Early insights into behavioral effects of circadian disruption during adolescence in a neurodevelopmental mouse model based on brain dysconnectivity

Ahmed Abderaouf Bouteldja

Supervised by: Nicolas Cermakian and Lalit Srivastava

Integrated Program in Neuroscience

McGill University, Montréal

October 2024

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science

Table of contents

Abstract

Schizophrenia (SZ) is a severe neuropsychiatric disorder affecting about 1% of the global population and is a leading cause of disability worldwide. Despite extensive research, its origins remain elusive due to its complex etiology and symptomatology. SZ is thought to arise from the interaction of genetic and environmental factors that disrupt early neurodevelopment, leading to altered brain connectivity that, in patients, appears before psychosis onset and correlates with symptom severity. Disrupted sleep and circadian rhythms are also consistently reported in SZ, though their causes and impact on symptoms are not fully understood.

To address these gaps, we utilized the neonatal ventral hippocampal lesion (NVHL) model, which mirrors SZ-related behavioral changes driven by brain dysconnectivity in key brain regions. Our study had two primary aims: first, to explore how brain dysconnectivity interacts with circadian disruption during adolescence to affect behavior, and second, to investigate the impact of brain dysconnectivity on circadian function.

To address the first aim, we generated NVHL male and female mice, exposing them to either chronic jet lag (CJL) or a standard 12h:12h light-dark (LD) protocol for four weeks during adolescence. Afterwards, all mice were returned to 12:12LD to ensure they are tested in the same circadian phase. Behavioral tests, including novel object recognition, elevated plus maze, open field, and three-chamber sociability and social novelty test, revealed that NVHL led to locomotor hyperactivity in both sexes and to reduced social novelty in females. Additionally, CJL resulted in decreased anxiety-like behavior and reduced sociability in females; however, contrary to our hypothesis, we did not find any significant interaction between NVHL and CJL.

For the second aim, mice were individually housed in running wheel cages to assess circadian rhythms under various light conditions: 12:12LD, constant darkness (DD), and constant light (LL). While NVHL mice exhibited normal rhythms under 12:12LD, significant changes were observed under constant light conditions. Under DD, both NVHL male and female mice exhibited increased fragmentation and decreased activity levels compared to the control mice. Furthermore, exposure to LL led to a large reduction in activity in NVHL mice, especially males, as well as increased fragmentation and greater day-to-day variability.

These findings highlight a complex relationship between brain dysconnectivity and circadian disruption during adolescence and suggest that the NVHL-induced brain dysconnectivity may lead to circadian dysregulation. In addition, sex-specific differences were present as females had more significant behavioral alterations compared to males. This study offers valuable insights into circadian rhythms in SZ within the context of brain dysconnectivity, laying the groundwork for future research to explore the underlying mechanisms.

Résumé

La schizophrénie (SZ) est un trouble neuropsychiatrique grave affectant environ 1 % de la population mondiale et constitue l'une des principales causes d'invalidité à l'échelle mondiale. Malgré des recherches approfondies, ses origines demeurent insaisissables en raison de son étiologie et de sa symptomatologie complexes. On pense que la SZ résulte de l'interaction entre des facteurs génétiques et environnementaux qui perturbent le neurodéveloppement précoce, entraînant une connectivité cérébrale altérée qui, chez les patients, apparaît avant l'apparition de la psychose et est corrélée à la gravité des symptômes. Des troubles du sommeil et des rythmes circadiens sont également systématiquement rapportés dans la SZ, bien que leurs causes et leur impact sur les symptômes ne soient pas encore entièrement compris.

Pour combler ces lacunes, nous avons utilisé le modèle de la lésion néonatale de l'hippocampe ventral (NVHL), qui reflète les changements comportementaux liés à la SZ, induits par une dysconnectivité cérébrale dans des régions cérébrales clés. Notre étude avait deux objectifs principaux : premièrement, explorer comment la dysconnectivité cérébrale interagit avec la perturbation circadienne pendant l'adolescence pour affecter le comportement, et deuxièmement, examiner l'impact de la dysconnectivité cérébrale sur la fonction circadienne.

Pour répondre au premier objectif, nous avons généré des souris NVHL mâles et femelles, les exposant soit à un décalage horaire chronique (CJL), soit à un protocole standard de lumière-obscurité (LD) 12h:12h pendant quatre semaines au cours de l'adolescence. Par la suite, toutes les souris ont été replacées en 12:12LD pour garantir qu'elles soient testées dans la même phase circadienne. Les tests comportementaux, y compris la reconnaissance d'objets nouveaux, le labyrinthe en croix surélevé, le champ ouvert et les tests de sociabilité et de nouveauté sociale à trois chambres, ont révélé que la NVHL a conduit à une hyperactivité locomotrice chez les deux

sexes et à une réduction de la nouveauté sociale chez les femelles. De plus, le CJL a entraîné une diminution des comportements anxieux et une réduction de la sociabilité chez les femelles ; cependant, contrairement à notre hypothèse, nous n'avons trouvé aucune interaction significative entre NVHL et CJL.

Pour le deuxième objectif, les souris ont été logées individuellement dans des cages équipées de roues d'exercice pour évaluer les rythmes circadiens dans différentes conditions lumineuses : 12:12LD, obscurité constante (DD) et lumière constante (LL). Alors que les souris NVHL présentaient des rythmes normaux sous 12:12LD, des changements significatifs ont été observés sous LL. En DD, les souris NVHL mâles et femelles présentaient une fragmentation accrue et des niveaux d'activité réduits par rapport aux souris témoins. De plus, l'exposition à LL a entraîné une forte réduction de l'activité chez les souris NVHL, en particulier chez les mâles, ainsi qu'une fragmentation accrue et une plus grande variabilité d'un jour à l'autre.

Ces résultats soulignent une relation complexe entre la dysconnectivité cérébrale et la perturbation circadienne au cours de l'adolescence et suggèrent que la dysconnectivité induite par la NVHL pourrait entraîner une dérégulation circadienne. En outre, des différences spécifiques liées au sexe ont été observées, les femelles présentant des altérations comportementales plus marquées par rapport aux mâles. Cette étude apporte des informations précieuses sur les rythmes circadiens dans la SZ dans le contexte de la dysconnectivité cérébrale, jetant les bases de futures recherches pour explorer les mécanismes sous-jacents.

Acknowledgements

I would like to thank the current and past lab members for their helpful discussions, for their assistance in my project, and most importantly, for making the lab a fun environment to work in. Namely, I would like to thank past lab members Tara Delorme, Shashank Srikanta, Namasthée Harris-Gauthier, Marie-Ève Cloutier, Priscilla Carvalho Cabral, and Sophia Stegeman for all their help in setting me up in the lab and teaching me the necessary techniques required to start my project. I would like to thank Danae Penichet, Maryam Malki, Sebastián Boy Waxman, Genavieve Maloney, Albert Pelinovsky, Juliana Meyerfreund, Jae-Eun Shin, Shoko Eguchi, Shreya Nandakumar, and Micah Provost for being great lab members who were always willing to help and collaborate. A special shoutout to Sebastián who greatly assisted me by a lending a hand (literally) in behavioral tests, even after sleeping in the lab due to an overnight experiment; to Danae, for being my partner in crime in many ways as we collaborated in various aspects of the lab; and to Genavieve, for keeping everything running smoothly as our research assistant (and for lending me a life-saving surgery kit). I would like to thank Sanjeev Bhardwaj for teaching me the neonatal stereotaxic surgeries and slicing with the cryostat. I would like to thank the animal facility staff, including Guylaine Gadoury, Tara Thomson, and Jessica Balbin, for all their help related to animal work/care. Last, but certainly not least, I would like to thank the past undergraduate students I mentored and worked with, including Lianne Marceau, who helped me a lot with behavioral testing, analyses, brain slicing, and other important tasks; Victoria Lu, who assisted in behavioral analyses and brain slicing; and Christopher Roy.

I would like to thank my supervisors Nicolas Cermakian and Lalit Srivastava. They say that the key to a successful graduate studies experience lies in having a good supervisor. Luckily for me, I had two great supervisors that made my experience pleasant, even at the most difficult times. I

would like to express my gratitude to Nico for providing continuous mentorship, support, and understanding during my time in his lab. Even when it seemed that everything was not going right, Nico was always supportive and proactive about the situation. In addition, his approach to mentoring his students, including in aspects related to presentations, writing, and networking, has significantly improved me as a researcher. I would like to extend that gratitude to Lalit, who always had an open door for me and provided valuable insight and mentorship. I would like to thank them both for reviewing and editing work related to my master's thesis project and fellowships/awards. I would like to also thank my advisory committee members Adrien Peyrache and Bruno Giros, as well as my mentor Cecilia Flores, for their helpful comments and advice during my committee meeting and seminar.

None of this work would have been possible without my wonderful and supportive family. I dedicate this work to them. To my mother, without whom I would not be where I am now, thank you for your relentless support, for always believing in me, and for providing me with everything I need to ensure my success. To my sister, for being my best friend who was always there for me at my lowest and highest times, I am grateful for you. And to my dad, whose advice, constant support, and perspective propelled me towards the path of success.

Contribution of authors

Ahmed Abderaouf Bouteldja participated in the experimental design and conducted all behavioral testing, with assistance from Sebastián Boy Waxman (graduate student) and Lianne Marceau (undergraduate student). He analyzed the behavioral data, supported by Lianne Marceau and Victoria Lu (undergraduate student), utilizing both automated software (elevated plus maze) and manual coding (novel object recognition, three-chamber test). In addition, he performed the neonatal mouse stereotaxic surgeries, as well as all related pre- and post-surgical work, including setting up breeders, litters, weaning, and husbandry. He carried out the brain collection procedure himself. He completed brain slicing and staining with the help of Lianne Marceau and Victoria Lu, and independently handled brain slice imaging. Ahmed also set up the wheel-running experiments and performed the associated analyses. Lastly, he conducted all statistical analyses himself.

Nicolas Cermakian contributed to the project by participating in the experimental design, providing guidance in data analysis, and offering expert interpretation of results related to circadian rhythms. His lab also supplied the essential equipment needed to conduct the experiments. Additionally, he reviewed and edited the master's thesis and prior work.

Lalit Srivastava was involved in the experimental design and result interpretation, drawing on his expertise in neurodevelopmental disorders and behavioral testing. His lab provided the tools required for the neonatal stereotaxic surgeries. He also reviewed and edited the master's thesis and earlier related work.

List of figures

List of abbreviations

AMYG	Amygdala
ANOVA	Analysis of variance
ARMS	At risk mental state
BMAL1	Brain and muscle ARNT-like1
CJL	Chronic jet lag
CLOCK	Circadian locomotor output cycles kaput
CRY1/2	Cryptochrome
DD	Constant darkness
DR	Discrimination ratio
DTNBP1	Dystrobrevin binding protein 1
EEG	Electroencephalogram
EPM	Elevated plus maze
HC-PF	Hippocampal-prefrontal
HPC	Hippocampus
IB	Ibotenic acid
IpRGCs	Intrinsically photosensitive retinal ganglion cells
IS	Interdaily stability
IV	Intradaily variability
LD	Light-dark
LL	Constant light
MIA	Maternal immune activation
MPFC	Medial prefrontal cortex
NAc	Nucleus accumbens
NDD	Neurodevelopmental disorder
NOR	Novel object recognition
NVHL	Neonatal ventral hippocampal lesion
OFT	Open field test
PBS	Phosphate-buffered saline
PER1/2/3	Promotors of period

PFC	Prefrontal cortex
PHb	Perihabenular nucleus
PND	Postnatal day
PPI	Prepulse inhibition
RA	Relative amplitude
ROUT	Robust regression and outlier removal
SCN	Suprachiasmatic nucleus
SOP	Standard operating procedure
SZ	Schizophrenia
UHR	Ultra-high risk
VHPC	Ventral hippocampus
VmPFC	Ventromedial prefrontal cortex
VTA	Ventral tegmental area
ZT	Zeitgeber time

Introduction:

Schizophrenia (SZ) is a debilitating and chronic psychiatric disorder that affects approximately 1% of the global population and is among the top causes of disability worldwide. The symptoms of SZ are classified into three main categories: positive symptoms (e.g., hallucinations and delusions), negative symptoms (e.g., social withdrawal and avolition), and cognitive symptoms (e.g., executive function and working memory deficits). Unfortunately, current treatments, which include typical and atypical antipsychotics, primarily address positive and negative symptoms, often with limited success, while cognitive and some negative symptoms remain largely untreated. Moreover, these medications are frequently associated with significant neurological and metabolic side effects, some of which can be severe, such as clozapine-induced agranulocytosis (Mijovic & MacCabe, 2020; Stepnicki et al., 2018). Consequently, SZ imposes a substantial burden not only on patients but also on their families and society at large. In fact, in 2004, the financial burden of healthcare and non-healthcare costs related to SZ in Canada was estimated at approximately 2.02 billion CAD (Goeree et al., 2005).

The difficulty in treating SZ can be attributed to its complex symptomatology, multifactorial origins, and high co-morbidity. SZ is believed to stem from abnormal neurodevelopment, which is triggered by a combination of genetic and environmental factors that disrupt brain development presumably starting from the prenatal period. This disrupted development leads to alterations in brain morphology and function, ultimately resulting in the wide range of behavioral deficits observed in patients. This theory is supported by neuroimaging studies that have documented progressive structural and functional changes in the brains of patients, which occur before the onset of psychosis (a hallmark symptom of SZ) and are correlated with behavioral deficits (Cannon et al., 2015; Ho et al., 2019).

Among the diverse symptoms of SZ, sleep disturbances are among the most consistently observed yet often overlooked. In fact, Cohrs et al. (2008) reported that up to 80% of SZ patients experience such disturbances. These sleep issues are likely partly driven by circadian dysregulation, as both preclinical and clinical studies suggest that disrupted circadian rhythms may be a fundamental feature of the disorder (Bouteldja et al., 2024). Given the critical role of proper sleep and circadian function—and the adverse consequences associated with their disruption under normal circumstances—these disturbed patterns may contribute additional risk to the disorder, exacerbating its overall severity.

Comprehensive review of relevant literature:

Circadian rhythms and health

Almost every natural thing follows a cycle. This includes the Earth's rotation around its own axis, resulting in a 24-hour cycle. Thus, it is of the organism's own interest to adapt to this cycle to maximize its evolutionary fitness, whereby it can anticipate daily environmental changes. This adaptation translated to endogenous and self-sustaining near 24-hour biological rhythms, known as circadian rhythms. In mammals, circadian rhythms are controlled by the hypothalamic suprachiasmatic nucleus (SCN), which is composed of a network of neurons that, when coupled, form a unified network (Ko & Takahashi, 2006). At the molecular level, circadian rhythms are generated through transcriptional-translational feedback loops involving the core clock machinery brain and muscle ARNT-like1 (BMAL1) and circadian locomotor output cycles kaput (CLOCK) proteins, which heterodimerize and interact with the enhancer box response elements in the promotors of period (*PER1/2/3*) and cryptochrome (*CRY1/2*) genes. The PER and CRY proteins then heterodimerize, translocate to the nucleus and repress the transcriptional activity of the BMAL1:CLOCK heterodimer. This near 24-h process is completed after PER and CRY proteins are degraded, thereby relieving BMAL1:CLOCK to restart the cycle (**Figure 1)**. Additionally, other molecular loops, as well as transcriptional, post-transcriptional, and post-translational regulators, are involved (for a review, see Cox and Takahashi, 2019). Moreover, through humoral and neural signals, the master clock in the SCN synchronizes peripheral clocks in other regions of the brain and body (Mohawk et al., 2012) and directly regulates various physiological and behavioral processes, such as body temperature, hormone secretion, and feeding (Marcheva et al., 2013)(**Figure 1)**. In addition to these functions, the central clock

regulates sleep by determining its timing, while a homeostatic component, consisting of a buildup in sleep pressure, dictates the need for sleep.

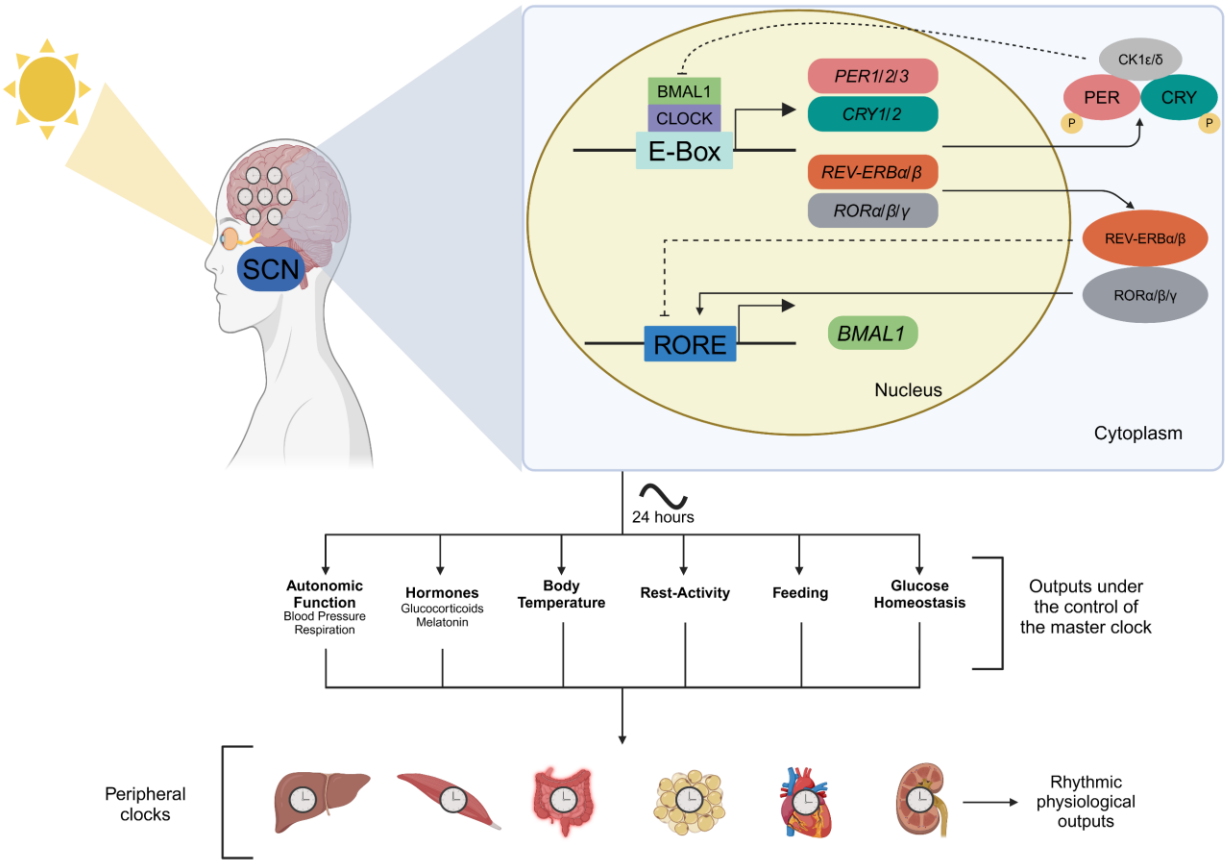


Figure 1. Circadian clocks and the functions they control. This figure was reproduced from Bouteldja et al. (2024) with approval from publisher. Created with BioRender.com.

While self-sustaining, an essential feature of circadian clocks is their capability to be reset by external environmental cues, including light, temperature, and food intake. Light, the most potent cue, acts on the SCN via the retinohypothalamic tract; consequently, it allows the organism to adapt to the external light-dark (LD) cycle (i.e., in alignment to solar day) (Duffy & Wright, 2005). The light signal transduced to the SCN is first received by a melanopsin-

expressing photoreceptive system consisting of intrinsically photosensitive retinal ganglion cells (ipRGCs) (Hattar et al., 2002). Light is not only essential for photoentrainment but also indirectly influences behavior through the SCN by modulating downstream pathways involved in sleep regulation, motivation, cognition, and mood (LeGates et al., 2014). Besides this indirect pathway, ipRGCs directly project to brain regions involved in circadian rhythm regulation, such as the subparaventricular zone, in mood regulation, such as the amygdala (AMYG), and in sleep regulation, such as the ventrolateral preoptic area (LeGates et al., 2014). Thus, light can not only have widespread effects on behavior through these two pathways, but it can also alter mood and cognition independent of changes in sleep architecture or clockwork machinery. Indeed, a previous study be LeGates and colleagues (2012) showed that aberrant light schedules led to impairments in mood and cognition without causing circadian arrhythmicity or sleep deprivation. A follow-up study by the same group revealed this effect on mood can be mediated through an SCN-independent pathway from a subset of ipRGCs to the perihabenular nucleus (PHb) of the dorsal thalamus, which projects to several mood-regulation areas (Fernandez et al., 2018). Furthermore, the ventromedial prefrontal cortex (vmPFC), which plays a central role in decision-making, emotional regulation, and cognition, receives input from the PHb. Intriguingly, when the input from the ipRGCs to the PHb is ablated, morphological, physiological, and molecular deficits follow in the vmPFC, without any changes in circadian function or mood (Lazzerini Ospri et al., 2024). This is an important finding as the vmPFC and its functions are particularly implicated in psychiatric disorders like SZ (Fan et al., 2013).

While the ability to entrain to light gives the organism the advantage of adapting to the external LD cycle, in today's age—where people are susceptible to artificial light at night, abnormal work schedules, and transmeridian travel—this feature can render people at a greater

risk of negative health consequences. The desynchrony between the internal clock and the external world not only impacts sleep—which itself can affect health—but also disrupts many processes under the clock's control, including neuroendocrine, immune, and metabolic functions, thereby increasing the risk of negative health consequences. Indeed, disruption of circadian rhythms has been linked to increased risks of cardiovascular disease, obesity, and psychiatric disorders (Brainard et al., 2015, Walker et al., 2020). This includes neurodevelopmental disorders (NDDs), which are characterized by behavioral deficits arising due to early abnormal neurodevelopment, leading to impairments in daily functioning. Patients with NDDs, such as autism spectrum disorder, attention-deficit/hyperactivity disorder, and SZ commonly display deficits in circadian system functioning (Bouteldja et al., 2024).

