ADVANCES IN
Sleep & Circadian
SCIENCE

2023 ASCS ABSTRACT BOOK

9

WITHDRAWN

10

Perceived Stress and Insomnia Symptoms Mediate the Relationship Between Discrimination and Depressive Symptoms

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Full Name and Credentials

Joanna Hobson, BS

Introduction (100 word limit)

People living with HIV (PWH) experience stigma and discrimination on a daily basis due to their health status, race, sexual orientation and etc. These experiences of discrimination have known implications for poor health (e.g. over-activation of stress regulatory systems) and mood disorders. The purpose of this study was to elucidate the impact of discrimination on current stress, insomnia symptoms and depression in 64 adults with and without HIV.

Methods (200 word limit)

Participants were invited to do a single study session where they completed the Perceived Stress Scale, the Everyday Discrimination Scale, Center for Epidemiological Studies Depression Scale (CESD), and Insomnia Severity Index.

Results (200 word limit)

Experiences of discrimination was associated with greater perceived stress ($p < .001$), insomnia symptoms ($p = .003$) and depressive symptoms ($p < .001$). Additionally, stress and insomnia symptoms helped explain the relationship between discrimination and depressive symptoms [95% CI .007 - .158]. Specifically, discrimination predicted greater stress ($t = 5.409$, $p = .000$), stress predicted greater insomnia symptoms ($t = 2.532$, $p = .013$), and insomnia symptoms predicted greater depressive symptoms ($t = 3.300$, $p = .001$).

Conclusions (100 word limit)

Examining the impact of upstream stressors (e.g. discrimination) on health outcomes are imperative for the management of comorbid HIV and pain.

Support

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11

Acute and long-term effects of light exposure on sleep and sleepiness in everyday life

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Full Name and Credentials

Altug Didikoglu

Introduction (100 word limit)

Light has immediate alerting effects and long-term impacts on sleep and sleepiness by being the fundamental environmental synchroniser of the biological clock. These relationships have comprehensively been examined in controlled conditions. Insufficient sleep is linked to several physical and mental health problems and disrupted alertness is associated with workplace safety and productivity issues, but the ways of measuring and supporting sleep and alertness in everyday life are limited. We used gold-standard wearable continuous light monitors combined with a smartphone-based data collection which allowed longitudinal behavioural records at any time in everyday life.

Methods (200 word limit)

We used wearable wristband light dosimeters, 'SpectraWear', which were manufactured at the University of Manchester and can measure photopic illuminance (lx) and α-opic equivalent daylight illuminances (EDI), including melanopic lx. Light monitors were used by 59 participants for 1 week (N=419 days). Individuals completed online surveys of baseline sociodemographic characteristics, general health, physical activity questionnaires, the Munich Chronotype questionnaire and the Pittsburgh Sleep Quality Index. They recorded their sleep and work schedules using a study-specific diary (N=478 days). They also reported their subjective sleepiness throughout the day using the Karolinska Sleepiness Scale 10-item version (KSS, N=1799). Light exposure patterns in everyday life were described using density plots and time-of-day distributions. Acute effects of light on sleepiness were assessed using linear mixed models with individuals as a random effect adjusted for time of day and time awake. We then assessed the impacts of light history on sleepiness and sleep latency. Lastly, we compared weekly and daily light exposures and sleep traits using linear regression models for grand averages of participants and linear mixed models for daily measures.

Results (200 word limit)

Participants were exposed to higher wavelengths more frequent than shorter wavelengths in everyday life. People had earlier light exposure and shorter bright light duration on workdays compared to free days. Immediate melanopic EDI was associated with alertness with 1 log lx unit of light exposure decreasing the KSS by 0.4 scores. Duration since the wake time mediated this effect with the alerting

effect of light being stronger in the mornings. We showed that the light history of the last 3 hours before waketime is a better predictor of morning alertness than the acute effect of morning light. We also showed that the average light exposure of the last hour before bedtime increases sleep latency. We finally showed that interdaily stability of light exposure, higher daytime and lower night light exposure may result in early wake and bedtimes.

Conclusions (100 word limit)

Overall, we showed in everyday life that light acutely and in the long term is associated with sleep and alertness. These findings demonstrated the feasibility of simultaneously working continuous wearable light dosimetry and free-choice behavioural recording for studies investigating the influences of light on physiology and behaviours. In addition, real-world light exposure tended to be lower in melanopic illuminance than photopic lux, indicating the necessity to measure light considering the spectral and biological properties.

Support

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12

Clustering bipolar disorder risk variants by their effect on sleep and circadian traits.

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Full Name and Credentials

Dr Lovemore Kunorozva

Introduction (100 word limit)

Bipolar disorder (BD) is a common, severe, and recurrent familial psychiatric disorder that causes unusual shifts in mood, energy, activity levels, concentration, and the ability to carry out day-to-day tasks. There are two types of BD (BDI and BDII), BDI causes mania and may cause depression, while BDII causes hypomania and depression. Sleep and circadian activity rhythms are closely associated with mood symptoms and disruption to these rhythms may exacerbate BD symptoms. Genetic studies (GWAS) have identified multiple risk variants for BD. The association between these BD risk variants with sleep and circadian traits is unknown.

Methods (200 word limit)

To enable new insights into disease-causing pathways, we clustered BD risk variants by their effect on sleep and circadian traits. We tested the association of 63 BD risk variants from a recent GWAS (Mullins et al. 2021) with traits related to sleep timing, quality, and quantity both self-reported and objectively measured using Clustergrammer for hierarchical clustering.

Results (200 word limit)

We found bipolar risk variants cluster into four bins based on their association with sleep and circadian traits. These clusters indicate bipolar genetic risk may function through four distinct mechanisms related to early chronotype, late chronotype, high sleep efficiency, and increased sleep duration.

Conclusions (100 word limit)

This finding may allow the classification of BD patients by genetic pathways potentially offering a way forward toward genetically informed diagnosis, surveillance, and management of BD patients using sleep and circadian interventions. Additionally, this confirms that genetic heterogeneity contributes to the clinical heterogeneity of bipolar, thus consideration of sleep and circadian traits' contribution to

psychopathologic components of BD may improve the genetic prediction of complex psychiatric disorders.

Support

N/A

13

Circadian timing contributes to the daily rhythm of ventilation in mice independently of metabolic rate

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Full Name and Credentials

Aaron Jones, B.A.

Introduction (100 word limit)

To optimize nutrient availability across the 24-hr day, animals have evolved daily rhythms in energy metabolism and, consequently, a daily rhythm in breathing to mediate metabolic gas exchange. Daily ventilatory rhythms have been implicated in a variety of respiratory diseases such as sleep apnea, asthma, COPD, COVID-19 and SUDEP. The daily rhythm in ventilation is organized by the master circadian clock, the suprachiasmatic nuclei (SCN). However, the extent that the ventilation rhythm is driven by SCN-imposed oxygen and carbon dioxide cycles (*i.e*. metabolic rate) versus other SCN-derived mechanisms is unclear.

Methods (200 word limit)

Here, we utilized a reverse feeding protocol to test the hypothesis that the circadian clock contributes to ventilatory rhythms independent of metabolic rate. Minute ventilation was recorded in wild-type C57Bl6/J mice using whole-body plethysmography, and metabolic rate was assessed using indirect calorimetry. To determine the extent that circadian regulation of oxygen and carbon dioxide sensing within the brainstem acts in collaboration with metabolic rate to organize the daily rhythm in minute ventilation, we additionally assessed ventilatory and metabolic rhythms in mice with the clock gene BMAL1 knocked out from Phox2b-expressing chemoreceptor cells (BKOP).

Results (200 word limit)

In wild-type mice, we found that under *ad libitum* and night-time feeding, the acrophases of food intake, oxygen consumption, carbon dioxide production, and minute ventilation remained tightly aligned to the dark phase and SCN timing. However, under exclusive day feeding, the daily ventilation rhythm became disrupted and phase-misaligned with oxygen and carbon dioxide cycles, indicating opposing influences of metabolism and central circadian timing. Similar to wild-type mice, BKOP mice exhibited tightly aligned acrophases of metabolic rate and minute ventilation under night feeding. However, in contrast to wild-type mice, day-fed BKOP mice retained a daily rhythm in minute ventilation that aligned primarily with carbon dioxide production. Collectively, these results indicate that both metabolic rate and intrinsic time-keeping within Phox2b-expressing chemoreceptor cells orchestrate the peak timing of ventilation.

Conclusions (100 word limit)

We conclude that the daily rhythm in minute ventilation is not merely driven by daily oxygen and carbon dioxide cycles, but instead influenced by other SCN-derived mechanisms such as clock gene regulation within the respiratory network.

Support

N/A

14

Estimating Circadian Rhythmicity across Psychosocial States

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Full Name and Credentials

Zlatan Krizan, Ph.D.

Introduction (100 word limit)

Sleep disruption is known to impact various psychosocial states, including emotional distress, positive affect, social engagement, and impulse-control. However, potential circadian oscillation across these states, as well as their synchronicity, have been comparatively neglected. To this end, the current investigation estimated circadian rhythms in five psychosocial states that underlie personality functioning, namely extroverted, agreeable, conscientious, neurotic, and open-minded behavior.

Methods (200 word limit)

Data were drawn from Wilson, Thompson, & Vazire (2017), where one-hundred and twenty-four young adults participated in a diary study on personality. Participants were 67% female with an average age of twenty, with 43% identifying as white, 19% as Asian, 8% as Black, 4% Hispanic, and 25% not reporting. Each participant completed six daily surveys across six days, every 2 hours from 11am-9pm. During each assessment, they comprehensively reported on ongoing psychosocial states. This resulted in six daily sets of 5 reports, namely behaving in an (1) extroverted/enthusiastic vs. quite/reserved way, (2) warm/sympathetic vs. critical/quarrelsome way, (3) dependable/self-disciplined vs. disorganized/careless way, (4) anxious/easy upset vs. calm/emotional stable way, and (5) open to new experiences vs. convention/uncreative way (spanning five domains of personality functioning). For each set of items, they rated how they were thinking, feeling, and behaving during the hour just preceding the survey on 5-point scales. Multi-level cosinor analysis modeled circadian fluctuations across the five psychosocial states, while the standardized difference between the apex and nadir (for each state) served as the effect size estimate of amplitude.

Results (200 word limit)

The cosinor analyses indicate substantive circadian rhythms across most psychosocial states. Strongest rhythms were observed for extroverted/enthusiastic behavior, with a trough around 9am and peak around 9pm (d=.56), and agreeable behavior, with an identical but slightly weaker rhythm (d=.39). Open-minded behavior showed a similar rhythm an hour behind that was less pronounced (d=.32). Conscientious behavior showed a peak at 1pm (d=.31), while neurotic distress/anxiety did not show a rhythm.

Conclusions (100 word limit)

This investigation estimated circadian rhythms across various domains of psychosocial functioning, finding that all aspects of functioning except distress showed predictable daily fluctuations. Social enthusiasm and cooperativeness was lowest in the morning and highest in the evening, while self-discipline was highest in early afternoon (similar to cognitive performance). The results were limited by an age-restricted sample and relatively coarse sampling of time-units. Overall, the findings suggest that all performance and engagement-related psychosocial states show similar daily fluctuations and call for identification of shared mechanisms across levels of experience and behavior.

Support

N/A

15

Examining Changes in Mouse Cortical Network Dynamics Due to Sleep Deprivation

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Full Name and Credentials

Delaney Beckner, BS

Introduction (100 word limit)

Society sometimes prevents humans from getting the requisite amount of sleep. This leads to many health problems, including an increase in reaction time and hindered attention. These cognitive impairments can lead to accidents, and are thought to have contributed to high-profile, deadly events. Research has revealed a possible mechanism for this phenomenon. Clusters of neurons have been shown to go into “off-states” during sleep deprivation in independent cortical regions via multi-unit analysis. These states have been associated with deficits in tasks relating to the region of the off neurons. This has been used as evidence of local sleep in rodents.

