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Circadian disruption affects initial learning but not cognitive flexibility in an automated set-shifting task in adult Long-Evans rats

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ABSTRACT

A sizeable percent of adults are subject to circadian disturbances such as shift work, which involves misalignment of time of light exposure, activity periods, sleep, and eating. Chronic adherence to disruptive circadian schedules can negatively impact cognitive functioning. Developing preclinical models of circadian disruption allow investigation of the relationship and underlying mechanisms between circadian disruption and cognitive functioning. We placed adult Long-Evans rats of both sexes on a 12:12 h light:dark schedule in which rats performed an automated operant-behavior task for 3 months, with daily testing occurring either 4 h after lights-on or lights-off. At the end of this period, rats were tested on an automated set-shifting task to compare the effects of the 2 testing schedules on cognitive flexibility, which is the focus of this report. Over the initial 3-month period, day-tested rats shifted to a diurnal activity schedule, with males shifting more effectively than females, while night-tested rats remained nocturnal. Upon beginning the set-shifting task, night-tested rats took longer to reach criterion performance in the initial, visual-cue detection stage as compared to day-tested rats. The groups did not differ in performance on subsequent egocentric-cue based and reversal phases. Sex-related differences in task performance unrelated to testing schedule, particularly longer latencies to lever press in females, were also detected. One possible explanation for our findings is that the night-tested rats also experienced a form of circadian disruption when they were exposed to ambient light during the daily testing sessions, and that the form they experienced was more detrimental to initial acquisition of the task than testing during the light phase. Subsequent experiments will incorporate a night-tested group that is not exposed to ambient light in order to better understand the effect seen in the night-tested rats in the current study.

1. Introduction

Circadian rhythms are intrinsically-generated oscillations in behavior and other aspects of physiology that allow organisms to align their daily functioning with the external environment [1]. As such, changes in temporal organization that differ from a species' normal, established daily rhythmicity can negatively impact cognitive functioning, as well as numerous other aspects of normal physiologic functioning [2]. Alterations of this nature are termed circadian disruption, conveying the detrimental nature of changes to normal circadian rhythmicity.

A common form of circadian disruption encountered in human populations is shift work, which is a term that encompasses a diverse set of work-time schedules, particularly those that fall outside of normal daytime hours [3,4]. Estimates of the percent of adult workers who chronically follow alternate, shift-work type schedules range from 20% up to 30% [4,5]. Of particular concern are those who experience work-related circadian disruption and yet must perform at peak cognitive ability to avoid potentially life-threatening mistakes; up to 50% of workers in protective services and 40% of healthcare workers experience shift work and other forms of circadian disruption [4]. While there is not a concordance between studies, problems with attention and executive functioning, which the prefrontal cortex is involved in mediating, are often reported in subjects experiencing longer-term circadian disruption [6,7]. A particularly important aspect of executive functioning, cognitive flexibility, is critical for workers in healthcare, protective services, and other occupations in order to optimally respond to changing situational demands [8]. Adult populations experiencing different forms of chronic circadian disruption exhibit impaired cognitive flexibility as assessed by a variety of methods [6,9–11]. Thus, it is important to employ preclinical models to investigate the relationship between circadian disruption and cognitive functioning.

Behavioral models of cognitive flexibility typically involve both

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attentional set shifts that are either intradimensional (the relevant, new aspect of the stimulus which the subject must attend to is within the same stimulus dimension) or extradimensional (within a different stimulus dimension), and reversals (a previously salient aspect of a stimulus that had to be disregarded becomes relevant again). Of the different models available to assess cognitive flexibility in rodents [12–14], we utilize a set-shifting task version performed in automated operant-behavior chambers [13]. Rats are initially required to learn a visual-cue based strategy to optimize performance, and then are required to make an extradimensional switch to an egocentric, spatial strategy, followed by a subsequent intradimensional spatial reversal. An advantage of this model is an emphasis on response conflict, in which the same visual and spatial cues are present throughout but change in relevancy [13].

Shift work can be challenging to model because it involves misalignment of times of light exposure, activity, sleep, and feeding [15]. In this study, we model shift work and examine its effects on cognitive flexibility by testing and feeding rats during the light phase of the light:dark cycle, and comparing the findings to rats tested during the dark phase. Additionally, rats were subject to the experimental manipulations for 3 months, which better models the chronic nature of shift work. Because the defining feature of shift work is to alter endogenous circadian cyclicity to meet the scheduling demands of a job, testing and feeding rats during the light phase mimics alterations in circadian misalignment seen with shift work [16]. We hypothesized that rats tested during the light phase would take longer to make both the extradimensional transition and the subsequent intradimensional reversal.

