5). The reversal phase was initiated in the next experimental session, wherein the image that when selected previously resulted in an error (e.g., the marble image in Figs. 2 and 3), now resulted in a correct response and was rewarded with a pellet. Reversal sessions continued until the performance criterion was met (at least 80 trials with 80% correct) or for a maximum of 15 reversal sessions.

2.4. EEG/Optrode Guide Placement Surgery

Between the PD1 and PD2 training session series, rats were anesthetized and craniotomized as before and EEG screws (P1 Technologies) were placed bilaterally above the prefrontal cortex

(approximately A/P 3.0, M/L ± 2.0 mm) and bilaterally behind bregma (approximately A/P -4.0, M/L ± 3.0 mm) for differential recordings. Guide cannulas were placed at the sites of the viral infusions (A/P 0.6, M/L ± 3.0 , D/V -3.5 mm) and fixed with light cure dental adhesive (Patterson Dental). Two EMG wires with metal paddles (P1 Technologies) were inserted bilaterally into the nuchal muscles. The electrodes were connected to a 6-pin connector (P1 Technologies) 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.

2.5 EEG Recording and Optogenetic Stimulation

At the end of the second PD2 session in which a rat had met training criteria, the dummy tether was removed, and a head stage instrumented with preamplifiers and friction fit optrodes connectors were attached. The LED optrodes were inserted into the bilateral guide cannulas and the rat was placed into a recording chamber where cortical EEG was recorded for the next 24 h (i.e., ZT10 on the last day of PD2 through ZT9 on the initial reversal day 1, REV1). During the 9 h prior to the first session of the reversal phase (i.e., ZT0–9), rats in the 9 SD group were sleep deprived, while the 0 SD and ChR2 groups were left undisturbed.

Beginning at ZT 9 on REV1, all rats (regardless of treatment group) received optogenetic stimulation. Stimulation parameters were 445 nm, 300 mA, 10 Hz, 20 ms PW, 20% duty cycle for 1 h using the Pinnacle 8487 system controlled by Sirenia software (Pinnacle Technologies). At ZT 10, the rats were removed from the recording chamber, transported to the touchscreen 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 recording chamber to track 3 h of recovery sleep. The next day, rats were returned to their home cage until the subsequent reversal session. Reversal sessions continued until performance criteria were met or for a maximum of 15 reversal sessions.

2.6 Sleep Deprivation

For the 9 SD rats, SD was achieved using an automated rotating bar (Pinnacle Technologies) on the cage floor, with the settings reported in Ward et al. (2017). Briefly, the bar rotated for 4 s randomly every 4–16 s, and changed rotation direction every 10–40 s. For the first 6 h, the automated SD device was used alone but for the remaining 4 h of the 10 h SD period, a trained

researcher was present to ensure that complete SD occurred. If the researcher observed the rat attempting to sleep (despite the rotating bar), they gently stroked the rats' whiskers with an artisan's paintbrush. This hybrid approach was taken because pilot data using the rotating bar system showed that one of four rats acclimated and was able to sleep in these chambers after 6 h, consistent with other reports (Wooden et al., 2014).

2.7 Histology

At the conclusion of the study, a small cohort of each group entered a follow-up experiment to confirm the effectiveness of optogenetic activation (Fig. S1). Regardless, all rats were anesthetized and perfused, and brains prepared for cryosectioning. Brains were blocked A/P ± 10.0 mm from the guide cannula cortical imprint and sectioned in the coronal plane at 40 μ m using a cryostat (ThermoFisher) and mounted to slides. A confocal microscope (Leica DMI8, TCS SPE) was used to verify AAV expression in dorsal striatal neurons and cannula/optrode placement (Fig. S2).

2.8 Data Collection and Processing

2.8.1 Behavioral Measures

The Bussey Saksida touchscreen operant platform has several IR sensors that track beam breaks as a time series of events (i.e., to record the rodents' behavior). Raw data from each trial was exported from the ABET database as a .csv file from which behavioral metrics were extracted using a custom MATLAB script.

We now detail the difference in terminology between the pre-named events from the ABET database and the behavioral metrics reported herein. Most PD training and reversal phase metrics are derived from two events: The 'Display Images' event timestamps when the two images are presented on the monitor. The 'Touch Down' event indicates when the animal responds to an image and the touch screen sensor is triggered. In each session, the initial trial begins with images being displayed and a 'Trial_Counter' event is incremented (Fig. 3). We use this to keep track of the number of total initial trials. If the rodent touches down on its assigned correct image, this will increment a 'Correct_Counter' and is used to keep track of the number of **correct responses**. However, if the rodent chooses the incorrect image this will increment a 'Correction_Trial_Counter' and after a delay will be presented with the images in the same

left/right positions as before. **Errors** are the number of trials in which the incorrect image was selected first. If the rodent continues to touch the incorrect image after the first error this will increment a 'Sequential_Correction_Trials' counter. We define **perseverative errors** as the total number of successive errors within *repeated* correction trials. **Perseveration bouts** are defined as the times that the rodent makes at least one perseverative error, regardless of how many times a perseverative error is repeated before a correction trial with a correct response. Correction trials continued until the rodent touched the correct image, after which a reward was dispensed and a new initial trial began. We define **response latency** as the average difference in time between the display image and touch down during all initial trials, correction trials, and sequential correction trials. We also tally the **total trials** to include all initial, correction, and sequential correction trials.

2.8.2 Behavioral Statistics

Once a rat reached criteria during the PD training or reversal phases, they discontinued future sessions in that phase and their final session metric carried forward. For behavioral outcomes, IBM SPSS version 28.0 statistics package performed mixed 2-way analysis of variance (ANOVA) tests on the within factor of *sessions* (1–15) and the between factor of *treatment* (Chr2 vs. 0 SD vs. 9 SD groups) were used to analyze total trials, correct responses, errors, perseverative errors and perseverative error bouts, and response latency. Fishers LSD was used for post hoc analyses of *treatment*. The number of sessions to meet criteria was analyzed using a one-way ANOVA.

2.8.3 EEG Measures

Biopotentials were sampled at 500 Hz and filtered at 0.5–40 Hz and 10–100 Hz for EEG and EMG signals, respectively, with Sirenia software (Pinnacle Technologies). The NREM, REM, and waking states were determined manually offline in 10-s epochs by trained individuals using SleepSign software (Kissei Comtec). NREM sleep was identified by high-amplitude EEG signals and low EMG activity. Regular low-amplitude EEG and minimal EMG activity characterized REM sleep. Wake periods were recognized by low-amplitude fast EEG and high-amplitude EMG activity. Prior to performing EEG spectral analyses, a multistep statistical thresholding approach (Davis et al., 2022; Lawrence-Sidebottom et al., 2021) was used for the unbiased removal of artifacts from scored files.

2.8.4 EEG Statistics

For REM, NREM, wake, and brief awakenings, state-specific parameters (viz. duration, latency, transitions, episode count, and frequency) and FFT spectral parameters (viz., NREM delta power, wake-, REM-, NREM-normalized power spectra from 1 to 20 Hz) spreadsheets were populated via a custom MATLAB algorithm and subjected to statistical analyses with Dell Statistica version 13.2 and GraphPad Prism 7.

Data were subjected to one-way ANOVAs with treatment (ChR2 vs. 0 SD vs. 9 SD) as a between-subjects factor. Where ANOVA indicated a significant main effect for treatment group, Fisher's LSD post hoc analyses were applied to determine whether 0 SD vs. ChR2, 0 SD vs. 9 SD, and ChR2 vs. 9 SD group differences were significant.

3. Results

3.1 Pairwise Discrimination Reversal

Both SD rats and ChR2-expressing rats subjected to optogenetic stimulation of the dorsal striatum prior to image reversal exhibited significant impairment of cognitive flexibility compared to control rats (not expressing ChR2) that also received light stimulation. These data indicate that optogenetic activation of the striatal indirect pathway immediately before the initial reversal session disrupts the ability of rats to adapt to changes in contingencies in a manner consistent with sleep-deprived rats (Fig. 4).