Methods (200 word limit)

Two mice (1 male, 1 female) were subjected to a transcranial injection of a viral vector, causing them to express GCaMP6f. The mice then had 1mm diameter lenses implanted into their medial prefrontal cortices. The mice had at least 5 minutes of baseline activity recorded using miniature fluorescent microscopes during both wake and sleep. The mice were then subjected to sleep-deprivation for 6 hours, and similar recordings were collected. The videos were then processed using Anaconda3 to identify neurons, characterize the activity of each, and analyze the correlations between neurons within the same field.

Results (200 word limit)

The recordings revealed complex changes in neuronal activity both between sleep and wake and between each before and after sleep-deprivation. Individual neurons and networks had different firing patterns during sleep and wake, which changed again when the mice were sleep-deprived.

Conclusions (100 word limit)

Sleep pressure alters cortical dynamics in complex manners that may explain associated deficits. This technology allows for direct observation of spatiotemporal relationships between individual neurons, allowing the dynamic changes to be completely characterized.

Support

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16

WITHDRAWN

17

N2 and wakefulness drive sleep satisfaction in adults

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Full Name and Credentials

Renske Lok, PhD

Introduction (100 word limit)

The measurable aspects of brain function derivable from polysomnography (PSG) that are correlated with perceived sleep satisfaction are poorly understood. Previous smaller studies have identified nearly every stage of sleep as being associated with sleep satisfaction. A more recent study of 1500 older individuals found a weak association of PSG-derived sleep efficiency with perceived sleep depth and restfulness. Using recent developments in automated sleep scoring, which remove the within- and between-rater error associated with human scoring, and machine learning models, we revisit whether whole-night PSG measurements are associated with sleep satisfaction.

Methods (200 word limit)

Random forest machine learning was used to investigate the relationship between a single night of PSG data from the Sleep Heart Health Study (N=3,165, community-dwelling middle-aged and older adults) and self-reported sleep satisfaction (restfulness, depth). PSG files were rescored using a novel automated algorithm that generates a sleep stage for each 15-s epoch and a stage matching probability. Data were also parsed into 20 minute-fragments based on time relative to wake time. Forty predictor variables were examined, including PSG-derived sleep [stages (percent, minutes, average probability), transitions, efficiency, respiratory distress index, latency], demographics [gender, race, ethnicity, age, marital status, education level], anthropometric [waist, body mass index], overall health scores [SF-36 Physical and Mental Component Scales, self-rated health], medications [sleeping pills, benzodiazepine, antidepressant, nicotine, caffeine, alcohol], and general mental health [self-rated stress, sleepiness]. Data were randomly split into sets of 75% (training) and 25% (testing) of the model.

Results (200 word limit)

Whole-night models explained 30% of subjective sleep depth and 27% of subjective sleep restfulness. The top four predictors of both models (minutes of N2, wake after sleep onset (WASO), sleep efficiency, age) captured 28% (restfulness) and 26% (depth) of the relative model variance. With increasing sleep satisfaction, there was a progressive increase in N2 and a decrease in WASO of similar magnitude (resulting in a progressive increase in sleep efficiency) without systematic changes in N1, N3, or REM. The reduction in WASO was most notably in a reduction in the number of longer bouts. The impact of age on sleep satisfaction did not follow a monotonic pattern. In comparing those with the best and worst subjective sleep experience, there is a range of approximately 30 minutes more N2, 30 minutes less WASO, an improvement of sleep efficiency of 7-8%, and an age span of 3-5 years. Random Forest

models derived from PSG fragments closer to the offset of sleep did not provide better explanatory power than the whole-night data set.

Conclusions (100 word limit)

In community-dwelling adults, the amount of wake, N2, overall sleep efficiency, and to a lesser extent, age are important predictors of self-reported sleep quality on a given night. Specifically, an increase in N2 with a concomitant decrease in wake duration after sleep onset, possibly through a reduction of the length of longer wake episodes, is associated with better self-reported sleep depth and restfulness. Interventions targeting N2 and wakefulness may be suitable for improving the self-reported sleep experience.

Support

N/A

18

Multidimensional sleep health prior to SARS-CoV-2 infection and risk of long COVID: a prospective cohort study

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Full Name and Credentials

Siwen Wang, M.D.

Introduction (100 word limit)

Multidimensional sleep health (e.g., early chronotype, adequate sleep duration, no insomnia, no snoring, and no excessive daytime sleepiness) and a combined healthy sleep score have been linked with COVID-19 severity and mortality. However, the relationship between multidimensional sleep health and risk of long COVID is unknown. We investigated whether sleep health before and during the pandemic, prior to SARS-CoV-2 infection, are associated with risk of long COVID.

Methods (200 word limit)

This study included 32,249 women from an ongoing longitudinal cohort, the Nurses' Health Study II. Participants reported sleep characteristics prior to the COVID-19 pandemic on a 2017 questionnaire (chronotype question was on a 2015 questionnaire) and were followed from April 2020 to November 2021 with a series of COVID-19-related health surveys (COVID-19 sub-study). We defined pre-pandemic healthy sleep score according to five healthy sleep dimensions: morning chronotype, 7–8 hours of sleep/day, low insomnia symptoms, no self-reported snoring, no frequent daytime dysfunction. On the first COVID-19 sub-study survey (returned April 2020–August 2020), past-7-day average daily sleep duration and sleep quality were queried. SARS-CoV-2 infection (confirmed by test) and CDC-defined long COVID (≥4 weeks of symptoms) were self-reported during follow-up. Among those who reported a positive SARS-CoV-2 test, we used Poisson regression to estimate relative risks (RRs) of long COVID with 1) pre-pandemic healthy sleep score and 2) sleep characteristics early in the pandemic, adjusting for demographics, other lifestyle factors, and comorbidities. Individuals who reported prior SARS-CoV-2 infection at the time of pandemic sleep assessment were excluded from analyses of pandemic sleep characteristics.

Results (200 word limit)

We documented 1,979 self-reported SARS-CoV-2 infections during follow-up. The mean (SD) participant age was 64.7 years (4.6), and 42.8% (n=846) were frontline healthcare workers. Of these, 870 (44.0%) developed long COVID. Pre-infection healthy sleep score was inversely associated with risk of long COVID (P trend<0.001). Compared to women who had a healthy sleep score of 0 or 1 (least healthy), those who scored 5 (most healthy) had a 31% lower risk of developing long COVID (RR=0.69, 95%

CI=0.51–0.92). Results were comparable when additionally adjusted for pre-infection depression and anxiety. Associations did not differ by healthcare worker status, body mass index, smoking history, physical activity, diet quality, alcohol consumption, or history of chronic diseases. A mutually adjusted model with five individual healthy sleep factors indicated that associations were mainly driven by sleep duration and daytime dysfunction. Women who had healthy sleep at both timepoints were at lowest risk of long COVID (Table 4, RR=0.62, 95% CI=0.49–0.79), compared to women who had a low-to-moderate sleep score (0–3) prior to the pandemic and had neither optimal sleep duration (i.e., 7–8 hours/day) nor 'fairly good' or 'good' sleep quality early in the pandemic.

Conclusions (100 word limit)

Pre-infection healthy sleep may be protective of long COVID. Future research should investigate whether interventions on sleep health may prevent long COVID or improve long COVID symptoms.

Support

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19

WITHDRAWN

20

Early-to-mid pregnancy sleep and circadian markers in relation to birth outcomes: an epigenetics pilot study

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Full Name and Credentials

Erica Jansen, PhD, MPH

Introduction (100 word limit)

Maternal sleep and circadian health during pregnancy are emerging as important predictors of infant outcomes. Yet, investigations of readily-accessible biomarkers, which could provide mechanistic clues, are scant. DNA methylation, which refers to the addition of a methyl group to a cytosine-guanine dinucleotide (CpG) site, is one way by which behaviors such as sleep duration and timing could impact gene expression and related health outcomes. We conducted a pilot study to investigate links between maternal leukocyte DNA methylation of the circadian genes *BMAL1*, *PER1*, and *MTNR1B* and birth outcomes within a pregnancy cohort.

Methods (200 word limit)

The study population included 96 women with singleton births who delivered at Von Voigtlander Hospital in Ann Arbor, MI. Women completed a questionnaire regarding health behaviors and sociodemographic information and provided a blood sample at least once during early-to-mid pregnancy (average gestation weeks= 14.2 weeks). Leukocyte DNA was isolated and DNA methylation (percent of methylated cells) at multiple CpG sites within *BMAL1*, *PER1*, and *MTNR1B* genes were quantified by pyrosequencing. Birth outcomes including gestational age at delivery, birthweight, and head circumference were abstracted from medical charts. Linear regression analyses were run between each CpG site and birth outcome, adjusting for important confounders (model-specific) including gestational age, infant sex, parity, smoking, and education. Sleep duration and timing were assessed as secondary exposures.

Results (200 word limit)

Women were on average 30.3 ± 5.3 years of age. The majority were White (81%), married (73%), and had at least some post-high school education (74%). Average self-reported sleep duration was 8.6 ± 1.6 hours and average sleep midpoint was 2:46 AM ± 79 minutes. Higher methylation of a CpG site in *PER1* was associated with smaller log-transformed head circumference (β=-0.02 with 95% CI -0.02 to 0.01; P, trend=0.04). Higher methylation of *MTNR1B* (averaged across all sites) was associated with lower log-transformed birthweight (-0.08 with 95% CI -0.16 to -0.01; P, trend=0.0495). In addition, longer sleep

duration was associated with higher birthweight (0.10 with 95% CI 0.02 to 0.18 comparing >9 hours to <8 hours; P, trend=0.04). Neither sleep duration nor sleep midpoint were related to DNA methylation of the selected circadian genes.

Conclusions (100 word limit)

This pilot investigation revealed that higher methylation of *PER1* and *MTNR1B* genes measured in early-to-mid pregnancy was related to smaller head circumference and birth weight of offspring, respectively. In contrast, longer maternal sleep duration was associated with higher birthweight. Results add to the growing evidence base that maternal circadian mechanisms may be involved in birth outcomes. Future investigations with larger sample sizes and a greater number of circadian genes are warranted.

Support

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21

Bright Light Therapy for CPAP-resistant OSA symptoms

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Full Name and Credentials

Isabella Soreca

Introduction (100 word limit)

Patients with sleep apnea often experience persistent sleep fragmentation and daytime sleepiness even when correctly using CPAP. Our study tested the patient's acceptability and efficacy of morning bright light therapy (BLT) to improve sleep, circadian rhythms and CPAP-resistant daytime symptoms in patients with sleep apnea

Methods (200 word limit)

In this within-subject cross-over study, 14 individual completed 4 weeks of BLT and 4 weeks of sham BLT in randomized order. Treatment effects were evaluated on actigraphy measured sleep parameters, sleepiness, depressive symptoms and sleep-related functional impairment, using multilevel mixed models.

Results (200 word limit)

Subjects experienced greater reduction in WASO with BLT compared to sham.

Bright light, compared to sham, did not increase the overall level of activity ($t = 0.64$, $c2(1) = 0.41$, $p = 0.523$). However, bright light did increase activity when days were shorter ($t = -41.38$, $c2(1) = 307.98$, $p <$ 10-15). It also increased the amplitude of circadian rest-activity rhythm ($t = 15.65$, $c2(1) = 244.92$, $p <$ 10-15). This effect was moderated by season: bright light amplified the circadian rest-activity rhythm particularly when days were short ($t = -7.5$, $c2(1) = 56.29$, $p <$ 10-13).