Circadian disruption has more severe consequences when it occurs during critical developmental stages, particularly in childhood and adolescence, as it interferes with neurodevelopment and leads to behavioral alterations that persist into adulthood (Westwood et al., 2023; Pifer et al., 2024). Adolescence, in particular, is characterized by rapid maturation and refinement of several brain regions (Konrad et al., 2013), including the SCN (Hagenauer & Lee, 2012), which makes the brain more susceptible to environmental disturbances. Mice exposed to chronic jet lag (CJL) during the neonatal period show impairments in memory, affect, and neuronal morphology in areas such as the hippocampus (HPC) when assessed in adulthood (Ameen et al., 2022). Similarly, exposure to CJL during adolescence leads to behavioral changes in both male and female adult mice (Cloutier et al., 2022). Beyond heightened vulnerability, adolescence is a distinct period—especially in modern times—when adolescents often experience misalignment between their internal circadian clock and social rhythms due to school schedules, nighttime electronics use, and an increased tendency towards eveningness (Touitou et

al., 2016); thus, this puts them at a higher risk of facing the deleterious effects of circadian disruption.

Circadian rhythms in schizophrenia

The pre-clinical and clinical studies investigating circadian dysregulation in NDDs suggest that it may be an inherent characteristic of the disorders (**Figure 2)**. In SZ, a study by Wulff and colleagues (2012) showed that, overall, SZ patients demonstrated disruptions in their daily activity rhythms ranging from decreased activity levels to complete arrhythmicity. In addition, a subgroup of these patients showed an almost 4-h phase delay in their peak melatonin concentration—the hormone that helps regulate sleep-wake timing and whose timing of release is regulated by the SCN—while the other subgroup showed decreased overall melatonin concentrations; likewise, a meta-analysis looking at peak melatonin concentrations in SZ versus controls reported significantly lower concentrations in those with SZ (Bastos et al., 2019).

Findings from mutant models of SZ similarly show changes in circadian phase and rhythmicity, including decreases in amplitude (measure of rhythm robustness) and increases in fragmentation and activity onset variability (Bouteldja et al., 2024). For example, a study from our group exploring circadian rhythms in *Sandy* mice, which carry a loss of function of SZ risk gene *Dtnbp1*, showed that *Sandy* mice had greater activity onset variability under all lighting conditions compared to controls (Bhardwaj et al., 2015). In addition to the changes in daily activity rhythms and melatonin rhythms/concentration reported in SZ, studies also report associations of clock gene variations with SZ, which, like other NDDs, has a significant heritable component (Gidziela et al., 2023). For example, Johansson and colleagues (2016) found altered expression of *CRY1* and *PER2* genes in fibroblasts, as well as reduced expressions of *CLOCK*, *PER2*, and *CRY1* in the mononuclear blood cells of SZ patients.

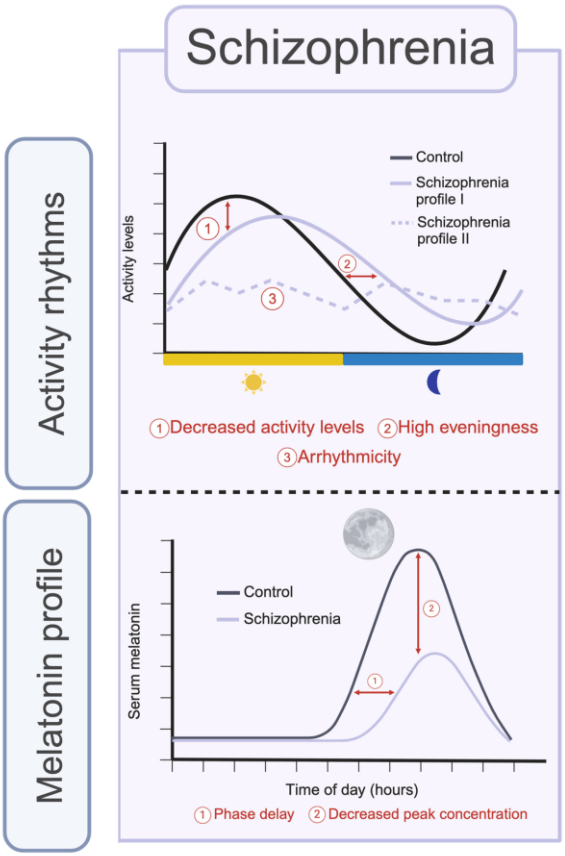


Figure 2. Summary of circadian dysregulation found in schizophrenia based on clinical studies. This figure was adapted from Bouteldja et al. (2024) with approval from publisher. Created with BioRender.com.

Disruptions in circadian functioning are not only believed to be an inherent characteristic of SZ but may also act as a risk factor for the disorder (**Figure 3**). Since the etiologies of NDDs are multifactorial and arise from multiple genetic and environmental risk factors, circadian disruption, particularly during key developmental periods, could contribute to the development of the disorder and worsening of symptoms. A previous study showed that SZ patients with lower relative amplitude (RA) demonstrated lower cognitive functioning compared to others; in addition, RA was found to be the strongest predictor of outcomes, irrespective of age,

highlighting the possible role that robust circadian rhythms play in affecting behavior severity (Bromundt et al., 2011). When assessing the predictive power of circadian rhythms on persecutory delusion—which is the most common type of delusion in SZ—a study found that RA and interdaily stability (IS; i.e., stability of rhythms from day to day) were significantly associated with persecutory symptoms, but not in the combined sample (Kammerer et al., 2021). These findings suggest that there is a heightened sensitivity to symptom worsening due to circadian dysfunction in healthy individuals but not in patients. Thus, circadian disruption may play a key role in the period preceding the emergence of psychosis.

Indeed, sleep disturbances have been reported in the prodromal phase of SZ, which refers to the period preceding the appearance of the characteristic symptoms of a disorder (e.g., psychosis). Several criteria were developed to identify individuals at risk of psychosis to better understand and ameliorate the course of the disorder, including individuals at ultra-high risk (UHR) state (Phillips et al., 2002), at risk mental state (ARMS) (Yung & McGorry, 1996), or clinical high risk for psychosis (Addington & Heinssen, 2012). Individuals identified as ARMS displayed lower amplitude, more rhythm fragmentation, and less synchrony to the LD cycle compared to controls (Castro et al., 2015). In another study, UHR subjects displayed more

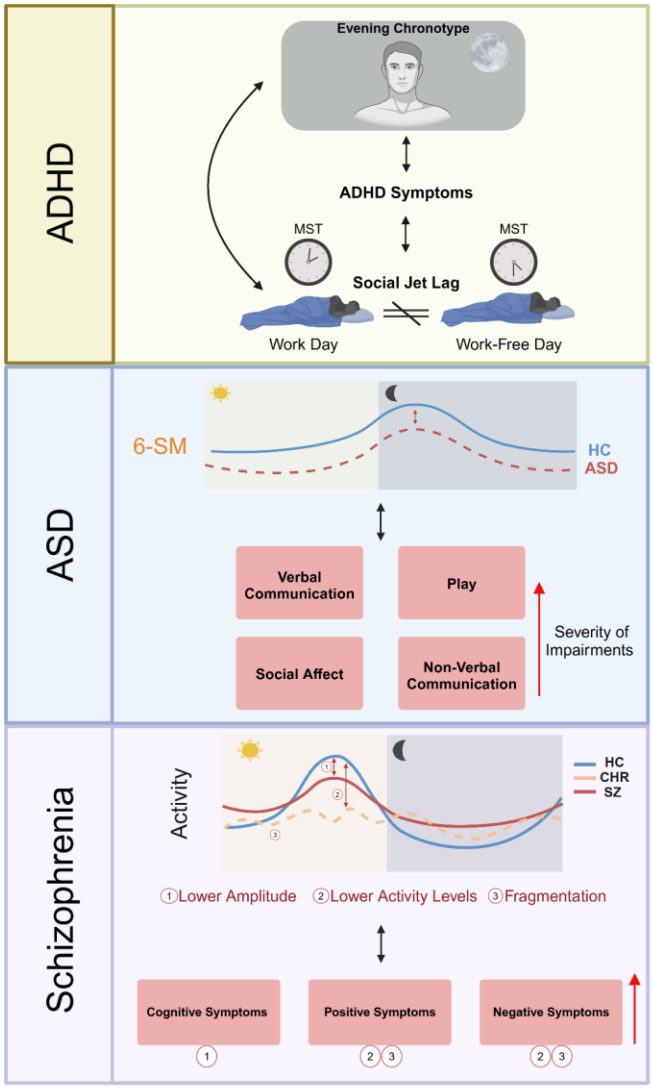


Figure 3. Summary of clinical findings exploring circadian disruption as a risk factor in schizophrenia. This figure was reproduced from Bouteldja et al. (2024) with approval from publisher. Created with BioRender.com.

rhythm fragmentation and later onset of nocturnal rest compared to controls (Lunsford-Avery et al., 2017)(**Figure 3**). Interestingly, changes in daily activity, RA, and intradaily variability (IV; i.e., measure of rhythm fragmentation) were predictive of greater positive and negative symptom severity at the 1-year follow up (Lunsford-Avery et al., 2017). In a study by another group, UHR

individuals who ended up converting to psychosis displayed disturbed circadian rhythms at baseline (Goncalves et al., 2016). Thus, these studies highlight the potential role that circadian disturbances may contribute to SZ symptom development and severity.

Animal studies exploring the effects of light disruption on SZ-relevant behaviors point towards the negative consequences of improper light exposure, particularly during key developmental periods, on the phenotypes analogous to the disorder. When subjected to constant light (LL), which was shown to desynchronize the SCN network (Ohta et al., 2005), *Sandy* mice displayed locomotor hyperactivity and prepulse inhibition (PPI) deficits (Bhardwaj et al., 2015)—a measure of sensorimotor gating whose dysfunction is a common feature of SZ (Kumari et al., 2000). Similarly, exposure to a CJL protocol (6 h advance of the LD cycle) during adolescence resulted in sociability deficits and sex-specific changes in anxiety-like behavior and PPI in *Sandy* mice (Cloutier et al., 2022). In another model of SZ based on maternal immune activation (MIA), which is associated with increased SZ risk in humans (Choudhury & Lennox, 2021), LL led to reduced sociability in MIA males and in changes in the morphological index of the microglia in the dentate gyrus (Delorme et al., 2023). These findings highlight the effects of abnormal light exposure on behavior and brain structure.

Brain dysconnectivity in schizophrenia

As mentioned, SZ is a multifactorial disorder, with genetic and environmental risk factors interacting with each other starting from early development, resulting in the emergence of characteristic symptoms (i.e., psychosis) in late adolescence/early adulthood. Sex differences are also apparent in the emergence, severity, and prevalence of symptoms. Men tend to have an earlier onset and slightly higher prevalence, while women experience more mood disturbances and men exhibit more negative symptoms (Li et al., 2016). Furthermore, it is believed that

normal neurodevelopment is disrupted at an early stage due to those risk factors, and that symptoms emerge as the affected regions undergo maturation during adolescence/early adulthood. Indeed, studies show that SZ patients demonstrate progressive changes in grey matter volume, at least until adolescence in cortical and hippocampal regions (Mancini et al., 2020; Nath et al., 2021), which are attributed to loss of cell complexity (e.g., dendritic branching), rather than cell loss (Coyle et al., 2016). Additionally, these changes are evident even in individuals identified as at risk for SZ (Ho et al., 2019), including CHR subjects, who show steeper rate of gray matter loss prior to converting to psychosis (Cannon et al., 2015). Correlative neuroimaging studies have also associated these progressive changes to worsened symptom severity (Ho et al., 2003; Mathalon et al., 2001).

In addition to changes in volume and thickness, SZ patients exhibit changes in structural (i.e., volume and anatomical connections between brain regions) and functional (i.e., patterns and strength of temporal associations of activation between distinct brain regions) connectivity changes in distinct brain regions compared to healthy controls. A systematic review by Pettersson-Yeo and colleagues (2011) reported reduced connectivity in patients compared to controls, with increased connectivity also observed, though less commonly. Strikingly, these changes were present not only in individuals with chronic SZ but also in those categorized as first-episode SZ, early-onset SZ, UHR, and those at high genetic risk. A recent meta-analysis, including 815 SZ patients and 790 healthy controls, similarly revealed both hypo- and hyper-connectivity changes between the groups (Cai et al., 2022). This evidence supports the notion that abnormal neurodevelopment underlies the etiology of SZ, which appears to be progressive, as seen in longitudinal studies (e.g., Ho et al., 2019), and with a heritable component (e.g., Rasetti et al., 2011).

Many brain areas have been linked to SZ, including regions in the prefrontal, temporal, and thalamic areas. However, the HPC, located in the medial temporal lobe, is one of the most consistently implicated regions in SZ. This region is primarily known for its role in memory, learning, and spatial navigation (Anand & Dhikav, 2012). Anatomically, the HPC is organized along a longitudinal axis which in primates extends from the anterior to the posterior lobe, corresponding to the ventral and dorsal poles, respectively, in rodents (Strange et al., 2014). While the HPC is renowned for its role in memory and learning, it also plays a crucial role in emotional and motivational behaviors, with the ventral hippocampus (vHPC) being primarily involved in these functions as reported in numerous rodent studies (Bannerman et al., 2004). Structurally, patients with SZ exhibit HPC volume reduction, with the anterior subfields particularly affected in the early stages (McHugo et al., 2024), as well as dysregulation of synaptic function, and they show altered GABAergic and glutamatergic transmission, increased metabolic activity, and dysconnectivity with other brain regions (Wegrzyn et al., 2022). It has been demonstrated that some of these changes, such as reductions in the HPC CA1 area and hypermetabolism, can precede and even predict the onset of psychosis (Ho et al., 2019; Schobel et al., 2013).

Among the disrupted pathways, the hippocampal-prefrontal (HC-PF) cortex pathway is particularly implicated in SZ. This circuit is crucial for many of the cognitive and emotional functions that are impaired in the disorder. Patients exhibit altered HC-PF connectivity both at resting state (Zhou et al., 2008) and when performing a working memory task (Meyer-Lindenberg et al., 2005). In fact, impaired HC-PF connectivity during working memory tasks is considered a potential endophenotype of SZ (Bahner & Meyer-Lindenberg, 2017). Interestingly, altered HC-PF connectivity was also reported in individuals identified as ARMS (Benetti et al.,

2009) and in siblings of patients with SZ (Rasetti et al., 2011). Animal studies also support altered HC-PF function in SZ. In mice with the chromosome 22q11.2 microdeletion—which is associated with a 30-fold increase in risk for SZ (Karayiorgou et al., 2010)—impaired HC-PF synchrony was reported, which was correlated with working memory deficits (Sigurdsson et al., 2010). In MIA rats, Dickerson et al. (2010) found impaired HC-PF synchrony, which was correlated with PPI deficits. In another neurodevelopmental mouse model, which involves the administration of methylazoxymethanol acetate during gestation, Philips et al. (2002) reported reduced HC-PF synchrony during sleep. Therefore, the HC-PF pathway is a crucial target for understanding the pathophysiology of SZ.

The neonatal ventral hippocampal lesion model

Although modeling a psychiatric disorder such as SZ in animals may be considered impractical, several animal models based on genetic and environmental factors have been attempted to understand aspects of SZ etiopathology. While genetic and pharmacological models capture specific aspects of the disorder, such as the involvement of a single gene or neurotransmitter system (e.g., dopamine), they often lack the neurodevelopmental component that is critical to understanding SZ. This gap highlighted the need for a more comprehensive neurodevelopmental model that could encompass the broader neurobiology related to SZ. In recent years, the neonatal ventral hippocampal lesion (NVHL) model developed by Lipska et al. (1993), has become one of the most well-characterized neurodevelopmental models to understand the etiopathology of SZ. This model was based on the neurodevelopmental theory of SZ, a widely accepted theory for the disorder's etiology (Weinberger, 1987). The NVHL model, originally constructed in rats, involves excitotoxic lesioning of the vHPC—a region selected based on analogous developmental anomalies observed in humans—on postnatal day (PND) 7 (Lipska et al., 1993).

PND 7 was chosen as it corresponds to the third trimester in human development, a period of high vulnerability in fetal hippocampal development believed to increase the risk for SZ (Scheibel & Conrad, 1993). As stated earlier, the vHPC is particularly important for emotional and motivational behaviors, as it projects to the PFC and limbic dopamine regions implicated in SZ.

Since no lesions in the hippocampi are seen in the brain scans of SZ patients, this model's strength does not lie in its construct validity, but rather in its face validity. Specifically, when the ventral hippocampi are lesioned during the neonatal period, but not at later stages, symptoms relevant to SZ (e.g., deficits in sociability, sensorimotor gating, working and spatial memory) appear during the adolescence/early adulthood period, reminiscent to what is seen in SZ patients (Tseng et al., 2009). Importantly, neurochemical, anatomical, and electrophysiological changes related to SZ are also observed in this model, including altered dopaminergic transmission, changes in cortical and striatal neuronal architecture, deficits in interneuron maturation, and altered spine density and dendritic length in the PFC (Flores et al., 2005; O'Donnell, 2012; Nath et al., 2023). As in SZ patients, NVHL animals display hypersensitivity to dopaminergic drugs (e.g., amphetamine) (Lipska et al., 1993), and the administration of antipsychotic drugs can reverse some of the behavioral and physiological changes seen in the model (Le Pen & Moreau, 2002; Richtand et al., 2006). As with other neurodevelopmental models, NVHL rats display altered HC-PF synchrony (Lee et al., 2012). Thus, the NVHL model acts as a neurodevelopmental model that can be leveraged to study SZ, as it captures several behavioral, cellular, and pharmacological changes observed in the disorder.

While the majority of behavioral NVHL studies have been performed in rats, Naert et al. (2013) were the first to assess the behavioral changes in mice. Similar to what was observed in

rats, their resulted showed that NVHL male mice (specifically in the "LARGE" lesion subtype) displayed locomotor hyperactivity, deficits in working memory, and hypersensitivity to amphetamine. However, no deficits in sensorimotor gating, sociability/social novelty, or spatial memory were reported. Preliminary data from Dr. Srivastava's lab showed that NVHL male mice exhibited locomotor hyperactivity, PPI deficits, and deficits in novel object recognition (NOR) (unpublished data).

To assess sleep disturbances—a common feature of SZ (Laskemoen et al., 2019)—Ahnaou et al. (2007) performed a polysomnographic study in NVHL rats. While no disturbances in sleep organization were observed, they found a slowing in electroencephalogram (EEG) patterns at pre-puberty (PND 35)—marked by increases in differences of absolute power at various frequency bands during wake and deep sleep—which the authors explained may be a result of early loss of cortical cholinergic afferents from the vHPC. This finding aligns with earlier reports of altered EEG power spectra in SZ (Morihisa et al., 1983). However, SZ patients also exhibit a broader range of sleep deficits, such as delayed sleep onset, shortened rapid-eye-movement latency, reduced deep sleep, and lower sleep efficiency (Ferrarelli, 2021). These disturbances have been observed prior to the onset of psychosis (Ruhrmann et al., 2010), in individuals at UHR (Poe et al., 2017) and have been identified as predictors of psychosis onset (Waite et al., 2020). Nevertheless, to date, no study has characterized circadian function, or the effects of circadian disruption, in the NVHL model. This is especially important as the circuit systems affected in the NVHL model, such as the mesocorticolimbic dopamine system, can interact with the SCN and modulate behaviour under its control (Grippo et al., 2017; Oishi & Lazarus, 2017).

Rationale and objectives:

While circadian behaviors in SZ have primarily been studied in genetic animal models (Delorme et al., 2020), a mechanistic link between circadian behavior and cortical brain deficits resulting directly from abnormal neurodevelopment remains unexplored. Given the neurodevelopmental nature of SZ, it is plausible that brain circuits involved in circadian-regulated processes (e.g., sleep/wake timing) are impacted during this developmental disruption. Moreover, the complexity and heterogeneity of brain connectivity changes in SZ make them challenging to fully capture and test. However, the NVHL model presents an opportunity to investigate how abnormal neurodevelopment-induced brain dysconnectivity affects circadian behaviors as this model captures various behavioral, cellular, and pharmacological changes observed in SZ resulting from brain dysconnectivity (Tseng et al., 2009). Additionally, considering SZ's multifactorial origins, the NVHL model allows for the examination of how brain dysconnectivity interacts with other risk factors—such as environmental circadian disruption (e.g., CJL) during critical developmental periods like adolescence.

Since the brain circuit systems affected in the NVHL model may be involved in overall circadian system functioning (e.g., Grippo et al., 2017), we hypothesized that NVHL mice will display changes in circadian function, as measured by daily rest-activity rhythms. Furthermore, we predicted that circadian disruption through exposure to CJL during adolescence will worsen the behavioral abnormalities observed in the model, given that CJL was shown to have a myriad of effects on brain functioning (e.g., Siddique et al., 2022). Finally, since sex differences are present in SZ (Abel et al., 2010), circadian rhythms (Duffy et al., 2011), and in the NVHL model (e.g., Silva-Gómez et al., 2003), we expected that sex-specific behavioral differences will be apparent.

To test these hypotheses, we aimed, utilizing the NVHL model, 1) to assess SZ-relevant behavioral changes following circadian disruption during adolescence, and 2) to investigate the effects of brain dysconnectivity on circadian behavior. Potential sex-specific differences were examined for both aims.

