

AWARD NUMBER: W81XWH-18-1-0100

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

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REPORT DATE: JUNE 2022

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE JUNE 2022		2. REPORT TYPE Annual		3. DATES COVERED 15MAY2021 - 14MAY2022	
4. TITLE AND SUBTITLE Investigating Striatal Attentional Circuits to Understand and Mitigate Deficits in Cognitive Flexibility Due to Sleep Loss				5a. CONTRACT NUMBER W81XWH-18-1-0100	
				5b. GRANT NUMBER BA170226	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Hans P.A. Van Dongen, Christopher J. Davis E-Mail: hvd@wsu.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Washington State University p/a Daniel Nordquist 240 French Admin Bldg, Pullman, WA 99164-1001				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Development Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
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. After extended closure of the human sleep laboratory due to the COVID-19 pandemic, the double-blind human study was resumed in Year 4. The parallel animal study involving development of transgenic rat models and behavioral and brain-based data collection is approaching completion.					
15. SUBJECT TERMS Sleep deprivation, performance impairment, attentional control, cognitive flexibility, resilience, striatum, caffeine, modafinil, optogenetic stimulation					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	51	19b. TELEPHONE NUMBER (include area code)

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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 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. The parallel animal study involving development of transgenic rat models and behavioral and brain-based data collection is approaching completion. A second 1-year no-cost extension was granted, extending the timeline of the project by another 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.	60	In progress
Data Analysis		
Milestone(s) Achieved: Aim 2 analyses completed.	60	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).	60	In progress
Data Analysis		
Milestone(s) Achieved: Aim 3 analyses completed.	60	n/a
Final Report Preparation		
Compilation of analyses from aims 1–3 and drafting of report and briefing	45-60	n/a
Presentation of study results to the DoD	60	n/a
Milestone(s) Achieved: study completed	60	In progress

What was accomplished under these goals?

During Year 4, the major activities to be completed to achieve the goal milestones were:

- Continue aim 2 data collection 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).
- Resume data collection for Aim 3 following human sleep laboratory closure due to the COVID-19 pandemic.

During Year 4, the specific objectives were to:

- Optimize concentration of the inhibitory opsin, halorhodopsin (Halo) viral construct in transgenic rats – completed.
- Collect behavioral and electroencephalogram (EEG) data for sleep deprivation mimic experiments in monotransgenic strain for Aim 2 – completed.
- Collect behavioral and EEG data for sleep deprivation rescue experiments in monotransgenic strain for Aim 2 – in progress.
- Collect data in double-blind study of human research participants for Aim 3 – in progress.

During Year 4, key outcomes included the following:

- The effectiveness of optogenetic activation and inhibition protocols 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, and these findings are now supported by statistical analyses.
- One manuscript detailing the rodent work was accepted for publication (Lawrence-Sidebottom et al., 2021); another is under review (Foakes et al., submitted).
- Human subjects data collection in the laboratory study of Aim 3 was resumed as soon as approval to reopen the laboratory was received (fall 2021).

While 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 in Year 2 already, novel and significant scientific progress can be reported for Year 4 based on results obtained in Aim 2. We report those results here.

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]. Adenosinergic cells in this region are known to support perseverative behavior [Kehagia et al., 2010; Wei et al., 2011; Yu et al., 2009], and their activation by adenosine 2a (A2a) receptors under conditions of SD may underlie cognitive inflexibility in this state. Moreover, both dopamine type 2 (DrD2) receptors [Satterfield et al., 2017; Tomasi et al., 2016; Volkow et al., 2012] and A2a receptor systems [Bodenmann et al., 2012; Rétey et al., 2007] have been implicated through genetic and pharmacological studies in humans to be involved in the cognitive deficits resulting from SD.

These two receptors interact antagonistically in a unique population of medium spiny neurons (MSNs) in the dorsal striatum [Farrell et al., 2013] that serves the indirect pathway of the basal ganglia. 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., 2016; 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]. Our aim was to query the intersection of these two functions in parallel to acquire a mechanistic understanding of their collective contribution to the cognitive utility of adaptive response efficiency following SD. We hypothesized that striatal MSNs expressing A2a-DrD2 receptors are necessary and sufficient to confer the negative impacts of SD on cognitive flexibility. More specifically, we posit that activating this circuit will put the brain in SD-like state evidenced by performance deficits and EEG signatures, and that inhibiting the circuit following acute SD will mitigate these outcomes.

We employed an image discrimination reversal learning paradigm to model SD-induced cognitive inflexibility in transgenic rats that express Cre recombinase-dependent viral DNA constructs to transfect MSNs of the dorsal striatum that express the A2a-DrD2 positive neurons. We used optogenetic techniques to activate these MSNs and putatively *mimic* the effects of SD

on reversal task performance in rats injected with channelrhodopsin (ChR2) as compared with rats injected with a control viral construct (mCherry). We also recorded cortical EEG to determine whether optogenetic activation of the dorsal striatum A2a-DrD2-positive neurons artificially induced characteristic SD recovery phenotypes including sleep rebound and elevated delta power from Fast Fourier Transforms (FFT) of the biopotentials. Next, we further interrogated this circuit with optogenetics to inactivate the A2a-DrD2-positive striatal neurons in SD rats using Halorhodopsin (Halo) to *rescue* reversal task performance and suppress sleep rebound and spectral signatures of sleep need and compare with mCherry injected SD-rats.

2. Methods

2.1. Subjects

Male breeder DrD2-Cre rats (LE-Tg(Drd2-icre)1Ottc) procured from the Rat Research and Resource Center (RRRC; 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 enrichment and maintained on a 12/12-hr light dark cycle at 24 ± 2 °C with access to food and water. After the completion of the experiments rats were anesthetized, perfused and brains fixed for histological verification of transfection efficiency and optrode tip placement.

2.2. 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 pairwise discrimination (PD) reversal task. For both SD mimic and rescue experiments handling and training sessions were initiated at Zeitgeber time (ZT) 10. To incentivize performance, 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 weekday 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 provided 10–15 banana flavored sugar pellets (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 in and out of the operant chambers. The training was composed of three phases: pre-training, PD training, and reversal (Fig. 1). Rats in the SD mimic and rescue experiments experienced the exact pre-training and PD training phase protocols.