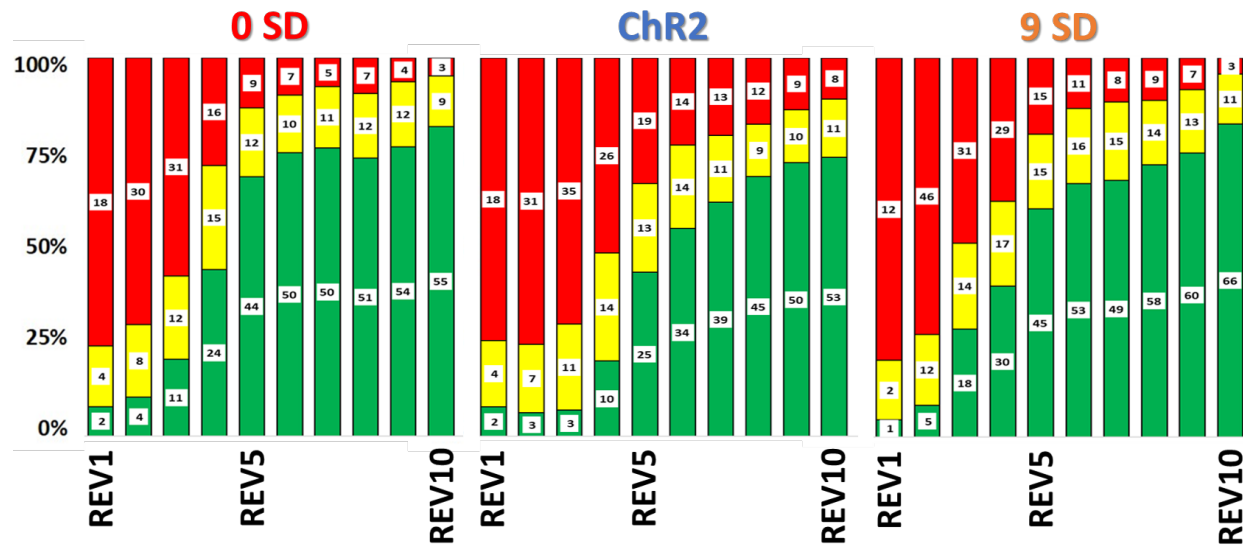


Figure 4. Performance across sequential reversal sessions (REV1–10). Proportional responding expressed as percent (actual mean session values labeled) indicate that compared with undisturbed rats (0 SD; left), optogenetically stimulated rats (ChR2; center) and rats sleep deprived for 9 h (9 SD; right) manifest more perseverative errors (red bars), comparable initial errors (yellow bars) and fewer correct responses (green bars).

Strobel et al. (2022) reported that the REV4 to REV6 sessions were the sessions at which SD rat response patterns separated from non-SD controls in surgically- and pharmacologically-naïve cohorts. This appears to be consistent with the session-to-session changes in Figure 4, so we analyzed these sessions independently to increase statistical power by reducing the number of measures incorporated in the ANOVA. While the average number of trials was not different (Fig. 5A), perseverative errors doubled in ChR2 ($P=0.009$) and 9 SD rats ($P=0.046$) compared to 0 SD rats ($F_{2,22}=4.63$, $P=0.021$; Fig. 5B). Total errors (initial + perseverative) were also higher in ChR2 ($P=0.017$) and 9 SD rats ($P=0.047$) with respect to 0 SD rats ($F_{2,22}=3.95$, $P=0.034$; Fig. 5C). Together, these findings support our hypothesis that activation of the DrD2+ medium spiny neurons in the dorsal striatum compromises adaptive decision-making in a manner analogous to sleep deprivation.

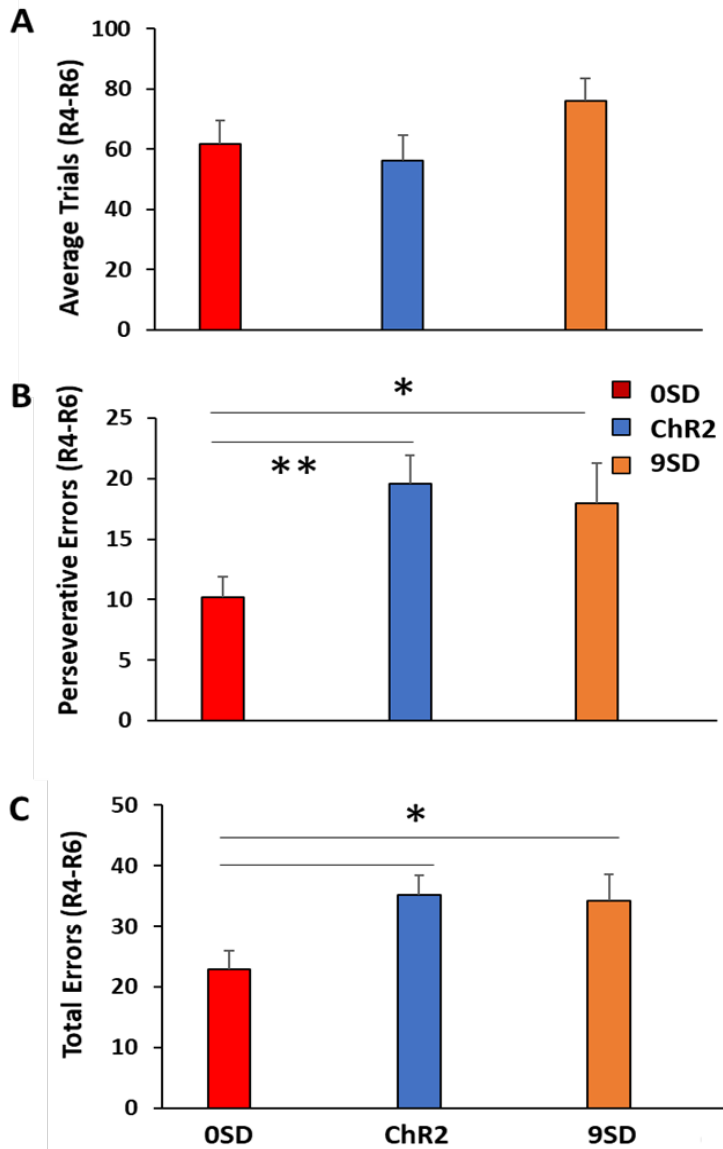


Figure 5. Analysis of discriminating reversal sessions supports the hypothesis that optogenetic activation of the striatal indirect pathway confers cognitive rigidity in rats in a manner consistent to sleep loss. (A) The average number of trials in reversal sessions 4–6 was not different between undisturbed rats (0 SD; $n=10$; red bars), optogenetically stimulated rats (ChR2; $n=9$; blue bars), or rats sleep deprived for 9 h (9 SD; $n=6$; orange bars). However, (B) perseverative errors and (C) total errors indicate elevated errors in ChR2 and 9 SD rats compared to 0 SD rats. Bars depict means and whiskers SEMs (*= $P<0.05$; **= $P<0.01$).

3.2 EEG

We 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 9 SD and ChR2 rats compared to 0 SD controls. Optogenetic stimulation of the dorsal striatum of ChR2 rats had significant effects on sleep timing and EEG spectral parameters compared to control rats that also received light stimulation.

3.2.1 Vigilance states prior to light stimulation

In the final 2 h immediately prior to optogenetic stimulation (ZT7–9), the behavioral intervention in the 9 SD rats resulted in significant changes in the percent time in wake ($F_{2,19}=7.55$, $P=0.004$), NREM ($F_{2,19}=9.59$, $P=0.007$), and REM sleep ($F_{2,19}=8.32$, $P=0.003$; Fig. 6). Post hoc analysis indicated the effectiveness of the hybrid SD method with the 9 SD rats awake 96.7% of the time, which was 21.6% and 28.8% more than ChR2 ($P=0.009$) and 0 SD ($P=0.001$) rats, respectively. The 9 SD rats had 16.0% less NREM ($P=0.016$) and 5.7% less REM ($P=0.003$) than ChR2 rats' baseline sleep, and 22.3% less NREM ($P=0.002$) and 6.6% less REM ($P=0.001$) than 0 SD rats' baseline sleep.