Methodology:

Animals

Male and female eight weeks old C57BL/6N mice were obtained from Charles River Laboratories. For cohort 1, 32 females and 16 males were used (to achieve a 2:1 female-to-male breeding ratio), while for cohort 2, 16 females and 16 males were used as it was determined that a 1:1 female-to-male ratio was more efficient for this project since only one female per cage got pregnant at a time during the breeding process for cohort 1. For cohort 3, the non-experimental mice obtained from cohort 2 were used to set up breeder cages, whereby males and females from separate litters were mated together. After their arrival, mice were then placed in ventilated cages under a standard laboratory lighting condition of 12:12 LD for one week to acclimate to their surroundings with *ad libitum* access to food and water. Males were kept in cages of two while females of five to disrupt their estrous cycles according to the Lee-Boot effect—a phenomenon that occurs when there is a suppression of estrous cycles when female mice are grouped together in large groups in the absence of a male stimulus (Van Der Lee & Boot, 1955). Three days prior to mating, soiled male bedding was placed in the female cages to increase the likelihood of copulation according to the Whitten effect (Whitten, 1956). On the breeding day, each individual male mouse was placed in their respective female cages around Zeitgeber Time (ZT) 6 (i.e., 6h after lights on). Mice were then left to mate overnight, and females were assessed between ZT 0 and ZT 1 for the presence of a vaginal plug. This process continued for three days, or until a plug was found. Once a plug was found, the female mouse was weighed and placed in her own cage. To increase the likelihood of obtaining litters on separate days to perform the stereotaxic surgeries on separate litters each day, the breeding process was dispersed over several weeks

(i.e., female 1 started breeding on day 1, female 2 on day 2, etc.). Cages of pregnant dams were checked daily each morning to confirm the presence of a litter. If a litter was found, the day of birth was marked as PND 0. The cages were then left undisturbed until the surgery day on PND 14. All procedures were approved by the Facility Animal Care Committee at the Douglas Research Centre, in accordance with the Canadian Council on Animal Care guidelines.

Stereotaxic Surgery

NVHL mice were generated as previously described (Nath et al., 2022). Litters of male and female pups were obtained from the C57BL/6N breeder cages. The pre-operative care, surgery procedure, and post-operative care were carried out according to McGill's Rodent Stereotaxic Surgery standard operating procedure (SOP) #202. The analgesia and anesthesia procedures were followed according to SOPs #101 and #110, respectively. At PND 14, pups within each litter were randomized to sham or lesion groups and anesthetized by isoflurane. An incision was made in the skin overlying the skull and 0.25 µL of ibotenic acid (IB; excitotoxic agent)(10 µg/µL, ABCAM), or phosphate-buffered saline (PBS, 1X, pH 7.4) in controls, was stereotaxically injected bilaterally into the vHPC at a rate of 0.05 µL/min at the following coordinates from bregma: anterior/posterior: −2.8; lateral: ±2.8; dorsal/ventral: −3.2. For cohort 2 and 3 specifically, in order to address the needle blockage that caused the solution to not get infused for all of our cohort 1 mice as per the lesion verification process (see later), once the needle was inserted to the Z-coordinate, it was taken out again and the needle opening was deblocked using the end of non-absorbable sterile suture threads (Ethicon Nylon Sutures 5-0 FS-2 19 mm Needle 18"). Then, this process was repeated three times before the solution was released. While this process was done for cohort 2, we still got a very low success rate. It was later determined that, upon the fourth time, the needle was being inserted to the Z-coordinate at a pace that caused it to

get blocked no matter how many times it was getting de-blocked after; thus, the solution was to insert the needle very slow and to also verify that the solution was being infused during the procedure. In cohort 3, when the needle and its tubing were prepared, this was done by inserting sterile water before adding either IB or PBS with clear air separation between the two. The level of water was marked so, as the solution was being infused, the water level can be monitored to see if it was indeed moving. If yes, then there was no needle blockage and the solution was being injected accordingly. If not, the needle was taken out again and deblocked using the suture thread. Upon the completion of injections, the pups were sutured, identified using an ear punch, and placed in a recovery cage with a heat pad underneath. Mice were kept in the cage until all surgeries were completed to ensure they properly recovered from surgeries and that the vet glue—whose smell is not well tolerated by parents—was dried. After the surgery day, mice were closely monitored to ensure their proper recovery. At PND 21, mice were weaned and weighed. Since the litters and surgeries were dispersed over several weeks, mice were divided into two groups to ensure that they were placed in the CJL or LD protocol no later than one week following weaning (as opposed to waiting until the last group of mice are weaned a couple of weeks later). Thus, each cohort consisted of two groups, with the exception of cohort 3, which consisted of multiple groups as the breeding and surgeries were more dispersed over time.

Chronic Jet Lag Protocol

After weaning, mice were randomly assigned to either the CJL or 12:12 LD condition per cage (**Figure 4**). They were placed in ventilated light-proof cabinets (Actimetrics, Wilmette, IL, USA), and kept for two days under 12:12 LD condition. On the third day, the CJL group were subjected to a 6-h phase advance every two days, for a total four weeks (Cloutier et al., 2022). The control group remained in 12:12 LD. Following completion of the four weeks, all mice were

placed in 12:12 LD for two weeks prior to behavioral testing, to ensure that they were tested under the same circadian phase. Males and females were housed separately, and cage changes occurred weekly.

Behavioral Testing

To account for the mortality rate (~ 10%) associated with the surgery and the chances that the lesioning procedure was not successful (e.g., due to needle blockage, location of lesion beyond vHPC, etc.), this study consisted of multiple cohorts to obtain sufficient sample sizes. Thus, to maintain consistency in experimental design across cohorts, behavioral tests were conducted in the same order and at the same ages for each cohort in the following order: NOR, elevated plus maze (EPM), open field test (OFT), three-chamber sociability and social novelty test, and PPI of acoustic startle (**Figure 4**). They were separated by at least two days of rest. However, due to a malfunction in the PPI machines after cohort 1, PPI was not tested in subsequent cohorts and was excluded from the analysis, especially as no mice with a successful lesion were obtained from cohort 1. The order of tests took into consideration the amount of stress induced by the tests and the effect of previous behavioral testing experience on exploratory behavior (Shoji & Miyakawa, 2021). All tests were performed starting at ZT1. For all tests, mice were habituated to the testing room 30 minutes prior to the testing. Tests were performed under dim light conditions (~15 lux) unless indicated otherwise. Experimenters were absent from the room during the testing period for all the tests. The behavioral tests are described below.

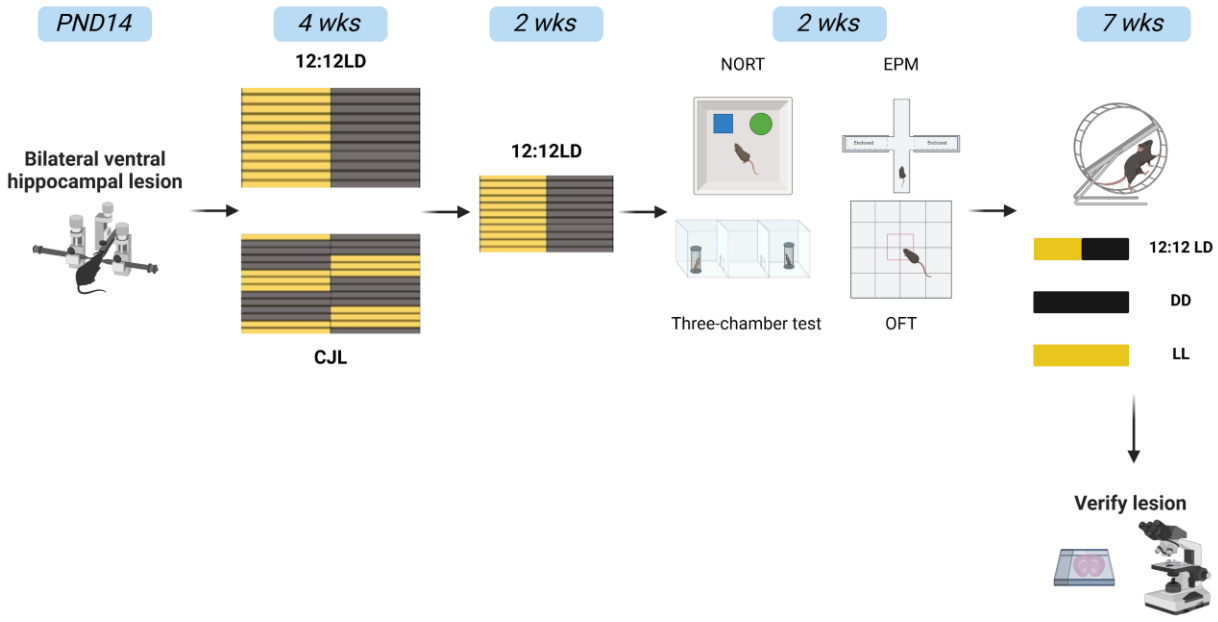


Figure 4. Experimental timeline. Created with BioRender.com.

Novel Object Recognition

NOR is a commonly used test to assess recognition learning and memory (model of declarative memory), based on the rodent's innate tendency to explore a novel object over a familiar one (Leger et al., 2013). This test was performed as previously described (Srikanta et al., 2021). On day one, mice were placed in empty grey box open field boxes [48cm X 48cm X 48 cm] for ten minutes to habituate to the apparatus (habituation phase). On day two, mice were placed in the boxes to freely explore for ten minutes with two identical objects (either two tall and translucent containers with geometric patterns or two round, matte-finish glass bottles with cork stoppers) (**Figure 5**) diagonally facing each other at opposite corners (whose location was randomly dispersed across trials) of the box (familiarization phase). Objects were selected based on

recommendations made in previous literature (Heyser & Chemero, 2012). Four hours later (intersession interval), mice were placed back in the boxes to freely explore for ten minutes, with one of the identical objects (a new set of objects was used to take into account for any odour left on used objects) replaced with a differently shaped one (novel object) (testing phase). Two overhead Swann cameras were used for recording. Object exploration was counted when the mouse's nose was directed towards the object, around 2 to 3 cm from it, while object climbing was excluded. Videos were manually scored by two experimenters blinded to the mouse group condition by using Chronotate (Philipsberg et al., 2023). Based on the rodent's natural tendency to prefer the novel object over the familiar one, recognition memory was assessed based on the mouse's ability to remember the encounter with the familiar object. The Discrimination Ratio (DR) was measured to calculate the ability to discriminate between the familiar and novel object. For the training segment, the ratio was calculated as the time spent exploring the object that was replaced by the novel one over the other familiar object:

$$DR = \frac{Time\ spent\ exploring\ novel\ object}{Time\ spent\ exploring\ familiar\ object}$$

Figure 5. Objects used part of the novel object recognition test.

Elevated Plus Maze

EPM is one of the most used behavioral tests to measure anxiety-like behavior, based on the rodent's tendency to avoid open spaces and to seek enclosed space (Walf & Frye, 2007). The apparatus was elevated 75 cm from the floor and consisted of a plus-shaped structure with two open and two closed arms (Length = 50 cm x Width = 5 cm per arm) painted black. The two closed arms were enclosed by 10 cm high walls on three sides. For the test, mice were placed on the center of the structure, while facing an open arm, and left to freely explore for five minutes. Increased time spent in the open arms is seen as decreased anxiety-like behavior. One overhead Swann camera was used for recording. Data were analyzed using ezTrack (Pennington et al., 2021). Mice that jumped off the structure or spent the entire time frozen on an open arm were excluded from analysis. The ratios of time spent in open over closed arms and number of entries (entry is counted when mouse enters with four limbs to area) to open over closed arms were measured to assess anxiety-like behavior:

$$Time\ Ratio = \frac{Time\ spent\ in\ open\ arms + \frac{Time\ spent\ in\ center}{2}}{Time\ spent\ in\ closed\ arms + \frac{Time\ spent\ in\ center}{2}}$$

$$Entry\ Ratio = \frac{Number\ of\ entries\ to\ open\ arms}{Number\ of\ entries\ to\ closed\ arms}$$

Open Field Test

OFT is a well-established method to measure spontaneous locomotor activity and anxiety-like behavior (Seibenhener & Wooten, 2015). The VersaMax Legacy Open Field setup (AccuScan Instruments, Inc., Columbus, OH, USA) was used for this test. For a total of 50 minutes, mice were placed to freely explore in VersaMax acrylic chambers (Length × Width × Height = 17.5 cm × 10 cm x 26 cm) equipped with infrared sensors that record and score locomotion-related

variables. Variables included total horizontal activity, total movement time, and thigmotaxis ratio (time in margin/time in center). Data were collected using the Versamax Software (version 4.0, 2004; AccuScan Instruments, Inc., Columbus, OH, USA).

Three-Chamber Sociability and Social Novelty Test

The three-chamber test is used to test sociability (preference of rodent over object) and social novelty (preference of new rodent over familiar one) (Yang et al., 2011). The apparatus consisted of a three-chambered plastic structure (Length = 26 cm x Width = 21.6 x Height = 21.6 cm) with small vertical openings between each chamber. Objects and strain-, sex-, and age-matched stranger mice were placed under wire mesh pen cup containers. On the evening prior to testing, stranger mice with a C57BL/6 background were habituated to both the wire mesh pen cup containers they were placed under and the structure itself. The testing component was divided into three consecutive stages, each lasting ten minutes: 1) habituation, 2) sociability, and 3) social novelty preference. In the habituation phase, the experimental mouse was placed in the middle chamber to start freely exploring, and the containers had two identical objects (small transparent bottles with rice grains inside) underneath. In the sociability phase, one of the objects was replaced with a stranger mouse. Finally, in the social novelty preference phase, the remaining object was replaced with the novel stranger mouse. Each phase was separated by five minutes of rest, during which the mouse was kept in the middle chamber. Two overhead Swann cameras were used for recording. Data were manually scored by two experimenters blinded to the mouse group condition by using Chronotate (Philipsberg et al., 2023). Exploration of object/mouse was considered when the mouse's nose was directed towards the cup, around 2 to 3 cm from it, while climbing on top of the cup was excluded from analysis. The sociability and the social novelty preference ratios were calculated:

$$Sociability\ ratio = \frac{Time\ spent\ interacting\ with\ mouse}{Time\ spent\ interacting\ with\ object}$$

$$Social\ novelty\ ratio = \frac{Time\ spent\ interacting\ with\ novel\ mouse}{Time\ spent\ interacting\ with\ familiar\ mouse}$$

Wheel-Running Activity Under Different Lighting Conditions

After the completion of behavioral tests, mice were individually housed in running wheel cages, which were placed in light-proof ventilated cabinets (**Figure 4**). After four days of entrainment in 12:12 LD, mice were exposed to three different lighting conditions, each for a period of two weeks, in the following order: 12:12 LD, constant darkness (DD), and LL. Light was controlled via ClockLab software, version 6 (Actimetrics, Wilmette, IL, USA). 12:12 LD was used to test the ability to entrain to external cues (i.e., LD cycle). DD was used to measure endogenous rhythms without an influence from an external light cue. Finally, LL, which was shown to desynchronize the SCN neuronal network, but not disrupt individual neuronal oscillators (Ohta et al., 2005), was utilized to uncover any underlying network connectivity issues already present in the SCN (i.e., an already weakened SCN neural network may respond more intensely to LL). ClockLab software was used to record and analyze wheel running data. The last ten days of each lighting condition was included in the analysis. The obtained variables included circadian period (tau; calculated using a chi-square periodogram); active period duration (alpha; refers to numbers of hours between activity onset and offset); total amount of daily activity; and percent day activity relative to total daily activity. An activity bout (specific period of sustained activity) analysis was also performed. The variables that were analyzed included total number of bouts, number of bouts per day, and the average bout length. Non-parametric variables were also derived; specifically, RA (robustness of rhythms); IS (stability of rhythms across days); and IV (level of rhythm fragmentation). For constant conditions (DD and LL), the subjective night was

calculated as the onset of activity plus half of the calculated tau (Delorme et al., 2021), while the remainder period was considered as the subjective day.

Lesion Verification

After the completion of the testing period, mice were euthanized, and their brains were collected, snap-frozen and stored in -80 ℃ (**Figure 4**). A cryostat microtome (Leica) was used to obtain 35 µm-thick coronal sections between Bregma -1.34 mm and -3.64 mm. The sections were mounted on Fisherbrand Tissue Path Superfrost Plus Gold Slides. The slides were stained using Nissl-staining, which allows for the visualization of the nuclei of neurons (Kádár et al., 2009). After coverslipping the slides using Permount, they were kept sitting for at least 48 hours prior to imaging. Slides were visualized and imaged using an automated brightfield and fluorescent microscope (Olympus BX63). Brain section images were blindly assessed and scored for lesion status. Specifically, a lesion (observed as a visible loss of neurons or presence of cavity) was considered successful if 1) the lesion was done bilaterally, and 2) the lesion was limited to the vHPC. Mice with unilateral lesions were excluded unless specifically indicated. Mice with no visible lesion were excluded from the analysis.

Statistical Analysis

GraphPad Prism (version 10 for Windows, GraphPad Software, San Diego, California USA), and ANOVA2 2020 (Dr. Joseph Rochford, McGill University, Montréal, QC Canada) were used to perform the statistical analyses. The data were graphed using GraphPad Prism. Two-way between-factor analysis of variance (ANOVA) was performed on normally distributed data sets with equal variance across groups. Two-tailed paired Student *t* tests were used to compare means between two groups. The Shapiro-Wilks test and the Levene's test were used to test for normality and homogeneity of variances, respectively. The ROUT method was used to identify outliers

where applicable. A value was considered an outlier if it was significantly different (Q = 1%) from the entire data set. Differences were considered significant when $p < 0.05$ and considered trending when $p < 0.1$.

Results:

Lesion Verification

This project involved a total of three cohorts. Brain slicing, staining, and imaging were conducted at the end of the experiment for each cohort to assess for lesion status. For cohort 1, which included a total of 14 males and 21 females, no lesions were found for any of the mice assigned in the IB groups (**Figure 6A**). As discussed above, it was later determined that this was due to needle blockage. To test the needle de-blockage procedure detailed in Methods, a male adult mouse was injected with purple dye in the same coordinates from bregma as the actual surgery: anterior/posterior: −2.8; lateral: ±2.8; dorsal/ventral: −3.2 (**Figure 6B**). This confirmed that the procedure was effective to de-block the needle. However, upon doing the lesion verification process for cohort 2, it was found that the majority of our IB mice did not have a successful lesion. Specifically, out of 18 females, only four (LD-IB) had a visible lesion (three unilateral/one bilateral). While in males, only two (LD-IB) had a visible lesion out of 22 (one unilateral/one bilateral) (**Figure 6C-D**). Upon further testing following these results, the de-blocking procedure was further improved as detailed in Methods. Now that the actual injection of solution could be monitored, it was confirmed that the blockage cause was due to the needle not being inserted very slowly into the brain. Thus, for cohort 3 mice, whose experimental procedure (including surgeries) is still ongoing, the injection of solution was verified while doing the surgeries. What remains to be confirmed is the extent of the lesion. Given these results, it is important to note that the results below are divided and presented in the following way: non-circadian behavior, which includes data from cohort 3, whose experimentation is still ongoing and pending lesion characterization; circadian behavior, which includes data from cohort 2,

specifically from mice with a verified lesion (both unilateral and bilateral lesions will be discussed).

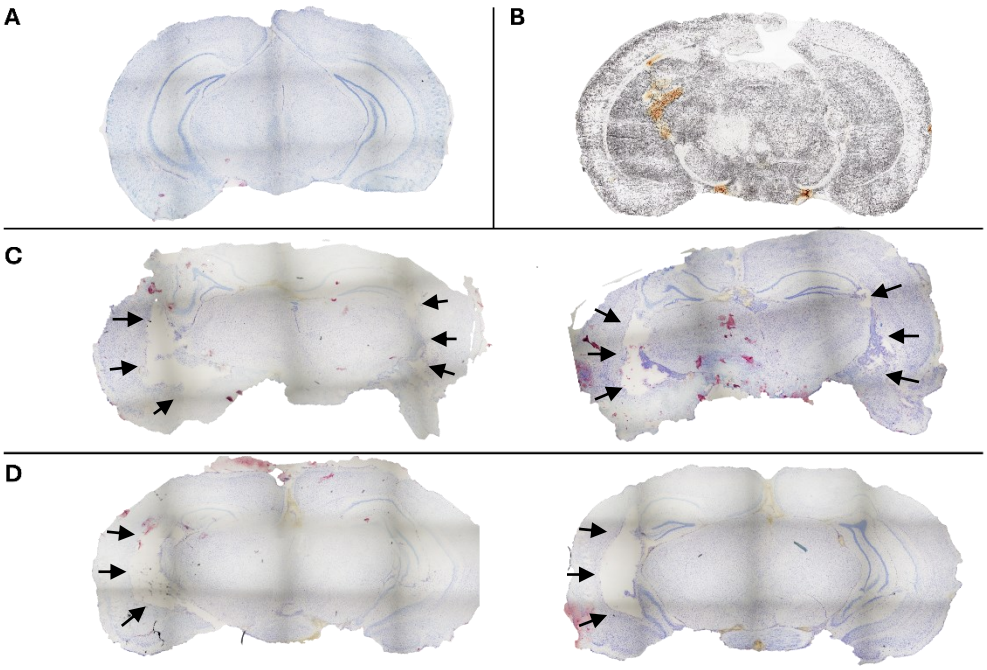


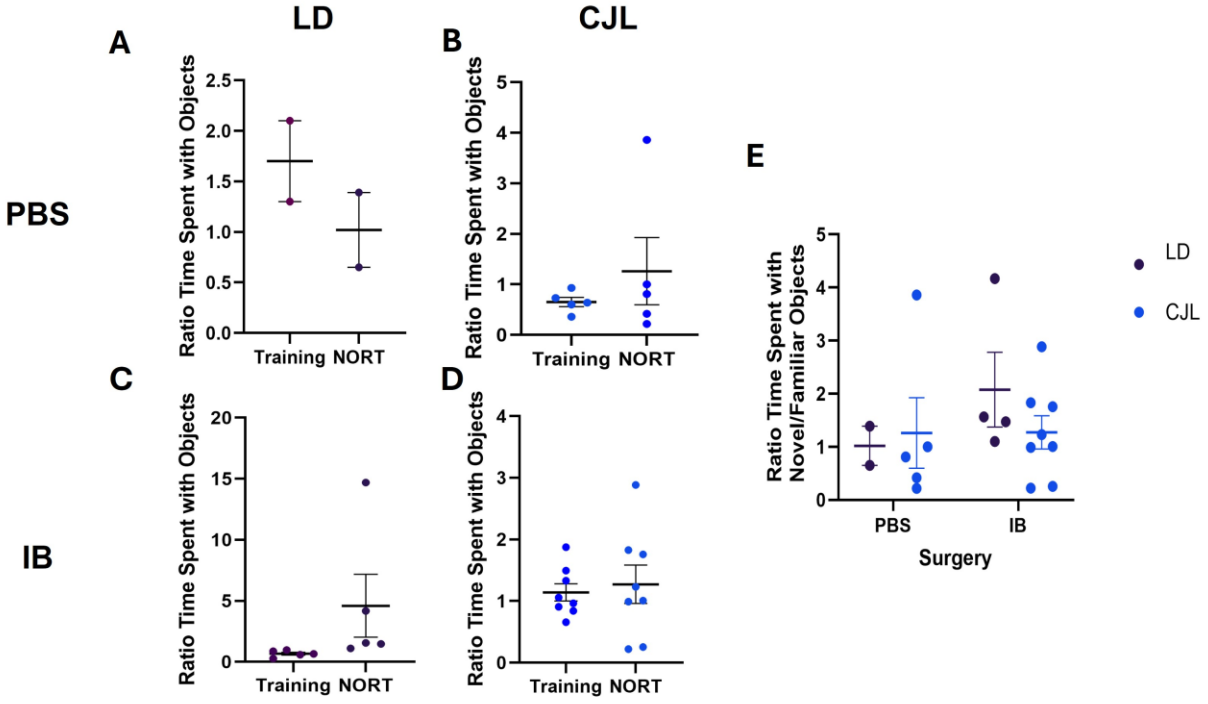
Figure 6. Lesion statuses in cohorts 1 and 2. Lesion status was verified using cresyl violet staining after obtaining 35 µm-thick coronal sections using cryostat. **A)** No lesions were found for mice in cohort 1 as the ventral hippocampus looked visually intact. **B)** De-blocking procedure was tested on an adult mouse by unilaterally injecting purple dye into the ventral hippocampus at the following coordinates: anterior/posterior: -2.8; lateral: ±2.8; dorsal/ventral: -3.2. **C)** Images showing bilateral lesion of the ventral hippocampus in a male seen as visible cavities. **D)** Images showing unilateral lesion of the ventral hippocampus in a male mouse seen as a visible cavity. Black arrows are pointing towards the area where the lesion is present.