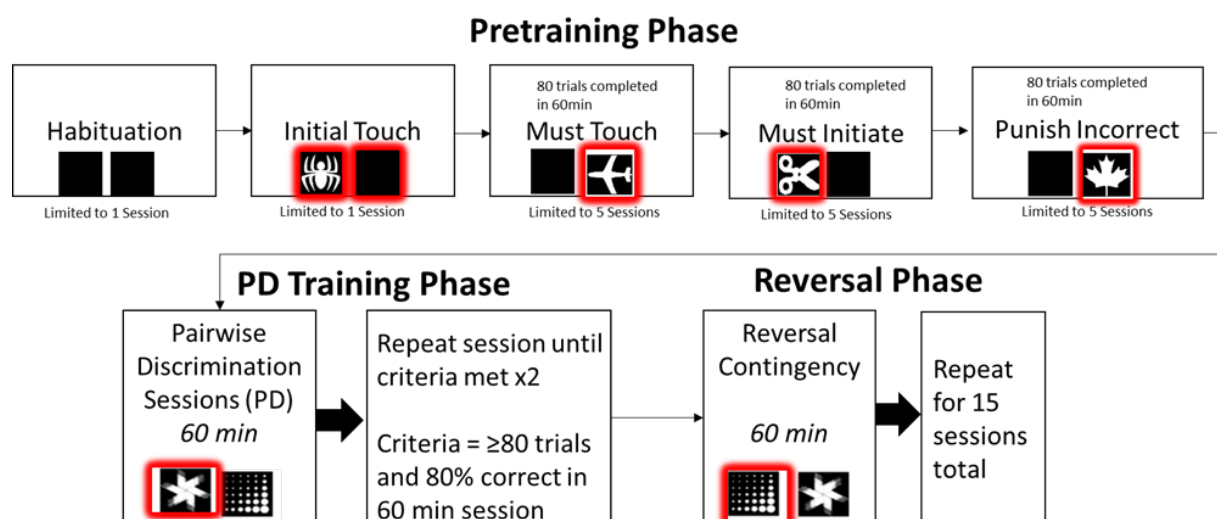


Figure 1. The three phases of the pairwise discrimination (PD) operant task. Red encircled images denote sugar pellet rewarded images in a subset of the rats (note that rewarded images were not cued by a red surround in the actual operant chambers). The opposite contingency for PD training and reversal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

2.2.1. Pre-training phase

Pre-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 side of the screen with the image, they would receive three pellets. If they touched the side of the screen without the image, they received one pellet. 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 $\times$ 3 pellets/correct image), each rat was therefore limited to a single instance of this session. The rats advanced to Must Touch, when they needed to touch the screen where an image was shown to receive the food reward (three pellets) and received no pellets when they touched the side of the screen without an image. The rats had to complete at least 80 trials to move to the next training step.

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 on the screen. As in the previous training phase, rats received three pellets for touching the image on the screen, and no pellets for touching the other side of the screen without an image. They had to complete at least 80 trials to move to the next stage. Punish Incorrect was the final stage of pre-training, wherein the rats had to incorporate previous response contingencies in addition to distinguishing the fully blank screen from when the screen contained a randomly presented image. The rats started the trial by touching the fully blank screen (no image presented), which caused the chamber light to come on and a tone to sound. The rats then needed to touch the screen where an image was presented. If an incorrect response was made (i.e., touched the side of the screen without the image), the rats entered a correction trial, in which the stimulus was presented on the same side again before continuing to the next actual trial. Once 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.

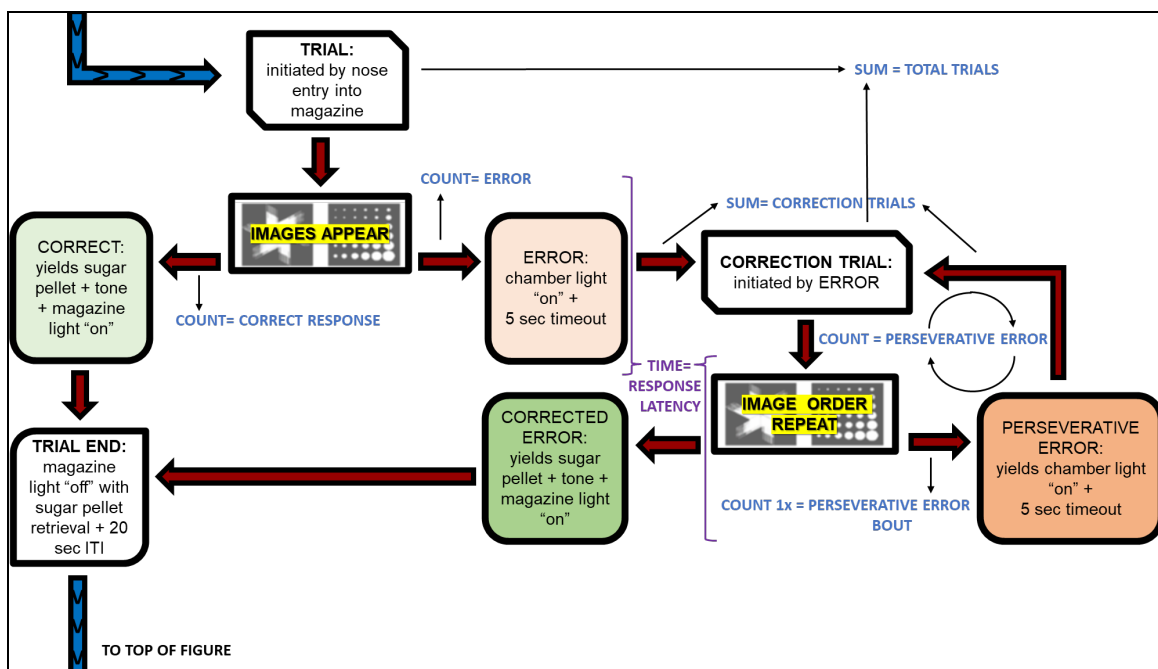


Figure 2. PD trial progression and dependent variable extractions. This figure depicts the execution of PD training and reversal phase trials. The blue and purple font depict the primary dependent variables and how they were extracted for analyses (described in the Data Analysis section).

2.2.2. PD training phase

At the beginning of PD training, the rats were randomly assigned to either “fan” or “marbles” as the correct image. The two images were presented side-by-side in each trial, and the rats only received a pellet for touching their assigned correct image. They 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. After surgical recovery, they reentered the PD Training phase at PD1.

2.2.3. PD reversal phase

Following the second session that a rat met PD training criteria, the rat was 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 optogenetic fiber probes attached. After EEG baseline recording, LED optogenetic stimulation (preceded by 9SD in SD Rescue studies) the Reversal phase was initiated wherein the image that when selected previously resulted in an error (e.g., the marble image in Figs. 1 and 2), now resulted in a correct response and was rewarded with a sugar pellet. Reversal sessions continued until performance criterion was met (at least 80 trials with 80% correct) or for a maximum of 15 reversal sessions.

2.3. SD Mimic Experiment

2.3.1. Virus injections

At 6 weeks of age, male DrD2+ rats (n=34; Fig. 3) 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 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 μ l per hemisphere of pAAV-EF1a-cDIO-hChR2(H134R)-mCherry, serotype 5 (n=17; ChR2 group) or pAAV-EF1a-cDIO-mCherry, Serotype 5 (n=17; Control group; UNC Vector Core). Viral vectors were infused at A/P 0.6, M/L $\pm$ 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 (3 mg/ml, 1 mg/kg, SC; ZooPharm) and then administered carprofen daily (5 mg/kg, PO) for 3 days.

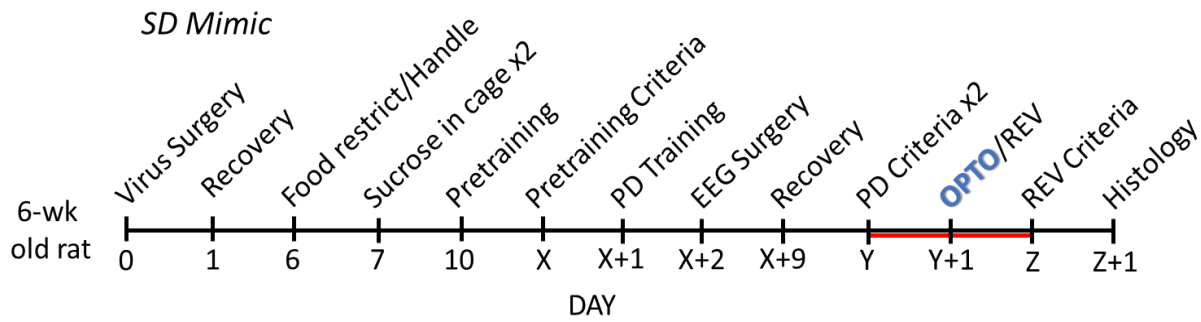


Figure 3. SD mimic experiment timeline of procedures and operant behavior. PD=pairwise discrimination, reversal REV, **OPTO**=1 hr excitatory blue light optogenetic stimulation (10 Hz) from ZT 9–10, SD=sleep deprivation, =EEG recording.