Across all conditions and time points, active treatment did not differ from sham for daytime sleepiness (X^2 (1)=1.51, p=0.220) however it was superior to sham in the active>sham sequence (Treatment * order:X^2 (1)=6.06, p=0.0138) and in early visits (Treatment * Visit: X^2 (1)=7.75, p=0.0054).

Across all conditions and time points, active treatment did not differ from sham for depressive symptoms (X^2 (1)= 1.50, p=0.22.), however it was superior to sham in the active>sham sequence (Treatment * order: X^2 (1)=6.06, p=0.0138) and when daylight was short (Treatment * Daylight: X^2 (1)=8.19, p=0.0042).

Conclusions (100 word limit)

Morning bright light was associated with consolidated sleep-wake cycles and reduced daytime sleepiness and depressive symptoms, in patients with OSA who did not fully respond to CPAP treatment. This study is the first to show a potential for a new application of a feasible, safe and low-cost intervention to improve sleep apnea outcomes.

Support

The study was funded by the Veterans Health Administration RR&D grant # I21 RX003304-02 (Soreca, PI)

22

Molecular Clock Dysfunction Within Leptin-Receptor Expressing Cells Increases Leptin Sensitivity in Mice

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Full Name and Credentials

Gabriella Marino

Introduction (100 word limit)

Metabolic homeostasis is largely influenced by the interaction between peripheral tissues and the central nervous system via key endocrine hormones, such as leptin. While prior evidence has demonstrated that clock proteins affect leptin signaling in a tissue-specific manner, minimal research has taken a whole-body approach to understanding the role of the molecular clock in leptin sensitivity.

Methods (200 word limit)

We generated a mouse model (BMAL1fl/fl;LepRcre+/?) in which the core clock gene BMAL1 is genetically deleted from all leptin receptor-expressing cells to determine the extent to which the local molecular clock affects behavioral leptin sensitivity. To measure leptin sensitivity, experimental animals and littermate controls (BMAL1+/+;LepRcre+/?) were fasted for 6 hours and administered an intraperitoneal injection of leptin (1 mg/kg) approximately an hour before dark onset. Behavioral leptin sensitivity was determined by subsequent food intake measured in 15-minute increments for 24 hours following leptin injection.

Results (200 word limit)

We found that BMAL1fl/fl;LepRcre+/? mice exhibit improved leptin sensitivity compared to control, with the most profound reduction in food intake occurring three hours after leptin injection. While preliminary, these data suggest that BMAL1 and/or the molecular clock transcriptional-translational feedback loop suppresses leptin sensitivity.

Conclusions (100 word limit)

Given the implications of leptin sensitivity on metabolic function, this work may provide unique insight on the role of the molecular clock in mediating whole-body leptin sensitivity and aid in our understanding of obesity.

Support

N/A

23

The decrease of glutamate concentration in the human ascending arousal system is correlated with the initiation and maintenance of sleep

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Brown University, PROVIDENCE, USA

Full Name and Credentials

Takashi Yamada, M.D. and Ph.D.

Introduction (100 word limit)

While the burden of insomnia is substantial, neural mechanisms underlying insomnia are poorly understood. Animal studies have suggested that the ascending arousal system is involved in sleep-wake regulation with glutamatergic and GABAergic signaling. Here, we tested whether the glutamatergic and GABAergic signaling in the ascending arousal system is involved in the initiation and maintenance of human sleep and whether the signaling is impaired in disturbed sleep by functional Magnetic Resonance Spectroscopy (MRS). We used the First-Night Effect (FNE), a transient sleep disturbance in the first sleep experiments, to create an insomnia model in healthy participants.

Methods (200 word limit)

Eighteen (11 females) healthy adults, aged 18-30, participated in the experiment with two afternoon-nap sessions (Day-1 and Day-2), separated by an approximately one-week interval. We presumed that the quality of sleep is poorer on Day-1 than Day-2. In each session, we asked participants to sleep inside the MRI bore with simultaneous polysomnography for 90 min. We repeated a 10-min MRS scan nine times to collect glutamate (Glx) and GABA concentrations in the ventromedial prefrontal cortex (vmPFC), a part of the ascending arousal system. We placed the voxel ($25x25x25mm^3$) before the genu of the corpus callosum for MRS.

We calculated how much Glx and GABA concentrations changed during NREM sleep relative to wakefulness as a baseline, after co-registration of MRS data and sleep stages were conducted. We obtained the sleep onset latency (SOL) for sleep initiation and the wake after sleep onset time (WASO), and slow wave sleep time (SWS) for sleep maintenance. Finally, we tested whether each of the Glx and GABA concentrations was correlated with each sleep parameter in each session.

Results (200 word limit)

The SOL was significantly shorter on Day-1 than Day-2, supporting the presumption that sleep was poorer on Day-1 than Day-2. On Day-2, the Glx concentration in the vmPFC was significantly reduced from baseline during NREM sleep. Moreover, the degree of Glx reduction during NREM sleep was

significantly positively correlated with the SOL and WASO but was negatively correlated with SWS, suggesting that the more significant decrease of Glx concentration is associated with the better initiation and maintenance of sleep on Day-2. On Day-1, however, no significant but slight reduction of Glx concentration during NREM sleep was observed. These results showed a significant difference in the degree of Glx reduction between Day-1 and Day-2. The degree of reduction of Glx during NREM sleep was correlated with the SOL but not with WASO or SWS on Day-1.

The GABA concentration during NREM sleep was significantly reduced from baseline on Day-2 but not on Day-1, showing a significant difference in the degree of GABA reduction between Day-1 and Day-2. However, the GABA concentration was not correlated with any of SOL, WASO, and SWS on Day-1 and Day-2.

Conclusions (100 word limit)

These results suggest that glutamatergic signaling, shown as a significant reduction of Glx, but not GABAergic signaling, in the human ascending arousal system, is involved in good sleep initiation and maintenance. Moreover, the results suggest that impaired glutamatergic signaling in the ascending arousal system is associated with poor sleep maintenance. The FNE may be used as a transient insomnia model in healthy young adults.

Support

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24

EFFECTS OF SOCIAL ISOLATION AND SPACE RADIATION ON FEAR EXTINCTION, NEUROINFLAMMATION, AND SLEEP IN RATS

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Full Name and Credentials

Austin Adkins, M.S.

Introduction (100 word limit)

The proposed Mars missions will expose astronauts to physical and psychological stressors including space radiation (SR) and social isolation (SI). These stressors, and other mission demands, may result in fragmented or reduced sleep. Stressors along with disrupted sleep can induce neuroinflammation. We assessed sleep duration, EEG power spectra (delta, <4 Hz and theta, 4-7 Hz), indices of neuroinflammation, and freezing behavior in rats exposed to ground-based analogs of SI and SR and trained in conditioned fear (CF), a model of stress-related learning. We separately analyzed rats classified as resilient (Res) or vulnerable (Vul) based on stress induced alterations in REM.

Methods (200 word limit)

Male, outbred, Wistar strain rats (8-9 months) were subjected to SI (visual barriers between cages, n=21) or individually housed (sham group, n=20). Separate groups of rats received SR (15 cGy GCRsim) and were individually housed (n=15) or subjected to SI (SI + SR, n=16). One sham group traveled with SR groups to control for potential effects of transit and were either individually housed (n=5) or subjected to SI (n=3). All rats were implanted with telemetry transmitters for recording EEG. For CF training, the rats were presented with 20 footshocks (ST: 0.8 mA; 0.5 s duration, 1.0 min ISI). Fear memory was assessed at one- (context (CTX)) and three-wks (extinction (EXT)) following training. Training took place during the 4th h of the light period followed by sleep recording. The rats were classified as Res or Vul based on percent change in REM amounts for the first 4 h of sleep recording following ST compared to baseline (%Change = Total REM ST/Total REM Baseline x 100) with Vul rats having 50% or greater decrease in REM and Res rats having smaller decreases (£ 50%), no change, or increases in REM. NanoString Neuroinflammation Panels were used to assess potential neuroimmune differences across groups.

Results (200 word limit)

SI, SR, and SI + SR produced significant alterations in sleep that were more pronounced when the Res and Vul phenotypes were considered. During the light period, baseline NREM amounts were lower in the SI Vul group compared to any other group. These differences persisted through CF, and both Res and Vul SI animals displayed lower NREM during EXT. Delta power during NREM was highest in the SI

groups following ST. There were no differences in baseline REM amounts for any group. Surprisingly, animals exposed to SR or SI + SR had higher total REM post-ST than any other group, and this persisted through EXT. The increase was due to an increase in the number of REM episodes and was associated with reduced theta power. Theta power was also decreased in the SI group but without significant REM disruption. Freezing behavior was increased in the irradiated groups compared to SI and Shams in all assessment periods, and did not extinguish. Studies examining the neuroimmune profiles are ongoing.

Conclusions (100 word limit)

SI, SR, and SI + SR significantly altered sleep in ways that may impact astronaut health and performance. SR fragmented REM and reduced theta power, and effects differed in Res and Vul rats. It also impaired fear extinction. SI altered sleep, but did not prevent extinction. Ongoing analyses will determine correlations between sleep and neuroinflammation to understand how they may be altered by inflight stressors. Differences in Res and Vul rats suggest that it may be possible to identify characteristics that may make astronauts differentially susceptible to SR.

Support

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25

Assessing Genetic Variation for Effects of Lithium on Circadian Clock Period and Mortality in Fruit Flies

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Full Name and Credentials

Noah L. Fryou

Introduction (100 word limit)

Lithium is the treatment of choice for bipolar disorder, but the mechanism for its therapeutic effect remains unknown, and it has a low threshold for toxic side-effects. Bipolar disorder is cyclical disorder with periodic onsets of illness including depressive and manic episode that vary in cycle length among patients. One of lithium's well-documented effects- a lengthened circadian clock period- is intriguing since a circadian system abnormality is a potential explanation for the cyclical nature of bipolar disorder.

Methods (200 word limit)

Although fruit flies are an efficient model organism for genetic analysis of circadian clock mechanisms, only a few Drosophila studies have documented circadian effects of lithium, and none have examined genetic variation. We used a random sample of eight inbred strains from the Drosophila Genetic Resource Panel (DGRP) to examine genetic variation for the response of circadian clock period to lithium, and toxicity.

Results (200 word limit)

Among the eight strains examined so far, there is no significant overall effect of lithium on circadian period, and only one significantly lengthens circadian clock period in response to lithium. There is a significant sex difference in the response to lithium for circadian period, with an increase females and a decrease in males. There are highly significant strain differences in mortality in response to lithium.

Conclusions (100 word limit)

Our results suggest that the set of approximately 200 DGRP strains will be useful for investigating genetic variation in circadian period changes and toxicity in response to lithium treatment.

Support

NA

26

The efficacy of weighted blankets on sleep and well-being among children with attention-deficit/hyperactivity disorder and sleeping difficulties

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Full Name and Credentials

Maria Lönn, Phd student

Introduction (100 word limit)

Difficulties with sleep initiation and sleep maintenance are common in children with attention-deficit/hyperactivity disorder (ADHD). Sleeping difficulties can have consequences on children's circadian rhythms with decreased daytime functioning and well-being but also family stress.

In Sweden, weighted blankets have been used as a sleep intervention in the child and adolescent mental health service (CAMHS) but have recently been discontinued due to lack of evidence.

The aim of this study was to evaluate the efficacy of weighted blankets on sleep and well-being among children with ADHD and sleeping difficulties.

Methods (200 word limit)

A randomized controlled trial with a cross-over design (4+4 weeks) was conducted at a CAMHS clinic in Sweden during 2019-2022. Children diagnosed with uncomplicated DSM-5 ADHD with verified sleeping difficulties according to three selected questions from the Children's Sleep Habits Questionnaire (CSHQ) were randomized to start with either a weighted blanket or a lighter control blanket. Data collection was performed in the child's home environment during measurement weeks (week 0, week 4 and week 8) using actigraphy (Motionware 1.2.47 Camntech), daily sleep diary, as well as questionnaires.