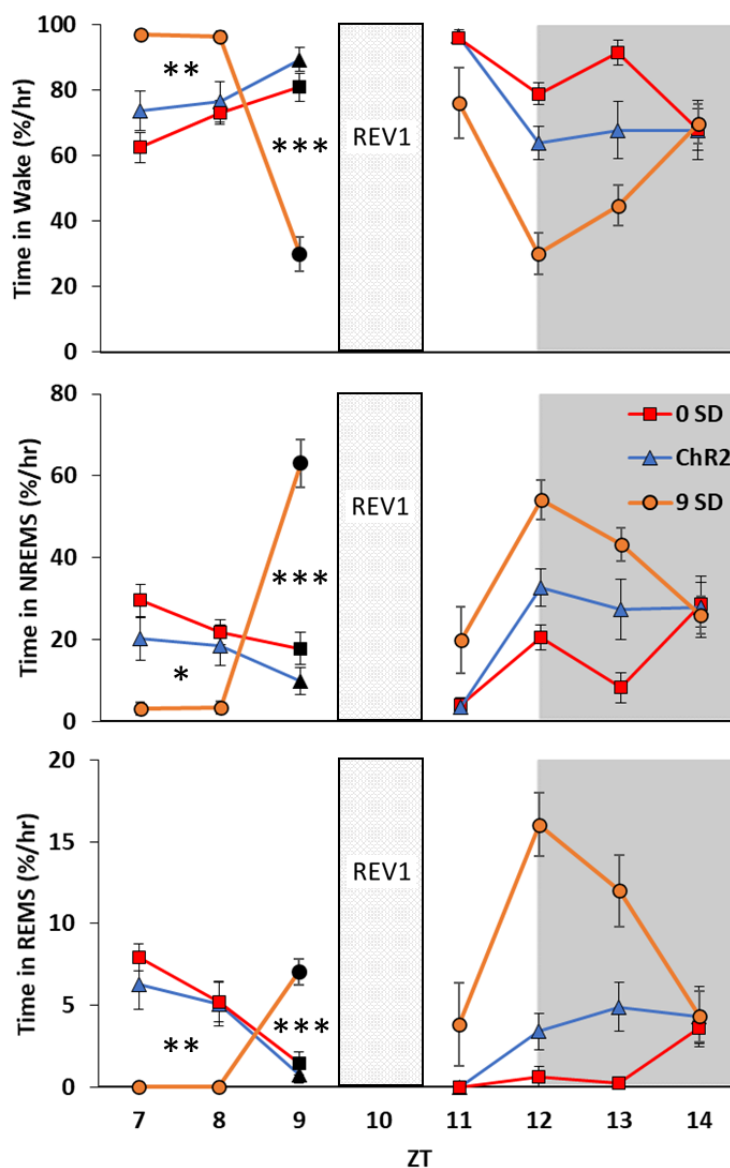


Figure 6. Percent time spent in each vigilance state prior to, during, and after light stimulation and the initial reversal session (REV1) in 0 SD (red squares; $n=8$), ChR2 (blue triangles; $n=9$), and 9 SD rats (orange circles; $n=6$). Black symbols at ZT9 represent the h in which light stimulation occurred. Symbols depict means and whiskers SEMs. The gray background indicates ambient darkness in the recording chamber. (*= $P<0.05$; **= $P<0.01$; ***= $P<0.001$; for statistically significant differences from the 9 SD group). Prior to REV1, no statistically significant differences existed between ChR2 and 0 SD rats.

3.2.2 Vigilance states during light stimulation

During optogenetic stimulation (ZT9–10), there were group differences detected in wake ($F_{2,19}=45.17$, $P<0.001$), NREM ($F_{2,19}=39.24$, $P<0.001$), and REM sleep ($F_{2,19}=24.29$, $P<0.001$; Fig. 6). In this hour, the 9 SD rats only spent an average of 29.9% of time in wake, compared to 89.4% in ChR2 rats ($P<0.001$) and 80.8% in 0 SD rats ($P<0.001$). NREM sleep in 9 SD rats was 53.1% higher than in ChR2 rats ($P<0.001$), and 45.3% higher than in 0 SD rats ($P<0.001$). Further, 9 SD rats also spent 7.1% of the hour in REM sleep compared to 0.7% in ChR2 rats ($P<0.001$) and 1.5% in 0 SD rats ($P<0.001$). Together these data suggest that 9 SD rats were already discharging sleep pressure when the light stimulation was occurring. This prompted the question as to what the rats were doing in the subsequent initial reversal session. While we did not record EEG during task performance, to determine sleep opportunity during the first reversal session (REV1), we summed 60 s bins without any IR beam break events recorded by the ABET system (e.g., back IR beam, front IR beam, food hopper beam, and touch screen beams) for rats in each group. The 9 SD rats had more such events ($F_{2,22}=6.29$, $P<0.007$; Fig. S3) than 0 SD ($p=0.014$) or ChR2 ($p=0.002$) rats. Nonetheless, the number of trials performed did not differ between groups during REV1, so to the extent that sleep occurred, it did not impact the level of task participation.

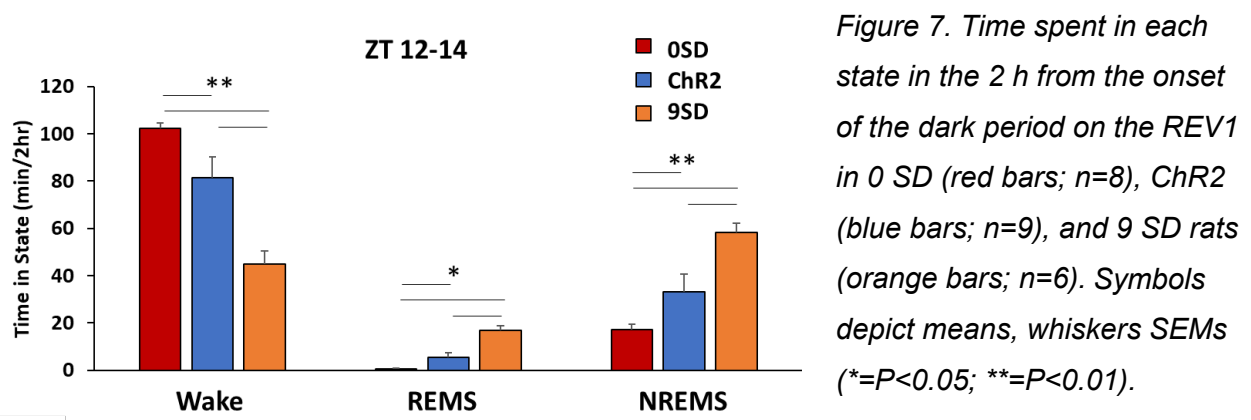
3.2.3 Vigilance states following light stimulation

In order to examine recovery sleep, we analyzed additional sleep/wake phenotypes at ZT12–14. None of the following differences between the experimental groups, which were observed at ZT12–14 after return to the recording subsequent to dorsal striatal light exposure at ZT10–11, were detected in baseline EEG data collected at the analogous time of day on the baseline day immediately prior to the day of optogenetic stimulation. The following effects are thus a consequence of the optogenetic stimulation of the DrD2-positive cells of the dorsal striatum.

Either of two outcomes could be taken as evidence that ChR2-induced changes mimicked the effect of SD on any given variable: 1) there were significant differences for 0 SD vs ChR2 and 0 SD vs 9 SD, but not for ChR2 vs 9 SD; and 2) the ChR2 group was significantly different from, and intermediate of, 0 SD and 9 SD.

Outcomes for several variables suggest that optogenetic stimulation of DrD2 cells mimics 9-h SD qualitatively, if not quantitatively. For the following variables, both experimental groups were

different from the 0 SD control, with ChR2 intermediate between 9 SD and 0 SD. Time awake was reduced by 20.93 min in the ChR2 rats and by 57.43 min in the 9 SD with respect to the 0 SD group ($F_{2,19}=27.04$, $P<0.001$; Fig. 7). Time in NREMS increased in both in ChR2 rats by 15.94 min and by 41.14 min in 9 SD rats compared to the 0 SD rats ($F_{2,19}=21.88$, $P<0.001$; Fig. 7). Likewise, time in REMS was elevated by 5.00 and 16.29 min in ChR2 and 9 SD rats, respectively, compared to 0 SD rats ($F_{2,19}=35.86$, $P<0.001$; Fig. 7).



The total number of REMS bouts across the 2-h session increased by 2.74 in the ChR2 rats and 8.83 in the 9 SD rats compared to the 0 SD group ($F_{2,19}=18.30$, $P<0.001$; Fig. 8). In the case of these variables, it is a reasonable presumption that a SD of shorter duration would equalize the ChR2 and 9 SD values. The intermediacy of ChR2 values should be regarded as sufficient evidence of mimicry of the SD effect, not as a lack of evidence.