Following virus injection surgery, all 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. Food restriction resumed 5 days following surgery.

2.3.2. EEG/optrode guide placement surgery

After the first PD training session, rats were anesthetized and craniotomized as before and EEG screws (P1 Technologies) 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 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. During subsequent PD and reversal sessions the rats were tethered to a dummy commutator.

2.3.3. Blue LED stimulation of striatal DrD2 neurons

Following the second session that a rat met PD training criteria, the rat was 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 optogenetic fiber probes was attached.

The LED optrodes (445 nm) were inserted into the bilateral guide cannulas and the rat placed into a recording chamber where cortical EEG was recorded. Recordings were sampled at 500 Hz using Sirenia software (Pinnacle Technology). At ZT 9 the following day, the rat (regardless of ChR2 or Control group assignment) received optogenetic stimulation to mimic SD.

Stimulation parameters were 300 mA, 10 Hz, 20 ms PW, 20% duty cycle for 1 hr 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 hr 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 criteria were met or for a maximum of 15 reversal sessions. Fifteen rats that did not meet performance criterion or lost a headcap were omitted from the study. This included eight rats from the ChR2 group and seven rats from the Control group. For the remaining rats, PD reversal data were analyzed, and EEG records were scored.

2.3.4. Validation of striatal optogenetic DrD2 activation

To verify the activity of the ChR2 construct, 4 ChR2 rats and 4 mCherry rats were subjected 10 Hz stimulation using blue LEDs from ZT 12–13. Rats were anesthetized with Euthasol (150 mg/kg, IP; Covetrus) and transcardially perfused with cold phosphate buffered saline (PBS) followed by 10% formalin. Brains were extracted and stored in 10% formalin for 24 hr 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.

Immunohistochemistry was used to determine whether neurons (identified with the *NeuN* neuronal marker) were DrD2 positive (identified with *mCherry* florescence) and the degree to which they were recently active via the intensity of the activity marker *cFos* (Fig. 4). 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 blocking buffer containing 5% BSA, 5% goat serum, 0.25% triton-x for 1 hr on an orbital shaker. Following three 5-min washes with PBS, the sections were placed in 4 mL of 1% sodium borohydride in PBS for 30 min. Sections were rinsed again in three PBS washes for 5 min each 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. Following 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). Sections were imaged using a Leica DMI8, TCS SPE confocal microscope. Images were analyzed for cell number and intensity using Pipsqueak ai (Rewire Neuro, Inc.).

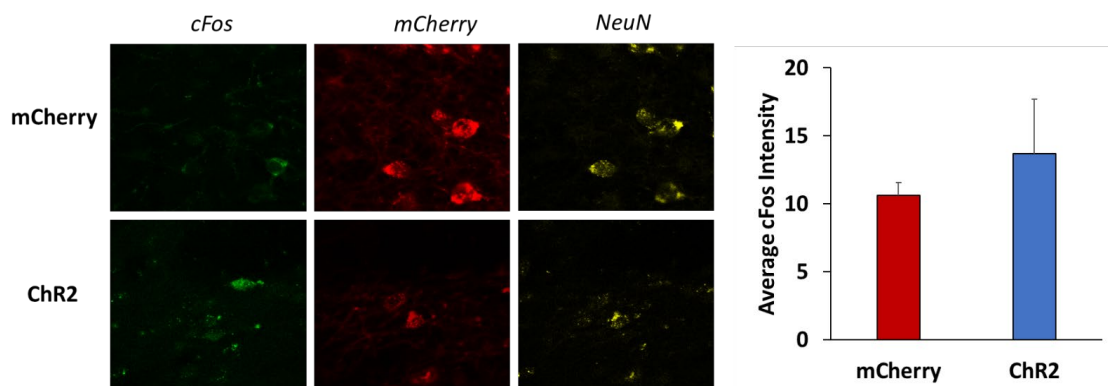


Figure 4. Immunohistochemistry to validate the effectiveness of blue light stimulation of the dorsal striatum in ChR2 rats (lower panel) versus mCherry rats (upper panel). Green=circuit activity marker (cFos), Red=DrD2 positive ChR2-mCherry or mCherry alone expression, Yellow=neuronal marker (NeuN) for cell-type specificity. The preliminary bar graph shows higher cFos activity following 1 hr of 10 Hz blue light stimulation of striatal DrD2-positive MSNs.

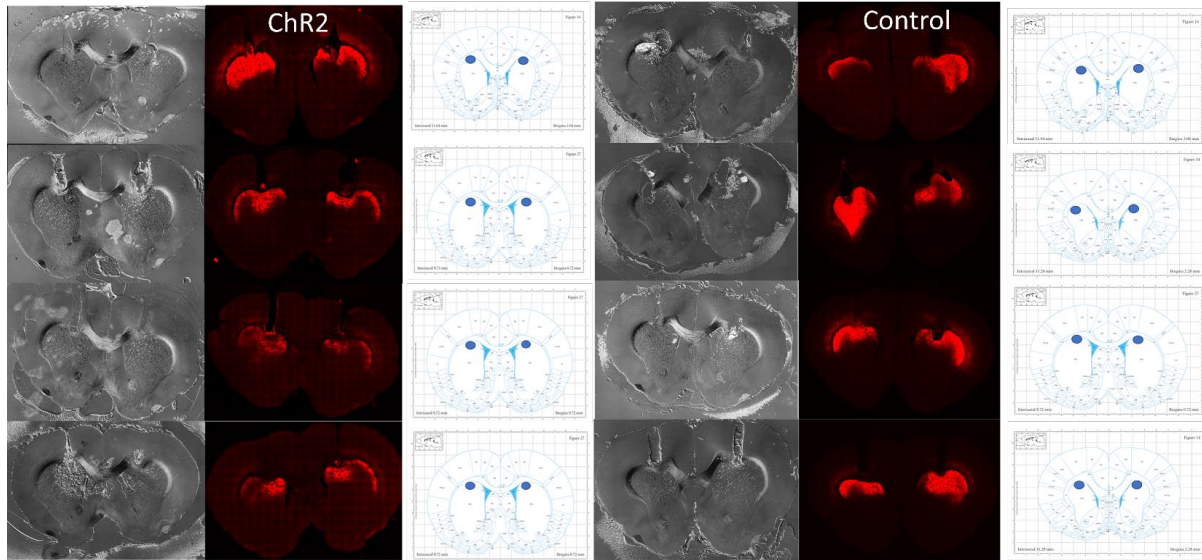


Figure 5. SD mimic study histological verification. After pairwise discrimination reversal criteria were met, DrD2+ rats transfected with pAAV-EF1a-cDIO-hChR2(H134R)-mCherry Serotype 5 virus (ChR2; left three columns) were euthanized and coronal slices of perfused brains were used to verify cannula track (gray slides), transfection efficiency (red fluorescing mCherry slides) and optrode tip estimate (blue circles) superimposed on corresponding pages of stereotaxic atlas. This was also performed with DrD2-Cre+ rats transfected with pAAV-EF1a-cDIO-hChR2(H134R)-mCherry Serotype 5 virus (Control; right three columns). While this figure depicts four rats per group, all rats used in the SD mimic study were verified in this manner. We are currently verifying Halo transfection and optrode placement in this manner.