Primary outcome was Total Sleep Time (TST) evaluated with actigraphy. Secondary outcomes included; Sleep Onset Latency (SOL), Wake After Sleep Onset (WASO) and Sleep Efficiency (SE) measured with actigraphy as well as child- and parent rated sleep and well-being. Parent-rated sleep outcomes included restlessness during sleep onset, restlessness during sleep and sleeping difficulties (CSHQ). Child rated sleep included Insomnia Severity Index (ISI).

Child-rated well-being was evaluated with health-related quality of life (EQ5DY) and Child Outcome Rating Scale (CORS). Paired t-test or Wilcoxon's signed test for non-parametric data was used to evaluate the effect of weighted blankets (α two-sided was set to 0.05).

Results (200 word limit)

The study included 94 children with ADHD with mean age 9.0, (sd 2.2), 53 boys and 41 girls. Adherence to both types of blankets (weighted blanket and lighter control blanket) was equally high (89.7% used the blankets during sleep for 6-7 days during measurement weeks). Low drop-out rate (n=3) and high adherence during the trial resulted in cross-over analysis of actigraph data (n=85), daily sleep diary (n=89), child questionnaire data (n=88) and parent questionnaire data (n=81).

Weighted blankets had a significant effect on TST (mean diff. 8.05 min, $p<0.05$) and SE (mean diff. 0.83%, $p<0.05$) but not on WASO ($p>0.05$) or SOL ($p>0.05$) when evaluating within subject differences of sleep during the period with weighted or control blanket. The daily sleep diary showed decreased restlessness during sleep (mean diff. -0.18, $p<0.05$) but not during sleep onset ($p>0.05$). Questionnaire outcomes showed significant effect on parent-rated CSHQ (mean diff: -1.05, $p<0.05$) but not on child-rated ISI ($p>0.05$).

No significant effect was found on child rated well-being according to EQ5DY or CORS ($p>0.05$) when comparing periods with weighted or control blankets.

Conclusions (100 word limit)

This RCT showed that children with ADHD and sleep problems experienced improved sleep time, sleep habits and less restlessness during sleep while using weighted blankets. Thus, weighted blankets are likely effective and an alternative to sleep medication. Use of weighted blankets as a sleep intervention within CAMHS has the potential to improve child sleep and decrease family stress. Consequence on child well-being may be more relevant in a long-term perspective and is yet to be determined.

Support

N/A

27

WITHDRAWN

28

Evidence for a stepped-care model integrating digital therapy for insomnia

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Full Name and Credentials

Chaewon Sagong

Introduction (100 word limit)

Digital cognitive behavioral therapy for insomnia (dCBT-I) has been shown to be effective, and confers the advantages of higher accessibility and affordability; however, tradeoffs include the loss of clinician support and the ability to personalize treatment. Furthermore, many individuals do not remit following dCBT-I, and thus may benefit from an increased dose of CBT-I. This study tested the efficacy of a stepped-care approach that combines dCBT-I (step 1) with face-to-face CBT-I (fCBT-I; step 2).

Methods (200 word limit)

1237 individuals with insomnia (DSM-5 diagnostic criteria) were randomized into two conditions at step 1: dCBT-I (N=613), or an online sleep education control (N=624). Participants in the dCBT-I condition who did not remit (ISI>9) were further randomized to either face-to-face CBT-I (N=103) or sleep education (N=104). Insomnia (Insomnia Severity Scale) was assessed at baseline, post-step 1, and post-step 2.

Results (200 word limit)

Those who received stepped-care (dCBT-I to fCBT-I) achieved comparable improvements in insomnia (pre-dCBT-I ISI: 18.7, SD=3.3; post-fCBT-I ISI: 7.5, SD=4.4) compared to those who remitted following only dCBT-I (pre-treatment ISI: 17.1, SD=3.0; post-treatment ISI: 6.6, SD=2.0). Furthermore, remission rates in the fCBT-I condition at step 2 (74.8%) was almost two-fold that of the control condition at step 2 (37.8%). Overall, adding a stepped-care component increased the remission rate by 2.18 times compared to dCBT-I alone.

Conclusions (100 word limit)

Preliminary evidence from this study provide suggest that a stepped-care approach that adds fCBT-I for non-remitters to dCBT-I is an efficacious model for insomnia treatment.

Support

Support for this study was provided from the National Institute of Mental Health R56MH115150 and R01MH122636 awarded to Dr. Christopher Drake.

30

Estimating cognitive scores, age, and sleep stages from full-night sleep EEG with a multi-task deep neural network.

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Full Name and Credentials

Wolfgang Ganglberger, MS

Introduction (100 word limit)

Impaired sleep quality, quantity and timing are associated with neurological and mental health disorders, potentially through disruption of functional and anatomical neuronal pathways. Sleep-wake cycles, and sleep state oscillations likely encode brain health, therefore, sleep and timing may provide accessible biomarkers for estimating brain health. One potential biomarker is sleep electroencephalogram (EEG)-based brain age, which could indicate abnormal or accelerated aging. Another potential method to build a biomarker is to directly estimate cognitive function from sleep. Here, we investigate how well age and neuropsychological test scores can be predicted from EEG using machine learning.

Methods (200 word limit)

We used data from 735 participants of the Framingham Heart Study (FHS). Each participant had at a minimum one polysomnography (PSG) and one neuropsychiatric evaluation for fluid intelligence (Wechsler Adult Intelligence Scale, NPS). Additionally, age at evaluation was available for each participant, resulting in 1244 PSG-age-NPS triplets. The EEG was transformed into the time-frequency domain with the multitaper method (2-second window length, 1-second step size). The resulting spectrograms were all zero-padded to 11 hours, harmonizing the numerical dimensions of the sleep representations across subjects.

We developed an artificial deep neural network with a “U-Net”-like architecture, i.e., consisting of an encoder and decoder part. The network’s input was a subject’s EEG spectrogram, and its tasks were to predict sleep stages for 30-second epochs, the subject’s age, and the subject’s neuropsychological test score (NPS). The model mainly consisted of convolution-batch normalization-max pooling/upsampling layers, with a total of 11 million parameters. Fully connected layers for the age and NPS prediction tasks consisted of 15,600 parameters. The loss functions used were cross entropy for the sleep staging task and mean squared error for the age and NPS tasks. We evaluated the model’s performance with 10-fold cross-validation.

Results (200 word limit)

Sleep staging: The accuracies (means and standard deviations across folds) per sleep stage were Wake 0.64 (0.02), N2 0.61 (0.02), N3 0.59 (0.04), NREM combined 0.79 (0.01), and REM 0.53 (0.06). Cohen's Kappa was k=0.54 (0.02). Predicting age: Pearson correlation between chronological age and predicted age was r= 0.60 (0.06). Predicting neuropsychological test score: Pearson correlation between the NPS and the predicted cognitive score was r= 0.25 (0.07). Hence, a multi-task deep learning network was able to predict sleep stages, age, and cognitive scores from full-night sleep EEG spectrograms with varying performance precision.

Conclusions (100 word limit)

While there was a strong correlation between predicted age and chronological age, predicted cognition scores correlated weakly to moderately with the actual scores. This study using data from 735 subjects, a relatively modest number regarding the machine learning approach chosen, may serve as proof-of-concept. It is likely that prediction performance for all tasks can be increased with a) larger and more variable datasets, and b) data containing consecutive nights of sleep, enabling encoding of sleep-wake cycle properties. The sleep-based, subject-specific estimates for age and cognition may serve as biomarkers for brain health, a hypothesis we will test in future studies.

Support

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31

The association between core body temperature and slow wave activity in cognitively normal older adults

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Full Name and Credentials

Ankit Parekh, PhD

Introduction (100 word limit)

Core body temperature (CBT) is believed to be associated with both sleep propensity and architecture. Circadian rhythm has a profound impact on CBT, and CBT is often characterized as an objective gold standard measure. Reduced CBT fluctuation amplitude particularly around sleep has been associated with neurodegenerative disorders. Slow wave sleep (SWS) contributes to both daytime functioning, physiological processes, and is thought to be influenced by CBT preceding sleep onset. Here we aim to examine the dynamics between change in CBT, measured using an ambulatory ingestible pill, and an objective SWS measure in a cohort of cognitively normal healthy older adults.

Methods (200 word limit)

19 cognitively normal elderly subjects (64 ± 6 yrs., 15 female) underwent a nocturnal polysomnography (NPSG) for 2 consecutive nights at the Mount Sinai Integrative Sleep Center (MSISC). The CorTemp ingestible sensor was used to measure CBT through its wireless transmission which is accurate to ±0.2°C. We used our previously validated algorithm to smooth nonlinear CBT data which had random gaps and outliers (DRAGO; Parekh et. al. IEEE SPMB, 2019). A decline in CBT was objectively defined as the absolute change in CBT from the first peak prior to lights out to the nadir after lights out. Slow wave activity (SWA), an objective measure of SWS, was calculated as the relative spectral power (F3-M2) in 0.5- 4 Hz in 5 second epochs. As in the case of CBT, we evaluated the percent change in SWA from lights out to the first peak after sleep onset.

Results (200 word limit)

Out of the 19 subjects, 4 had bad quality EEG and 3 had bad quality CBT data, reducing the effective sample size to 12 (2M/10F; mean duration of CBT recording = 47.5 ± 14.1 hours; mean total sleep time = 7.2 ± .7 hours, AHI3A = 9.34 ± 7.78). We observed that magnitude of decline in CBT was associated with the percent increase in SWA (rho = 0.79, $p < 0.01$). The mean decline in CBT was 0.49 ± 0.33°C and the mean increase in SWA was 98.39 ± 38.81% (mean ± std.).

Conclusions (100 word limit)

To our knowledge, this is the first report of simultaneous CBT measurement using ingestible telemetry together with objectively defined SWS. Our observations are in line with previous studies using gold standard measurements of CBT. We used objective measures for CBT as well as SWS, which is a strength compared to prior studies. Our data supports further validation of using ambulatory CBT measurements to characterize the dynamics between sleep and circadian rhythms. Here, individuals with obstructive sleep apnea were not excluded and hence its effect on the relationship between CBT and SWS remains to be tested.

Support

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32

Determinants of non-completion of sleep apnea testing during pregnancy

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Full Name and Credentials

Mihaela Bazalakova MD PhD

Introduction (100 word limit)

Completion of testing during pregnancy for those who screen positive for obstructive sleep apnea (OSA) is imperative for the timely diagnosis and appropriate treatment of OSA, as the latter may reduce the risk of developing hypertensive disorders of pregnancy or other adverse pregnancy outcomes. In order to identify potential barriers, we assessed predictors of non-completion of sleep apnea testing by women identified to be at high risk of OSA by screening during pregnancy. We hypothesized that non-completion of sleep apnea testing would be predicted by insurance status and obstetric factors, such as estimated gestational age (EGA) at time of testing.

Methods (200 word limit)

We performed a retrospective analysis of the first 500 women in our sleep pregnancy database which includes both pregnant and preconception patients who screened positive for OSA; here, those screened preconception were excluded. Multivariable Poisson regression was used to determine which factors were independently associated with non-completion.

Results (200 word limit)

Of 445 referred pregnant women, 214 (48.1%) completed sleep apnea testing. Factors associated with a higher incidence of testing non-completion on univariate analysis included race, payor, non-partnered status, type 2 diabetes mellitus, higher parity, one or more living children, history of preterm birth in a prior pregnancy, history of preeclampsia in a prior pregnancy, and referral in the third trimester of pregnancy. Symptoms of loud snoring or witnessed apneas were both associated with increased incidence of sleep apnea testing completion. Multivariable Poisson regression demonstrated that having public insurance predicted non-completion of sleep apnea testing during pregnancy.