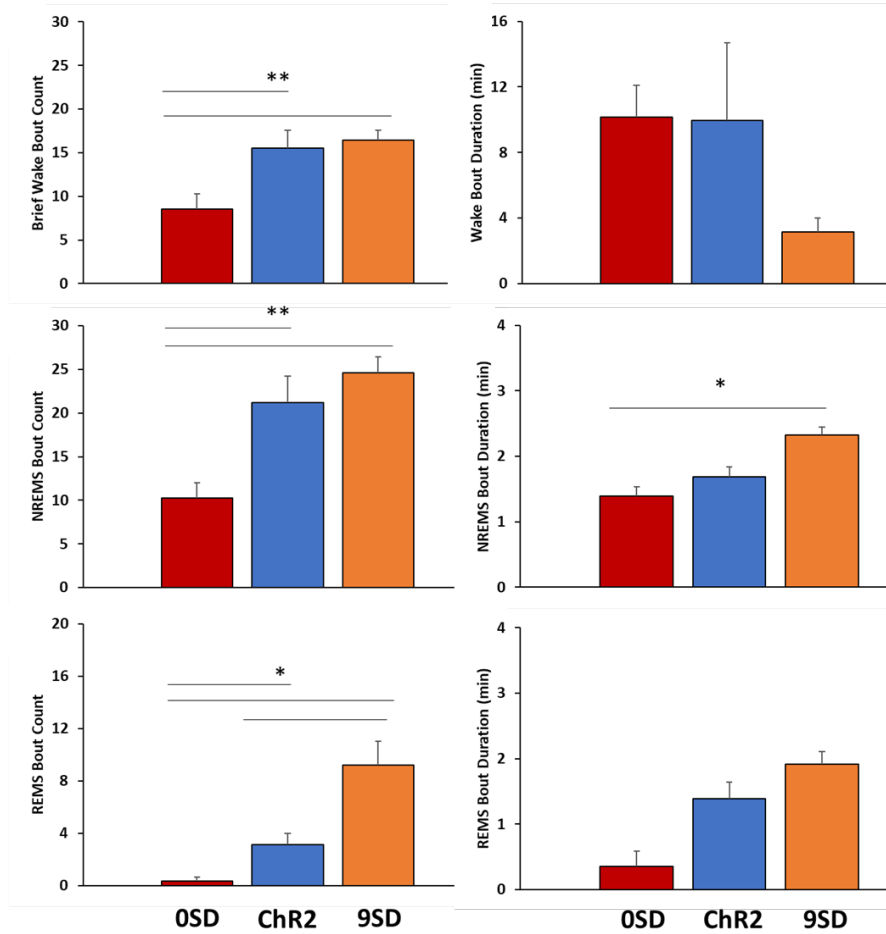


Figure 8. State-specific bout frequencies and durations in the 2 h from the onset of the dark period on REV1 in 0 SD (red bars; $n=8$), ChR2 (blue bars; $n=9$), and 9 SD rats (orange bars; $n=6$). Panels include the number of bouts (left column) and duration of bouts (right column) of wake (top row), NREMS (middle row), and REMS (bottom row). Symbols depict means, whiskers SEMs (*= $P<0.05$; **= $P<0.01$).

For the following variables, there were significant differences for 0 SD vs ChR2 and 0 SD vs 9 SD, but not for ChR2 vs 9 SD. Latency to 1 min of REMS was reduced to 86.68 min in ChR2 rats and 14.03 min in 9 SD rats compared to 0 SD rats ($F_{2,19}=12.17$, $P<0.001$; Fig. 9). There were, on average, 10.97 more NREMS bouts in ChR2 rats and 14.35 more in 9 SD rats, relative to 0 SD rats ($F_{2,19}=8.43$, $P=0.002$; Fig. 8). The number of brief awakenings increased in both the ChR2 and 9 SD groups by 7.06 and 7.90, respectively, compared with the 0 SD group ($F_{2,19}=5.35$, $P=0.014$; Fig. 8).

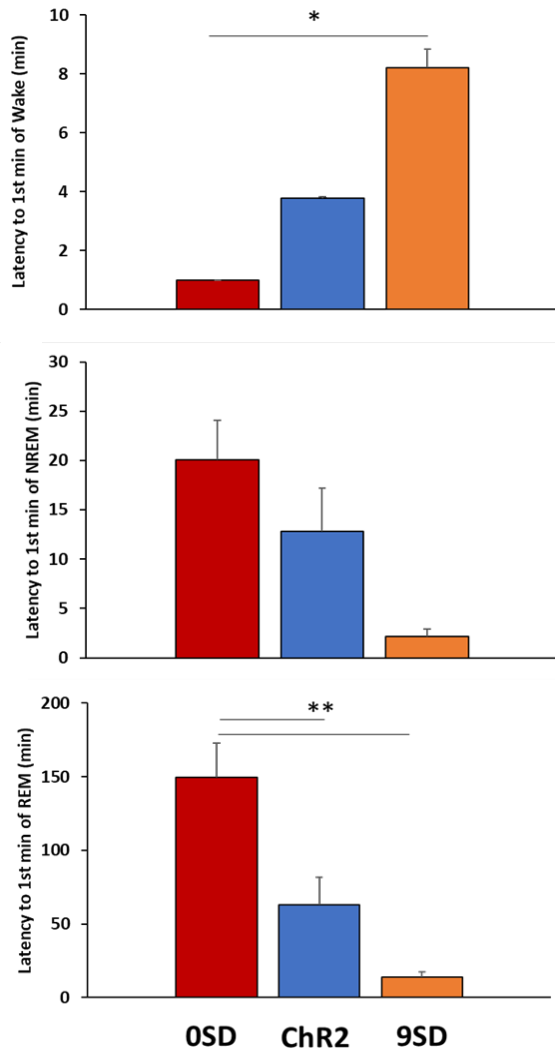


Figure 9. Latencies to the onset of Wake (top), NREM (middle), and REM (bottom) in the 2 h from the onset of the dark period on REV1 in 0 SD (red bars; $n=8$), ChR2 (blue bars; $n=9$), and 9 SD rats (orange bars; $n=6$). Symbols depict means, whiskers SEMs (*= $P<0.05$; **= $P<0.01$).

Finally, normalized NREMS EEG Spectra (1–20 Hz) exhibited subtle and opposing effects of 9 SD and ChR2 (Fig. 10). However, the 0.5–4.0 Hz delta power was suggestive of reduced homeostatic sleep drive in the 9 SD rats, but elevated homeostatic sleep drive in ChR2 relative to 0 SD rats ($F_{2,19}=9.96$, $P=0.001$; Fig. 10, inset). Collectively, sleep timing and sleep EEG spectral power values demonstrated elevation of sleep propensity and homeostatic sleep drive as a consequence of optogenetic stimulation of the DrD2 population in the dorsal striatum.

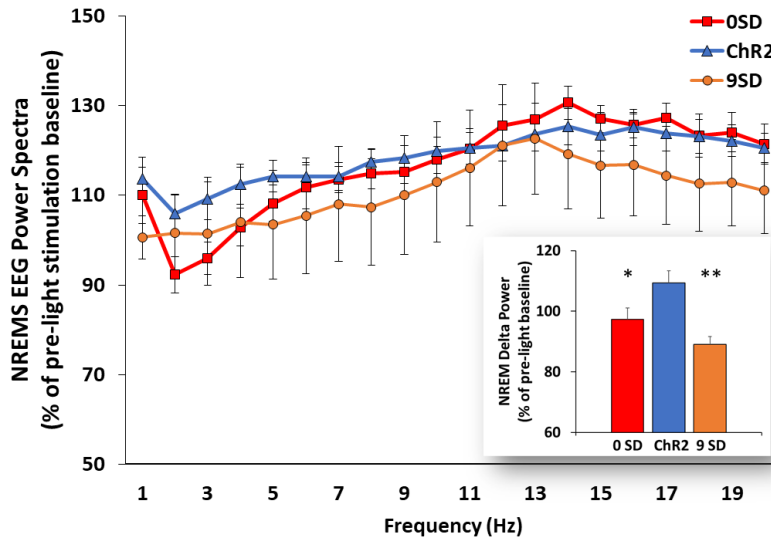


Figure 10. NREM spectral content and delta power in the 2 hours from the onset of the dark period on REV1 (*= $P<0.05$; **= $P<0.01$).

In summary, sleep timing values demonstrated an elevation of sleep propensity and homeostatic sleep drive as a consequence of either SD or optogenetic stimulation of the DrD2 population in the dorsal striatum. We conclude that, similar to SD, neuronal activity in the DrD2-positive population of the dorsal striatum promotes the accumulation of sleep need. This accumulation is, in turn, manifested at the behavioral level, but not as a reduction in motivated behavior, as task participation was not significantly reduced. Rather, the manifestation was cognitive rigidity, in which perseverative behavior was slow to reverse, even in the face of unrewarded responses for the previously rewarded behavior.

The fact that indirect pathway MSN activation is sufficient to mimic SD-induced cognitive rigidity suggests that the indirect pathway serves an active role in the suppression of cognitive flexibility in the SD state. We hypothesize that this active role is a protective mechanism that mitigates inevitable deficits in cortical processing that emerge over time awake, not a manifestation of pathology secondary to sleep loss. In the SD state, increased cognitive rigidity serves to reduce the likelihood of a maladaptive reversal, as would occur as a result of a psychomotor vigilance lapse. Two scenarios whereby this maladaptive reversal could occur when the reversal is not warranted (i.e., the original stimulus-reward contingency remains intact) are as follows: 1) Reward-producing behavior is unsuccessfully attempted due to a psychomotor lapse and consequently there is no reward delivery. 2) Reward-producing behavior is engaged successfully and results in reward delivery, but a lapse prevents reward detection/ consumption. In either case, the subject perceives incorrectly, as a consequence of lapses at the level of operant behavior performance (scenario 1) or reward detection/consumption (scenario 2) that

the reward was not delivered when the operant behavior was performed. As a result of the inaccurate perception of a failed trial, the subject then changes from the correct behavioral strategy to a novel, unsuccessful one. This strategic failure due to psychomotor lapses (not related to dorsal striatal function) would be assuaged by dorsal striatal-enforced rigidity.