Non-Circadian Behavior – Cohort 3

Recognition Memory

Recognition memory was tested using NORT. The differences between the object exploration ratios during the training and the testing (marked as "NORT" in graph) segments were assessed for each group using two-tailed paired Student *t*-tests. Unlike expected, the tests did not yield

any significant differences between the segments for any of the groups (**Figure 7**). However, with the exception of the LD-PBS group in males and the LD-PBS and CJL-PBS groups in females, which showed a decrease in NORT ratio compared to the training segment, the results did show a visible trend of an increase in ratios during the NORT segment compared to the training segment as would be expected, despite not trending towards statistical significance (**Figure 7**). Thus, the lack of significance is likely attributed to the lack of statistical power and high variability. When comparing the discrimination ratio of all groups, a two-factor (Lighting x Surgery) between-subject ANOVA yielded a significant main effect of surgery on ratio of time spent exploring the novel object over the familiar one in females, $F(1, 22) = 4.54$, $p < 0.05$, whereby the IB female mice had increased recognition memory compared to their sham counterparts (**Figure 7J**). An outlier identified using the ROUT test (1 LD-IB male) was removed from the analysis of the discrimination ratio between all the groups (**Figure 7E**).



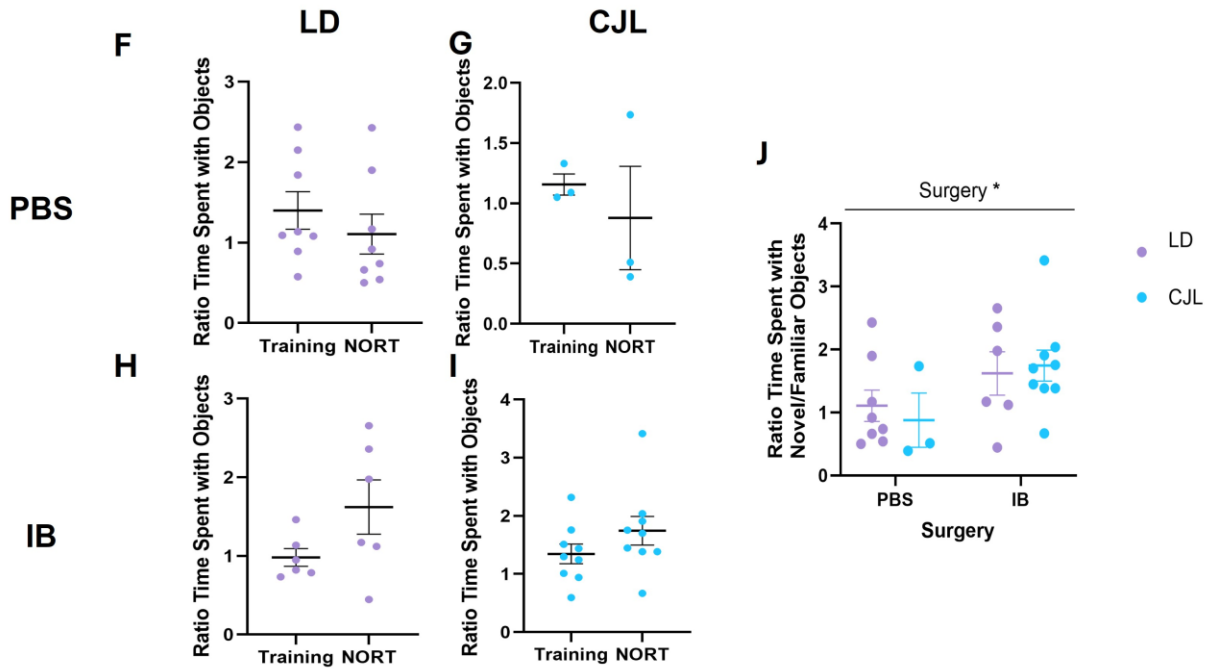


Figure 7. Enhanced recognition memory in lesioned female mice. The novel object recognition test was used to assess recognition memory. The differences in proportion of time spent with the objects in the training and testing ("NORT") phases were measured using paired Student *t*-tests for the PBS-LD (**A**), PBS-CJL (**B**), IB-LD (**C**), and IB-CJL(**D**) groups in males and for the PBS-LD (**F**), PBS-CJL (**G**), IB-LD (**H**), and IB-CJL (**I**) groups in females. Two-way between-factor (Lighting x Surgery) ANOVAs were performed to analyze the discrimination ratios between the novel object and the familiar object in males (**E**) and females (**J**). Data points represent individual mice and are presented as mean ± SEM. Abbreviations: LD: 12:12 light-dark; CJL: chronic jet lag; PBS: phosphate-buffered saline; IB: ibotenic acid. *$p < 0.05$.

Anxiety-like Behavior

The EPM test was used to assess anxiety-like behavior. A two-factor (Lighting x Surgery) between-subject ANOVA yielded a significant main effect of lighting on ratio of time spent in the open arms over the closed ones in females, $F(1, 21) = 11.76$, $p < 0.01$. However, the data did not meet the homogeneity of variances assumption after failing Levene's test ($p < 0.05$). To address this, the data were transformed using the square root transformation, which led to all the assumptions being met. Upon doing a two-factor (Lighting x Surgery) between-subject ANOVA

on these data, the analysis revealed a main effect of lighting on ratio of time spent in the open arms over the closed ones in females, $F(1, 21) = 9.23$, $p < 0.01$, whereby the CJL groups had increased ratios compared to their LD counterparts (**Figure 8C**). This effect was mainly driven by the PBS-CJL group, which is reflected in the trending increase in ratio it had compared to the IB group ($p = 0.0855$) (**Figure 8C**). No significant differences were found in males. Three male mice were excluded from the analyses as two (1 LD-PBS + 1 LD-IB) were frozen on an open arm for the entire recording and one (IB-CJL) jumped from the structure.

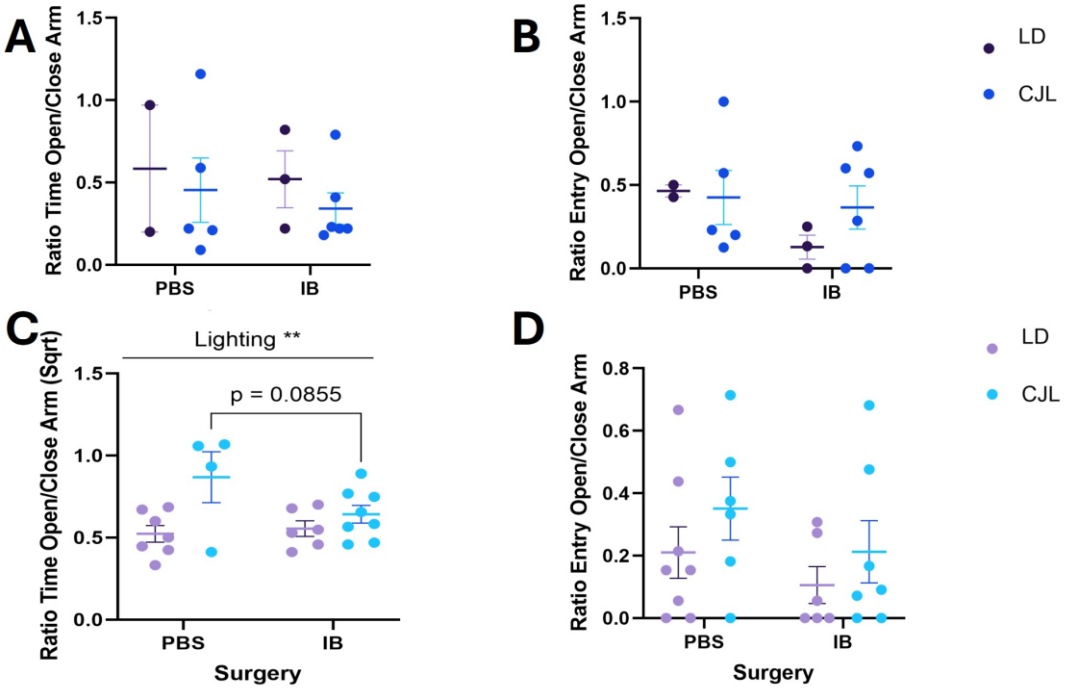


Figure 8. Decreased anxiety-like behavior caused by chronic jet lag in sham females. The ratio of time spent in open over closed arms and ratio of entry to open over closed arms were measured in both males (**A-B**) and females (**C-D**). Two-factor between-subject (Lighting x Surgery) ANOVAs were performed. Data points represent individual mice and are presented as mean ± SEM. Square root data transformation was performed on ratio time open/close arms in females in order to meet all ANOVA assumptions. Abbreviations: LD: 12:12 light-dark; CJL: chronic jet lag; PBS: phosphate-buffered saline; IB: ibotenic acid. ***p < 0.01.*

Locomotor Activity

The open field test was used to measure spontaneous locomotor activity and anxiety-like behavior. A two-factor (Lighting x Surgery) between-subject ANOVA yielded a significant main effect of surgery in males on total horizontal activity, $F(1, 17) = 7.73$, $p < 0.05$, and total movement time, $F(1, 17) = 5.40$, $p < 0.05$ (**Figure 9A-B**). A two-factor (Lighting x Surgery) between-subject ANOVA yielded a significant interaction effect on thigmotaxis (time spent in margin over center) in males, $F(1, 17) = 13.88$, $p < 0.01$ (**Figure 9C**). Simple main effects test conducted between lighting conditions at each surgery condition revealed that the PBS group exposed to CJL spent significantly less time in margins compared to their LD counterparts, $F(1, 17) = 19.80$, $p < 0.001$ (**Figure 9C**). Simple main effects test conducted between surgery conditions at each lighting condition revealed that the LD group injected with PBS spent significantly more time in margins compared to their IB counterparts, $F(1, 17) = 20.81$, $p < 0.001$ (**Figure 9C**). A two-factor (Lighting x Surgery) between-subject ANOVA yielded a significant main effect of surgery in females on total horizontal activity, $F(1, 25) = 7.53$, $p < 0.05$, and total movement time, $F(1, 25) = 5.88$, $p < 0.05$ (**Figure 9D-E**). However, the total horizontal activity data did not meet the homogeneity of variances assumption after failing Levene's test ($p < 0.05$). To address this, the data were transformed using the square root transformation, which led to all the assumptions being met and the main effect of surgery still being significant, $F(1, 25) = 7.36$, $p < 0.05$ (**Figure 9D**). No differences in thigmotaxis were found in females.

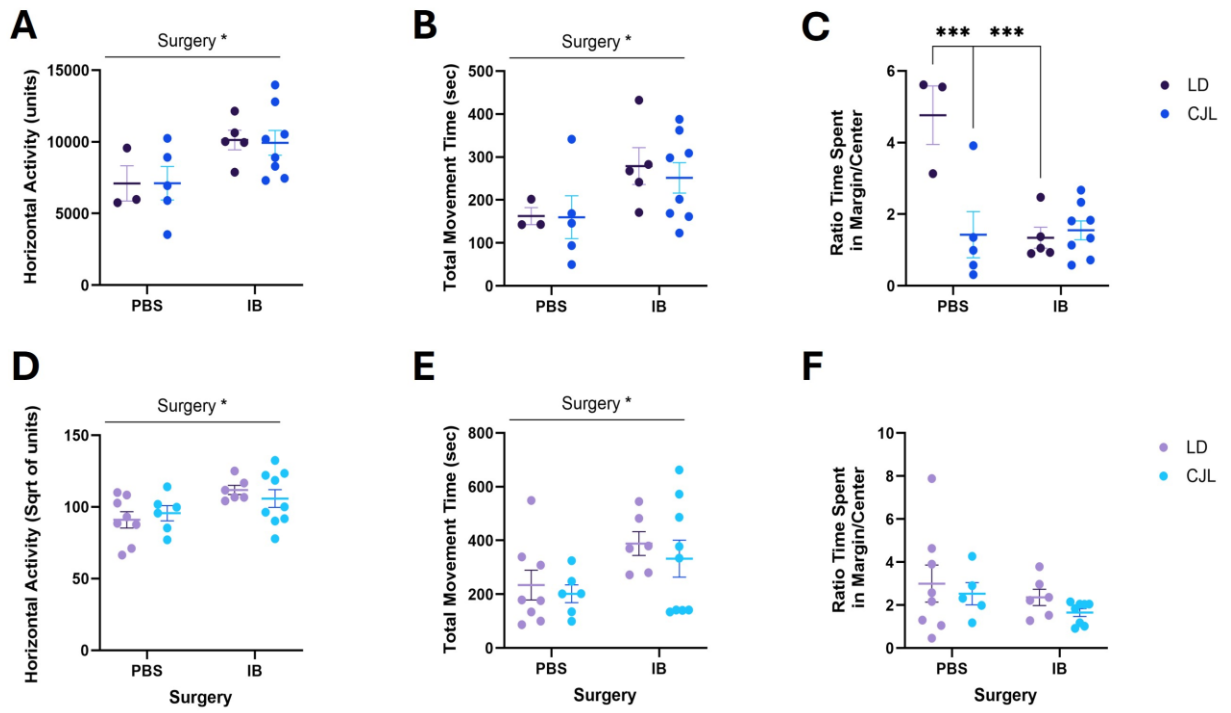


Figure 9. Hyperactivity in lesioned mice and decreased anxiety-like behavior caused by chronic jet lag and lesion in males. 1) Horizontal activity, total movement time, and thigmotaxis (ratio of time spent in margin over center) were measured in males (**A-C**) and females (**D-F**). Two-way between-factor (Lighting x Surgery) ANOVAs were performed to analyze horizontal activity, total movement time, and thigmotaxis ratio. Data points represent individual mice and are presented as mean ± SEM. Square root data transformation was performed on horizontal activity data in females in order to meet all ANOVA assumptions. Abbreviations: LD: 12:12 light-dark; CJL: chronic jet lag; PBS: phosphate-buffered saline; IB: ibotenic acid. *$p < 0.05$. ***$p<0.001$

Sociability and Social Novelty

The three-chamber test was used to measure sociability and social novelty. A two-factor (Lighting x Surgery) between-subject ANOVA yielded a significant interaction effect on sociability in males, $F(1, 16) = 12.39$, $p < 0.01$ (**Figure 10A**). Simple main effects test conducted between lighting conditions at each surgery condition revealed that the PBS group exposed to

CJL had decreased sociability compared to their LD counterparts, $F(1, 16) = 12.95$, $p < 0.001$ (**Figure 10A**). Simple main effects test conducted between surgery conditions at each lighting condition revealed that the LD group injected with IB had decreased sociability compared to their PBS counterparts, $F(1, 16) = 22.04$, $p < 0.001$ (**Figure 10A**). No differences in social novelty in males were found. A two-factor (Lighting x Surgery) between-subject ANOVA yielded a trending main effect of lighting on sociability in females, $F(1, 23) = 14.24$, $p = 0.0534$ (**Figure 10C**), after adjusting for outliers using ROUT (2 mice from the CJL-PBS group removed). A two-factor (Lighting x Surgery) between-subject ANOVA yielded a trending main effect of surgery on social novelty in females, $F(1, 23) = 10.96$, $p = 0.0802$ (**Figure 10D**), after adjusting for outliers using ROUT (1 mouse from PBS-LD + 1 mouse from PBS-CJL).

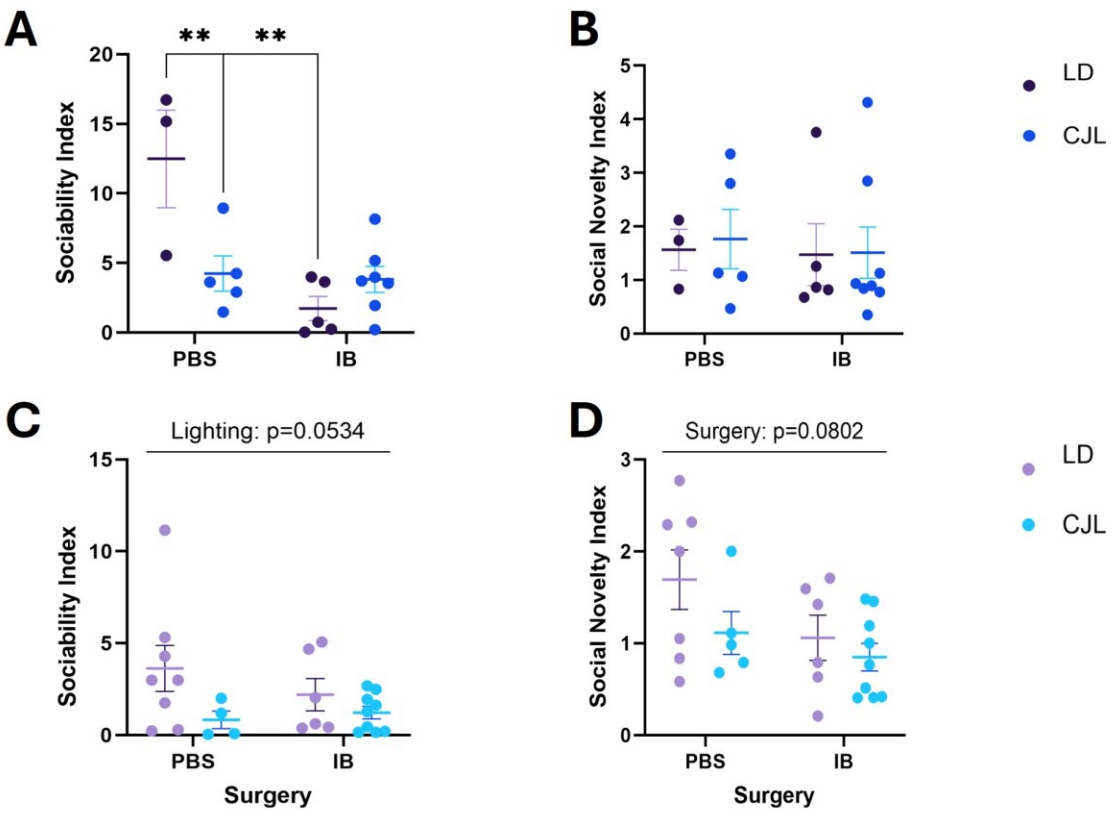


Figure 10. Decreased sociability caused by chronic jet lag and lesion in males. 1) Sociability and social novelty were measured in males (**A-B**) and females (**C-D**). Two-way between-factor (Lighting x Surgery) ANOVAs were performed to analyze sociability and social novelty. Data points represent individual mice and are presented as mean ± SEM. Abbreviations: LD: 12:12 light-dark; CJL: chronic jet lag; PBS: phosphate-buffered saline; IB: ibotenic acid. ***p<0.01*.

Circadian Behavior – Cohort 2 (Case Study)

Due to the low number of successful lesions in cohort 2 and the fact that most mice in cohort 3 are still in running wheels, the following results focus on the running wheel locomotor activity of cohort 2 mice with either unilateral or bilateral lesions of the vHPC. All of these mice were under

12:12LD during adolescence; thus, the possible effects of CJL will not be discussed thereafter. For analysis purposes, mice with either a unilateral or bilateral lesion are grouped together as "IB," unless otherwise specified. Additionally, any observed changes (e.g., increases or decreases) between groups are purely descriptive and have not undergone statistical assessment. The running wheel activity under 12:12 LD, DD, and LL for this group is examined and described below.

General locomotor variables

Under the 12:12 LD cycle, both male and female mice, regardless of surgery or lesion laterality, entrained well to the LD cycle, with most of their activity confined to the dark phase (**Figures 11-12**). All mice had a period of 24 hours (±0.02) and no differences in *alpha*. However, IB mice exhibited lower daytime activity counts, indicating their activity was more restricted to the dark phase compared to PBS mice. Upon transitioning to DD, more pronounced changes in activity rhythms emerged. Specifically, IB males showed an apparent increase in *alpha* and a decrease in total activity counts compared to PBS males (**Figure 13B-C**), while IB females exhibited a similar trend (**Figures 13E-F**). The most significant changes occurred when the mice were exposed to LL. Notably, IB male mice, regardless of lesion laterality, experienced a marked reduction in overall activity level (**Figure 13I**). This pattern was also observed in females (**Figure 13L**). Male IB mice also had an apparent reduction in period (**Figure 13G**).