Conclusions (100 word limit)

In this small study, after controlling for potentially confounding factors, public insurance was an independent predictor of not-completion of sleep apnea testing during pregnancy. Accordingly, we plan to next target interventions at improving patient education and access to sleep apnea testing for those in whom this risk factor is identified.

Support

None.

33

Exploring the temporal dynamics of sleep architecture following consecutive nights of pre-sleep alcohol administration using generalized additive models (GAMs)

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Full Name and Credentials

Katie McCullar

Introduction (100 word limit)

Alcohol is the most used and abused psychoactive substance in the world, yet our understanding of how it alters brain function and behavior is incomplete. Research has indicated that drinking alcohol before bed can alter sleep, including shorter sleep duration, lower sleep efficiency, and sleep architecture disruption. Sleep dynamics following alcohol remain poorly characterized. Moreover, the effect of consecutive nights of alcohol consumption on sleep remains to be elucidated. Thus, this study combines overnight sleep measurement with alcohol administration to investigate how alcohol use alters epoch-by-epoch progression of sleep architecture using generalized additive models (GAMs).

Methods (200 word limit)

Thirty (15F; ages=22-57, mean=33yr) adults who self-reported moderate drinking were instructed to maintain a consistent sleep schedule (8-9h time in bed) for a least 7 nights monitored by actigraphy before entering a cross-over within-subjects design involving two sets (separated by>3 days) of 3 consecutive nights of in-lab polysomnography (PSG). For all nights in each condition, participants drank one of two beverages—mixer-only or mixer+alcohol (target breath alcohol content (BrAC) of 0.08 mg/l) across 45 mins ending 1 h before lights out. PSGs were staged in 30-sec epochs according to Rechtschaffen and Kales (1968). The following sleep variables were derived: slow wave sleep (SWS) as a percent of sleep period across the full night (%SWS), full-night percent REM (%REM); each computed across thirds of the sleep period, i.e., %SWS T1-3 and % REM T1-3; Sleep Latency; and REM latency. Generalized additive modeling (GAM) was used to produce cumulative frequency plots of sleep stages with 30 second epochs to obtain high-resolution images of sleep architecture across the night. Mixed-methods analysis examined beverage type, study night, and the interaction of night and beverage type for the sleep variables of interest and was used to validate the trends found using the GAM models.

Results (200 word limit)

Comparing each alcohol night versus the mixer nights, the GAM models showed greater accumulation of SWS with alcohol from ~1.5 hours to ~6 hours into the sleep episode and slower for REM sleep with alcohol from ~1.5 hours to 6 hours Night 1 and ~1.5 hours to 5.5 hours for Nights 2 and 3. Of interest, the GAM models indicate that the effects of alcohol on REM sleep dissipate across the consecutive nights of alcohol consumption. The mixed-method analysis of epoch-staged data supported each of these findings and revealed a main effect of alcohol on: % SWS T1-T3, REML, % REM T1, % REM, and a main effect of night on: REML, % REM T2, and sleep latency. No significant interactions were discovered.

Conclusions (100 word limit)

Alcohol increased the rate of accumulation for SWS and decreased the rate of accumulation for REM sleep. Alcohol also decreased the total amount of REM sleep following one night of alcohol exposure. The later effect was less pronounced on the second and third nights of alcohol exposure. The effect of alcohol on REM sleep variables, i.e., lessening across the consecutive nights of alcohol consumption may indicate habituation.

Support

R01AA025593, 5P20GM139743-02

34

Ontogenesis of the molecular response to sleep loss

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Full Name and Credentials

Christine Muheim, PhD

Introduction (100 word limit)

Sleep deprivation (SD) results in profound cellular and molecular changes in the brain, potentially resulting in, or aggravating, neurodevelopmental and psychiatric disorders. In adult rodents, transcriptomic studies after SD provided key insights into the molecular consequences of sleep loss. However, less is known about the transcriptomic response to SD during development. Considering that developing animals do not respond to SD the same as adults (no changes in EEG slow wave activity), suggests that molecular changes caused by SD may also be different. The objective of the study was to evaluate the cortical response to SD using RNA-seq across postnatal development.

Methods (200 word limit)

Genome-wide gene expression after SD in the pre-frontal cortex (PFC) was measured at four ages: when the adult homeostatic response to SD is absent (postnatal day 16, P16), emerging (P24), adult like (P30), or fully established (P70-90). Cortical PFC RNA was extracted after 3h or 5h of SD, age matched control samples were collected at the same circadian time. Quantification of transcript expression from RNA-seq data was performed using Salmon and normalization of unwanted variables was performed using RUV-seq. Differential expression analysis was performed using edgeR. Functional enrichment analysis of genes differentially expressed (FDR<0.05) was done using DAVID v2021, using the following databases as sources of functional information: Uniprot Molecular Function (MF) and Biological Process (BP), and KEGG pathways (PW). Statistical enrichment was determined relative to all genes expressed in the mouse PFC with EASE score (modified Fisher's exact P-value) of 0.05. To cluster functional terms, a fuzzy clustering algorithm was run using a similarity threshold of 0.2-0.25 and an overlap of at least 3 genes.

Results (200 word limit)

We found that SD had different effects on PFC gene expression depending on developmental age. At the youngest age (P16), only a small fraction of genes was differentially regulated by SD. This pattern changed across development, with greater numbers of gene changes with increased age. Across all ages, there were a set of conserved genes, always differentially expressed after SD. Genes in that group were related to the wnt pathway and transcription in general. In ages where an adult like response to SD is

detectable, we found genes related to cellular stress response highly enriched. We also found effects unique to certain ages; at P24 SD affected genes related to development while at P90 the vast majority of differentially expressed genes (DEGs) could be mapped to metabolic pathways.

Conclusions (100 word limit)

These findings suggest that the cost of wakefulness—and possibly the driving force for sleep need—changes across postnatal development. With the appearance of an adult like response to SD we see an increase of the molecular response to cell stress. This potentially implies that sleep need reflects on a more global level the need for the activation of molecular repair mechanisms. Most interestingly however, our data shows that the Wnt signaling pathway is tied to sleep regulation even in the absence of a coordinated cortical manifestation of sleep need.

Support

N/A

35

Alcohol synergizes with cholinergic neuron depression to cause long-lasting sleep deficits

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Full Name and Credentials

Maggie Chvilicek, BS, BA

Introduction (100 word limit)

Insomnia is reported in 72% of patients in AUD treatment and is a high predictor of relapse, making it a valuable target to improve treatment outcomes. Despite extensive evidence of the phenomenon from self-reports and controlled experiments in humans and animal models, nothing is known about the biological mechanisms of how alcohol induces sleep deficits. *Drosophila melanogaster* show behavioral and mechanistic conservation of sleep and alcohol responses, offering an excellent approach to unraveling the mechanisms of their interaction. Our goal was to establish *Drosophila* as a model for alcohol-induced sleep deficits and to identify the neuronal mechanisms mediating these effects.

Methods (200 word limit)

We exposed flies to either 95% vaporized ethanol or water as a control and then placed them in the *Drosophila* Activity Monitor (DAM) for sleep analysis. The DAM simultaneously records locomotor activity from individual flies as infrared beam breaks. We quantified sleep as periods without any beam-breaks lasting 5 minutes or longer, as established previously. We measured sleep duration during the daytime and nighttime as well as sleep latency, i.e. the time between lights-off and the first sleep bout.

Results (200 word limit)

Following a single, high dose of alcohol, wildtype flies showed significant loss of nighttime sleep and significant increases in sleep latency compared to water-exposed controls. These effects persist for several days and depend on a high dose of alcohol in which flies become behaviorally sedated since even multiple low-dose alcohol exposures do not impact sleep. To investigate the biological mechanisms regulating these effects, we tested the roles of different transmitter-producing neuron populations. We genetically silenced specific neurons in a temperature-dependent manner either concurrently with alcohol exposure or prior to alcohol exposure. In doing so, we showed that silencing acetylcholine neurons during alcohol exposure dramatically increased sleep latency and reduced nighttime sleep duration compared to control flies, suggesting the involvement of cholinergic neurons in mediating alcohol's effects on sleep.

Conclusions (100 word limit)

Here, we establish *Drosophila* as an excellent model to study the mechanisms of alcohol-induced sleep loss since flies recapitulate human alcohol-induced sleep deficits that persist even in the absence of continued alcohol use. Our study's results offer first insights into the neuronal mechanisms of alcohol-induced sleep loss, with cholinergic neurons playing a central role. We propose a model in which high doses of alcohol suppress cholinergic signaling to consequently impact sleep latency and duration, even long after alcohol has been metabolized, indicating long-lasting neuronal adaptations with negative consequences for sleep.

Support

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36

WITHDRAWN

37

Machine learning analyses reveal circadian features predictive of risk for sleep disturbance

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Full Name and Credentials

Professor Krista K Ingram

Introduction (100 word limit)

Sleep disturbances often cooccur with mood disorders, with poor sleep quality affecting over a quarter of the global population. Recent advances in sleep and circadian biology suggest poor sleep quality is linked to disruptions in circadian rhythms, including significant associations between sleep features and circadian clock gene variants.

Methods (200 word limit)

Here, we employ machine learning techniques, combined with statistical approaches, in a deeply phenotyped population to explore associations between clock genotypes, circadian phenotypes (diurnal preference and circadian phase), and risk for sleep disturbance symptoms.

Results (200 word limit)

As found in previous studies, evening chronotypes report high levels of sleep disturbance symptoms. Using molecular chronotyping by measuring circadian phase, we extend these findings and show that individuals with a mismatch between circadian phase and diurnal preference report higher levels of sleep disturbance. We also report novel synergistic interactions in genotype combinations of Period 3, Clock and Cryptochrome variants (PER3B (rs17031614)/ CRY1(rs228716) and CLOCK3111 (rs1801260)/ CRY2 (rs10838524)) that yield strong associations with sleep disturbance, particularly in males.

Conclusions (100 word limit)

Our results indicate both direct and indirect mechanisms impact sleep quality; sex-specific clock genotype combinations predictive of sleep disturbance may represent direct effects of clock gene function on downstream pathways involved in sleep physiology. In addition, the mediation of clock gene effects on sleep disturbance indicates circadian influences on sleep quality. Unraveling the complex molecular mechanisms at the intersection of circadian and sleep physiology is vital for understanding how genetic and behavioral factors influencing circadian phenotypes impact sleep quality. Such studies provide potential targets for further study and inform efforts to improve non-invasive therapeutics for sleep and circadian disorders.

Support

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38

Sleep apnea-related hypoxemia, not sleep fragmentation, are associated with white matter hyperintensities in older adults

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Full Name and Credentials

Destiny Berisha, B.E.

Introduction (100 word limit)

Sleep apnea is a sleep breathing disorder that increases risk for Alzheimer's disease- related pathology potentially through cerebrovascular pathology, likely exacerbated by two features of sleep apnea: intermittent hypoxia and sleep fragmentation. White matter hyperintensities (WMH), a marker of small vessel cerebrovascular disease, increase in aging and in AD. Here, we tested the hypothesis that apnea-related hypoxemia severity, and not sleep fragmentation, are associated with WMH volume in older adults.

Methods (200 word limit)

Thirty-two older adults (73.4±5.3 years, 19 female, AHI=15.2±18.9) were evaluated with overnight polysomnography (PSG). Apnea severity was quantified using the Apnea-Hypopnea Index (AHI), Respiratory Disturbance Index (RDI), and blood oxygen desaturation (Oxygen Desaturation Index (ODI), duration, frequency of ≥4% desaturations, and desaturation nadir) stratified by sleep stages. T1-MPRAGE and T2-FLAIR scans were acquired using a 3T Siemens scanner. Total WMH volumes were derived using a semi-automated algorithm. Overnight memory retention was assessed using the change in the lure discrimination index (LDI) for the emotional version of the Mnemonic Discrimination Task.