2.3.5. Histology

At the conclusion of the study, rats were anesthetized, perfused, and the brains prepared for cryosectioning as described above. Brains were blocked A/P ± 10.0 mm from the guide cannula 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. 5).

2.4. SD Rescue Experiment

2.4.1. Virus injections

At 6 weeks of age, male DrD2+ rats (n=45 to date; Fig. 6) were anesthetized and craniotomized as described above for virus infusion at the same coordinates, except SD rescue rats received 3 μ l per hemisphere of pAAV-EF1a-DIO-eNpHR3.0-mCherry, serotype 5 (n=19 to date; Halo

group) or pAAV-EF1a-cDIO-mCherry, Serotype 5 (n=26 to date; Control group). Post-operative treatment, recovery, and food restriction occurred as above.

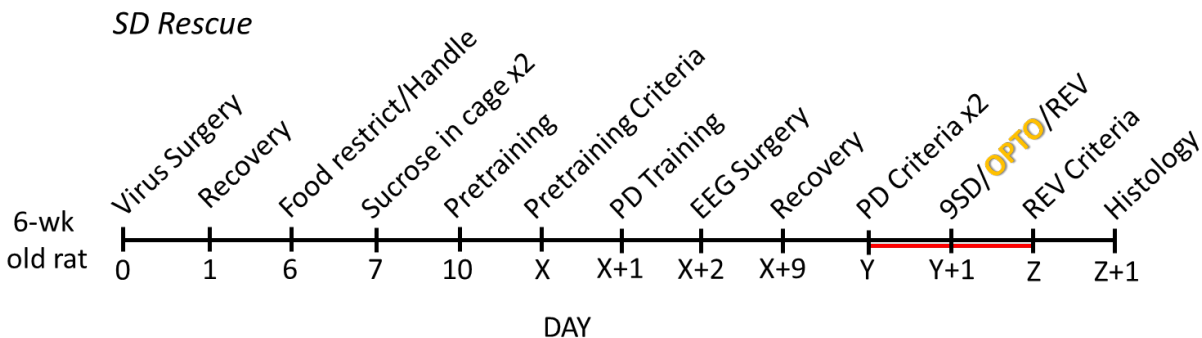


Figure 6. *SD Rescue experiment timeline of procedures and operant behavior. PD=pairwise discrimination, reversal REV, 9SD=sleep deprivation from ZT 0–9, OPTO=1 hr inhibitory yellow light optogenetic stimulation (50 Hz) from ZT 9–10, —=EEG recording.*

2.4.2. EEG/optrode guide placement surgery

After the first PD training session, rats were instrumented with bilateral EEG electrodes and optrodes as described above.

2.4.3. Yellow LED suppression of striatal DrD2 neurons

As in the SD mimic experiment, following the second PD training session that a rat met criteria it was removed, and the headstage was attached. The yellow LED optrodes (590 nm) were inserted into the bilateral guide cannulas. The rat was then placed into a recording chamber equipped with a rotary bar automated SD system (Pinnacle Technology) with the bar set to turn on for 4s randomly every 8–16 sec, changing direction every 10–40 sec beginning the following day at ZT 0 for nine hours and cortical EEG was recorded. Preliminary data suggested that after the first 5 hr of SD in these systems the sleep bouts began to appear, so a research assistant supplemented with gentle handling SD for the last four hours of the study to ensure prolonged wakefulness. At ZT 9 the day the rats received optogenetic stimulation at 300 mA, 50 Hz, 20 ms PW, 20% duty cycle for 1 hr. At ZT 10, the rats were removed from the recording chamber, transported to the operant chamber, and connected to the dummy commutator, and underwent the first reversal session. Following the 1 hr reversal session, the rats were returned to the recording chamber to record recovery sleep and returned to their home cage until the reversal session on the next day. Reversal sessions continued until performance criterion was met (at least 80 trials with 80% correct) or for a maximum of 15 reversal sessions. Eight of

eleven rats did not meet performance criterion at any phase or lost a headcap and were omitted from the study.

2.4.4. Validation of optogenetic striatal DrD2 suppression

Striatal *cFos* activation is observed in rats following ZT 3–6 sleep deprivation (Semba et al., 2001). To verify the suppression of striatal DrD2 activity by the Halo construct, 4 Halo rats and 4 control mCherry rats were sleep deprived by the rotating bar method from ZT 3–6 and to suppress this striatal *cFos* signal we rats were optogenetically stimulated at 50 Hz using yellow LEDs for the last hour of the SD. Rats were then perfused, and the brains prepared for cryosectioning and immunochemistry as described above to determine neuronal (*NeuN*) DrD2 (*mCherry*) and the levels of activity (*cFos*; Fig. 7).

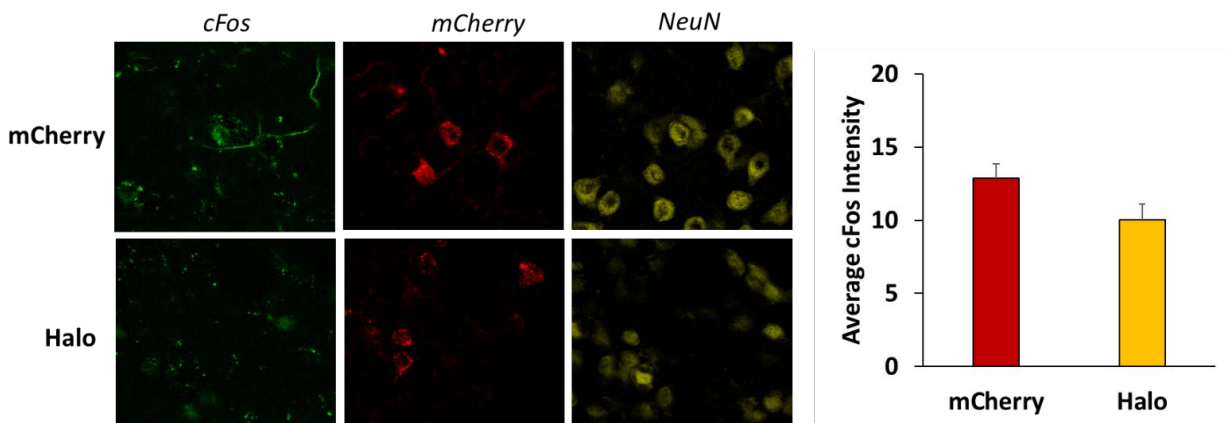


Figure 7. Immunohistochemistry to validate the effectiveness of yellow light stimulation of the dorsal striatum in Halo (lower panel) rats versus mCherry rats (upper panel). Green=circuit activity marker (*cFos*), Red=DrD2 positive Halo-mCherry or mCherry alone expression, Yellow=neuronal marker (*NeuN*) for cell-type specificity. The preliminary bar graph shows lower *cFos* activity following SD and 1 hr of 50 Hz yellow light stimulation of striatal DrD2-positive MSNs.