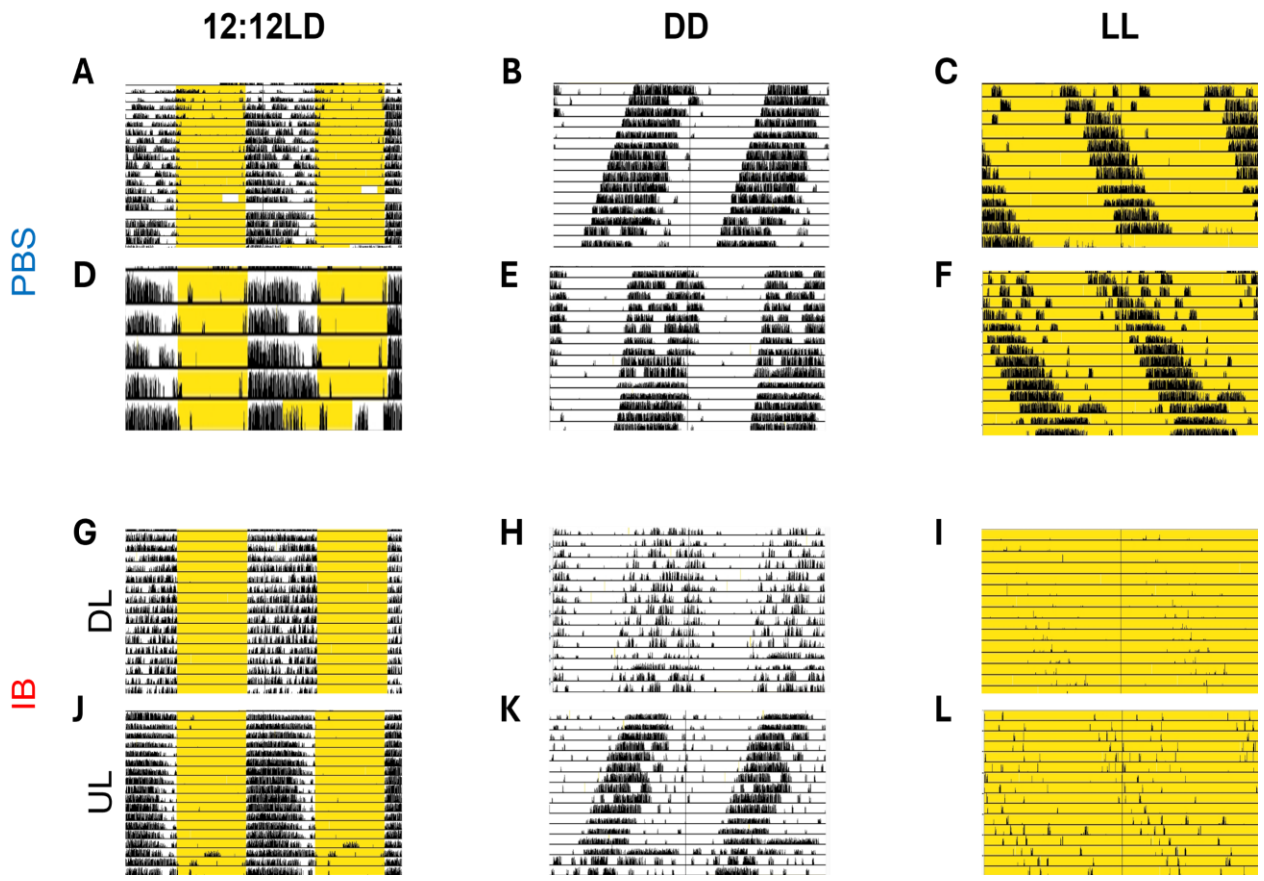


Figure 11. Marked reduction in running wheel activity and increased fragmentation under constant light in male IB mice. Actograms demonstrating running wheel activity of PBS (**A-F**) and IB (**G-L**) male mice under 12:12LD, DD, and LL. Each row represents an individual mouse. Days are vertically stacked one on the other, time (in hours) is shown across the x-axis, and data are double plotted to facilitate visualization. Abbreviations: DL: double lesion; UL; unilateral lesion; LD: light-dark; DD: constant darkness; LL: constant light; PBS: phosphate-buffered saline; IB: ibotenic acid.

Non-parametric variables

Non-parametric variables were assessed under all conditions. During the 12:12 LD cycle, RA, IS, and IV were comparable across groups, though one IB female exhibited a noticeable increase in IV compared to the other mice. In DD, male IB mice showed greater IV and lower RA compared to the PBS group (**Figure 14A-B**), which was similarly the case for females (**Figure 14C-D**). Under LL, male IB mice exhibited an increase in IV compared to the PBS group (**Figure 14F**), with a similar trend observed in females (**Figure 14H**).

Bout analysis

Bout analysis was conducted across all conditions. In the 12:12 LD cycle, there was considerable variability in the total number of bouts, with some mice displaying a high number of bouts and others fewer. Consequently, there was large variability in both average bout length and bouts per day. In DD, the high IV observed in IB males corresponded with a greater total number of bouts and a shorter average bout length compared to PBS groups (**Figure 15A-B**). A similar pattern was seen in females (**Figure 15C-D**). Under LL, male IB mice showed reduced average bout length, while females showed reduced average bout length and decreased number of bouts (**Figure 15G-H**).

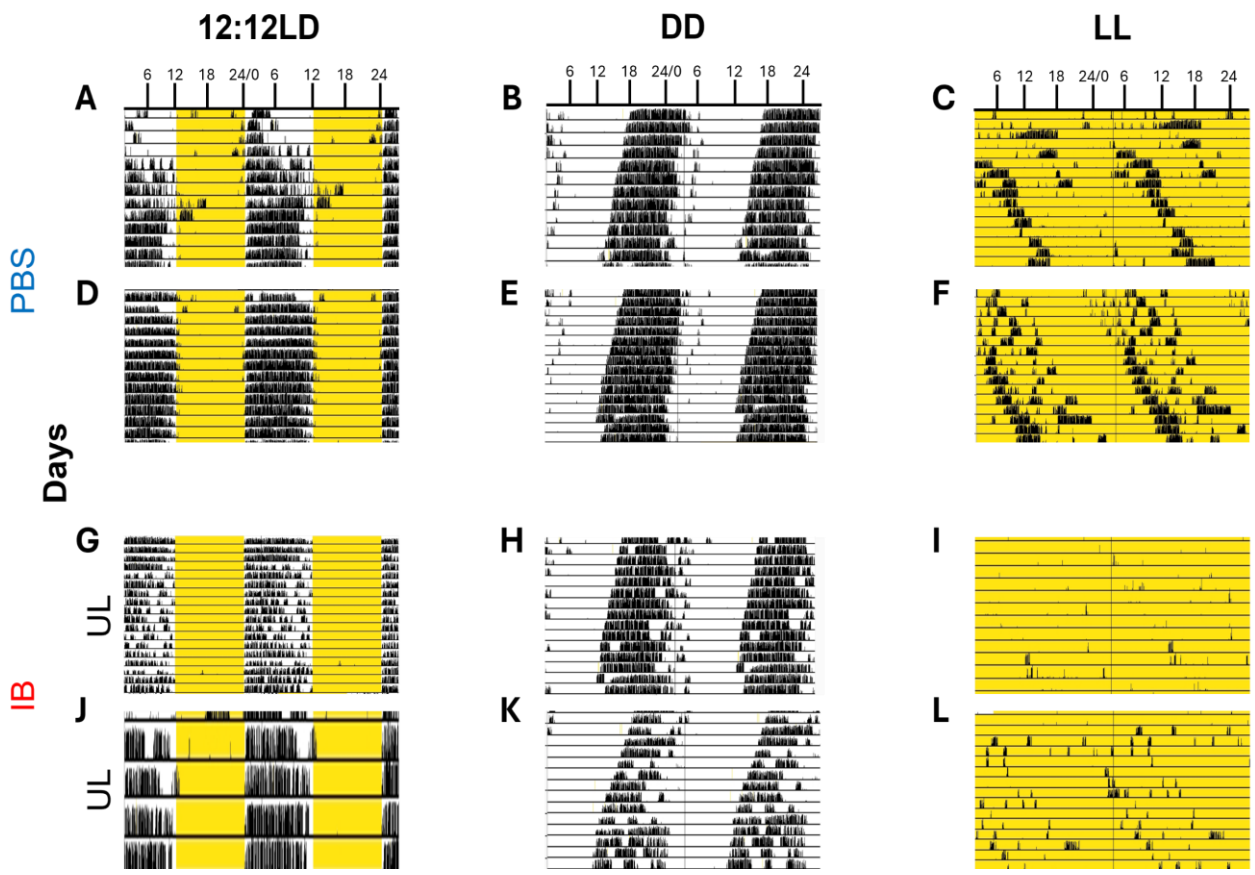


Figure 12. Marked reduction in running wheel activity and increased fragmentation under constant light in female IB mice. Actograms demonstrating running wheel activity of PBS (**A-F**) and IB (**G-L**) female mice under 12:12LD, DD, and LL. Each row represents an individual mouse. Days are vertically stacked one on the other, time (in hours) is shown across the x-axis, and data are double plotted to facilitate visualization. Abbreviations: UL; unilateral lesion; LD: light-dark; DD: constant darkness; LL: constant light PBS: phosphate-buffered saline; IB: ibotenic acid.

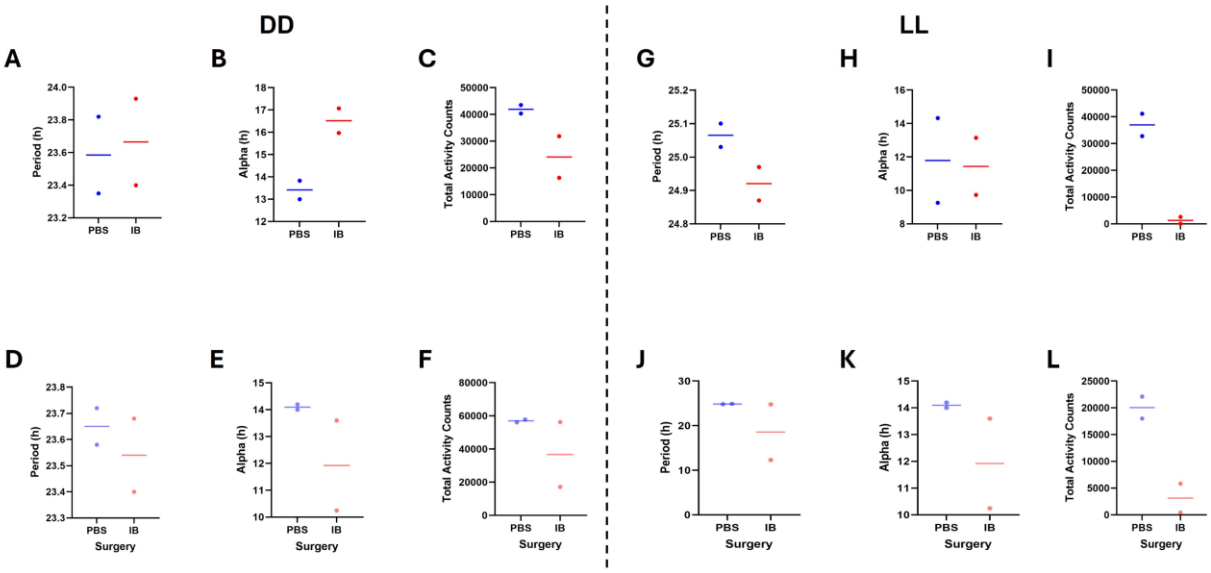


Figure 13. Lesioned mice exhibit large reduction in total activity under LL. Period (h), alpha (h), and total day activity (counts) were assessed for both males (top row) and females (bottom row) under DD and LL. Due to the low sample size, data were not statistically analyzed but are graphed to demonstrate observed changes. Data points represent individual mice and are presented as median. Abbreviations: DD: constant darkness; LL: constant light; PBS: phosphate-buffered saline; IB: ibotenic acid.

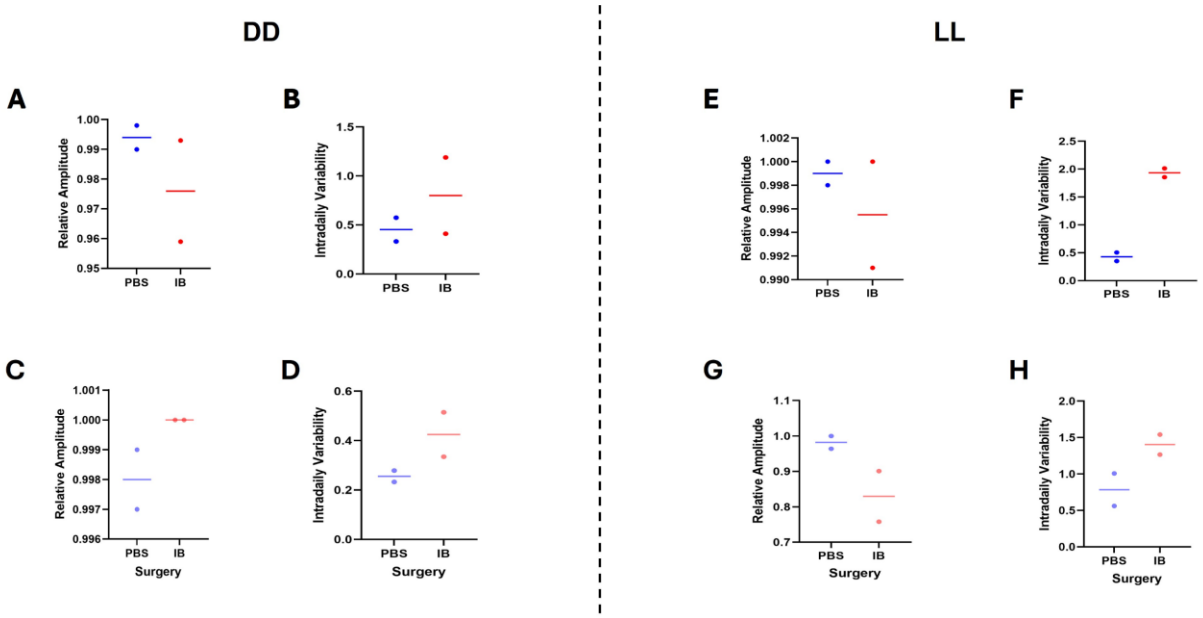


Figure 14. Lesioned mice exhibit increased fragmentation under constant conditions. Relative amplitude and intradaily variability were assessed for both males (top row) and females (bottom row) under DD and LL. Due to the low sample size, data were not statistically analyzed but are graphed to demonstrate observed changes. Data points represent individual mice and are presented as median. Abbreviations: DD: constant darkness; LL: constant light; PBS: phosphate-buffered saline; IB: ibotenic acid.

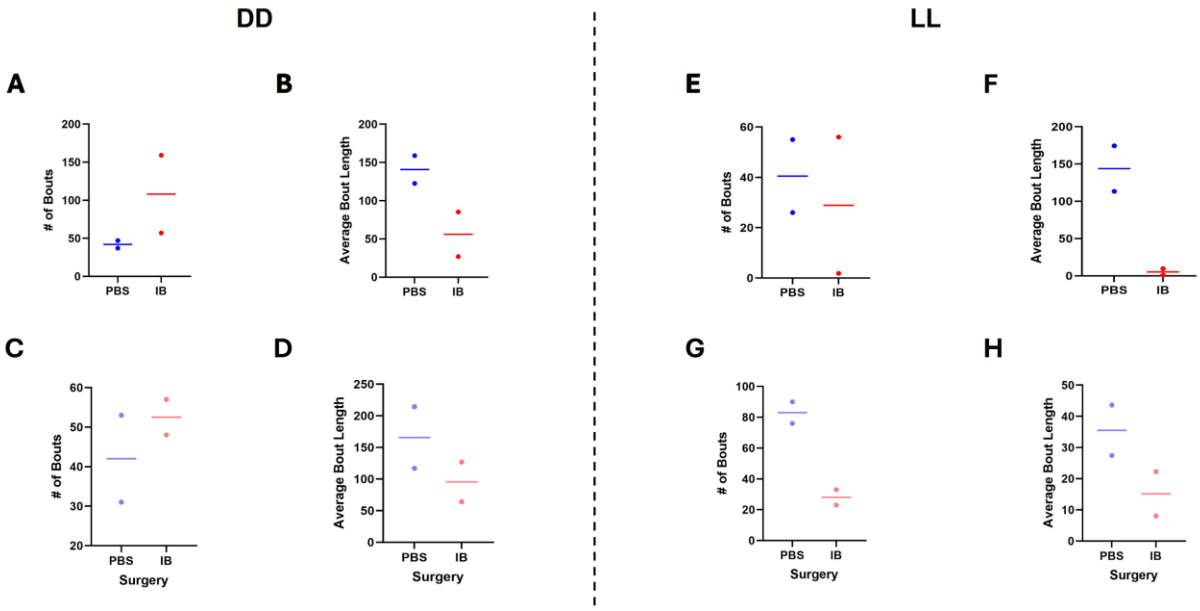


Figure 15. Lesioned mice exhibit increased activity bouts under DD. Total number of bouts and average bout length were assessed for both males (top row) and females (bottom row) under DD and LL. Due to the low sample size, data were not statistically analyzed but are graphed to demonstrate observed changes. Data points represent individual mice and are presented as median. Abbreviations: DD: constant darkness; LL: constant light; PBS: phosphate-buffered saline; IB: ibotenic acid.

Discussion:

SZ is a neuropsychiatric disorder that can be highly debilitating for patients and is among the leading causes of disability worldwide. Its largely unknown pathological origins and poor treatment outcomes underscore the necessity to further our understanding of its etiology. It is widely believed that SZ stems from abnormal neurodevelopment beginning in the prenatal period, as evidenced by structural and functional brain connectivity differences in patients compared to healthy controls that appear prior to the classical presentation of the disorder (Pettersson-Yeo et al., 2011). Additionally, patients consistently show disruptions in sleep (Cohrs, 2008), likely driven in part by alterations in circadian function (Bouteldja et al., 2024). However, the underlying causes of these sleep and circadian disruptions remain unclear. Furthermore, since proper sleep and circadian rhythms are critical for maintaining health, and their dysfunction can lead to cognitive, mood, and overall health impairments, it is essential to determine the extent to which these disruptions contribute to symptom development and severity in SZ patients. Thus, by utilizing the NVHL mouse model, this project aimed to investigate how brain dysconnectivity in brain areas affected in SZ can interact with circadian disruption itself as a risk factor, and whether it can contribute to circadian dysregulation.

Schizophrenia-relevant behavioral changes following circadian disruption during adolescence:

For our first aim, we sought to examine the effects of circadian disruption during adolescence on cognition, affect, locomotor activity, and sociability, as well as social novelty in NVHL mice. We chose these behavioral aspects due to their relevance to SZ. Namely, recognition memory deficits are commonly observed in SZ patients (Pelletier et al., 2005), anxiety is a frequent

symptom (Temmingh & Stein, 2015), and increased locomotor activity may model psychomotor agitation seen in some patients (Elsaid et al., 2019). Additionally, locomotor activity provides insight into the mesolimbic and nigrostriatal dopamine systems, which are involved in regulation of voluntary physical activity (Ruiz-Tejada et al., 2022). Finally, SZ patients typically exhibit social functioning deficits (Langdon et al., 2014). We hypothesized that exposure to CJL during adolescence would worsen the behavioral abnormalities typically observed in the NVHL model, contributing to an additive effect on the morphological and functional brain changes already present.

We employed the NORT to assess recognition memory. To our knowledge, this is the first study to explore object recognition memory specifically in NVHL mice. Contrary to expectations, males did not show significant differences in the time spent exploring the novel versus the familiar object compared to the training phase in any group, suggesting impaired recognition memory. However, except for the LD-PBS group, there was a general trend towards a preference for the novel object. A similar trend was observed in females within the IB groups. We attribute the lack of statistical significance to the limited sample size and high variability in both sexes. This is particularly evident since the absence of recognition memory cannot be ascribed to the mice's age, as they were in early adulthood (PND 80–90), a developmental stage where robust recognition memory is generally observed in both males and females (Cyrenne & Brown, 2011). Additionally, key brain regions involved in object recognition memory, particularly the perirhinal cortex and the hippocampal CA1 region (Abel et al., 2010), are expected to be fully matured at that age.

Interestingly, in females, lesioned mice—regardless of light exposure—exhibited improved recognition memory compared to PBS groups. However, upon closer examination, this

finding seems driven by the PBS groups' lack of recognition memory, as their ratio values hovered around "1", indicating no clear preference for the novel object over the familiar one. In addition, an increase in recognition memory cannot be explained by a lesion of the vHPC. At the very least, we would expect no significant differences in recognition memory, as the vHPC is primarily associated with stress and emotional regulation (Henke, 1990), whereas the dorsal HPCplays a key role in spatial memory (Moser et al., 1995). Furthermore, vHPC lesions that encompass nearly 50% of hippocampal volume have been shown to spare object recognition memory function (Broadbent et al., 2004). However, this anatomical distinction in memory function is not entirely clear, as the vHPC has also been implicated in spatial memory processes (Broadbent et al., 2004; Ferbinteanu et al., 2003). For instance, working memory deficits have been observed when the vHPC is lesioned during the neonatal period in both rats (Lipska et al., 2002) and mice (Naert et al., 2013). Interestingly, these impairments did not appear when the lesions were performed in adulthood, highlighting the critical role of proper maturation of HP-PF circuits for working memory (Wang & Cai, 2006).

While we did not observe any changes in recognition memory driven by CJL, a previous study from our lab using the same paradigm reported a slight reduction in recognition memory in male wild-type mice caused by CJL (Cloutier et al., 2022). In addition, in another study that employed different CJL and NORT protocols, deficits in NORT (after a 60-minute retention interval) were found following 8 weeks of a 6-hour phase advance in the LD cycle, which was associated with a decrease in hippocampal neurogenesis (Horsey et al., 2019). Thus, while aberrant light exposure is known to have an effect on cognition (LeGates et al., 2012), the duration and type of CJL protocol—and by extension, any light exposure paradigm—as well as the subject’s sensitivity to it may determine if that effect is properly exerted.