How might the DrD2/A2a positive neurons gauge time awake and increase their activity? This population is defined by the coincident expression of DrD2 and A2a receptors, which act antagonistically at the biochemical level through their convergent, opposing effects on the activity of the cyclic adenosine monophosphate (cAMP)-synthesizing enzyme adenylyl cyclase within the cell. DrD2 receptors activate the Gi pathway, resulting in reduced adenylyl cyclase activity and reduced intracellular (cAMP) concentration. This effect is counteracted by A2a receptor-induced Gs activation [reviewed by Kim & Palmiter, 2008]. DrD2 receptors are downregulated in the human striatum as a result of SD [Volkow et al., 2012], but there is no known evidence for A2a receptor downregulation as a function of sleep state. On the contrary, the adenosinergic tone is elevated in the brain with SD [reviewed by Basheer et al., 2004]. Thus, the collective reduction in DrD2 availability and increased adenosinergic tone during wake is expected to increase intracellular cAMP concentration in DrD2-A2a MSNs over time spent awake. Increased cAMP concentration would increase excitability and output of these GABAergic MSN, and thereby decrease the activity of downstream effector neurons, resulting in cognitive rigidity.

The work reported here used genetically encoded optogenetic constructs to confirm that the excitation of these cells is sufficient to promote cognitive rigidity. Nonetheless, several pieces of information relevant to this conceptual model remain to be addressed. For instance, neither elevated adenylyl cyclase activity nor increased excitability of these cells over time spent awake has been measured directly *in vivo* or *in vitro*. The dependence of SD-dependent cognitive rigidity on elevated adenosinergic tone or reduced DrD2 availability in the dorsal striatum, specifically, has not been demonstrated. The mechanisms whereby DrD2 availability becomes reduced over time spent awake are not known. The results reported here suggest that continued focus on the DrD2/A2a population in the dorsal striatum as a mediator of cognitive rigidity in sleep deprivation is warranted in future work.

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Supplemental Information

S1. Validation of Optogenetics

In addition to the optogenetic-induced behavioral and electrophysiological changes reported, we used immunohistochemistry and the neuronal activity marker cFos to demonstrate the striatal activation of the ChR2 construct. Thus, 1–2 weeks after the conclusion of the main study, 4 rats from the ChR2 group, and 5 rats from the 0 SD group received another LED stimulation from ZT9–10. Rats were anesthetized with Euthasol (150 mg/kg, IP; Covetrus) and transcardially perfused with cold PBS followed by 10% formalin. Brains were extracted and stored in 10% formalin for 24 h at 4 °C before being transferred to 30% sucrose in PBS and stored at 4 °C. Brains were sectioned at 40 µm using a ThermoFisher Cryostar Nx70. Coronal brain sections were placed into PBS.

The temporal dynamics of cFos in the rat striatum with sleep loss are complex, cFos immunoreactivity is not observed following SD from ZT6–9 and is negligible when extending SD to ZT3–9, however, SD from ZT3–6 shows some cFos immunoreactivity (Semba et al., 2001).

To provide light stimulation in a period where cFos is likely inducible with SD, 4 rats from the 9 SD group received SD by the rotating bar method from ZT3–6 and were optogenetically stimulated for the last h of the SD (ZT 5–6) and were then perfused, and the brains prepared for immunohistochemistry as above.

ChR2 rats subjected to 1-h LED stimulation had higher cFos immunoreactivity compared to that of 0 SD rats subjected to 1-h LED stimulation or 3 SD rats subjected to 1-h LED stimulation (Fig S1). We anticipated that compared with 0 SD rats (or even ChR2 rats), 3 SD rats would exhibit higher cFos immunoreactivity from SD. This was not the case and may be explained by strain differences and/or the type of SD method. This does not detract from the key finding that optogenetic stimulation did not activate striatal MSNs in either of the two ChR2-negative virus control groups 0 SD and 3 SD.

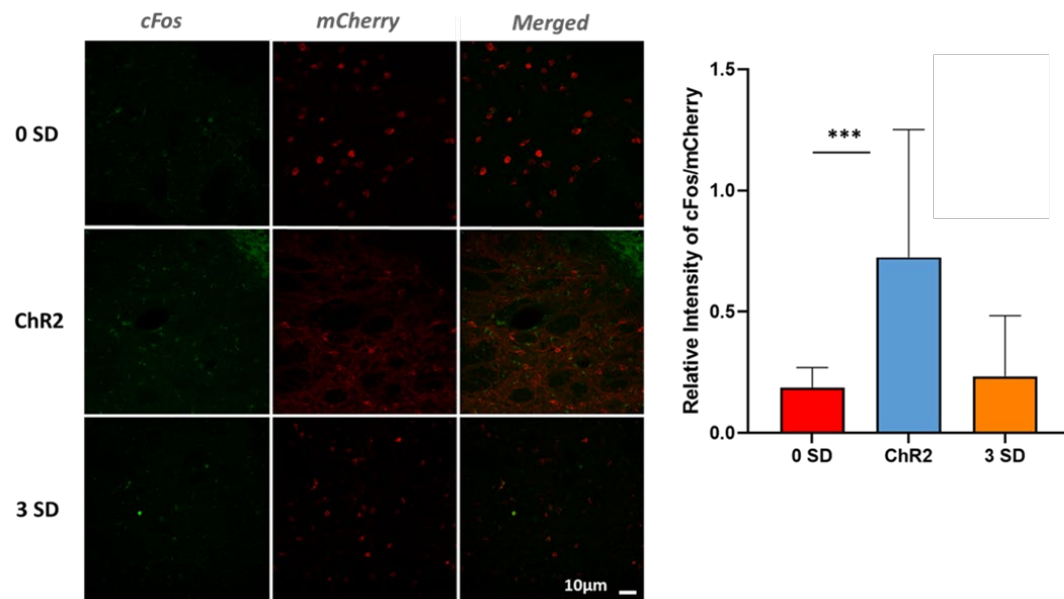


*Figure S1. Immunohistochemistry showing the effect of light stimulation of the dorsal striatum on the cellular activity marker cFos. Images are from 0 SD rats (n=5; upper row), ChR2 rats (n=4; middle row), and 3 SD rats (n=4; bottom row). Stains represent an activity marker (cFos=green; left column) and DrD2-positive expression (mCherry=red; middle column), with a merged overlay in the right column. The bar graph indicates higher ratiometric cFos/mCherry activity following light stimulation of striatal DrD2-positive MSNs transfected with ChR2 (blue bar), compared to 0 SD (red bar) and 3 SD (orange bar) controls (***) $P < 0.001$.*

S1.1 Immunohistochemistry Methods

Immunohistochemistry was used to identify recently active DrD2-positive cells (identified with *mCherry* fluorescence) via the intensity of the activity marker *cFos* (Fig. S1). Free-floating coronal sections were placed in nets in a 6-well plate and washed three times in PBS for 5 min each. Sections were then submerged in a blocking buffer containing 5% BSA, 5% goat serum, and 0.25% triton-x for 1 h on an orbital shaker. Sections were washed three times with PBS (5-min/wash), and placed in 4 mL of 1% sodium borohydride in PBS for 30 min. Sections were again rinsed in PBS three times (5 min/wash) before being transferred into microfuge tubes containing mouse anti-*cFos* (Phosphosolutions; 1:1000), rabbit anti-NeuN (ThermoFisher Scientific; 1:500), 2% BSA and 0.25% triton-x and placed at 4°C on a rotator for 72 h. Following primary incubation, sections were returned to the nets and washed three times with PBS for 5 min each. Sections were bathed in secondary antibody containing goat anti-mouse 488 (ThermoFisher Scientific; 1:2000), donkey anti-rabbit 647 (ThermoFisher Scientific; 1:1000), 2% BSA, 0.25% triton-x overnight at 4°C on an orbital shaker. After three final washes in PBS (5 min each) sections were positioned on slides until dry and were covered in VECTASHIELD® HardSet™ Antifade Mounting Medium with DAPI (H-1500-10). Slides were imaged using a Leica DMI8, TCS SPE confocal microscope. Images were analyzed for cell number and intensity using Pipsqueak ai (Rewire Neuro, Inc).

S2. Histological confirmation of virus location, transfection efficiency, optrode placement

After pairwise discrimination reversal criteria were met or 15 sessions completed without successfully meeting criteria, DrD2+ rats transfected with pAAV-EF1a-cDIO-hChR2(H134R)-*mCherry* Serotype 5 virus (ChR2; Fig. S2, left three columns) were euthanized and coronal slices of perfused brains were used to verify cannula track (gray slides), transfection efficiency (red fluorescing *mCherry* slides) and optrode tip estimate (blue circles superimposed on corresponding pages of the stereotaxic atlas). This procedure was also performed with DrD2-Cre+ rats transfected with pAAV-EF1a-cDIO-hChR2(H134R)-*mCherry* Serotype 5 virus (0 SD control; Fig. S2, right three columns). While this figure depicts four rats per group, all rats used in the study were verified in this manner.