2.5. Data Analyses

The Bussey Saksida touchscreen operant platform is composed of several IR sensors that when triggered are recorded as a time series of events to track 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.

2.5.1. Terminology

Here we 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. 2). 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. *Perseverative 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. Correction trials continued until the rodent touches the correct image after which a reward is dispensed, and a new initial trial began. We define *response latency* as the average difference in time between 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. Although it should be noted that the *percent correct trials* are calculated as the ratio of correct responses only to the number of initial trials.

2.5.2. Behavioral statistics

For behavioral outcomes IBM SPSS version 28.0 statistics package performed mixed 2-way ANOVAS on the within factor of *sessions* (1–15) and the between factor of *virus* (ChR2 group vs. mCherry group) were used to analyze cumulative trial totals, correct responses, % correct responses, errors, perseverative errors and perseverative error bouts, and response latency. The number of sessions to meet criteria was analyzed using Student t-test.

2.5.3. Vigilance state 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, 1–20 Hz) spreadsheets were populated via a custom Matlab algorithm and subjected to statistical analyses with Dell Statistica version 13.2 and GraphPad Prism 7.

3. Results

3.1. SD Mimic Experiment

Optogenetic stimulation of the dorsal striatum of ChR2 rats prior to image reversal had significant adverse effects on cognitive flexibility compared to control rats that also received light stimulation. Our 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. The differences in cumulative total trials (Fig. 8A) or correct trials (Fig. 8B) were not statistically different. However, the cumulative errors, perseveration errors, and perseveration error bouts and response latencies following image reversal had statistically significant session x group interactions ($F_{14,238}=4.01$, $P<0.001$; $F_{14,238}=5.72$, $P<0.001$; $F_{14,238}=2.76$, $P<0.001$; $F_{14,238}=2.31$, $P=0.005$, respectively). Specifically, ChR2 rats had a 32% increase in errors (Fig. 8C; t-test, $P=0.012$), a 35% increase in perseverative errors (Fig. 8D; t-test, $P=0.009$), and 28% increase in perseverative error bouts (Fig. 8E; t-test, $P=0.027$), compared to mCherry rats during the reversal phase. The pairwise comparison was not significant in cumulative response latency (Fig. 8F; t-test, $P=0.182$). Given that overall total trial number (Fig. 8A) and the R1 trial response latency (Fig. 8G) don't differ suggests that the dysfunction is indeed cognitive as opposed to fatigue, motor deficits or mere response inhibition per the multifunctional aspects of dopamine signaling the striatum.

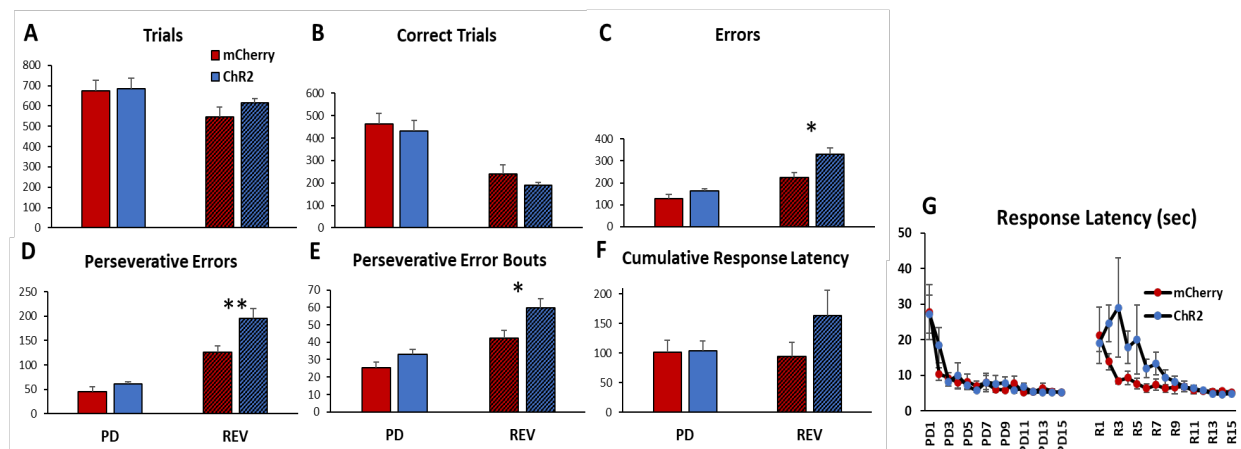


Figure 8. Optogenetic activation of the striatal indirect pathway disrupts cognitive flexibility in rats. After activation of striatal DrD2+ medium spiny neurons and image reversal (REV, darker

colored bars), ChR2 rats ($n=9$; blue bars and symbols) did not change the cumulative total number of trials (**A**) or correct trials (**B**) relative to control mCherry rats ($n=10$; crimson bars and symbols). However, in characteristic indices of cognitive flexibility, ChR2 rats manifest more errors (**C**), perseverative errors (**D**) and perseverative error bouts (**E**) than their mCherry controls. Although not statistically significant in pairwise comparisons ChR2 rats appeared to have more response variability in the response latency (**F**, **G**) compared to control mCherry rats ($n=10$; crimson bars and symbols). Moreover, there were no between group (mCherry vs. ChR2) differences in the pairwise discrimination training phase (PD, lighter colored bars), suggesting that these changes were due to optogenetic activation of DrD2+ striatal circuitry. Data are expressed as group mean $\pm$ SEM, * = $p<0.05$, ** = $p<0.01$.

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. 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 ChR2 rats compared to controls. EEG data were scored in 10 s epochs as wake, NREM or REM sleep. In ChR2 rats, optogenetic activation of the striatal indirect pathway led to a 48% increase in sleep rebound in the 3 hr period following the 10 Hz optogenetic stimulation and the initial reversal session compared to control virus rats that also received the stimulation.

Based on the above findings we analyzed additional sleep/wake phenotypes at ZT 12–14 and none of the following differences between the experimental groups, which were observed at ZT 12–14 after return to the home cage subsequent to dorsal striatal light exposure at ZT 10–11, 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.

Sleep timing and EEG spectral parameters indicated increased propensity for sleep as a consequence of optogenetic stimulation at a time of day (ZT 12–14) when wake normally predominates in nocturnal rats. The time spent awake was significantly reduced by 25% (Fig. 9A; t-test, $P=0.021$) and the time spent asleep increased by 2.5-fold (Fig. 9A; t-test, $P=0.024$) in ChR2 rats relative to mCherry. The total number of state transitions ($F_{2,26}=3.78$, $P=0.036$), and the number of wake-to-sleep transitions ($F_{2,26}=3.67$, $P=0.039$) were significantly elevated in ChR2 rats relative to mCherry rats. Latency to one consecutive minute of wake was delayed by

2.7 minutes in ChR2 rats (3.77 ± 1.46 min) relative to mCherry rats (1.00 ± 1.55 min; t-test, $P=0.032$). Mean wake episode duration was reduced by 50% in ChR2 rats relative to mCherry rats ($F_{2,26}=3.41$, $P=0.049$). ChR2 rats exhibited 2-fold more slow wave sleep bouts than mCherry rats during the first 2-hr interval of the 6-hr post-stimulation polysomnographic recording ($F_{2,30}=7.93$, $P=0.002$). ChR2 rats exhibited a 2.5-fold increase in the number of brief awakenings relative to mCherry rats ($F_{2,30}=8.37$, $p<0.001$), as a function of their increased propensity for sleep.