2. Materials and methods

2.1. Subjects

Forty-eight Long-Evans rats of both sexes were purchased in 3 separate cohorts of 16 rats each (8 male and 8 female) from Harlan Laboratories (Indianapolis, IN) at approximately 70 days of age. Rats were single-housed in polycarbonate cages with wood-chip bedding (Beta Chip, Northeastern Products Corp., Warrensburg, NY) in a temperature- and humidity-controlled room (22 °C, 40–55% humidity) on a 12 h light:12 h dark (12:12 LD) cycle. After a 1-week acclimation period, the rats were transferred to cages housed inside light-tight, temperature- and humidity-controlled chambers, with up to 4 cages per chamber, which allowed for manipulation of LD cycles for smaller groups. White light inside the chambers (average intensity 290 lx) was controlled by digital timers. Each home cage was equipped with a stainless steel running wheel to monitor activity. Rats had access to running wheels at all times while in their home cages. Running wheel revolutions were registered by magnetic detectors attached to the running wheels.

Rats were fed a primary diet of 2020X Teklad Rodent Diet (Harlan). TestDiet sucrose pellets (AIN-76A, 45 mg each, St. Louis, MO) were used for food-based reinforcement of operant-behavior testing. Once rats entered the light-tight chambers, food restriction was initiated to maintain motivation for performing the behavioral task. Body weights were reduced to targeted weight of 85% of the free-fed weights. Then, target weights were incrementally increased by 5–10 g every 2 weeks, with a maximum of 250 ± 10 g for females and 350 ± 10 g for males, to allow for growth. Daily feeding occurred 30 min after behavior testing was completed and rats were returned to their home cages. Tap water was provided ad libitum.

Rats were equally divided into 2 experimental groups based on light phase at time of behavioral testing in their LD cycle: day-tested rats were tested 4 h after lights-on (zeitgeber time or ZT4), and night-tested rats were tested 4 h after lights-off (ZT16). All rats were exposed to ambient light during transport to and from the testing room (average intensity 385 lx), and once inside the testing room (average intensity 271 lx). While in the operant-testing chambers, rats were exposed to light from the house light and cue lights (2.8 watt bulbs, average intensity 6 lx in the center of each chamber). Rats were brought to the testing room 10 min prior to the beginning of testing, tested for 30 min (Phases 1 to 3) or 60 min (Phases 4 to 6), and then remained in the testing room for 5 min after testing was complete while the next group of rats was put into the testing chambers. Thus, exposure to light during each daily session lasted approximately 45 min during Phases 1 to 3 and 75 min during Phases 4 to 6.

All procedures were approved by the Institutional Animal Care and Use Committee at University of Illinois Urbana-Champaign and were in accordance with the guidelines of the Public Health Service Policy on Humane Care and Use of Laboratory Animals [17].

2.2. Testing apparatus and set-shifting task

Two weeks after transfer to the light-tight chambers, behavioral testing commenced. Rats were first tested on an attention task (results not yet published) for 11 weeks, before beginning the set-shifting task. Training and testing was performed 6 days per week in 10 operant behavior-conditioning chambers, which were housed in sound-insulated and ventilated cubicles (Med Associates Inc., St. Albans, VT). In the middle of one wall was a pellet trough with a head-entry detector. A retractable lever was located on both sides of the trough. Cue lights were evenly spaced above each lever and the trough. A house light was mounted on the top center of the opposite wall. The set-shifting programs were modified from ones developed by Dr. Stan Floresco [18,19], and were programmed using Medstate Notation programming language (Med Associates).

There were 9 male day-tested, 10 female day-tested, 11 male night-tested, 11 female night-tested (41 total) which completed the set-shifting task in each group; all of these rats were included in the data analysis. Three of the original 48 rats were removed from the study during the previous attention task due to failure to meet minimal criteria for learning the task. Four other rats were not included in the analysis because their data sets for set-shifting were incomplete. Set-shifting was completed in 10 days (35 rats), 11 days (3 rats), 12 days (2 rats), or 13 days (1 rat), respectively, depending on the rate of progress

Table 1
Timeline for experiment, including a brief description of each phase and the time it took the average rat to complete.

Phase	Time to complete	Phase description
Acclimation	1 week	Acclimate to facility after arrival.
Enter light-tight chambers	2 weeks	Enter home cages in light-tight chambers and begin food restriction.
Attention task	11 weeks	Rats tested on attention task (not reported in this manuscript).
Phase 1	2 days	Train to press single levers.
Phase 2	5 days	Train to press both levers.
Phase 3	Last day of phase 2	Determine side preference.
Phase 4	1 day	Visual Cue discrimination. Forming initial attentional set.
Phase 5	1 day	Response phase. Shift set to new egocentric cue dimension. Assesses cognitive flexibility.
Phase 6	1 day	Reversal phase. Shift set within egocentric cue dimension to opposite lever. Assesses cognitive flexibility.