We used the EPM test to assess anxiety-like behavior. In males, we found no significant differences in the ratio of time spent in the open versus closed arms or in the ratio of entries to open versus closed arms. In females, however, CJL led to a significant increase in time spent in the open arms, particularly in the PBS group, suggesting reduced anxiety-like behavior. This result aligns with the findings of Cloutier et al. (2022), who reported that CJL increased open-arm entries, a phenotype observed primarily in Sandy male mice. However, other studies using different CJL paradigms, such as Horsey et al. (2019), reported opposite effects, with CJL increasing anxiety-like behavior. Furthermore, Acosta et al. (2023) also observed increased anxiety-like behavior following an 8-week CJL protocol, though these changes were detected in the OFT than the EPM, and testing was conducted during the CJL protocol, not after re-entrainment to the LD cycle to ensure they are tested during the same phase and that the observed effects are not acute. These varying results underscore the influence of specific light paradigms on behavioral outcomes.

Contrary to expectations, our lesioned mice did not exhibit the reduced anxiety-like behavior typically seen in NVHL rodents in the EPM. Previous studies in both rats (Beninger et al., 2009; Wood et al., 2003) and mice (Naert et al., 2013) have shown that NVHL animals tend to spend more time in the open arms, a behavior attributed to disrupted connections from the vHPC to the medial prefrontal cortex (mPFC), leading to increased impulsivity and risk-taking (St Onge & Floresco, 2010). Indeed, neonatal lesions of the mPFC lead to reduced anxiety-like behavior (Schwabe et al., 2006). Several factors may explain the lack of effect in our IB mice. First, technical issues related to the EPM test itself could have influenced the results. For instance, some IB mice froze in the open arms, indicating fear, which led to their exclusion from the analysis. Variations in handling, room lighting, environmental conditions (e.g., temperature,

humidity), habituation time, background noise, and specific testing protocols can all introduce variability and affect the reliability of behavioral outcomes (Sukoff Rizzo & Silverman, 2016). Additionally, the small sample size and individual variability within groups could have contributed to the lack of significant findings.

Another key factor is the incomplete confirmation of lesion status. The extent, laterality, and symmetry of the lesions are yet to be fully validated, and these variables, along with the lesioning method, environmental context, and mouse strain used, can all impact the behavioral phenotype (Tseng et al., 2009). For example, Naert et al. (2013), who observed decreased anxiety-like behavior in NVHL mice, performed the lesions at PND7 using an electrolytic method, whereas our study employed IB lesions at PND14. The lesioning method and timing may influence the behavioral outcomes, as Naert et al. (2013) did not observe certain deficits typically associated with NVHL, such as impaired PPI.

We assessed spontaneous locomotor activity and anxiety-like behavior using the OFT. As anticipated, both male and female IB mice displayed significant hyperactivity compared to their PBS counterparts. This locomotor hyperactivity in response to a novel environment is a well-documented characteristic of NVHL rats (Sams-Dodd et al., 1997) and mice (Naert et al., 2013), arising from structural and functional disruptions in prefrontal cortical neurons (O'Donnell, 2012). These alterations affect the mesocorticolimbic dopamine system (Tseng et al., 2006), with antipsychotic treatment—targeting dopamine receptors—able to reverse the hyperactivity induced by NVHL (Lipska et al., 1993; Rueter et al., 2004).

Interestingly, CJL did not induce any locomotor changes in our study. The SCN and its projection targets, such as the lateral hypothalamus, dorsal raphe nucleus, and nucleus accumbens (NAc), can influence locomotor activity (Barbacka-Surowiak & Gut, 2001; Heiss et

al., 2024; Zhu et al., 2016). Goa and colleagues (2020) found that brain glucose metabolism was reduced across multiple brain regions following 34 days of CJL, and they also observed altered gene expression in the PFC and NAc after 10 days of CJL. Siddique et al. (2022) corroborated these findings, further identifying changes in clock gene expression in the raphe nucleus, NAc, and hypothalamus. These studies demonstrate that CJL disrupts the metabolic and molecular function of brain areas involved in key behaviors like locomotion. However, it remains unclear whether these effects are primarily acute, driven by rapid changes in LD cycles, or whether they persist once animals are re-entrained to a standard lighting regimen, potentially explaining the absence of changes in locomotion in this study.

When examining anxiety-like behavior in the OFT, we found that both CJL and IB resulted in reduced anxiety-like behavior in males, as indicated by less time spent in the margins, compared to the LD-PBS group. This finding aligns with previous reports of reduced anxiety-like behavior in NVHL rodents (Beninger et al., 2009; Naert et al., 2013; Wood et al., 2003). However, it contrasts with the increased anxiety-like behavior typically observed following CJL (Acosta et al., 2023; Horsey et al., 2019). Given the large variability and small sample size in the LD-PBS group, we interpret this result cautiously. It is possible that the outcome is driven by an anomalously high amount of time spent in the margins by the LD-PBS group rather than a true decrease in anxiety-like behavior in the CJL and IB groups. Further investigation with a larger sample size is needed to confirm this interpretation.

We assessed sociability and social novelty using the three-chamber test. In males, we observed a pattern similar to the reduced anxiety-like behavior seen in the OFT: mice exposed to CJL and IB showed decreased sociability compared to the LD-PBS group. The effects of NVHL on sociability are mixed, with some studies reporting deficits (Becker & Grecksch, 2000; Becker

et al., 1999; Sams-Dodd et al., 1997), while others have found no changes (Daenen et al., 2002; Drouin-Ouellet et al., 2011; Naert et al., 2013). Notably, in the only mouse behavioral study to date to our knowledge, Naert et al. (2013) reported enhanced sociability in NVHL mice compared to controls. Neurobiologically, both the PFC and AMYG—regions crucial for social behavior—are disrupted in the NVHL model (O'Donnell, 2012; Vazquez-Roque et al., 2014), which would predict deficits in sociability. These discrepancies in findings could be due to the various modifying factors that influence the outcomes of NVHL lesions.

In females, we found a trending effect of CJL on sociability, with CJL leading to decreased sociability compared to PBS-treated mice. This aligns with previous findings from our lab, which showed that CJL decreases sociability in both males and females (Cloutier et al., 2022). Aberrant light exposure through disrupted light-dark cycles has been shown to induce morphological changes and altered gene expression in the PFC, a region critical for social behavior (Karatsoreos et al., 2011; Siddique et al., 2022).

Regarding social novelty, we found a trending effect of surgery in females, where the NVHL resulted in reduced social novelty compared to the PBS groups, suggesting impaired social memory. To our knowledge, the only other study examining social novelty in NVHL mice (Naert et al., 2013) reported no differences between groups; however, their study was conducted in males, in which we also found no changes. We anticipated decreased social novelty, given that brain areas involved in social memory, such as the mPFC and AMYG (Shivakumar et al., 2024), exhibit altered functionality following NVHL lesions (O'Donnell, 2012; Vazquez-Roque et al., 2014).

Interestingly, previous research has shown that female rats retain social recognition for longer periods than males (Bluthe & Dantzer, 1990). These sex differences in social recognition

may arise from neural circuits involving the extrahypothalamic pathways of arginine-vasopressin and oxytocin (Ferguson et al., 2002). It is possible that sex-specific developmental changes in the PFC, HPC, and AMYG, driven by gonadal hormones, contribute to the differential effects of NVHL on social novelty. Indeed, sex differences in morphology and cell signaling within the corticolimbic system, influenced by perinatal and pubertal gonadal hormones, could modulate susceptibility to environmental insults, leading to divergent behavioral outcomes (Premachandran et al., 2020).

In summary, we aimed to assess the impact of circadian disruption during adolescence on behavior in NVHL mice. Contrary to our hypothesis, CJL did not exacerbate the behavioral deficits caused by NVHL, and no significant interaction was observed between the two factors. Our findings indicate that NVHL led to locomotor hyperactivity in both sexes and a reduction in social novelty specifically in females. Additionally, CJL resulted in decreased anxiety-like behavior and reduced sociability in females. These preliminary findings suggest that the long-term effects of exposure to aberrant lighting during adolescence on behavior are complex and may not interact with disrupted neurodevelopment as initially predicted. However, due to the small sample size and high variability in our data, the generalizability of these results is limited. A clearer understanding will emerge once all subjects in the cohort have been fully tested.

The effects of brain circuit dysconnectivity on locomotor activity rhythms:

For our second aim, we sought to investigate the effects of NVHL on circadian function by examining locomotor activity rhythms. We hypothesized that the brain dysconnectivity

characteristic of this model would lead to alterations in locomotor activity rhythms under different lighting conditions.

To date, only one study has examined sleep patterns in the NVHL model to our knowledge. Ahnaou et al. (2007) reported that although the overall organization of sleep—reflected by the time spent in each sleep stage—remained stable, there was a marked slowing in EEG power both at pre-puberty (PND 35) and post-puberty (PND 56). While sleep timing is controlled by a circadian component, it is also governed by a homeostatic sleep drive and other wake- and sleep-promoting systems. Therefore, solely analyzing sleep patterns is not sufficient to characterize circadian function and other more direct measures are needed. In rodents, wheel-running behavior is a well-established approach to assess circadian activity, as locomotor output is regulated by the SCN (Kramer et al., 2001).

We assessed circadian function in NVHL mice by measuring their running wheel activity under different lighting conditions. Under 12:12 LD, all mice entrained well to the LD cycle; however, upon exposure to constant conditions, notable differences emerged between the groups. In DD, male IB mice exhibited more fragmented activity and extended periods of activity compared to PBS mice, with bilateral lesions showing more pronounced effects. Similarly, females with unilateral lesions displayed more fragmented activity than their PBS counterparts, though the effect appeared less intense. The most striking observations occurred under LL. In males, IB mice displayed much lower activity levels compared to the PBS group, with the bilaterally lesioned mouse showing almost no activity. Additionally, activity in these mice was more fragmented than in controls. A similar trend was observed in females, where lesioned mice showed lower activity levels, though one mouse displayed a less pronounced phenotype.

As mentioned, LL induces locomotor arrhythmicity by desynchronizing individual cellular oscillators within the SCN without disrupting their intrinsic rhythms (Ohta et al., 2005). However, only a subset of mice in LL becomes behaviorally arrhythmic, while others either remain rhythmic with a prolonged activity period or exhibit split locomotor activity with two activity bouts per 24 hours (Ohta et al., 2005). In rhythmic mice, the SCN output is more robust due to greater synchrony among individual oscillators, whereas, in split-activity mice, the left and right SCN oscillators seem to oscillate in antiphase (Ohta et al., 2005).

Previous studies utilizing LL generally report that most mice fall into the rhythmic group with prolonged running periods and increased IV (Bhardwaj et al., 2015; Chabot et al., 2012; Delorme et al., 2021; Ikeda et al., 2000; Meyer-Bernstein & Morin, 1996; Tapia-Osorio et al., 2013), while others describe complete arrhythmicity (Rosenwasser & Fixaris, 2013). LL typically reduces trough-to-peak amplitude in activity, as low as 1.04-fold according to Ohta et al. (2005), due to SCN desynchrony. However, to our knowledge, no studies have reported activity levels as low as those seen in IB mice. If locomotor output amplitude reflects SCN network coherence and desynchrony, our findings suggest that IB may disrupt SCN network integrity, increasing its susceptibility to LL exposure.

A key candidate for the potential disruption of SCN network integrity by IB is the mesocorticolimbic system. This dopaminergic network, which includes the PFC, ventral tegmental area (VTA), and NAc, is known to have altered function in response to NVHL (Tseng et al., 2009). Dopamine, the SCN, and the circadian system are closely interlinked. Nearly all aspects of dopamine signaling, such as receptor expression and transport, exhibit circadian rhythms (Castaneda et al., 2004). In addition, brain areas part of the mesocorticolimbic system, including the VTA and NAc, are also under circadian control (Becker-Krail et al., 2022).

Conversely, dopamine can modulate SCN function by acting on dopamine receptors within the SCN, affecting photoentrainment, and the SCN is directly innervated by dopaminergic neurons from the VTA (Grippo et al., 2017). Beyond the SCN, dopamine plays a role in regulating circadian rhythms in the retina, modulating light responsiveness and its transmission to the SCN (Mendoza & Challet, 2014).

Preclinical studies provide evidence for dopamine's role in altering SCN function. Fifel and Cooper (2014) demonstrated that mice with progressive degeneration of midbrain dopamine neurons showed normal entrainment to a 12:12 LD cycle but exhibited increased IV and, in some cases, complete arrhythmicity under constant conditions. Interestingly, these mice did not display differences in overall locomotor activity compared to controls. In a related study, dopamine depletion by lesioning the nigrostriatal system in rhesus monkeys resulted in disrupted or abolished locomotor rhythms in LL (Fifel et al., 2014).

It is also possible that IB exerts its effects outside of the SCN. In the same study by Fifel et al. (2014), melatonin and cortisol levels remained unaltered in lesioned rhesus monkeys, suggesting intact SCN function. Therefore, altered dopamine function may be affecting locomotor activity in regions downstream of the SCN. For example, lesioning dopamine fibers in the medial forebrain altered PER2 expression in several motor-related regions, such as the dorsal striatum, without disrupting PER2 rhythms in the SCN (Gravotta et al., 2011; Hood et al., 2010). These lesions led to arrhythmicity and reduced overall activity in constant darkness, while proper entrainment to the 12:12 LD cycle was maintained (Gravotta et al., 2011). Thus, in line with Fifel and Cooper's (2014) study, the altered dopaminergic function induced by IB could impair dopamine-driven circadian control in motor-related regions like the dorsal striatum or VTA, resulting in the observed deficits in locomotor activity under constant conditions.

Beyond these potential effects, it is crucial to consider that IB could have impacted nearby regions of the vHPC, contributing to the observed phenotype. One such region is the AMYG, which is in close proximity to the vHPC and plays a key role in both emotional processing and dopaminergic function, thereby modulating stress and anxiety-like responses. Thus, its involvement with dopaminergic areas like the VTA may impact stress response, motivation, and locomotor output (Beier et al., 2015). For instance, the AMYG could enhance stress reactivity to LL exposure, thereby contributing to the large reduction in overall activity.

Interestingly, our results suggest that even a unilateral lesion produced deficits, though to a lesser extent than bilateral lesions. This suggests that the unilateral NVHL induces profound impairments in brain connectivity, particularly within the mesocorticolimbic system, that cannot be fully compensated by the intact hemisphere. The vHPC's critical role in linking cortical and subcortical circuits, including its influence on areas involved in motor control and emotional regulation, may explain why unilateral lesions still produce significant circadian and behavioral disruptions.

In summary, this study represents the first assessment of circadian behavior in the NVHL model. Although the current analysis includes a limited number of mice, the findings suggest that NVHL may lead to circadian disruptions, potentially linked to alterations in the dopamine system. While these initial results are promising, further confirmation will be needed with the inclusion of more subjects to solidify these conclusions.

Conclusion:

In conclusion, this study investigated how circadian disruption during adolescence and brain dysconnectivity influence behavior and affect circadian function using the NVHL mouse model. We assessed cognition, affect, locomotion, and sociability after CJL exposure, and analyzed wheel-running behavior to evaluate circadian rhythms.

For the first aim, we predicted an interaction between CJL and NVHL but found no significant effects. Instead, NVHL caused hyperactivity in both sexes and reduced social novelty in females, while CJL decreased anxiety-like behavior and sociability in females. For the second aim, we hypothesized NVHL would disrupt circadian function. Preliminary results from a small sample indicated NVHL alters circadian rhythms under constant lighting in both sexes. The interpretation of these findings is limited by the low sample size and pending confirmation of lesion status. Nevertheless, the observed circadian dysregulation associated with NVHL offers valuable insight into how the affected neural pathways may influence circadian function within the context of schizophrenia. As the study progresses and additional subjects are assessed, a more complete picture will likely emerge.

Should these preliminary results be corroborated upon completion of the study, follow-up experiments should aim to investigate the underlying mechanisms driving these alterations, particularly under constant light conditions. For example, exploring the light-responsive pathways or assessing dopamine signaling in response to light pulses could shed light on why lesioning the vHPC can have these effects. This can be achieved by exposing NVHL and control mice to a light pulse following a period in DD, then extracting their brains at the same circadian time. Immunohistochemistry (IHC) can then be used to measure c-Fos expression (a marker of neuronal activation) in various light-responsive regions (e.g., SCN and neurons along the

retinohypothalamic tract), as well as dopamine-related markers (e.g., tyrosine hydroxylase and dopamine transporter) in dopaminergic areas such as the VTA and NAc. Furthermore, dopamine rhythms can be measured by extracting the brain at different circadian time points following DD and performing IHC.

Additionally, the SCN of *Per2*-Luciferase mice—which allow for measuring *Per2* gene expression activity through bioluminescence—with an NVHL can be dissected and explanted following LL to assess for clock gene expression at the network level by using a Lumicycle. Further, *Per2* expression at the neuronal level can be measured using a high-resolution bioluminescence microscope, which would allow to measure individual cellular rhythms within SCN. Finally, although no significant interactions were found between NVHL and CJL in this study, exploring alternative paradigms of circadian disruption may reveal interactions that were not captured by the current model.

Bibliography

Abel, K. M., Drake, R., & Goldstein, J. M. (2010). Sex differences in schizophrenia. *Int Rev Psychiatry*, *22*(5), 417-428. https://doi.org/10.3109/09540261.2010.515205

Acosta, J., Crespo, M. T., Plano, S. A., Golombek, D. A., Chiesa, J. J., & Agostino, P. V. (2023). Chronic jet lag reduces motivation and affects other mood-related behaviors in male mice. *Front Physiol*, *14*, 1225134. https://doi.org/10.3389/fphys.2023.1225134

Addington, J., & Heinssen, R. (2012). Prediction and prevention of psychosis in youth at clinical high risk. *Annu Rev Clin Psychol*, *8*, 269-289. https://doi.org/10.1146/annurev-clinpsy-032511-143146

Ahnaou, A., Nayak, S., Heylen, A., Ashton, D., & Drinkenburg, W. H. (2007). Sleep and EEG profile in neonatal hippocampal lesion model of schizophrenia. *Physiol Behav*, *92*(3), 461-467. https://doi.org/10.1016/j.physbeh.2007.04.020

Ameen, R. W., Warshawski, A., Fu, L., & Antle, M. C. (2022). Early life circadian rhythm disruption in mice alters brain and behavior in adulthood. *Sci Rep*, *12*(1), 7366. https://doi.org/10.1038/s41598-022-11335-0

Anand, K. S., & Dhikav, V. (2012). Hippocampus in health and disease: An overview. *Ann Indian Acad Neurol*, *15*(4), 239-246. https://doi.org/10.4103/0972-2327.104323

Antunes, M., & Biala, G. (2012). The novel object recognition memory: neurobiology, test procedure, and its modifications. *Cogn Process*, *13*(2), 93-110. https://doi.org/10.1007/s10339-011-0430-z

Bahner, F., & Meyer-Lindenberg, A. (2017). Hippocampal-prefrontal connectivity as a translational phenotype for schizophrenia. *Eur Neuropsychopharmacol*, *27*(2), 93-106. https://doi.org/10.1016/j.euroneuro.2016.12.007

Bannerman, D. M., Rawlins, J. N., McHugh, S. B., Deacon, R. M., Yee, B. K., Bast, T., Zhang, W. N., Pothuizen, H. H., & Feldon, J. (2004). Regional dissociations within the hippocampus--memory and anxiety. *Neurosci Biobehav Rev*, *28*(3), 273-283. https://doi.org/10.1016/j.neubiorev.2004.03.004

Barbacka-Surowiak, G., & Gut, M. (2001). The effect of dorsal raphe nucleus (DRN) lesions on the locomotor activity rhythm in mice. *Folia Biol (Krakow)*, *49*(1-2), 77-84. https://www.ncbi.nlm.nih.gov/pubmed/11732169

Bastos, M. A. V., Jr., Oliveira Bastos, P. R. H., Portella, R. B., Soares, L. F. G., Conde, R. B., Rodrigues, P. M. F., Jr., & Lucchetti, G. (2019). Pineal gland and schizophrenia: A systematic review and meta-analysis. *Psychoneuroendocrinology*, *104*, 100-114. https://doi.org/10.1016/j.psyneuen.2019.02.024

Becker, A., & Grecksch, G. (2000). Social memory is impaired in neonatally ibotenic acid lesioned rats. *Behav Brain Res*, *109*(1), 137-140. https://doi.org/10.1016/s0166-4328(99)00163-1

Becker, A., Grecksch, G., Bernstein, H. G., Hollt, V., & Bogerts, B. (1999). Social behaviour in rats lesioned with ibotenic acid in the hippocampus: quantitative and qualitative analysis. *Psychopharmacology (Berl)*, *144*(4), 333-338. https://doi.org/10.1007/s002130051015

Becker-Krail, D. D., Walker, W. H., 2nd, & Nelson, R. J. (2022). The Ventral Tegmental Area and Nucleus Accumbens as Circadian Oscillators: Implications for Drug Abuse and Substance Use Disorders. *Front Physiol*, *13*, 886704. https://doi.org/10.3389/fphys.2022.886704

Beier, K. T., Steinberg, E. E., DeLoach, K. E., Xie, S., Miyamichi, K., Schwarz, L., Gao, X. J., Kremer, E. J., Malenka, R. C., & Luo, L. (2015). Circuit Architecture of VTA Dopamine Neurons Revealed by Systematic Input-Output Mapping. *Cell*, *162*(3), 622-634. https://doi.org/10.1016/j.cell.2015.07.015

Benetti, S., Mechelli, A., Picchioni, M., Broome, M., Williams, S., & McGuire, P. (2009). Functional integration between the posterior hippocampus and prefrontal cortex is impaired in both first episode schizophrenia and the at risk mental state. *Brain*, *132*(Pt 9), 2426-2436. https://doi.org/10.1093/brain/awp098

Beninger, R. J., Tuerke, K. J., Forsyth, J. K., Giles, A., Xue, L., Boegman, R. J., & Jhamandas, K. (2009). Neonatal ventral hippocampal lesions in male and female rats: effects on water maze, locomotor activity, plus-maze and prefrontal cortical GABA and glutamate release in adulthood. *Behav Brain Res*, *202*(2), 198-209. https://doi.org/10.1016/j.bbr.2009.03.044