REM propensity was also elevated as a consequence of optogenetic stimulation. Time spent in REM sleep was significantly elevated in ChR2 rats relative to mCherry rats (Fig. 9A; t-test, $P=0.040$). Latency to REM sleep (t-test, $P=0.047$) and 1-min consecutive REM sleep (t-test, $P=0.013$) were both reduced by about 1.5 hr in ChR2 rats relative to mCherry rats. The number of REMS bouts were also elevated in the first 2 hr of recording in ChR2 rats (average approximately 3 bouts over 2 hrs) relative to mCherry rats (modal value 0 bouts; t-test, $P=0.023$). REM bout duration was also increased in ChR2 rats relative to mCherry rats, by approximately 2.5-fold (t-test, $P=0.035$).

Following stimulation and PD reversal, EEG spectral power at frequencies less than 20 Hz, a measure of homeostatic sleep drive, were elevated in ChR2 rats relative to mCherry rats during all three states. Specifically, the statistically significant elevations in ChR2 rats occurred at 13 Hz during wake (Fig. 9B, $F_{19,285}=1.63$, $P=0.048$), at 1 Hz during REMS (Fig. 9D, $F_{19,133}=3.17$, $P<0.001$), and at 2–4 Hz during NREMS (Fig. 9C, $F_{19,285}=2.27$, $P=0.002$). Delta power (1–4 Hz power) during NREM sleep, the most robust and reliable measure of homeostatic sleep pressure, was elevated in ChR2 rats relative to mCherry rats (Fig. 10C inset, $F_{19,285}=3.66$, $P=0.039$). Whereas delta power values were below the average daily delta power values in mCherry rats (as expected at ZT 12–14 when homeostatic sleep pressure is at its daily nadir), delta power was elevated to 110% of the daily average in ChR2 rats after optogenetic stimulation.

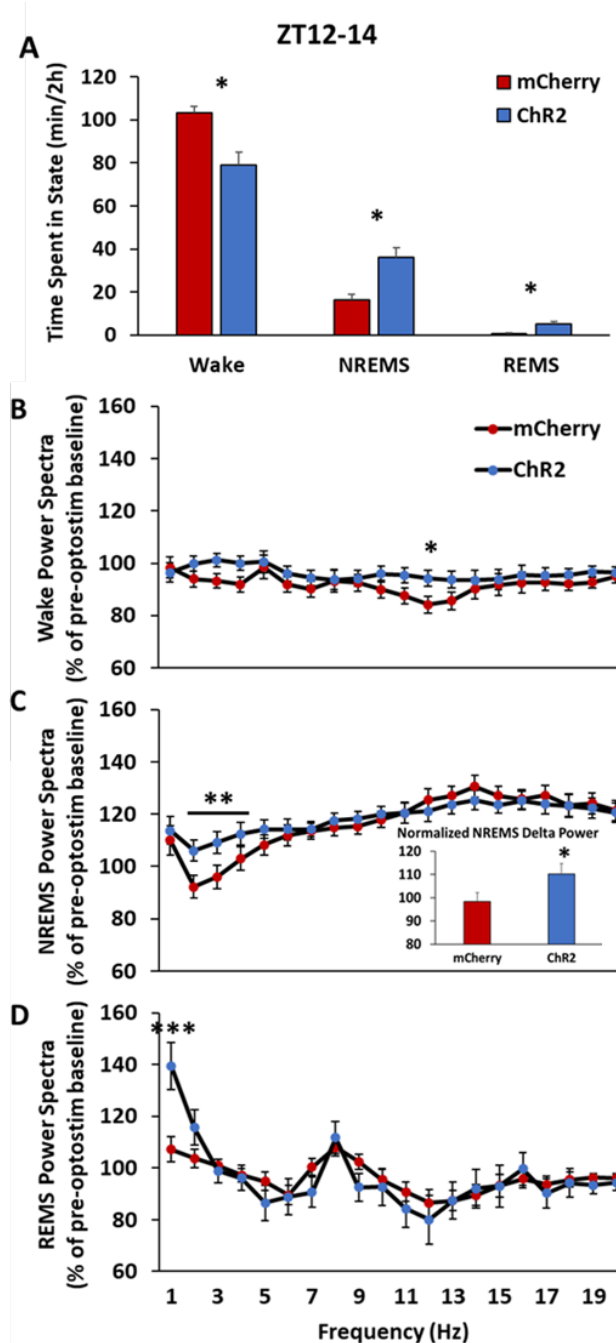


Figure 9. Optogenetic activation of the striatal indirect pathway produces a sleep rebound. At ZT 12–14 (1–3 hr after PD reversal) following optogenetic stimulation of striatal DrD2+ medium spiny neurons, ChR2 rats (blue bars; $n=8$) manifest decreased wakefulness increased NREMS and REMS (A) compared to mCherry controls (crimson symbols; $n=8$). EEG spectral power was elevated in ChR2 rats relative to mCherry rats during wake (B), NREMS (C), NREMS delta power (1–4 Hz, C inset) and REMS (D). Data are expressed as group mean $\pm$ SEM, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

3.2. SD Rescue Experiment

Current progress for this experiment is reported below (Fig. 10).