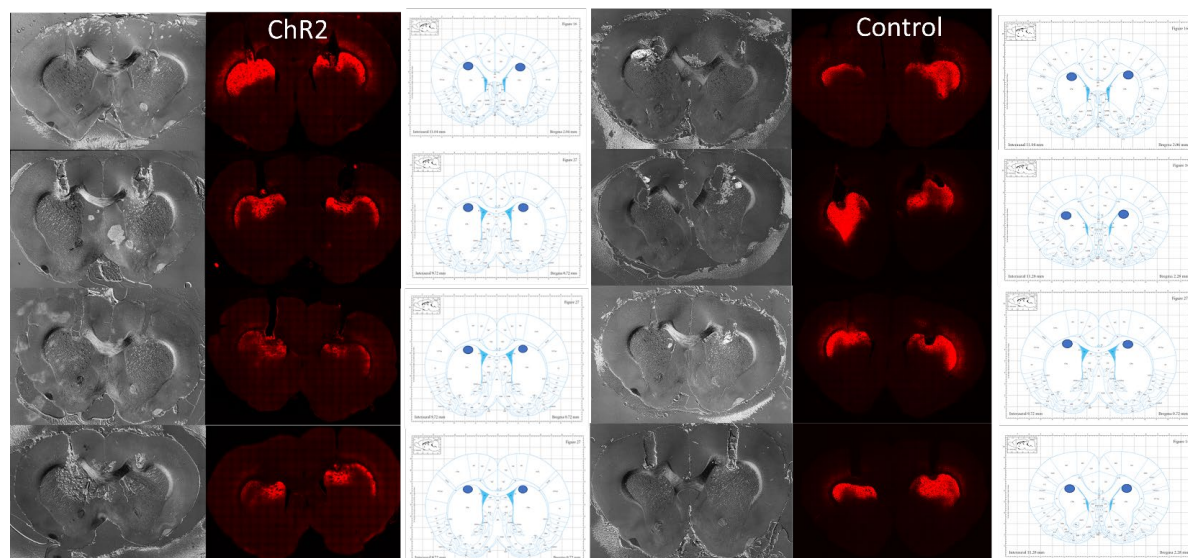


Figure S2. Histological confirmation of viral construct expression.

During Year 5, other achievements included:

During to the COVID-19 pandemic, the human sleep laboratory was closed throughout Year 3 and into Year 4, and no human subject experimentation for Aim 3 could be conducted. The laboratory reopened in the fall of 2021, and although human subject enrollment was resumed immediately, recruitment was challenging due to ongoing labor shortages, which affected the availability of our target population (i.e., healthy young adults). At the end of Year 5 we had 63 out of our target sample size of N=90, or 70%. An additional 1-year no-cost extension has been requested and approved for the completion of the human study of Aim 3. See the table below for enrollment projections.

Project Year	Year 2		Year 3				Year 4				Year 5				Year 6			
Target Enrollment (per quarter)	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Extension Need		Pandemic study					Study resumption, post-pandemic								Study completion			
N randomized	10	13*	0	0	0	0	4	9	15	15	10	5	6	10	10	10	10	0
N completed	6	7	0	0	0	0	3	5	11	11	8	4	3	8	8	8	8	0
Target Enrollment (cumulative completed)	6	13	13	13	13	13	16	21	32	43	51	55	58	66	74	82	90	90

We published two manuscripts based on results obtained during preparatory work for Aim 2 (PDF copies enclosed).

We published three manuscripts based 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. In Year 5 specifically, a Ph.D. student in the Experimental Psychology program at Washington State University, Amanda Hudson, continued her involvement 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 worked on the project under the direct mentorship of the Co-PI (Honn) of the project.

- Also in Year 5, a Ph.D. student in the Neuroscience program, Rachael Muck, and a Ph.D. student in the Experimental Psychology program, Kirsie Lundholm (both at Washington State University), developed research skills through their involvement with this project that will pay dividends in each of their dissertation research projects. This included collection of blood samples from participants (as both students are trained phlebotomists) followed by the processing and genotyping of the blood samples. These two graduate students worked on the project under the direct mentorship of the research faculty: Ms. Muck was supervised by Van Dongen (PI) and Satterfield (Assistant Research Professor), and Ms. Lundholm was supervised by Honn (Co-PI) and Satterfield.

How were the results disseminated to communities of interest?

Dr. Courtney Kurinec presented preliminary results from a cognitive task in the study showing that unitization of word pairs improves memory for associations during sleep deprivation, at the Associated Professional Sleep Societies meeting (SLEEP 2022), Charlotte, NC, June 2022.

Dr. Van Dongen (PI) presented on functional measurements and biomarker assessments of fatigue to predict the temporal dynamics of sleep loss-induced performance impairment at the US DoD-sponsored MQ Military Biomarker Symposium at Macquarie University, Sydney, Australia, June 2022; and on resilience to performance deficits from sleep loss at the DARPA Forward conference at Washington State University, Pullman, WA, September 2022.

Dr. Christopher Davis (Co-I) was invited to present a seminar entitled “Cognitive rigidity: Poor sleep, poor performance” at the Brigham Young University, Department of Cognitive and Behavioral Neuroscience, Provo, UT, October 2022.

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

For the animal studies: During Year 6 of the project (under no-cost extension), we will publish the full set of results of Aims 1 and 2 of the project.

For the human study: During Year 6 of the project (under no-cost extension), we will complete the human subjects study of Aim 3 (90 healthy adults completed), unblind to drug condition and genotype, perform data analyses, and prepare at least two manuscripts detailing our findings.

4. IMPACT

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

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

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?

Increased public recognition of reduced cognitive flexibility as a distinct and profound consequence of sleep loss, driven in part by our past and ongoing outreach and dissemination activities, is changing the narrative about sleep loss, fatigue, and operational performance and safety across a wide range of military and civilian settings. This is an important indirect benefit to society related to the project.

5. CHANGES/PROBLEMS

Changes in approach and reasons for change

Dr. Lynn Caldwell of NAMRU-D, who served as an advisor for Aim 3 of the project through a subcontract, has retired from the military. She has agreed to continue to serve as an advisor as a civilian consultant, at no further cost to the project.

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

We have incurred considerable delays in the human study of Aim 3 as the human sleep laboratory was closed for 1.5 years due to the COVID-19 pandemic. Subsequent recruitment has been slow due to the ongoing post-pandemic worker shortage, which has adversely affected the availability of the population we draw from. A third 1-year no-cost extension has been granted to complete the project.

Changes that had a significant impact on expenditures

The funds obligated to this project have been expended. Discretionary funds at Washington State University are available to complete the project in Year 6.

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

Foakes C, Lawrence-Sidebottom D, Dralega AT, Harvey DO, Schmidt MA, Davis CJ (2022). The rat Lux Actuating Search Task (LAST) and effects of sleep deprivation on task reversal performance. *Neurobiology of Sleep and Circadian Rhythms* 13: 100081.

Kelly MR, Yuen F, Satterfield BC, Auchus RJ, Gaddameedhi S, Van Dongen HPA, Liu PY (2022). Endogenous diurnal patterns of adrenal and gonadal hormones during a 24-hour constant routine after simulated shift work. *Journal of the Endocrine Society* 12: bvac153.

Kombala CJ, Agrawal N, Sveistyte A, Karatsoreos IN, Van Dongen HPA, Brandvold KR (2023). Profiling rhythmicity of bile salt hydrolase activity in the gut lumen with a rapid fluorescence assay. *Organic & Biomolecular Chemistry* 21: 4028–4038.

Kurinec CA, Stenson AR, Hinson JM, Whitney P, Van Dongen HPA (2022). Electrodermal activity is sensitive to sleep deprivation but does not moderate the effect of total sleep deprivation on affect. *Frontiers in Behavioral Neuroscience* 16: 885302.

Strobel BK, Schmidt MA, Harvey DO, Davis CJ (2022). Image discrimination reversal learning is impaired by sleep deprivation in rats: Cognitive rigidity or fatigue? *Frontiers in Systems Neuroscience* 16: 1052441.

Books or other non-periodical, one-time publications

Nothing to report.

Other publications, conference papers, and presentations

Kurinec C, Whitney P, Hinson J, Satterfield B, Honn K, Van Dongen H (2022). Unitization improves memory for associations during sleep deprivation. *Sleep*, 45 (Suppl. 1): A48 (conference abstract).

Satterfield B, Finlay M, Skeiky L, Lawrence-Sidebottom D, Schmidt M, Wisor J, Van Dongen H (2022). ARC genotype modulates EEG spectral power following total sleep deprivation. *Sleep*, 45 (Suppl. 1): A11 (conference abstract).

Van Dongen H, Koritala B, Porter K, Arshad O, Gajula R, Mitchell H, Arman T, Manjanatha M, Teeguarden J, McDermott J, Gaddameedhi S (2022). Circadian dysregulation of human DNA repair genes and elevated DNA damage in simulated night shift schedule. *Sleep*, 45 (Suppl. 1): A11 (conference abstract).