Bhardwaj, S. K., Stojkovic, K., Kiessling, S., Srivastava, L. K., & Cermakian, N. (2015). Constant light uncovers behavioral effects of a mutation in the schizophrenia risk gene Dtnbp1 in mice. *Behav Brain Res*, *284*, 58-68. https://doi.org/10.1016/j.bbr.2015.01.048

Bluthe, R. M., & Dantzer, R. (1990). Social recognition does not involve vasopressinergic neurotransmission in female rats. *Brain Res*, *535*(2), 301-304. https://doi.org/10.1016/0006-8993(90)91613-l

Bouteldja, A. A., Penichet, D., Srivastava, L. K., & Cermakian, N. (2024). The circadian system: A neglected player in neurodevelopmental disorders. *Eur J Neurosci*, *60*(2), 3858-3890. https://doi.org/10.1111/ejn.16423

Brainard, J., Gobel, M., Scott, B., Koeppen, M., & Eckle, T. (2015). Health implications of disrupted circadian rhythms and the potential for daylight as therapy. *Anesthesiology*, *122*(5), 1170-1175. https://doi.org/10.1097/ALN.0000000000000596

Broadbent, N. J., Squire, L. R., & Clark, R. E. (2004). Spatial memory, recognition memory, and the hippocampus. *Proc Natl Acad Sci U S A*, *101*(40), 14515-14520. https://doi.org/10.1073/pnas.0406344101

Bromundt, V., Koster, M., Georgiev-Kill, A., Opwis, K., Wirz-Justice, A., Stoppe, G., & Cajochen, C. (2011). Sleep-wake cycles and cognitive functioning in schizophrenia. *Br J Psychiatry*, *198*(4), 269-276. https://doi.org/10.1192/bjp.bp.110.078022

Cai, M., Wang, R., Liu, M., Du, X., Xue, K., Ji, Y., Wang, Z., Zhang, Y., Guo, L., Qin, W., Zhu, W., Fu, J., & Liu, F. (2022). Disrupted local functional connectivity in schizophrenia: An updated and extended meta-analysis. *Schizophrenia (Heidelb)*, *8*(1), 93. https://doi.org/10.1038/s41537-022-00311-2

Cannon, T. D., Chung, Y., He, G., Sun, D., Jacobson, A., van Erp, T. G., McEwen, S., Addington, J., Bearden, C. E., Cadenhead, K., Cornblatt, B., Mathalon, D. H., McGlashan, T., Perkins, D., Jeffries, C., Seidman, L. J., Tsuang, M., Walker, E., Woods, S. W.,…North American Prodrome Longitudinal Study, C. (2015). Progressive reduction in cortical thickness as psychosis develops: a multisite longitudinal neuroimaging study of youth at elevated clinical risk. *Biol Psychiatry*, *77*(2), 147-157. https://doi.org/10.1016/j.biopsych.2014.05.023

Castaneda, T. R., de Prado, B. M., Prieto, D., & Mora, F. (2004). Circadian rhythms of dopamine, glutamate and GABA in the striatum and nucleus accumbens of the awake rat: modulation by light. *J Pineal Res*, *36*(3), 177-185. https://doi.org/10.1046/j.1600-079x.2003.00114.x

Castro, J., Zanini, M., Goncalves Bda, S., Coelho, F. M., Bressan, R., Bittencourt, L., Gadelha, A., Brietzke, E., & Tufik, S. (2015). Circadian rest-activity rhythm in individuals at risk for psychosis and bipolar disorder. *Schizophr Res*, *168*(1-2), 50-55. https://doi.org/10.1016/j.schres.2015.07.024

Chabot, C. C., Connolly, D. M., & Waring, B. B. (2012). The effects of lighting conditions and food restriction paradigms on locomotor activity of common spiny mice, Acomys cahirinus. *J Circadian Rhythms*, *10*(1), 6. https://doi.org/10.1186/1740-3391-10-6

Choudhury, Z., & Lennox, B. (2021). Maternal Immune Activation and Schizophrenia-Evidence for an Immune Priming Disorder. *Front Psychiatry*, *12*, 585742. https://doi.org/10.3389/fpsyt.2021.585742

Cinalli, D. A., Jr., Cohen, S. J., Guthrie, K., & Stackman, R. W., Jr. (2020). Object Recognition Memory: Distinct Yet Complementary Roles of the Mouse CA1 and Perirhinal Cortex. *Front Mol Neurosci*, *13*, 527543. https://doi.org/10.3389/fnmol.2020.527543

Cloutier, M. E., Srivastava, L. K., & Cermakian, N. (2022). Exposure to Circadian Disruption During Adolescence Interacts With a Genetic Risk Factor to Modify Schizophrenia-relevant Behaviors in a Sex-dependent Manner. *J Biol Rhythms*, *37*(6), 655-672. https://doi.org/10.1177/07487304221125363

Cohrs, S. (2008). Sleep disturbances in patients with schizophrenia : impact and effect of antipsychotics. *CNS Drugs*, *22*(11), 939-962. https://doi.org/10.2165/00023210-200822110-00004

Cox, K. H., & Takahashi, J. S. (2019). Circadian clock genes and the transcriptional architecture of the clock mechanism. *J Mol Endocrinol*, *63*(4), R93-R102. https://doi.org/10.1530/JME-19-0153

Coyle, J. T., Balu, D. T., Puhl, M. D., & Konopaske, G. T. (2016). History of the Concept of Disconnectivity in Schizophrenia. *Harv Rev Psychiatry*, *24*(2), 80-86. https://doi.org/10.1097/HRP.0000000000000102

Cyrenne, D. L., & Brown, G. R. (2011). Ontogeny of sex differences in response to novel objects from adolescence to adulthood in lister-hooded rats. *Dev Psychobiol*, *53*(7), 670-676. https://doi.org/10.1002/dev.20542

Daenen, E. W., Wolterink, G., Gerrits, M. A., & Van Ree, J. M. (2002). The effects of neonatal lesions in the amygdala or ventral hippocampus on social behaviour later in life. *Behav Brain Res*, *136*(2), 571-582. https://doi.org/10.1016/s0166-4328(02)00223-1

Delorme, T. C., Ozell-Landry, W., Cermakian, N., & Srivastava, L. K. (2023). Behavioral and cellular responses to circadian disruption and prenatal immune activation in mice. *Sci Rep*, *13*(1), 7791. https://doi.org/10.1038/s41598-023-34363-w

Delorme, T. C., Srivastava, L. K., & Cermakian, N. (2020). Are Circadian Disturbances a Core Pathophysiological Component of Schizophrenia? *J Biol Rhythms*, *35*(4), 325-339. https://doi.org/10.1177/0748730420929448

Delorme, T. C., Srivastava, L. K., & Cermakian, N. (2021). Altered circadian rhythms in a mouse model of neurodevelopmental disorders based on prenatal maternal immune activation. *Brain Behav Immun*, *93*, 119-131. https://doi.org/10.1016/j.bbi.2020.12.030

Dickerson, D. D., Wolff, A. R., & Bilkey, D. K. (2010). Abnormal long-range neural synchrony in a maternal immune activation animal model of schizophrenia. *J Neurosci*, *30*(37), 12424-12431. https://doi.org/10.1523/JNEUROSCI.3046-10.2010

Drouin-Ouellet, J., Brownell, A. L., Saint-Pierre, M., Fasano, C., Emond, V., Trudeau, L. E., Levesque, D., & Cicchetti, F. (2011). Neuroinflammation is associated with changes in glial mGluR5 expression and the development of neonatal excitotoxic lesions. *Glia*, *59*(2), 188-199. https://doi.org/10.1002/glia.21086

Duffy, J. F., Cain, S. W., Chang, A. M., Phillips, A. J., Munch, M. Y., Gronfier, C., Wyatt, J. K., Dijk, D. J., Wright, K. P., Jr., & Czeisler, C. A. (2011). Sex difference in the near-24-hour intrinsic period of the human circadian timing system. *Proc Natl Acad Sci U S A*, *108 Suppl 3*(Suppl 3), 15602-15608. https://doi.org/10.1073/pnas.1010666108

Duffy, J. F., & Wright, K. P., Jr. (2005). Entrainment of the human circadian system by light. *J Biol Rhythms*, *20*(4), 326-338. https://doi.org/10.1177/0748730405277983

Elsaid, S., Kloiber, S., & Le Foll, B. (2019). Effects of cannabidiol (CBD) in neuropsychiatric disorders: A review of pre-clinical and clinical findings. *Prog Mol Biol Transl Sci*, *167*, 25-75. https://doi.org/10.1016/bs.pmbts.2019.06.005

Fan, F. M., Tan, S. P., Yang, F. D., Tan, Y. L., Zhao, Y. L., Chen, N., Li, B. B., Song, C. S., Wang, Y. H., Jin, Z., Zhou, D. F., Milham, M. P., Zou, Y. Z., & Zuo, X. N. (2013). Ventral medial prefrontal functional connectivity and emotion regulation in chronic schizophrenia: a pilot study. *Neurosci Bull*, *29*(1), 59-74. https://doi.org/10.1007/s12264-013-1300-8

Ferbinteanu, J., Ray, C., & McDonald, R. J. (2003). Both dorsal and ventral hippocampus contribute to spatial learning in Long-Evans rats. *Neurosci Lett*, *345*(2), 131-135. https://doi.org/10.1016/s0304-3940(03)00473-7

Ferguson, J. N., Young, L. J., & Insel, T. R. (2002). The neuroendocrine basis of social recognition. *Front Neuroendocrinol*, *23*(2), 200-224. https://doi.org/10.1006/frne.2002.0229

Fernandez, D. C., Fogerson, P. M., Lazzerini Ospri, L., Thomsen, M. B., Layne, R. M., Severin, D., Zhan, J., Singer, J. H., Kirkwood, A., Zhao, H., Berson, D. M., & Hattar, S. (2018). Light Affects Mood and Learning through Distinct Retina-Brain Pathways. *Cell*, *175*(1), 71-84 e18. https://doi.org/10.1016/j.cell.2018.08.004

Ferrarelli, F. (2021). Sleep Abnormalities in Schizophrenia: State of the Art and Next Steps. *Am J Psychiatry*, *178*(10), 903-913. https://doi.org/10.1176/appi.ajp.2020.20070968

Fifel, K., & Cooper, H. M. (2014). Loss of dopamine disrupts circadian rhythms in a mouse model of Parkinson's disease. *Neurobiol Dis*, *71*, 359-369. https://doi.org/10.1016/j.nbd.2014.08.024

Fifel, K., Vezoli, J., Dzahini, K., Claustrat, B., Leviel, V., Kennedy, H., Procyk, E., Dkhissi-Benyahya, O., Gronfier, C., & Cooper, H. M. (2014). Alteration of daily and circadian rhythms following dopamine depletion in MPTP treated non-human primates. *PLoS One*, *9*(1), e86240. https://doi.org/10.1371/journal.pone.0086240

Flores, G., Alquicer, G., Silva-Gomez, A. B., Zaldivar, G., Stewart, J., Quirion, R., & Srivastava, L. K. (2005). Alterations in dendritic morphology of prefrontal cortical and nucleus accumbens neurons in post-pubertal rats after neonatal excitotoxic lesions of the ventral hippocampus. *Neuroscience*, *133*(2), 463-470. https://doi.org/10.1016/j.neuroscience.2005.02.021

Gao, Q., Khan, S., & Zhang, L. (2020). Brain activity and transcriptional profiling in mice under chronic jet lag. *Sci Data*, *7*(1), 361. https://doi.org/10.1038/s41597-020-00709-6

Gidziela, A., Ahmadzadeh, Y. I., Michelini, G., Allegrini, A. G., Agnew-Blais, J., Lau, L. Y., Duret, M., Procopio, F., Daly, E., Ronald, A., Rimfeld, K., & Malanchini, M. (2023). A meta-analysis of genetic effects associated with neurodevelopmental disorders and co-occurring conditions. *Nat Hum Behav*, *7*(4), 642-656. https://doi.org/10.1038/s41562-023-01530-y

Goeree, R., Farahati, F., Burke, N., Blackhouse, G., O'Reilly, D., Pyne, J., & Tarride, J. E. (2005). The economic burden of schizophrenia in Canada in 2004. *Curr Med Res Opin*, *21*(12), 2017-2028. https://doi.org/10.1185/030079905X75087

Goncalves, B., Castro, J., Zanini, M. A., Bittencourt, L., Gadelha, A., Cunha, G. R., Coelho, F. M., Tufik, S., Bressan, R. A., & Brietzke, E. (2016). Circadian rhythm disturbances and conversion to psychosis in ultra high-risk youth. *Braz J Psychiatry*, *38*(2), 177-179. https://doi.org/10.1590/1516-4446-2015-1859

Gravotta, L., Gavrila, A. M., Hood, S., & Amir, S. (2011). Global depletion of dopamine using intracerebroventricular 6-hydroxydopamine injection disrupts normal circadian wheel-running patterns and PERIOD2 expression in the rat forebrain. *J Mol Neurosci*, *45*(2), 162-171. https://doi.org/10.1007/s12031-011-9520-8

Grippo, R. M., Purohit, A. M., Zhang, Q., Zweifel, L. S., & Guler, A. D. (2017). Direct Midbrain Dopamine Input to the Suprachiasmatic Nucleus Accelerates Circadian Entrainment. *Curr Biol*, *27*(16), 2465-2475 e2463. https://doi.org/10.1016/j.cub.2017.06.084

Hagenauer, M. H., & Lee, T. M. (2012). The neuroendocrine control of the circadian system: adolescent chronotype. *Front Neuroendocrinol*, *33*(3), 211-229. https://doi.org/10.1016/j.yfrne.2012.04.003

Hattar, S., Liao, H. W., Takao, M., Berson, D. M., & Yau, K. W. (2002). Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. *Science*, *295*(5557), 1065-1070. https://doi.org/10.1126/science.1069609

Heiss, J. E., Zhong, P., Lee, S. M., Yamanaka, A., & Kilduff, T. S. (2024). Distinct lateral hypothalamic CaMKIIalpha neuronal populations regulate wakefulness and locomotor activity. *Proc Natl Acad Sci U S A*, *121*(16), e2316150121. https://doi.org/10.1073/pnas.2316150121

Henke, P. G. (1990). Hippocampal pathway to the amygdala and stress ulcer development. *Brain Res Bull*, *25*(5), 691-695. https://doi.org/10.1016/0361-9230(90)90044-z

Heyser, C. J., & Chemero, A. (2012). Novel object exploration in mice: not all objects are created equal. *Behav Processes*, *89*(3), 232-238. https://doi.org/10.1016/j.beproc.2011.12.004

Hilker, R., Helenius, D., Fagerlund, B., Skytthe, A., Christensen, K., Werge, T. M., Nordentoft, M., & Glenthoj, B. (2018). Heritability of Schizophrenia and Schizophrenia Spectrum Based on the Nationwide Danish Twin Register. *Biol Psychiatry*, *83*(6), 492-498. https://doi.org/10.1016/j.biopsych.2017.08.017

Ho, B. C., Andreasen, N. C., Nopoulos, P., Arndt, S., Magnotta, V., & Flaum, M. (2003). Progressive structural brain abnormalities and their relationship to clinical outcome: a longitudinal magnetic resonance imaging study early in schizophrenia. *Arch Gen Psychiatry*, *60*(6), 585-594. https://doi.org/10.1001/archpsyc.60.6.585

Ho, N. F., Holt, D. J., Cheung, M., Iglesias, J. E., Goh, A., Wang, M., Lim, J. K. W., de Souza, J., Poh, J. S., See, Y. M., Adcock, R. A., Wood, S. J., Chee, M. W. L., Lee, J., & Zhou, J. (2019). Correction: Progressive decline in hippocampal CA1 volume in individuals at ultra-high-risk for psychosis who do not remit: findings from the longitudinal youth at risk study. *Neuropsychopharmacology*, *44*(12), 2144. https://doi.org/10.1038/s41386-019-0477-6

Hood, S., Cassidy, P., Cossette, M. P., Weigl, Y., Verwey, M., Robinson, B., Stewart, J., & Amir, S. (2010). Endogenous dopamine regulates the rhythm of expression of the clock protein PER2 in the rat dorsal striatum via daily activation of D2 dopamine receptors. *J Neurosci*, *30*(42), 14046-14058. https://doi.org/10.1523/JNEUROSCI.2128-10.2010

Horsey, E. A., Maletta, T., Turner, H., Cole, C., Lehmann, H., & Fournier, N. M. (2019). Chronic Jet Lag Simulation Decreases Hippocampal Neurogenesis and Enhances Depressive Behaviors and Cognitive Deficits in Adult Male Rats. *Front Behav Neurosci*, *13*, 272. https://doi.org/10.3389/fnbeh.2019.00272

Ikeda, M., Sagara, M., & Inoue, S. (2000). Continuous exposure to dim illumination uncouples temporal patterns of sleep, body temperature, locomotion and drinking behavior in the rat. *Neurosci Lett*, *279*(3), 185-189. https://doi.org/10.1016/s0304-3940(99)00943-x

Jonsson, L., Anckarsater, H., Zettergren, A., Westberg, L., Walum, H., Lundstrom, S., Larsson, H., Lichtenstein, P., & Melke, J. (2014). Association between ASMT and autistic-like traits in children from a Swedish nationwide cohort. *Psychiatr Genet*, *24*(1), 21-27. https://doi.org/10.1097/YPG.0000000000000010

Kadar, A., Wittmann, G., Liposits, Z., & Fekete, C. (2009). Improved method for combination of immunocytochemistry and Nissl staining. *J Neurosci Methods*, *184*(1), 115-118. https://doi.org/10.1016/j.jneumeth.2009.07.010

Kammerer, M. K., Mehl, S., Ludwig, L., & Lincoln, T. M. (2021). Sleep and circadian rhythm disruption predict persecutory symptom severity in day-to-day life: A combined actigraphy and experience sampling study. *J Abnorm Psychol*, *130*(1), 78-88. https://doi.org/10.1037/abn0000645

Karatsoreos, I. N., Bhagat, S., Bloss, E. B., Morrison, J. H., & McEwen, B. S. (2011). Disruption of circadian clocks has ramifications for metabolism, brain, and behavior. *Proc Natl Acad Sci U S A*, *108*(4), 1657-1662. https://doi.org/10.1073/pnas.1018375108

Karayiorgou, M., Simon, T. J., & Gogos, J. A. (2010). 22q11.2 microdeletions: linking DNA structural variation to brain dysfunction and schizophrenia. *Nat Rev Neurosci*, *11*(6), 402-416. https://doi.org/10.1038/nrn2841

Ko, C. H., & Takahashi, J. S. (2006). Molecular components of the mammalian circadian clock. *Hum Mol Genet*, *15 Spec No 2*, R271-277. https://doi.org/10.1093/hmg/ddl207

Konrad, K., Firk, C., & Uhlhaas, P. J. (2013). Brain development during adolescence: neuroscientific insights into this developmental period. *Dtsch Arztebl Int*, *110*(25), 425-431. https://doi.org/10.3238/arztebl.2013.0425

Kramer, A., Yang, F. C., Snodgrass, P., Li, X., Scammell, T. E., Davis, F. C., & Weitz, C. J. (2001). Regulation of daily locomotor activity and sleep by hypothalamic EGF receptor signaling. *Science*, *294*(5551), 2511-2515. https://doi.org/10.1126/science.1067716

Kravitz, A. V., & Matikainen-Ankney, B. A. (2020). Motor Control: Memory and Motor Control in the Dorsal Striatum. *Curr Biol*, *30*(22), R1366-R1368. https://doi.org/10.1016/j.cub.2020.09.018

Kumari, V., Soni, W., Mathew, V. M., & Sharma, T. (2000). Prepulse inhibition of the startle response in men with schizophrenia: effects of age of onset of illness, symptoms, and medication. *Arch Gen Psychiatry*, *57*(6), 609-614. https://doi.org/10.1001/archpsyc.57.6.609

Langdon, R., Connors, M. H., & Connaughton, E. (2014). Social cognition and social judgment in schizophrenia. *Schizophr Res Cogn*, *1*(4), 171-174. https://doi.org/10.1016/j.scog.2014.10.001

Laskemoen, J. F., Simonsen, C., Buchmann, C., Barrett, E. A., Bjella, T., Lagerberg, T. V., Vedal, T. J., Andreassen, O. A., Melle, I., & Aas, M. (2019). Sleep disturbances in schizophrenia spectrum

and bipolar disorders - a transdiagnostic perspective. *Compr Psychiatry*, *91*, 6-12. https://doi.org/10.1016/j.comppsych.2019.02.006

Lazzerini Ospri, L., Zhan, J. J., Thomsen, M. B., Wang, H., Komal, R., Tang, Q., Messanvi, F., du Hoffmann, J., Cravedi, K., Chudasama, Y., Hattar, S., & Zhao, H. (2024). Light affects the prefrontal cortex via intrinsically photosensitive retinal ganglion cells. *Sci Adv*, *10*(13), eadh9251. https://doi.org/10.1126/sciadv.adh9251

Le Pen, G., & Moreau, J. L. (2002). Disruption of prepulse inhibition of startle reflex in a neurodevelopmental model of schizophrenia: reversal by clozapine, olanzapine and risperidone but not by haloperidol. *Neuropsychopharmacology*, *27*(1), 1-11. https://doi.org/10.1016/S0893-133X(01)00383-9

Lee, H., Dvorak, D., Kao, H. Y., Duffy, A. M., Scharfman, H. E., & Fenton, A. A. (2012). Early cognitive experience prevents adult deficits in a neurodevelopmental schizophrenia model. *Neuron*, *75*(4), 714-724. https://doi.org/10.1016/j.neuron.2012.06.016

LeGates, T. A., Altimus, C. M., Wang, H., Lee, H. K., Yang, S., Zhao, H., Kirkwood, A., Weber, E. T., & Hattar, S. (2012). Aberrant light directly impairs mood and learning through melanopsin-expressing neurons. *Nature*, *491*(7425), 594-598. https://doi.org/10.1038/nature11673