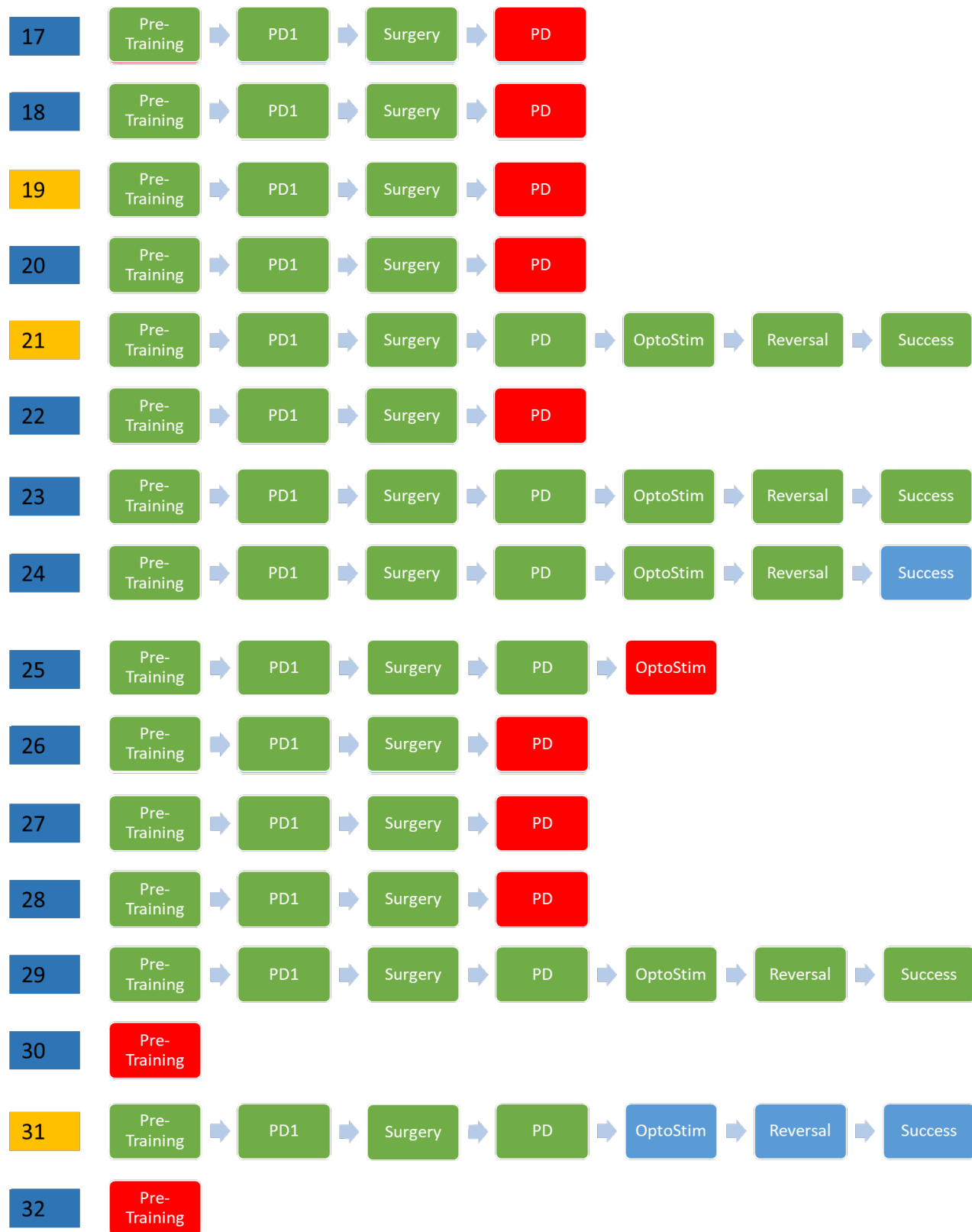




Figure 10. Individual progress of DRD2+ rats for the SD rescue experiment. Blue numbered rats are mCherry controls (n=26) and yellow numbered rats are from in the Halo group (n=19). Green background indicates completed events, light blue indicates events to be completed (n=7), and red indicates events that warranted removal of a rat from the study.

4. Interim Conclusions

In the SD mimic experiment, the 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 neuronal population in the dorsal striatum. Further, the increased errors, perseverative errors, perseverative bouts, and response latency in the pairwise discrimination reversal phase supported our hypothesis that activation of the DrD2+ medium spiny neurons in the dorsal striatum compromises adaptive decision making. Our nearly completed SD rescue experiment will serve to demonstrate causality in the interpretation of our findings.

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Zhang JP, Xu Q, Yuan XS, Cherasse Y, Schiffmann SN, de Kerchove d'Exaerde A, Qu WM, Urade Y, Lazarus M, Huang ZL, Li RX. Projections of nucleus accumbens adenosine A2A receptor neurons in the mouse brain and their implications in mediating sleep-wake regulation. *Front Neuroanat* 2013, 7:43.

During Year 4, 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 human subject enrollment was resumed immediately. Although human subject recruitment is challenging due to ongoing labor shortages, which affect availability of our target population (i.e., healthy young adults), at the end of Year 4 we had 43 subjects completed (30 more than before the laboratory reopened) out of our target sample size of N=90, or close to 50%. This puts us back on track for completion of the Aim 3 study.

We published one manuscript based on results obtained during preparatory work for Aim 2 (PDF copy enclosed).

We published two 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 4 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 has been involved in the project under the direct mentorship of the Co-PI (Honn) of the project.

How were the results disseminated to communities of interest?

After a productive Year 3 for dissemination, Year 4 was heavily focused on making up for lost time due to the COVID-19 pandemic, leaving little time for other activities. That being said, the PI (Van Dongen) presented on sleep, sleep deprivation, and human performance in an Essential Topics in Translational Sleep Medicine videoconference seminar organized by the University of Arizona College of Medicine in September 2021; and on the impact of sleep deprivation on cognitive flexibility and emotional control at the World Sleep 2022 congress in Rome, Italy, in March 2022.

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

For the animal studies (Aim 2): During Year 5 of the project (under no-cost extension), we will complete the rescue studies in order to finish the project. In addition to a manuscript currently under review, we will submit at least one additional manuscript detailing our work.

For the human study (Aim 3): During Year 5 of the project (under no-cost extension), we will complete the human subjects study (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 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?

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

- 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 omitted the polysomnographic recording of sleep, as the hands-on procedure involved in electrode application increases the risk of transmission of COVID-19. We replaced polysomnography with an additional obstructive sleep apnea risk questionnaire during screening and additional wrist actigraphy during the laboratory study, which suffices as a sleep measurement technology for addressing Aim 3.

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 remained closed until the fall of 2021. Under no-cost extension we have now resumed all research activities and intensified our human research efforts to make up for lost time. A second 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 Years 3 and 4. The remaining funds are needed to complete the study during the second 1-year no-cost extension that was granted to finish the project. We do not expect to require any additional 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

Hayter EA, Wehrens SMT, Van Dongen HPA, Stangherlin A, Gaddameedhi S, Crooks E, Barron NJ, Venetucci LA, O'Neill JS, Brown TM, Skene DJ, Trafford AW, Bechtold DA (2021). Distinct circadian mechanisms govern cardiac rhythms and susceptibility to arrhythmia. *Nature Communications* 12: 2472.

Kyle JE, Bramer LM, Claborne D, Stratton KG, Bloodsworth KJ, Teeguarden JG, Gaddameedhi S, Metz TO, Van Dongen HPA (2022). Simulated night-shift schedule disrupts the plasma lipidome and reveals early markers of cardiovascular disease risk. *Nature and Science of Sleep* 14: 981–994.

Lawrence-Sidebottom D, Schmidt MA, Harvey DO, Van Dongen HPA, Davis CJ (2021). Floor vibrations for motivation and feedback in the rat vibration actuating search task. *PLoS ONE* 16: e0257980.

Books or other non-periodical, one-time publications

Nothing to report.

Other publications, conference papers, and presentations

Van Dongen HPA, Balkin TJ, Hursh SR, Dorrian J (2022). Performance deficits during sleep loss and their operational consequences. In Kryger M, Roth T, Goldstein CA, Dement WC

(Eds.), Principles and Practice of Sleep Medicine (7th ed.). Elsevier, Philadelphia: 742–749 (book chapter).

Van Dongen HPA (September 2021). Sleep, sleep deprivation, and human performance. Essential Topics in Translational Sleep Medicine seminar, University of Arizona College of Medicine; via videoconference (invited lecture).

Van Dongen HPA (March 2022). Impact of sleep deprivation on cognitive flexibility and emotional control. World Sleep 2022; Rome, Italy (oral presentation).