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.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	<i>Hans P.A. Van Dongen, Ph.D.</i>
Project Role:	<i>PI</i>
Researcher Identifier:	<i>ORCID ID: 0000-0002-4678-2971</i>
Nearest person month worked:	<i>1</i>
Contribution to Project:	<i>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.</i>
Funding Support:	

Name:	<i>Chris Davis, Ph.D.</i>
Project Role:	<i>Co-PI</i>
Researcher Identifier:	<i>ORCID ID: 0000-0002-9613-928X</i>
Nearest person month worked:	<i>2</i>
Contribution to Project:	<i>Dr. Davis directed operations and workforce for the rat behavioral research, prepared reports, and wrote, edited and submitted manuscripts (Aim 2).</i>
Funding Support:	

Name:	<i>Kimberly A. Honn, Ph.D.</i>
Project Role:	<i>Co-PI</i>
Researcher Identifier:	<i>ORCID ID: 0000-0001-8911-6277</i>
Nearest person month worked:	<i>1</i>
Contribution to Project:	<i>Dr. Honn led the human subjects laboratory study of Aim 3 and took responsibility for regulatory compliance.</i>
Funding Support:	

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

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

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

Name:	<i>Jonathan Wisor, Ph.D.</i>
Project Role:	<i>Co-PI</i>
Researcher Identifier:	<i>ORCID ID: 0000-0003-4948-4379</i>
Nearest person month worked:	1
Contribution to Project:	<i>Dr. Wisor analyzed the optogenetic experimentation data and ran vigilance and sleep statistics for Aim 2.</i>
Funding Support:	

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

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

Name:	<i>Courtney Kurinec, Ph.D.</i>
Project Role:	<i>Assistant Research Professor</i>
Researcher Identifier:	<i>Washington State University ID: 11693086</i>
Nearest person month worked:	1
Contribution to Project:	<i>Dr. Kurinec assisted with management of the day-to-day operation of the human laboratory study for Aim 3.</i>
Funding Support:	

Name:	<i>Amanda Hudson</i>
Project Role:	<i>Ph.D. Student</i>
Researcher Identifier:	<i>Washington State University ID: 11624214</i>
Nearest person month worked:	1
Contribution to Project:	<i>Ms. Hudson implemented performance testing for the human study of Aim 3.</i>
Funding Support:	

Name:	<i>Emily Moslener</i>
Project Role:	<i>Ph.D. Student</i>
Researcher Identifier:	<i>Washington State University ID: 11765573</i>
Nearest person month worked:	1
Contribution to Project:	<i>Ms. Moslener performed data management for the human study of Aim 3.</i>
Funding Support:	

Name:	<i>Kirsie Lundholm</i>
Project Role:	<i>Ph.D. Student</i>
Researcher Identifier:	<i>Washington State University ID: 11723261</i>
Nearest person month worked:	1
Contribution to Project:	<i>Ms. Lundholm provided screening and behavioral monitoring support for the human study of Aim 3.</i>
Funding Support:	

Name:	<i>Lillian Skeiky</i>
Project Role:	<i>Ph.D. Student</i>
Researcher Identifier:	<i>Washington State University ID: 11656455</i>
Nearest person month worked:	1
Contribution to Project:	<i>Ms. Skeiky provided logistic support for the human study of Aim 3.</i>
Funding Support:	

Name:	<i>Rachael Muck</i>
Project Role:	<i>Ph.D. Student</i>
Researcher Identifier:	<i>Washington State University ID: 11441657</i>
Nearest person month worked:	1
Contribution to Project:	<i>Ms. Muck worked on genotyping for the human study of Aim 3, and she interacted with the pharmacy providing double-blind study medication.</i>
Funding Support:	

Name:	<i>Sofia Fluke</i>
Project Role:	<i>Ph.D. Student</i>
Researcher Identifier:	<i>Washington State University ID: 11726590</i>
Nearest person month worked:	<i>1</i>
Contribution to Project:	<i>Ms. Fluke provided behavioral monitoring support for the human study of Aim 3.</i>
Funding Support:	

Name:	<i>Matthew E. Layton, M.D., Ph.D.</i>
Project Role:	<i>Physician of Record</i>
Researcher Identifier:	<i>ORCID ID: 0000-0002-3287-9203</i>
Nearest person month worked:	<i>1</i>
Contribution to Project:	<i>Dr. Layton served as medical director for the study and implemented procedures for the pharmacogenetic interventions in the human subjects of Aim 3.</i>
Funding Support:	

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

Name:	<i>Dawn DePriest, Ph.D., FNP-C</i>
Project Role:	<i>Medical Oversight</i>
Researcher Identifier:	<i>Washington State University ID: 11458230</i>
Nearest person month worked:	<i>1</i>
Contribution to Project:	<i>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.</i>
Funding Support:	

Name:	<i>Sue Weeks, MN, FNP-C, PMHNP-BC, DNP</i>
Project Role:	<i>Medical Oversight</i>
Researcher Identifier:	<i>Washington State University ID: 11458230</i>
Nearest person month worked:	<i>1</i>
Contribution to Project:	<i>Together with Drs. Joseph and DePriest, Dr. Weeks performed medical exams during screening and oversaw subject health and well-being in the human subjects study of Aim 3.</i>
Funding Support:	

Name:	<i>Stephen James, Ph.D.</i>
Project Role:	<i>Researcher</i>
Researcher Identifier:	<i>ORCID ID: 0000-0003-4139-7967</i>
Nearest person month worked:	<i>1</i>
Contribution to Project:	<i>Dr. James maintained simulator equipment and software.</i>
Funding Support:	

Name:	<i>Michelle Schmidt</i>
Project Role:	<i>Research Technician</i>
Researcher Identifier:	<i>Washington State University ID: 11126756</i>
Nearest person month worked:	<i>2</i>
Contribution to Project:	<i>Ms. Schmidt completed histology to determine viral transfection for constructs and generated figures.</i>
Funding Support:	

Name:	<i>Daniel Harvey</i>
Project Role:	<i>Research Technician</i>
Researcher Identifier:	<i>Washington State University ID: 10245277</i>
Nearest person month worked:	<i>2</i>
Contribution to Project:	<i>Mr. Harvey wrote code for data analysis of the animal experiments and generated figures.</i>
Funding Support:	

Name:	<i>Naomi Teeter</i>
Project Role:	<i>Research Coordinator</i>
Researcher Identifier:	<i>Washington State University ID: 11792273</i>
Nearest person month worked:	1
Contribution to Project:	<i>Ms. Teeter assisted with scheduling research assistants, obtaining informed consent, and overseeing the laboratory study for Aim 3.</i>
Funding Support:	

Name:	<i>Myles Finlay, RPSGT</i>
Project Role:	<i>Registered Polysomnographic Technologist</i>
Researcher Identifier:	<i>Washington State University ID: 11546225</i>
Nearest person month worked:	1
Contribution to Project:	<i>Mr. Finlay oversaw sleep monitoring in the human subjects study of Aim 3, including assessment of baseline sleep to verify inclusion criteria.</i>
Funding Support:	

Name:	<i>Mark McCauley</i>
Project Role:	<i>Research Engineer</i>
Researcher Identifier:	<i>Washington State University ID: 11331753</i>
Nearest person month worked:	1
Contribution to Project:	<i>Mr. McCauley assisted with training research assistants and trouble-shooting technical issues for the simulated driving and shooting tasks in the research protocol for the human study of Aim 3.</i>
Funding Support:	

Name:	<i>Jonah Scott</i>
Project Role:	<i>Research Assistant</i>
Researcher Identifier:	<i>Washington State University ID: 11698308</i>
Nearest person month worked:	1
Contribution to Project:	<i>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.</i>
Funding Support:	

Name:	<i>Tye Arrington-Fox</i>
Project Role:	<i>Research Assistant</i>
Researcher Identifier:	<i>Washington State University ID: 11791760</i>
Nearest person month worked:	1
Contribution to Project:	<i>Mr. Arrington-Fox 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.</i>
Funding Support:	

Name:	<i>Braden Bell</i>
Project Role:	<i>Research Assistant</i>
Researcher Identifier:	<i>Washington State University ID: 11829377</i>
Nearest person month worked:	1
Contribution to Project:	<i>Mr. Bell 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.</i>
Funding Support:	

Name:	<i>Lindsey Bell</i>
Project Role:	<i>Research Assistant</i>
Researcher Identifier:	<i>Washington State University ID: 11686355</i>
Nearest person month worked:	1
Contribution to Project:	<i>Ms. Bell 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.</i>
Funding Support:	

Name:	<i>Anthony Bennett</i>
Project Role:	<i>Research Assistant</i>
Researcher Identifier:	<i>Washington State University ID: 11814077</i>
Nearest person month worked:	1
Contribution to Project:	<i>Mr. Bennett 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.</i>
Funding Support:	

Name:	Blake Budke
Project Role:	<i>Research Assistant</i>
Researcher Identifier:	<i>Washington State University ID: 11588945</i>
Nearest person month worked:	1
Contribution to Project:	<i>Mr. Budke 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.</i>
Funding Support:	