LeGates, T. A., Fernandez, D. C., & Hattar, S. (2014). Light as a central modulator of circadian rhythms, sleep and affect. *Nat Rev Neurosci*, *15*(7), 443-454. https://doi.org/10.1038/nrn3743

Leger, M., Quiedeville, A., Bouet, V., Haelewyn, B., Boulouard, M., Schumann-Bard, P., & Freret, T. (2013). Object recognition test in mice. *Nat Protoc*, *8*(12), 2531-2537. https://doi.org/10.1038/nprot.2013.155

Li, R., Ma, X., Wang, G., Yang, J., & Wang, C. (2016). Why sex differences in schizophrenia? *J Transl Neurosci (Beijing)*, *1*(1), 37-42. https://www.ncbi.nlm.nih.gov/pubmed/29152382

Lipska, B. K., Aultman, J. M., Verma, A., Weinberger, D. R., & Moghaddam, B. (2002). Neonatal damage of the ventral hippocampus impairs working memory in the rat. *Neuropsychopharmacology*, *27*(1), 47-54. https://doi.org/10.1016/S0893-133X(02)00282-8

Lipska, B. K., Jaskiw, G. E., & Weinberger, D. R. (1993). Postpubertal emergence of hyperresponsiveness to stress and to amphetamine after neonatal excitotoxic hippocampal damage: a potential animal model of schizophrenia. *Neuropsychopharmacology*, *9*(1), 67-75. https://doi.org/10.1038/npp.1993.44

Lipska, B. K., & Weinberger, D. R. (1994). Subchronic treatment with haloperidol and clozapine in rats with neonatal excitotoxic hippocampal damage. *Neuropsychopharmacology*, *10*(3), 199-205. https://doi.org/10.1038/npp.1994.22

Lunsford-Avery, J. R., Goncalves, B., Brietzke, E., Bressan, R. A., Gadelha, A., Auerbach, R. P., & Mittal, V. A. (2017). Adolescents at clinical-high risk for psychosis: Circadian rhythm disturbances predict worsened prognosis at 1-year follow-up. *Schizophr Res*, *189*, 37-42. https://doi.org/10.1016/j.schres.2017.01.051

Mancini, V., Sandini, C., Padula, M. C., Zoller, D., Schneider, M., Schaer, M., & Eliez, S. (2020). Positive psychotic symptoms are associated with divergent developmental trajectories of hippocampal volume during late adolescence in patients with 22q11DS. *Mol Psychiatry*, *25*(11), 2844-2859. https://doi.org/10.1038/s41380-019-0443-z

Marcheva, B., Ramsey, K. M., Peek, C. B., Affinati, A., Maury, E., & Bass, J. (2013). Circadian clocks and metabolism. *Handb Exp Pharmacol*(217), 127-155. https://doi.org/10.1007/978-3-642-25950-0_6

Mathalon, D. H., Sullivan, E. V., Lim, K. O., & Pfefferbaum, A. (2001). Progressive brain volume changes and the clinical course of schizophrenia in men: a longitudinal magnetic resonance imaging study. *Arch Gen Psychiatry*, *58*(2), 148-157. https://doi.org/10.1001/archpsyc.58.2.148

McHugo, M., Roeske, M. J., Vandekar, S. N., Armstrong, K., Avery, S. N., & Heckers, S. (2024). Smaller anterior hippocampal subfields in the early stage of psychosis. *Transl Psychiatry*, *14*(1), 69. https://doi.org/10.1038/s41398-023-02719-5

Mendoza, J., & Challet, E. (2014). Circadian insights into dopamine mechanisms. *Neuroscience*, *282*, 230-242. https://doi.org/10.1016/j.neuroscience.2014.07.081

Meyer-Bernstein, E. L., & Morin, L. P. (1996). Differential serotonergic innervation of the suprachiasmatic nucleus and the intergeniculate leaflet and its role in circadian rhythm modulation. *J Neurosci*, *16*(6), 2097-2111. https://doi.org/10.1523/JNEUROSCI.16-06-02097.1996

Meyer-Lindenberg, A. S., Olsen, R. K., Kohn, P. D., Brown, T., Egan, M. F., Weinberger, D. R., & Berman, K. F. (2005). Regionally specific disturbance of dorsolateral prefrontal-hippocampal functional connectivity in schizophrenia. *Arch Gen Psychiatry*, *62*(4), 379-386. https://doi.org/10.1001/archpsyc.62.4.379

Mijovic, A., & MacCabe, J. H. (2020). Clozapine-induced agranulocytosis. *Ann Hematol*, *99*(11), 2477-2482. https://doi.org/10.1007/s00277-020-04215-y

Mohawk, J. A., Green, C. B., & Takahashi, J. S. (2012). Central and peripheral circadian clocks in mammals. *Annu Rev Neurosci*, *35*, 445-462. https://doi.org/10.1146/annurev-neuro-060909-153128

Morihisa, J. M., Duffy, F. H., & Wyatt, R. J. (1983). Brain electrical activity mapping (BEAM) in schizophrenic patients. *Arch Gen Psychiatry*, *40*(7), 719-728. https://doi.org/10.1001/archpsyc.1983.01790060017002

Moser, M. B., Moser, E. I., Forrest, E., Andersen, P., & Morris, R. G. (1995). Spatial learning with a minislab in the dorsal hippocampus. *Proc Natl Acad Sci U S A*, *92*(21), 9697-9701. https://doi.org/10.1073/pnas.92.21.9697

Naert, A., Gantois, I., Laeremans, A., Vreysen, S., Van den Bergh, G., Arckens, L., Callaerts-Vegh, Z., & D'Hooge, R. (2013). Behavioural alterations relevant to developmental brain disorders in mice with neonatally induced ventral hippocampal lesions. *Brain Res Bull*, *94*, 71-81. https://doi.org/10.1016/j.brainresbull.2013.01.008

Nath, M., Bhardwaj, S. K., Srivastava, L. K., & Wong, T. P. (2023). Altered excitatory and decreased inhibitory transmission in the prefrontal cortex of male mice with early developmental disruption to the ventral hippocampus. *Cereb Cortex*, *33*(3), 865-880. https://doi.org/10.1093/cercor/bhac107

Nath, M., Wong, T. P., & Srivastava, L. K. (2021). Neurodevelopmental insights into circuit dysconnectivity in schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry*, *104*, 110047. https://doi.org/10.1016/j.pnpbp.2020.110047

O'Donnell, P. (2012). Cortical disinhibition in the neonatal ventral hippocampal lesion model of schizophrenia: new vistas on possible therapeutic approaches. *Pharmacol Ther*, *133*(1), 19-25. https://doi.org/10.1016/j.pharmthera.2011.07.005

Ohta, H., Yamazaki, S., & McMahon, D. G. (2005). Constant light desynchronizes mammalian clock neurons. *Nat Neurosci*, *8*(3), 267-269. https://doi.org/10.1038/nn1395

Oishi, Y., & Lazarus, M. (2017). The control of sleep and wakefulness by mesolimbic dopamine systems. *Neurosci Res*, *118*, 66-73. https://doi.org/10.1016/j.neures.2017.04.008

Pelletier, M., Achim, A. M., Montoya, A., Lal, S., & Lepage, M. (2005). Cognitive and clinical moderators of recognition memory in schizophrenia: a meta-analysis. *Schizophr Res*, *74*(2-3), 233-252. https://doi.org/10.1016/j.schres.2004.08.017

Pennington, Z. T., Diego, K. S., Francisco, T. R., LaBanca, A. R., Lamsifer, S. I., Liobimova, O., Shuman, T., & Cai, D. J. (2021). ezTrack-A Step-by-Step Guide to Behavior Tracking. *Curr Protoc*, *1*(10), e255. https://doi.org/10.1002/cpz1.255

Pettersson-Yeo, W., Allen, P., Benetti, S., McGuire, P., & Mechelli, A. (2011). Dysconnectivity in schizophrenia: where are we now? *Neurosci Biobehav Rev*, *35*(5), 1110-1124. https://doi.org/10.1016/j.neubiorev.2010.11.004

Philipsberg, P. A., Christenson Wick, Z., Diego, K. S., Vaughan, N., Galas, A., Jurkowski, A., Feng, Y., Vetere, L. M., Chen, L., Soler, I., Cai, D. J., & Shuman, T. (2023). Chronotate: An open-source tool for manual timestamping and quantification of animal behavior. *Neurosci Lett*, *814*, 137461. https://doi.org/10.1016/j.neulet.2023.137461

Phillips, K. G., Bartsch, U., McCarthy, A. P., Edgar, D. M., Tricklebank, M. D., Wafford, K. A., & Jones, M. W. (2012). Decoupling of sleep-dependent cortical and hippocampal interactions in a neurodevelopmental model of schizophrenia. *Neuron*, *76*(3), 526-533. https://doi.org/10.1016/j.neuron.2012.09.016

Phillips, L. J., Leicester, S. B., O'Dwyer, L. E., Francey, S. M., Koutsogiannis, J., Abdel-Baki, A., Kelly, D., Jones, S., Vay, C., Yung, A. R., & McGorry, P. D. (2002). The PACE Clinic: identification and management of young people at "ultra" high risk of psychosis. *J Psychiatr Pract*, *8*(5), 255-269. https://doi.org/10.1097/00131746-200209000-00002

Pifer, G. C., Ferrara, N. C., & Kwapis, J. L. (2024). Long-lasting effects of disturbing the circadian rhythm or sleep in adolescence. *Brain Res Bull*, *213*, 110978. https://doi.org/10.1016/j.brainresbull.2024.110978

Poe, S. L., Brucato, G., Bruno, N., Arndt, L. Y., Ben-David, S., Gill, K. E., Colibazzi, T., Kantrowitz, J. T., Corcoran, C. M., & Girgis, R. R. (2017). Sleep disturbances in individuals at clinical high risk for psychosis. *Psychiatry Res*, *249*, 240-243. https://doi.org/10.1016/j.psychres.2016.12.029

Premachandran, H., Zhao, M., & Arruda-Carvalho, M. (2020). Sex Differences in the Development of the Rodent Corticolimbic System. *Front Neurosci*, *14*, 583477. https://doi.org/10.3389/fnins.2020.583477

Rasetti, R., Sambataro, F., Chen, Q., Callicott, J. H., Mattay, V. S., & Weinberger, D. R. (2011). Altered cortical network dynamics: a potential intermediate phenotype for schizophrenia and association with ZNF804A. *Arch Gen Psychiatry*, *68*(12), 1207-1217. https://doi.org/10.1001/archgenpsychiatry.2011.103

Richtand, N. M., Taylor, B., Welge, J. A., Ahlbrand, R., Ostrander, M. M., Burr, J., Hayes, S., Coolen, L. M., Pritchard, L. M., Logue, A., Herman, J. P., & McNamara, R. K. (2006). Risperidone pretreatment prevents elevated locomotor activity following neonatal hippocampal lesions. *Neuropsychopharmacology*, *31*(1), 77-89. https://doi.org/10.1038/sj.npp.1300791

Rosenwasser, A. M., & Fixaris, M. C. (2013). Chronobiology of alcohol: studies in C57BL/6J and DBA/2J inbred mice. *Physiol Behav*, *110-111*, 140-147. https://doi.org/10.1016/j.physbeh.2013.01.001

Rueter, L. E., Ballard, M. E., Gallagher, K. B., Basso, A. M., Curzon, P., & Kohlhaas, K. L. (2004). Chronic low dose risperidone and clozapine alleviate positive but not negative symptoms in the rat neonatal ventral hippocampal lesion model of schizophrenia. *Psychopharmacology (Berl)*, *176*(3-4), 312-319. https://doi.org/10.1007/s00213-004-1897-4

Ruhrmann, S., Schultze-Lutter, F., Salokangas, R. K., Heinimaa, M., Linszen, D., Dingemans, P., Birchwood, M., Patterson, P., Juckel, G., Heinz, A., Morrison, A., Lewis, S., von Reventlow, H. G., & Klosterkotter, J. (2010). Prediction of psychosis in adolescents and young adults at high risk: results from the prospective European prediction of psychosis study. *Arch Gen Psychiatry*, *67*(3), 241-251. https://doi.org/10.1001/archgenpsychiatry.2009.206

Ruiz-Tejada, A., Neisewander, J., & Katsanos, C. S. (2022). Regulation of Voluntary Physical Activity Behavior: A Review of Evidence Involving Dopaminergic Pathways in the Brain. *Brain Sci*, *12*(3). https://doi.org/10.3390/brainsci12030333

Sams-Dodd, F., Lipska, B. K., & Weinberger, D. R. (1997). Neonatal lesions of the rat ventral hippocampus result in hyperlocomotion and deficits in social behaviour in adulthood. *Psychopharmacology (Berl)*, *132*(3), 303-310. https://doi.org/10.1007/s002130050349

Scheibel, A. B., & Conrad, A. S. (1993). Hippocampal dysgenesis in mutant mouse and schizophrenic man: is there a relationship? *Schizophr Bull*, *19*(1), 21-33. https://doi.org/10.1093/schbul/19.1.21

Schobel, S. A., Chaudhury, N. H., Khan, U. A., Paniagua, B., Styner, M. A., Asllani, I., Inbar, B. P., Corcoran, C. M., Lieberman, J. A., Moore, H., & Small, S. A. (2013). Imaging patients with psychosis and a mouse model establishes a spreading pattern of hippocampal dysfunction and implicates glutamate as a driver. *Neuron*, *78*(1), 81-93. https://doi.org/10.1016/j.neuron.2013.02.011

Schwabe, K., Klein, S., & Koch, M. (2006). Behavioural effects of neonatal lesions of the medial prefrontal cortex and subchronic pubertal treatment with phencyclidine of adult rats. *Behav Brain Res*, *168*(1), 150-160. https://doi.org/10.1016/j.bbr.2005.11.005

Seibenhener, M. L., & Wooten, M. C. (2015). Use of the Open Field Maze to measure locomotor and anxiety-like behavior in mice. *J Vis Exp*(96), e52434. https://doi.org/10.3791/52434

Shivakumar, A. B., Mehak, S. F., Jijimon, F., & Gangadharan, G. (2024). Extrahippocampal Contributions to Social Memory: The Role of Septal Nuclei. *Biol Psychiatry*. https://doi.org/10.1016/j.biopsych.2024.04.018

Shoji, H., & Miyakawa, T. (2021). Effects of test experience, closed-arm wall color, and illumination level on behavior and plasma corticosterone response in an elevated plus maze in male C57BL/6J mice: a challenge against conventional interpretation of the test. *Mol Brain*, *14*(1), 34. https://doi.org/10.1186/s13041-020-00721-2

Siddique, R., Awan, F. M., Nabi, G., Khan, S., & Xue, M. (2022). Chronic jet lag-like conditions dysregulate molecular profiles of neurological disorders in nucleus accumbens and prefrontal cortex. *Front Neuroinform*, *16*, 1031448. https://doi.org/10.3389/fninf.2022.1031448

Sigurdsson, T., Stark, K. L., Karayiorgou, M., Gogos, J. A., & Gordon, J. A. (2010). Impaired hippocampal-prefrontal synchrony in a genetic mouse model of schizophrenia. *Nature*, *464*(7289), 763-767. https://doi.org/10.1038/nature08855

Silva-Gomez, A. B., Bermudez, M., Quirion, R., Srivastava, L. K., Picazo, O., & Flores, G. (2003). Comparative behavioral changes between male and female postpubertal rats following neonatal excitotoxic lesions of the ventral hippocampus. *Brain Res*, *973*(2), 285-292. https://doi.org/10.1016/s0006-8993(03)02537-x

Srikanta, S. B., Stojkovic, K., & Cermakian, N. (2021). Behavioral phenotyping of mice lacking the deubiquitinase USP2. *PLoS One*, *16*(2), e0241403. https://doi.org/10.1371/journal.pone.0241403

St Onge, J. R., & Floresco, S. B. (2010). Prefrontal cortical contribution to risk-based decision making. *Cereb Cortex*, *20*(8), 1816-1828. https://doi.org/10.1093/cercor/bhp250

Stepnicki, P., Kondej, M., & Kaczor, A. A. (2018). Current Concepts and Treatments of Schizophrenia. *Molecules*, *23*(8). https://doi.org/10.3390/molecules23082087

Strange, B. A., Witter, M. P., Lein, E. S., & Moser, E. I. (2014). Functional organization of the hippocampal longitudinal axis. *Nat Rev Neurosci*, *15*(10), 655-669. https://doi.org/10.1038/nrn3785

Sukoff Rizzo, S. J., & Silverman, J. L. (2016). Methodological Considerations for Optimizing and Validating Behavioral Assays. *Curr Protoc Mouse Biol*, *6*(4), 364-379. https://doi.org/10.1002/cpmo.17

Tapia-Osorio, A., Salgado-Delgado, R., Angeles-Castellanos, M., & Escobar, C. (2013). Disruption of circadian rhythms due to chronic constant light leads to depressive and anxiety-like behaviors in the rat. *Behav Brain Res*, *252*, 1-9. https://doi.org/10.1016/j.bbr.2013.05.028

Temmingh, H., & Stein, D. J. (2015). Anxiety in Patients with Schizophrenia: Epidemiology and Management. *CNS Drugs*, *29*(10), 819-832. https://doi.org/10.1007/s40263-015-0282-7

Touitou, Y., Touitou, D., & Reinberg, A. (2016). Disruption of adolescents' circadian clock: The vicious circle of media use, exposure to light at night, sleep loss and risk behaviors. *J Physiol Paris*, *110*(4 Pt B), 467-479. https://doi.org/10.1016/j.jphysparis.2017.05.001

Tseng, K. Y., Amin, F., Lewis, B. L., & O'Donnell, P. (2006). Altered prefrontal cortical metabolic response to mesocortical activation in adult animals with a neonatal ventral hippocampal lesion. *Biol Psychiatry*, *60*(6), 585-590. https://doi.org/10.1016/j.biopsych.2006.03.030

Tseng, K. Y., Chambers, R. A., & Lipska, B. K. (2009). The neonatal ventral hippocampal lesion as a heuristic neurodevelopmental model of schizophrenia. *Behav Brain Res*, *204*(2), 295-305. https://doi.org/10.1016/j.bbr.2008.11.039

Van Der Lee, S., & Boot, L. M. (1955). Spontaneous pseudopregnancy in mice. *Acta Physiol Pharmacol Neerl*, *4*(3), 442-444. https://www.ncbi.nlm.nih.gov/pubmed/13301816

Vazquez-Roque, R. A., Ubhi, K., Masliah, E., & Flores, G. (2014). Chronic cerebrolysin administration attenuates neuronal abnormalities in the basolateral amygdala induced by neonatal ventral hippocampus lesion in the rat. *Synapse*, *68*(1), 31-38. https://doi.org/10.1002/syn.21718

Waite, F., Sheaves, B., Isham, L., Reeve, S., & Freeman, D. (2020). Sleep and schizophrenia: From epiphenomenon to treatable causal target. *Schizophr Res*, *221*, 44-56. https://doi.org/10.1016/j.schres.2019.11.014

Walf, A. A., & Frye, C. A. (2007). The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nat Protoc*, *2*(2), 322-328. https://doi.org/10.1038/nprot.2007.44

Walker, W. H., 2nd, Walton, J. C., DeVries, A. C., & Nelson, R. J. (2020). Circadian rhythm disruption and mental health. *Transl Psychiatry*, *10*(1), 28. https://doi.org/10.1038/s41398-020-0694-0

Wang, G. W., & Cai, J. X. (2006). Disconnection of the hippocampal-prefrontal cortical circuits impairs spatial working memory performance in rats. *Behav Brain Res*, *175*(2), 329-336. https://doi.org/10.1016/j.bbr.2006.09.002

Wegrzyn, D., Juckel, G., & Faissner, A. (2022). Structural and Functional Deviations of the Hippocampus in Schizophrenia and Schizophrenia Animal Models. *Int J Mol Sci*, *23*(10). https://doi.org/10.3390/ijms23105482

Weinberger, D. R. (1987). Implications of normal brain development for the pathogenesis of schizophrenia. *Arch Gen Psychiatry*, *44*(7), 660-669. https://doi.org/10.1001/archpsyc.1987.01800190080012

Westwood, E., Smith, S., Mann, D., Pattinson, C., Allan, A., & Staton, S. (2023). The effects of light in children: A systematic review. *Journal of Environmental Psychology*, *89*, 102062. https://doi.org/10.1016/j.jenvp.2023.102062

Whitten, W. K. (1956). Modification of the oestrous cycle of the mouse by external stimuli associated with the male. *J Endocrinol*, *13*(4), 399-404. https://doi.org/10.1677/joe.0.0130399

Wood, G. K., Quirion, R., & Srivastava, L. K. (2003). Early environment contributes to developmental disruption of MPFC after neonatal ventral hippocampal lesions in rats. *Synapse*, *50*(3), 223-232. https://doi.org/10.1002/syn.10265

Wulff, K., Dijk, D. J., Middleton, B., Foster, R. G., & Joyce, E. M. (2012). Sleep and circadian rhythm disruption in schizophrenia. *Br J Psychiatry*, *200*(4), 308-316. https://doi.org/10.1192/bjp.bp.111.096321

Yang, M., Silverman, J. L., & Crawley, J. N. (2011). Automated three-chambered social approach task for mice. *Curr Protoc Neurosci*, *Chapter 8*, Unit 8 26. https://doi.org/10.1002/0471142301.ns0826s56

Yung, A. R., & McGorry, P. D. (1996). The prodromal phase of first-episode psychosis: past and current conceptualizations. *Schizophr Bull*, *22*(2), 353-370. https://doi.org/10.1093/schbul/22.2.353

Zhou, Y., Shu, N., Liu, Y., Song, M., Hao, Y., Liu, H., Yu, C., Liu, Z., & Jiang, T. (2008). Altered resting-state functional connectivity and anatomical connectivity of hippocampus in schizophrenia. *Schizophr Res*, *100*(1-3), 120-132. https://doi.org/10.1016/j.schres.2007.11.039

Zhu, X., Ottenheimer, D., & DiLeone, R. J. (2016). Activity of D1/2 Receptor Expressing Neurons in the Nucleus Accumbens Regulates Running, Locomotion, and Food Intake. *Front Behav Neurosci*, *10*, 66. https://doi.org/10.3389/fnbeh.2016.00066