Results (200 word limit)

Traditional measures of apnea severity and sleep fragmentation were not significantly associated with global WMH volume. However, both max duration of oxygen desaturation events (r=0.44, p=0.011) and max oxygen desaturation percentage (in rapid-eye movement (REM) and in total sleep: r=0.48, p=0.005, and r=0.44, p=0.01), were significantly associated with total WMH volume. Similar relationships were found with time spent below 90% and 80% oxygen saturation (Kendall's tau=0.33, p=0.02 and tau=0.275, p=0.03). These relationships survived adjustment for sex, age, and hyperlipidemia and/or hypertension medication at time of PSG. Global WMH volume was also negatively associated with overnight memory retention (r=-0.38, p=0.03).

Conclusions (100 word limit)

These findings suggest that hypoxemia severity, compared to sleep fragmentation, are more strongly associated with global WMH volume. As WMH volume was associated with overnight memory retention, this may suggest a pathway in which OSA increases WMH, which is subsequently associated with tau burden, worsening sleep-dependent memory.

Support

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39

A graph theoretical approach to study sleep-dependent memory consolidation in older adults

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Full Name and Credentials

Miranda Chappel-Farley, M.S.

Introduction (100 word limit)

The functional connectome of the human brain exhibits modular organization. In memory consolidation theory, reactivation of a memory trace during NREM sleep involves reinstatement of a hippocampal index that binds attributes of an experience existing within cortical modules. This reinstatement of activity facilitates associations between modules to promote consolidation. Surprisingly, graph theory—the mathematical study of networks—has been rarely applied to examine memory consolidation theory. Here, we examined whether network modularity (Q), an index of module segregation, and eigenvector centrality (EC), a metric of a node's influence over a network, are associated with memory consolidation and sleep architecture.

Methods (200 word limit)

Thirty-four older adults (μ=72.3±5.8 years, 26 Females) completed overnight polysomnography to assess sleep and performed the emotional version of the Mnemonic Discrimination Task, which assesses the ability to discriminate among similar negative, neutral, and positive images—prior to and following overnight sleep. The Lure Discrimination Index (LDI) measures this discrimination ability and was calculated before and after sleep, with overnight change in LDI measuring sleep-dependent memory consolidation. Structural and resting-state functional MRI data (partial acquisition focusing on medial temporal lobe regions) were collected using a 3T Siemens Magnetom Prisma Scanner. All neuroimaging data were preprocessed with the CONN toolbox. The Brainnetome Atlas was used to define network nodes (151 regions) and weighted signed adjacency matrices were derived. Q and hippocampal EC were computed using the Brain Connectivity Toolbox. Amygdala EC was also explored due to the emotional nature of the memory task. Parahippocampal and ventromedial parietooccipital sulcus (vmPOS) EC were selected as control regions. Q was computed by running 150 iterations of the Louvain Modularity Maximization algorithm to generate consensus partitions across four different resolution parameters corresponding to the 50th percentile of connection weights.

Results (200 word limit)

After adjusting for age, biological sex, and apnea-hypopnea index (AHI), greater Q (across all four resolution parameters) was negatively associated with overnight change in LDI irrespective of emotional valence (r's: -0.311 to -0.307, p's <0.05) and a greater percentage of NREM 3 sleep (r's: 0.362-0.367, p's<0.05). After controlling for the aforementioned covariates, hippocampal EC was not associated with overnight change in LDI but was positively associated with LDI at both immediate and delayed test when collapsing across emotional valence (all r's >= 0.38, p's<0.05). When looking at valence-specific memory performance, hippocampal EC was positively associated with LDI for positive and neutral stimuli at immediate test (r's >=0.32, p's<0.05), and LDI for negative stimuli at delayed test (r's >=0.34, p's<0.05). After adjusting for covariates, amygdala EC was positively associated with LDI at for emotional stimuli only at delayed test (r's >=0.34, p's<=0.05). Parahippocampal and vmPOS EC were not significantly associated with any memory or sleep architecture measure.

Conclusions (100 word limit)

These findings suggest that greater interregional connectedness and more network integration may support memory consolidation during sleep. Additionally, a more topologically influential hippocampus aids in memory function in general, whereas a more well-connected amygdala supports emotional memory performance following sleep. Notably, these relationships with memory performance were specific to hippocampus and amygdala, as no significant associations were observed with both proximal and distal control regions. Taken together, these results provide support for two major themes of memory consolidation theory: interregional communication and hippocampal influence on network organization.

Support

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40

WITHDRAWN

41

WITHDRAWN

42

Identification of longitudinal use trajectories of sedative-hypnotic medications among fee-for-service Medicare beneficiaries: 2016-2018

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Full Name and Credentials

Christopher N. Kaufmann, PhD, MHS

Introduction (100 word limit)

Use of sedative-hypnotic medications (including benzodiazepines and other hypnotics) is common, particularly in older adults. While indicated for short-term use for insomnia and anxiety disorders, chronic use of these agents is prevalent raising safety concerns for adverse health outcomes (e.g., falls, fractures, and cognitive impairment). While past studies showed an increasing trend in overall use of these agents, none have characterized longitudinal use trajectories that may be associated with problematic use patterns and adverse outcomes. This study aimed to characterize the dose and duration patterns of sedative-hypnotic medication use.

Methods (200 word limit)

In a retrospective cohort study using a 15% national sample of Medicare data, we identified fee-for-service Medicare beneficiaries initiating sedative-hypnotics in 2016-2018. We identified three mutually exclusive cohorts: a) benzodiazepine (BZD; e.g., diazepam) users, b) non-BZD hypnotic (e.g., zolpidem) users, and c) concurrent users (e.g., users of both BZDs and non-BZD hypnotics). We excluded beneficiaries diagnosed with malignant cancer or received hospice or nursing home care during the study period, or were diagnosed with dementia in the 6 months before initiating use (index date). We examined the 1-year trajectory period of prescriptions for these agents from the index date. For each eligible beneficiary, we calculated average weekly diazepam milligram equivalent (DME) dose based on dispensing data. For purposes of interpretation, we considered a DME>15 as high-dose, DME of 5-15 as moderate-dose, and DME<5 as low-dose use. We employed group-based multi-trajectory models to identify distinct dose and duration patterns in the 12 months after medication initiation.

Results (200 word limit)

Among 159,553 eligible beneficiaries (mean age=72.8 years [range 65-106], female=67.6%, White=87.9%), 77% (n=123,336) were BZD users, 18% (n=28,256) were non-BZD hypnotic users, and 5% (n=7,961) were concurrent users. For both BZD and non-BZD hypnotic users, we identified 4 distinct

trajectories: 1) brief high-dose use followed by discontinuation (45.9% of the BZD cohort; 37.9% of the non-BZD hypnotic cohort), 2) brief high-dose use followed by consistent low-dose use (34.5%; 45.9%), 3) chronic moderate-dose use (10.7%; 5.1%), and 4) chronic high-dose use (8.9%; 11.1%). For concurrent users, we identified 3 distinct trajectories: 1) brief concurrent moderate-dose use followed by low-dose use (62.0%), 2) chronic high-dose BZD use with moderate-dose non-BZD hypnotic use (18.5%), and 3) chronic moderate-dose BZD use with high-dose non-BZD hypnotic use (19.5%).

Conclusions (100 word limit)

Among Medicare fee-for-service beneficiaries, trajectory patterns in use of common sedative-hypnotic medications varied substantially. Future research should examine whether these patterns are associated with poorer health outcomes such as falls/fractures, cognitive impairment, and functional decline.

Support

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43

Parent, Home Environment, and Feeding Predictors of Circadian Rest-Activity Rhythm Entrainment among Young Infants

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Full Name and Credentials

Megan E. Petrov, PhD

Introduction (100 word limit)

Lighting, feeding mode, and maternal sleep contribute to infants achieving a circadian rest-activity rhythm within the first few months of life. However, less is known about other feeding conditions and the home environment as entrainment factors. Mother-reported data suggests differences in sleep and circadian rhythm outcomes by whether breastmilk is directly fed or expressed. Further, few investigations have examined the support, structure, and stimulation provided in the infant home as related to circadian rest-activity rhythmicity. Our aim was to explore whether feeding conditions, parent, and home environment factors are associated with circadian rest-activity rhythm at 8 weeks of life.

Methods (200 word limit)

English or Spanish speaking mothers (n=37, 33.1±4.6y, 27.0% Hispanic, 32.4% < bachelor's degree) and their full-term (≥37wk), singleton infants of normal weight (2.5-4kg) without major complications were recruited from the Phoenix, Arizona metropolitan area. At 8 weeks of age, infants wore a Micro Motionlogger (Ambulatory Monitoring Inc.) on their left ankle for five 24hr periods. Circadian rhythm analysis was performed in Action4 (v1.16) on actigrams with at least four valid 24hr periods (≥21hr) to produce cosinor and nonparametric circadian rhythm analysis (CRA) metrics including autocorrelation with 24hr lag-time, mesor, magnitude, acrophase, R2, interdaily stability, intradaily variability, and relative amplitude. Mothers reported on their current feeding mode (exclusively breastfed [n=22] vs. mixed and formula fed [n=15]), frequency of breastmilk feedings, and breastmilk mode (breast only [n=15] vs. pumped partial or fully [n=17]). Trained staff observed the infant's home environment using the Home Observation for Measurement of the Environment (HOME) Inventory total score and six subscale scores assessing parent/home environment responsivity, acceptance, learning materials, organization, involvement, and variety. Bivariate correlations examined associations among CRA metrics, maternal education, HOME Inventory scores, and breastmilk feeding frequency. Independent-sample t-tests examined differences in CRA metrics by feeding mode and breastmilk mode.

Results (200 word limit)

Infants, by 8 weeks of age, had the following CRA metrics (M±SD): 24hr autocorrelation of 0.25±0.12, mesor of 115.0±14.5, magnitude of 69.0±18.7, R2 of 0.43±0.10, acrophase of 14:18±1:45, interdaily

stability of 0.58±0.13, intradaily variability of 0.94±0.21, and relative amplitude of 0.62± 0.11. Less maternal education (Spearman r =-0.49, p 0.002), less presence and quality of learning materials in the home (r=-0.40, p =0.015), and being mixed or formula fed (M[SD]: 14:59±1:52) relative to exclusively breastfed (M[SD]: 13:50±1:32; t(35)=-2.04, p =0.049) were all associated with later acrophase time. None of these factors as well as the other HOME Inventory subscales were associated with other CRA metrics. However, there was a trend toward greater HOME Inventory total score and mean 24hour autocorrelation (r=.31, p=0.06). Among those providing breastmilk, there was no difference in CRA metrics between mothers who provided milk via pumping at least partially compared to the direct breast only. Lastly, greater number of breastmilk feedings per day were associated with greater intradaily variability (r=0.41, p=0.018)

Conclusions (100 word limit)

Among normally developing young infants, feeding conditions and the family home environment were not significantly related to the strength and daily regularity of their circadian rest-activity rhythmicity. However, indicators of lower socioeconomic status were related to later timing of peak activity suggesting these infants were being entrained toward a delayed circadian phase. Children with delayed circadian phase or evening chronotype tend to have more sleep disturbances, and are at greater risk for poor metabolic, mental health, and academic outcomes.