Name:	<i>Tayler Clifton</i>
Project Role:	<i>Research Assistant</i>
Researcher Identifier:	<i>Washington State University ID: 11828903</i>
Nearest person month worked:	1
Contribution to Project:	<i>Ms. Clifton 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.</i>
Funding Support:	

Name:	<i>Sara Delane</i>
Project Role:	<i>Research Assistant</i>
Researcher Identifier:	<i>Washington State University ID: 11622588</i>
Nearest person month worked:	1
Contribution to Project:	<i>Ms. Delane 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.</i>
Funding Support:	

Name:	<i>Freya Fanson</i>
Project Role:	<i>Research Assistant</i>
Researcher Identifier:	<i>Washington State University ID: 11821629</i>
Nearest person month worked:	1
Contribution to Project:	<i>Ms. Fanson 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.</i>
Funding Support:	

Name:	<i>Isabelle Howard</i>
Project Role:	<i>Research Assistant</i>
Researcher Identifier:	<i>Washington State University ID: 11829384</i>
Nearest person month worked:	1
Contribution to Project:	<i>Ms. Howard 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.</i>
Funding Support:	

Name:	<i>Gwen Jaramillo</i>
Project Role:	<i>Research Assistant</i>
Researcher Identifier:	<i>Washington State University ID: 11830381</i>
Nearest person month worked:	1
Contribution to Project:	<i>Ms. Jaramillo 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.</i>
Funding Support:	

Name:	<i>Ashlyn Kovacevich</i>
Project Role:	<i>Research Assistant</i>
Researcher Identifier:	<i>Washington State University ID: 11819440</i>
Nearest person month worked:	1
Contribution to Project:	<i>Ms. Kovacevich 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.</i>
Funding Support:	

Name:	<i>Sadie Kruger</i>
Project Role:	<i>Research Assistant</i>
Researcher Identifier:	<i>Washington State University ID: 11847621</i>
Nearest person month worked:	1
Contribution to Project:	<i>Ms. Kruger 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.</i>
Funding Support:	

Name:	<i>Evan Nessen</i>
Project Role:	<i>Research Assistant</i>
Researcher Identifier:	<i>Washington State University ID: 11821943</i>
Nearest person month worked:	1
Contribution to Project:	<i>Mr. Nessen 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.</i>
Funding Support:	

Name:	<i>Sofia Novochekhova</i>
Project Role:	<i>Research Assistant</i>
Researcher Identifier:	<i>Washington State University ID: 11821945</i>
Nearest person month worked:	1
Contribution to Project:	<i>Ms. Novochekhova 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.</i>
Funding Support:	

Name:	<i>Mariana Pacheco-Arcaya</i>
Project Role:	<i>Research Assistant</i>
Researcher Identifier:	<i>Washington State University ID: 11803213</i>
Nearest person month worked:	1
Contribution to Project:	<i>Ms. Pacheco-Arcaya 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.</i>
Funding Support:	

Name:	<i>Brooke Rogers</i>
Project Role:	<i>Research Assistant</i>
Researcher Identifier:	<i>Washington State University ID: 11790319</i>
Nearest person month worked:	1
Contribution to Project:	<i>Ms. Rogers 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.</i>
Funding Support:	

Name:	<i>Shelda Salomon</i>
Project Role:	<i>Research Assistant</i>
Researcher Identifier:	<i>Washington State University ID: 11672053</i>
Nearest person month worked:	1
Contribution to Project:	<i>Ms. Salomon 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.</i>
Funding Support:	

Name:	<i>Anthony Scholes</i>
Project Role:	<i>Research Assistant</i>
Researcher Identifier:	<i>Washington State University ID: 11812127</i>
Nearest person month worked:	1
Contribution to Project:	<i>Mr. Scholes 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.</i>
Funding Support:	

Name:	<i>Rebecca Simmons</i>
Project Role:	<i>Research Assistant</i>
Researcher Identifier:	<i>Washington State University ID: 11792459</i>
Nearest person month worked:	1
Contribution to Project:	<i>Ms. Simmons 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.</i>
Funding Support:	

Name:	<i>Claire Sparano</i>
Project Role:	<i>Research Assistant</i>
Researcher Identifier:	<i>Washington State University ID: 11829384</i>
Nearest person month worked:	1
Contribution to Project:	<i>Ms. Sparano 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.</i>
Funding Support:	

Name:	<i>Shen Tsao</i>
Project Role:	<i>Research Assistant</i>
Researcher Identifier:	<i>Washington State University ID: 11513719</i>
Nearest person month worked:	1
Contribution to Project:	<i>Ms. Tsao 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.</i>
Funding Support:	

Name:	<i>Jiayi Ena Wang</i>
Project Role:	<i>Research Assistant</i>
Researcher Identifier:	<i>Washington State University ID: 11696023</i>
Nearest person month worked:	1
Contribution to Project:	<i>Ms. Wang 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.</i>
Funding Support:	

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

Army Research Office / Multidisciplinary University Research Initiatives Program (subcontract under University of Michigan), “Understanding and Predicting Cognitive Fatigue across Multiple Timescales, Distinct Aspects of Cognition, and Different Individuals with Multiscale Whole Cortex Models” (PI: Forger D), September 2022–September 2025.

The new project did not stem from results obtained in the present project, and there is no overlap between the new project and the current project. Effort on the new project has been offset by reduced effort on completed and closed grants (see below).

Grants closed:

Office of Naval Research / Defense University Research Instrumentation Program, “Clinical Sleep Research to Characterize, Predict, and Ameliorate Operationally Relevant Sleep and Performance Deficits in Sleep Disordered Populations” (PI: Hansen DA), April 2022–March 2023. This was an instrumentation grant to help equip a new clinical sleep research laboratory within the Sleep and Performance Research Center’s human sleep research facility.

Australian Research Council, “Individualised Predictions of Circadian Timing, Sleep, and Performance” (PI: Phillips A), November 2021–August 2022. This grant involved providing expertise regarding individualization of biomathematical model predictions in operational settings.

Jazz Pharmaceuticals (industry grant), “Solriamfetol’s Effect on Cognitive Health in Apnea Participants during a Randomized Placebo-Controlled Study (SHARP): A 5-Week Double-Blind, Placebo-Controlled, Randomized, Crossover, Multicenter Study of Solriamfetol in Improving Cognitive Function in Participants with Excessive Daytime Sleepiness Associated with Obstructive Sleep Apnea plus Impaired Cognitive Function” (PI: Hansen DA), July 2021–June 2022. This was a Phase IV clinical trial of solriamfetol for cognitive performance improvement in treated sleep apnea patients with residual daytime sleepiness.

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 was subcontracted as a DoD consultant for the project.

7. SPECIAL REPORTING REQUIREMENTS

Quad Chart

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

PI: Hans P.A. Van Dongen Org: Washington State University

BA170226 / W81XWH1810100

Award Amount: \$2,797,841

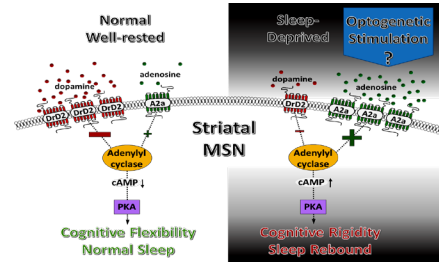
Study/Product Aims

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.



The dynamic interplay between adenosine and dopamine signaling regulates the excitability of striatal medium spiny neurons (MSNs). Elevated adenosine tone and reduced dopamine tone over time awake augments the excitability of GABAergic MSNs in the indirect pathway, which enhances their inhibitory feedback onto prefrontal executive circuitry, resulting in cognitive rigidity and sleep rebound.

Accomplishments: Aims 1 and 2 have been completed. To date, for the human study of Aim 3, 63 out of 90 subjects have completed the experiment.

Timeline and Cost

Activities	CY	18	19	20	21	22	23	24
1. Development of rodent model								
2. Optogenetic experiments								
3. Human genotype differences								
Estimated Budget (\$K)		\$445K	\$910K	\$955K	\$488K	NCE	NCE	NCE

Updated: 2023 May 14

Goals/Milestones

CY23 Goals – Publish paper documenting the overall results of the rat study (Aims 1 and 2); continue with the human subject laboratory sleep deprivation experiments (Aim 3).

Comments/Challenges/Issues/Concerns

- The human subject recruitment rate continued to be below pre-pandemic levels due to labor shortages affecting availability of our subject pool. We have been granted an additional 1-year no-cost extension (NCE) to complete the study and address Aim 3.

Budget Expenditure to Date

Projected Expenditure: \$2,798K.

Actual Expenditure: \$2,796K.

Other resources are available to complete the project.

8. APPENDICES

Foakes C, Lawrence-Sidebottom D, Dralega AT, Harvey DO, Schmidt MA, Davis CJ (2022). The rat Lux Actuating Search Task (LAST) and effects of sleep deprivation on task reversal performance. *Neurobiology of Sleep and Circadian Rhythms* 13: 100081.

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