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>3</i>
Contribution to Project:	<i>Dr. Davis directed operations and workforce for the rat behavioral research, prepared reports, 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 directed the optogenetic experimentation and ran vigilance 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 Research 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>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 assisted Ms. Hudson with 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:	<i>1</i>
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:	<i>1</i>
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>M.S. Student</i>
Researcher Identifier:	<i>Washington State University ID: 11441657</i>
Nearest person month worked:	<i>1</i>
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>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:	3
Contribution to Project:	<i>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.</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:	3
Contribution to Project:	<i>Mr. Harvey implemented the behavioral paradigm for the rat experiments of Aim 2. He also maintained equipment and wrote code for data analysis.</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>James Baldwin</i>
Project Role:	<i>Research Assistant</i>
Researcher Identifier:	<i>Washington State University ID: 11792776</i>
Nearest person month worked:	1
Contribution to Project:	<i>Mr. Baldwin 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:	<i>Blake Budke</i>
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>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>Kaya Duncan</i>
Project Role:	<i>Research Assistant</i>
Researcher Identifier:	<i>Washington State University ID: 11789557</i>
Nearest person month worked:	1
Contribution to Project:	<i>Ms. Duncan 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>Chris Jacobs</i>
Project Role:	<i>Research Assistant</i>
Researcher Identifier:	<i>Washington State University ID: 11744009</i>
Nearest person month worked:	1
Contribution to Project:	<i>Mr. Jacobs 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>Bea Magsino</i>
Project Role:	<i>Research Assistant</i>
Researcher Identifier:	<i>Washington State University ID: 11788333</i>
Nearest person month worked:	1
Contribution to Project:	<i>Ms. Magsino 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>Sophia Reynolds</i>
Project Role:	<i>Research Assistant</i>
Researcher Identifier:	<i>Washington State University ID: 11787682</i>
Nearest person month worked:	1
Contribution to Project:	<i>Ms. Reynolds 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>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>Hannah Sparber</i>
Project Role:	<i>Research Assistant</i>
Researcher Identifier:	<i>Washington State University ID: 11619309</i>
Nearest person month worked:	1
Contribution to Project:	<i>Ms. Sparber 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>Thao Maria Truong</i>
Project Role:	<i>Research Assistant</i>
Researcher Identifier:	<i>Washington State University ID: 11793198</i>
Nearest person month worked:	1
Contribution to Project:	<i>Ms. Truong 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>James Wolff</i>
Project Role:	<i>Research Assistant</i>
Researcher Identifier:	<i>Washington State University ID: 11789561</i>
Nearest person month worked:	1
Contribution to Project:	<i>Mr. Wolff 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>Taylor Wright</i>
Project Role:	<i>Research Assistant</i>
Researcher Identifier:	<i>Washington State University ID: 11789049</i>
Nearest person month worked:	1
Contribution to Project:	<i>Ms. Wright 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:

Andy Hill Cancer Research Endowment Fund, “Night Shift Work as a Carcinogen: Molecular Mechanisms, Biomarkers, and Therapeutic Targets” (PI: Satterfield BC), September 2021–August 2023. This is a study of the molecular mechanisms underlying elevated cancer risk in active-duty night shift workers.

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

Health Sciences and Services Authority of Spokane County, “Night Shift Work as a Carcinogen: Molecular Mechanisms, Biomarkers, and Therapeutic Targets” (PI: Satterfield BC), September 2021–September 2023. This is fund matching for the Andy Hill Cancer Research Endowment Fund grant described above.

Heel (industry grant), “Neurexan Mitigates Trauma-Induced Sleep Disturbances and Improves Fear-Associated Memory Impairments in a Rodent Model of PTSD” (PI: Davis CJ), January 2021–December 2022. This grant is to determine the extent to which single or multiple doses of Neurexan can alleviate sleep disruption in a PTSD model and examine the effects of Neurexan on fear conditioning behavior.

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 is a Phase IV clinical trial of solriamfetol for cognitive performance improvement in treated sleep apnea patients with residual daytime sleepiness.

Medical Technology Enterprise Consortium, “Sustaining and Enhancing Post Mission Analysis of Side-Scan Sonar Data” (PI: Honn KA), October 2021–January 2026. This research involves a collaboration with the Naval Health Research Center to investigate the assessment of side-scan sonar data from unmanned underwater vehicles deployed to detect underwater mines.

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

National Safety Council, “Collecting Empirical Data to Provide a Quantitative, Objective Framework to Aid in the Interpretation of Alertness Impairments Related to Fatigue, Alcohol, Marijuana, and Opiates” (PI: Mollicone DJ), April 2022–March 2023. This project involves assessment of the impairments induced by psychoactive substances in interaction with sleep deprivation.

Naval Postgraduate School, “Circadian Entrainment and Performance Recovery” (PI: Honn KA), August 2021–August 2022. This grant involves investigation of tools to speed up circadian adjustment and overcome performance impairment following rapid reversal of work schedules.

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 is an instrumentation grant to help equip a new clinical sleep research laboratory within the Sleep and Performance Research Center’s human sleep research facility.

Trackthatssleep (industry grant), “Postpartum Maternal Sleep Project” (PI: Hansen DA), June 2022–November 2023. This contract involves monitoring of maternal sleep in the weeks after giving birth to assess the time course of sleep deficiency.

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 4 and 5.

Grants closed:

Nothing to report.

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.

7. 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

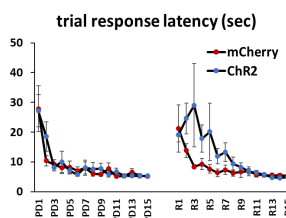
Award Amount: \$2,797,841

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.



Optogenetic activation of the striatal indirect pathway disrupts cognitive flexibility in rats like sleep loss does. Transgenic ChR2 rats had more response variability in response latency than mCherry control rats. There were no between-group differences in the pairwise discrimination training phase (left), indicating that the changes post reversal (right) were due to optogenetic activation of DrD2+ striatal circuitry.

Accomplishments: We obtained additional evidence in support of our overarching hypothesis regarding the role of striatal circuitry in cognitive flexibility impairment due to sleep loss (see figure). We are nearly done with the final animal study, a rescue experiment, to complete Aim 2. For the human studies, 43 subjects (48%) completed the laboratory experiment as of 2022 May 14 for Aim 3.

Timeline and Cost

Activities	CY	18	19	20	21	22	23
1. Development of rodent model		completed					
2. Optogenetic experiments							
3. Human genotype differences							
Estimated Budget (\$K)		\$445K	\$910K	\$955K	\$488K	no-cost	no-cost

Updated: 2022 Jun 13

Goals/Milestones

CY22 Goals – Finish rescue experiments of reduced cognitive flexibility due to sleep loss in transgenic rodents (Aim 2); continue human subject laboratory sleep deprivation experiments (Aim 3).

Comments/Challenges/Issues/Concerns

- A second 1-year no-cost extension was approved for the project to address COVID-19 pandemic-related project delays.
- The human subject recruitment rate is still below pre-pandemic levels due to labor shortages limiting availability of the target subject pool, but we are on track to make up for lost time and complete the project.

Budget Expenditure to Date

Projected Expenditure: \$2,798K.

Actual Expenditure: \$2,727K.

Other resources are available to complete the project if needed due to the pandemic-induced delays.

8. APPENDICES

Hayter EA, Wehrens SMT, Van Dongen HPA, Stangherlin A, Gaddameedhi S, Crooks E, Barron NJ, Venetucci LA, O'Neill JS, Brown TM, Skene DJ, Trafford AW, Bechtold DA (2021). Distinct circadian mechanisms govern cardiac rhythms and susceptibility to arrhythmia. *Nature Communications* 12: 2472.

Kyle JE, Bramer LM, Claborne D, Stratton KG, Bloodsworth KJ, Teeguarden JG, Gaddameedhi S, Metz TO, Van Dongen HPA (2022). Simulated night-shift schedule disrupts the plasma lipidome and reveals early markers of cardiovascular disease risk. *Nature and Science of Sleep* 14: 981–994.

Lawrence-Sidebottom D, Schmidt MA, Harvey DO, Van Dongen HPA, Davis CJ (2021). Floor vibrations for motivation and feedback in the rat vibration actuating search task. *PLoS ONE* 16: e0257980.