Support

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44

Associations between diabetes and sleep architecture: findings from the Baependi Heart Study

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Full Name and Credentials

Daniel Chen

Introduction (100 word limit)

Sleep disturbances are common clinical sequelae of type 2 diabetes (T2DM). The distribution of sleep stages, known as sleep architecture, is an important characteristic of sleep with implications for health. For example, one experimental study that suppressed NREM stage 3 (N3) observed impairments in glucose metabolism. However, there are few studies investigating how sleep architecture is altered in T2DM. Our aim was to compare sleep architecture among people with diabetes, prediabetes or neither in a large observational study. Our hypotheses is that people with diabetes and prediabetes would have poorer sleep architecture (less N3 and REM and more wake).

Methods (200 word limit)

This sample is from the Baependi Heart Study (BHS), a family-based cohort of adults in a rural area of southeastern Brazil. Participants underwent one night of at-home polysomnography (PSG) recordings and provided a fasting morning blood sample. We used the blood samples to assay HbA1c and fasting blood glucose. The PSG recordings were scored by qualified PSG technologists in Chicago and PSG data were summarized into amount and percentage of each sleep stage. Diabetes was defined as individuals who: 1) had a fasting blood glucose (FBG)>=126 OR 2) had HbA1c>=6.5 OR 3) were taking diabetic medication, and prediabetes was defined as individuals who 1) had [(5.7≤HbA1c<6.5) OR (100≤FBG<126)] AND 2) were not taking diabetic medication. All other participants were considered "healthy". We excluded participants that had an apnea-hypopnea index (AHI)>=30 from these analyses to reduce confounding due to OSA. Further, we compared sleep stages among these 3 groups in unadjusted and adjusted models and we additionally restricted to AHI<15 to further reduce confounding by OSA.

Results (200 word limit)

There were 1,152 participants in our sample; 112 had diabetes and 254 had prediabetes. In models adjusting for age, gender, BMI and AHI, people with prediabetes had significantly less REM sleep (-5.67min, p = 0.016) and REM percentage (-1.26%, p = 0.015) than healthy participants. Those with

diabetes had less total sleep (-.21 hours, p=.055) and less REM sleep (-6.2 minutes, p=.06), although not statistically significant. Additionally, we found that people with diabetes had significantly higher percentage of N3 than healthy participants (2.12%, p = 0.020). When we excluded participants with moderate OSA from the sample (AHI > 15), we found similar differences between groups.

Conclusions (100 word limit)

People with prediabetes and diabetes appeared to obtain slightly less REM sleep on average, even after accounting for sleep-disordered breathing. The underlying mechanism are unknown, but could be related to less total sleep time, particularly in those with diabetes. Whether less REM sleep has any health implications remains to be determined.

Support

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45

Prevalence of obstructive sleep apnea in commercial vehicle drivers and its correlation with road traffic accident and metabolic syndrome: A cross-sectional study.

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Full Name and Credentials

Dr. Surya Prakash

Introduction (100 word limit)

This study will attempt to evaluate the prevalence and association of OSA among commercial vehicle drivers from North India. Similarly, to date, no published study has been done to look for the association between metabolic syndrome and systemic inflammation in this group from India. There is no data regarding the relationship between OSA and traffic accidents in India. If data suggest a high prevalence of OSA and links it to road traffic accidents then specific interventions can be done to decrease the incidence of road traffic accidents.

Methods (200 word limit)

In this cross-sectional study, we evaluated 2200 commercial vehicle drivers. Out of 2200 drivers, we have screened 1915 subjects [1276 (67%) were low risk and 639 (33%) were high risk for OSA]. All drivers were commercial vehicle license holders. The subject's ages ranged from 30-60 years. Long-distance cab, truck, or bus driver was included in this study. Berlin and STOP-BANG questionnaires were used to assess high-risk groups for OSA amongst these drivers. All high-risk groups identified by the questionnaires underwent an overnight polysomnography test. Anthropometric Assessment: Body mass index (BMI), neck circumference, waist circumference (WC) and hip circumference (HC), and waist-hip ratio (WHR) were measured. Categorical data were analyzed by Chi-squared test, with Fisher correction when appropriate, and expressed as absolute numbers (%).

Results (200 word limit)

According to the STOP-BANG and Berlin questionnaires, the prevalence of sleep disorders was 29%. Blood pressure ($p<0.05$), body mass index ($p=0.0001$), and all circumferences profiles were significantly higher in the high-risk group as compared to the low-risk group.

In 639 high-risk subjects, polysomnography has been done in 181 subjects. Out of this, 151 (83.43 %) had OSA and 30 (16.57%) did not have OSA. Systolic and diastolic blood pressure ($p<0.05$) was significantly higher in the OSA group as compared to the non-OSA group. Mean values of BMI ($p=0.0001$), fat mass ($p=0.002$), %body fat ($p=0.002$), all circumferences ($p<0.05$) and skin folds thickness

($p<0.05$) were significantly higher in OSA subjects. Similarly, lipids ($p<0.05$) and liver function tests ($p<0.05$) and markers of insulin resistance ($p<0.05$) were significantly higher in OSA subjects as compared to non-OSA subjects. The mean values of high sensitive C-reactive protein ($p<0.0001$), hepacidine ($p=0.01$), interleukin 6 ($p=0.05$), interleukin 8 ($p=0.007$), interleukin 10 ($p=0.005$), VCAM ($p=0.04$), P-selectin ($p=0.001$), leptin ($p=0.02$), 8 epi- prostaglandin F2 alpha ($p=0.003$) was significantly higher in OSA subjects. We did not find any significant difference in serum ICAM, TNF alpha, thioredoxin in any groups ($P>0.05$).

Conclusions (100 word limit)

Untreated OSA is common in the Indian commercial driver population. This can lead to an increased risk of both fatal and non-fatal motor vehicle crashes. There is currently no mandate for effective screening, but effective screening tools do exist. If diagnosed with OSA, the literature demonstrates that effective treatment with CPAP therapy can decrease the risk of vehicle crashes and associated morbidity, mortality and cost.

Support

N/A

46

alpha1 containing GABAA receptors of thalamocortical relay neurons give sleep spindles their waxing and waning shape.

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Full Name and Credentials

David S Uygun, PHD

Introduction (100 word limit)

The thalamus is critical to the regulation of electroencephalographic (EEG) waveforms in both wakefulness and sleep. However, the circuitry underlying these oscillations is incompletely understood. Advances in in vivo CRISPR-Cas9 gene editing methods enable high-throughput and high precision gene targeting, within focused brain circuitry and neuronal subtypes. We used this technology to study the role of synaptic GABA inhibition onto the thalamocortical (TC) relay neurons in regulating sleep oscillations.

Methods (200 word limit)

To target TC neurons, we bred vesicular glutamate transporter subtype 2 mice expressing Cre recombinase (vGlut2-cre mice) with lox-stop-lox-Cas9 mice to generate vGlut2-Cas9 offspring. The resulting mice express Cas9 in vGlut2+ cells, including the majority of TC relay neurons. We then generated single-guide RNAs to target the alpha1 subunit of GABAA receptors, which is the synapse-localizing GABAA receptor isoform in wild-type TC relay neurons. Sleep recordings were conducted before and after introducing the knockdown (KD) of alpha1 subunits in a repeated measures design.

Results (200 word limit)

Compared with baseline (BL), KD of alpha1 in TC relay neurons reduced 10-15 Hz (sigma; the frequency band of spindles) power in spindle enriched NREM sleep , and altered the morphology of the spindles, reducing their amplitude (BL: 2.48±0.17 mV vs alpha1KD: 2.16±0.12 mV), duration (BL: 1.97±0.02 s vs alpha1KD: 1.82±0.04 s) and characteristic shape (N=14; pending histologic validation). There was a trend-level reduction in delta power but no changes in other frequency bands during NREM sleep.

Conclusions (100 word limit)

Sleep spindles have become a candidate target in disease because they are associated with learning and diminished by aberrant mental health. Our work suggests properly tuned spindles require synaptic GABAA receptors on TC relay neurons, where thalamic reticular nucleus outputs are received. These receptors may therefore be a target for pharmaceutical manipulation of spindles.

Support

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NIH support: R01 NS119227 (RB)

47

Retinal responsivity is associated with circadian phase and circadian alignment during the summer, but not during the winter

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Full Name and Credentials

Delainey Wescott, MS

Introduction (100 word limit)

Altered responsivity to light may be a physiological vulnerability for disrupted photoentrainment that may lead to circadian disruptions. The retina projects light information to the circadian clock through melanopsin-containing retinal ganglion cells (mRGCs). The post illumination pupil response (PIPR) measures the responsivity of mRGCs by isolating the sustained firing response of mRGCs. The current study investigated how season may affect this mechanistic pathway.

Methods (200 word limit)

Participants (N=72, age_m=35.9, age_{sd}=12.9) across a seasonal continuum (seasonal depression=21, subsyndromal seasonal depression=17, nonseasonal, never depressed controls=34) were invited for a winter and a summer visit (90 total assessments). Pupillometry assessments occurred between 10am-3pm and included alternating blue and red flashes of light. The PIPR was calculated as the net percent baseline pupil diameter 6 seconds post light stimulus and 10-30 seconds post stimulus. Circadian phase was assessed using Dim Light Melatonin Onset (DLMO). Salvia melatonin samples were collected every 30min over 6-hours on Friday evenings. PIPR and DLMO assessments occurred within the same week. Circadian alignment was calculated as the DLMO-midsleep phase angle. Midsleep was measured by actigraphy across 1-3 nights prior to DLMO. Two multilevel models were used to test the interactions of retinal responsivity and season on circadian phase and circadian alignment. Models included participant ID as a random intercept to account for repeated assessments across seasons. Age, gender, and circadian time of PIPR assessment were covariates.

Results (200 word limit)

Season of assessment significantly moderated the relationship between retinal responsivity and circadian phase (b=8.15; p=0.03), such that individuals with greater retinal responsivity showed later phase in summer. The relationship between responsivity and phase was blunted during the winter. There was a similar interaction between retinal responsivity and circadian alignment (b= -6.39; p=0.03), such that individuals with greater responsivity had shorter phase angles during the summer, with a blunted relationship during the winter.

Conclusions (100 word limit)

These findings suggest that seasonal changes in environmental light levels may impact the retina's responses to light and downstream circadian functioning. When there is sufficient light during the summer, individuals who are more responsive to light show delayed circadian timing, potentially due to the delaying effects of evening light. During the winter, the relationship between retinal responsivity and circadian phase was blunted, possibly due to lower overall environmental light. Understanding how environmental changes in light exposure interact with individual differences in retinal physiology is critical for developing targeted interventions for circadian disruptions.

Support

K.A.R. MH103313

48

Effect of Sleep Enhancement via Rocking on the Mouse Model of Humanized APPxPS1 Knock-in (KI) Induced Alzheimer's Disease (AD)

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Full Name and Credentials

Anjana Subramoniam

Introduction (100 word limit)

Research has shown that patients with AD experience sleep and circadian rhythm disturbances, often preceding cognitive impairments by many years, contributing to AD pathology.Efforts have been made to improve sleep in AD patients through pharmaceutical, electrical, and other means.However, more sleep enhancement techniques are warranted.Rocking during sleep has been observed to promote sleep through vestibular stimulation in both healthy humans and Wild Type (WT) mice.However, sleep enhancement via rocking has not been attempted in AD mouse models.Hence,we examine the effect of rocking on APP/PS1-KI mouse model.We hypothesize that sleep enhancement through rocking can potentially alter the progression of AD.

Methods (200 word limit)

All animal procedures were performed with IACUC approval at the University of Kentucky. APPxPS1 KI male mice (9-11 months) (which mimics the human AD condition with gradual expression of amyloid beta and cognitive impairment at nine months) were kept under a 12h:12h light/dark cycle and had access to food and water ad libitum. They were implanted with EEG/EMG electrodes and allowed to recover for two weeks. The mice were acclimated for two days, followed by EEG/EMG data acquisition during one day each of baseline, rocking, and recovery in that order. On the rocking day, a reciprocating elliptical-motion platform (HS-260 control – IKA Shakers) was utilized to laterally rock individually housed mice at a frequency of 1 Hz for ten hours (9 AM - 7 PM) during the light period (12h:12h light:dark cycle with lights on at 7 AM). The experiment was repeated thrice for each mouse. However, one baseline day had to be excluded due to data loss. After scoring vigilance state (Wake, NREM, REM) in 4-second epochs from the EEG/EMG data using standard criteria, mean bout duration, and proportion of time spent in each vigilance state were computed and compared for the three conditions.

Results (200 word limit)

We employed APPxPS1 KI mice to test the idea that rocking can mitigate sleep disturbances and cognitive impairments in AD-related neuropathology by increasing sleep depth and bout duration. We observed significant improvement in % NREM sleep during rocking compared to baseline ($p<0.015$) and post-rocking days ($p<0.001$) during the light period. A non-significant increase in mean NREM sleep bout

duration was also observed. Reciprocally, during the light period, a significant decrement in % REM sleep was observed for rocking compared to baseline ($p<0.001$) and post-rocking days ($p<0.001$). A significant reduction in the mean REM sleep bout duration was observed during rocking compared to baseline ($p<0.015$) and post-rocking days ($p<0.001$).

Conclusions (100 word limit)

Rocking can have the advantage of being easily translatable and non-invasive, and without side-effects in humans. To our knowledge, this is the first time that the effect of rocking on sleep was examined in an AD mouse model. Our results demonstrate that rocking increases NREM sleep. We plan to conduct future studies looking at the impact of rocking on AD neuropathology and cognition.

Support

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49

Relations of Sleep and Gut Metabolites in Colorectal Cancer Patients and Their Sleep-Partner Caregivers: A Preliminary Investigation

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Full Name and Credentials

Youngmee Kim, PhD

Introduction (100 word limit)

Cancer affects not only the patients, but also family members. Sleep disturbance is common among cancer patients and their family caregivers at a higher rate than their healthy adult counterparts. Growing recent evidence has also shown that imbalanced gut microbiota composition (gut dysbiosis) has been associated with the same morbidities of chronic sleep disturbance, suggesting the bidirectional relation between sleep disorders and gut dysbiosis. In addition, accumulating evidence shows the dyadic, cross-over effects of various cancer experiences of patients on their family caregivers' health outcomes, and vice versa. The interdependent sleep patterns in patient-caregiver pairs may affect their gut health.

Methods (200 word limit)

This pilot study examined the degree to which mutual sleep regulatory patterns between cancer patients and their sleep-partner caregivers was associated with the epigenic regulatory markers of gut metabolome. Patients who were diagnosed with colorectal cancer (n= 20, 56.6 years old, 34% female) and their heterosexual sleep partner caregivers (n= 20, 55.1 years old) completed a sleep diary (Sleep Consensus Diary) daily for 14 consecutive days and collected stool samples using fecotainers twice in a 10-day interval during the 14 days. Validating the methods, unsupervised principal components analysis showed tighter herd of metabolites among caregivers than patients, suggesting greater variance in the patients' metabolites; two patients, measured on two occasions (but not their caregivers) were outliers. Heatmap analysis revealed that these two patient outliers had 100-fold higher conjugated bile acids than all other patients and caregivers, suggesting a considerable suppression of their gut microbiome.

Results (200 word limit)

The untargeted metabolomics analysis identified the short chain fatty acid, butyrate, which plays a role of epigenetic regulator in the colon and other tissues. Levels of butyrate were correlated between two assessment times ($r = .63$) and between patients and their caregivers ($.11 < r < .27$), but did not significantly differ from each other ($t \leq .655$, $p \geq .484$). The two patients with the highest fecal conjugated bile acids also had the lowest butyrate levels. The metabolite library also revealed that most of the observable tryptophan metabolites followed a similar pattern with butyrate. Longer sleep

duration was marginally related to higher levels of butyrate (greater epigenic regulation) for both patients and caregivers, $r > .41$, $p < .08$. Although not statistically significant (due in part to small sample size), greater sleep efficiency was correlated with higher levels of butyrate ($r \geq .23$, $p \leq .37$), whereas longer sleep onset latency was correlated with lower levels of butyrate ($r \geq -.32$, $p \leq .18$) for both patients and caregivers. Furthermore, caregivers' greater sleep efficiency ($r = .52$, $p < .03$) and shorter sleep onset latency ($r = -.44$, $p < .07$) were correlated with their patients' higher levels of butyrate.

Conclusions (100 word limit)

These preliminary yet novel results hint the interplay between sleep and gut metabolites, warranting further investigation of the link between sleep and gut health at not only individual but also dyadic levels in a larger sample. The information about the association of mutual sleep regulation and gut health in colorectal cancer patients and their sleep partners who share similar psychological stressors of cancer and lifestyle behaviors, such as diet, does not currently exist. Such knowledge, however, about the downstream impact of mutual influence on sleep regulation and subsequent longer-term health has significant implication for this at-risk population.

Support

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50

Interactions between mutations in Shank3 and sex of sleep architecture and regulation

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Full Name and Credentials

Elizabeth Medina

Introduction (100 word limit)

Insomnia occurs at a higher rate in Autism Spectrum Disorders (ASD) than in typical development, affecting up to 93% of individuals. We have previously shown that a mouse model with a mutation in a high confidence ASD gene, Shank3, recapitulate the ASD sleep phenotype. ASD is diagnosis in more frequently in males than females at a rate of 4:1, however ASD the mechanisms contributing to this remain unknown. The objective of this study is to investigate sex-specific bias on sleep architecture using polysomnography in a rodent model of the neurodevelopmental disorder ASD.

Methods (200 word limit)

Adult (age 10-12weeks old) male and female Shank3ΔC and wildtype(WT) littermates were implanted with electroencephalographic (EEG) and electromyographic (EMG) electrodes. 48 hours of recording was collected using the Intan RHD2000 Interface. Animals were allowed 24 hours of undisturbed baseline, before 5 hours of sleep deprivation (SD) via gentle handling and allowed 19 hours of recovery sleep. Data was down sampled to 250 Hz and EEG and EMG data were high- and low-pass filtered at 0.05 and 50 Hz and 15 and 30 Hz, respectively. Time in rapid eye movement sleep (REM) and non-rapid eye movement sleep (NREM) and wakefulness was assessed visually by EEG and EMG waveform and activity in 4 second epochs using SleepSign. Matlab and SPSS were used for data analysis and visualization. Latency to sleep following SD was defined as the amount of time elapsed from release of SD to the first 28 seconds bout of non-rapid eye movement (NREM). NREM delta power (1-4 Hz) after SD was normalized relative to baseline NREM power.

Results (200 word limit)

We provide evidence that in adult mice sleep architecture and the homeostatic response to SD are modulated by both sex and the ASD relevant mutation, Shank3ΔC. In WT mice, females spend less time in awake in the dark period compared to males both at baseline and in recovery sleep. However, in response to SD, females WT mice have an increase in homeostatic sleep pressure (NREM delta). These sex differences are differentially altered in adult Shank3ΔC mutant mice. In baseline measurements,

female Shank3ΔC mice spend less time awake during the dark period compared to male counterparts, opposite of the sex effect displayed in WT mice.

In response to SD, female Shank3ΔC mice display a significant increase in homeostatic sleep pressure but spend more time in awake in the light period compared to Shank3ΔC males. Mutant Shank3ΔC mice males (both male and females), take significantly longer to fall asleep following SD despite accumulating equal or more sleep pressure than WT mice.

Conclusions (100 word limit)

Overall, our studies demonstrates sex differences in sleep architecture in adult mice at undisturbed baseline and in response to SD. Moreover a Shank3 mutation seems to affect female mice more severely after SD, as measured by sleep amounts, homeostatic sleep pressure, and sleep latency following SD. A genetic mouse model of ASD with a sex dependent phenotype, may help to better understand the mechanism contributing to the gender bias in ASD.

Support

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51

WITHDRAWN

52

RAI1 gene associated with sleep/circadian phenotypes enriched in Autism Spectrum Disorder WGS set

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Full Name and Credentials

Sandra Paulina Smieszek, PhD

Introduction (100 word limit)

Autism Spectrum Disorder (ASD) comprises a complex of neurodevelopmental disorders primarily characterized by deficits in verbal communication, impaired social interactions and repetitive behaviors. Smith–Magenis syndrome is a rare genetic disorder that results from an interstitial deletion of 17p11.2 and, in rare cases, from RAI1 gene variants. Haploinsufficiency of RAI1 is the primary cause of the neurobehavioral and metabolic phenotype in SMS. Individuals with SMS present with a distinct pattern of mild to moderate intellectual disability, and, almost uniformly, significant sleep disturbances. Alterations in RAI1 copy number have been also linked to a number of neurodevelopmental disorders including ASD.

Methods (200 word limit)

This study aimed to characterize the frequency of RAI1 genetic aberrations associated with Smith-Magenis Syndrome (SMS), in a large cohort of Autism Spectrum Disorder (ASD) whole genome sequencing samples. We conducted a large-scale association analysis of the ASD MSSNG whole genome sequencing data to elucidate the prevalence of RAI1 SNVs and CNVs in the ASD population. We accessed the MSSNG database hosting over 11,000 genomes (6080 probands) and queried both SNVs and CNVs. Specifically, we focused on the prevalence of the classic deletions, microdeletions of (exon 3) and of the causative variants.

Results (200 word limit)

We report a 2.5x enrichment of the major deletion and a >5x enrichment of the frameshift variants as compared to the known prevalence of SMS 1/15,000. Additionally, we report significant enrichment of RAI1 rare missense variants in ASD subjects with respect to controls (54 variants/6080 ASD subjects and 6 variants/2541 controls, p-value<0.002, OR 3.78, CI 1.62-8-81). The SMS phenotype and associated sleep disturbances are mainly caused by RAI1 haploinsufficiency. Sleep disturbances as seen in SMS may overlap in ASD, especially in patients with consequential variants in the RAI1 gene.

Conclusions (100 word limit)

Both ASD patients and SMS patients suffer from sleep disturbances. Currently, the prevailing theory is that there is an underlying circadian pathophysiology causing sleep disturbances in SMS associated with RAI1 haploinsufficiency, as these patients exhibit low overall melatonin concentrations and abnormal timing of peak plasma melatonin concentrations. This abnormal inverted circadian rhythm is estimated to occur in 95% of individuals with SMS. The sleep disturbance seen in individuals with SMS may be also the underlying mechanism in at least a subset of individuals with ASD, especially in those individuals with consequential variants in the RAI1 gene.

Support

NA

53

Caffeine consumption in *Drosophila* increases sleep fragmentation with age and leads to misalignment of the circadian clock.

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Full Name and Credentials

Aishwarya Segu

Introduction (100 word limit)

Sleep is one of the five factors involved in maintaining good health. Although sleep plays a major role in homeostasis its health is often disregarded. Humans use multiple psycho-stimulants to further disturb this sleep homeostasis. Caffeine, is one such psycho-stimulants known to affect sleep by causing hyperactivity. Furthermore sleep changes with age. The impact of caffeine on sleep across different ages is not understood and our study showed that with increased age, sleep duration does not change much, but sleep fragmentation increases. Secondly, we also looked at the prolonged exposure to caffeine and observed that it affects the circadian clock.

Methods (200 word limit)

Fly stocks:

Flies were maintained in 25°C with 75% RH in 12:12 light:dark condition.

Treatments:

Short exposure to caffeine: Flies of different ages namely 1,10, 20 and 30 were treated with 0.5, 0.75 and 1 mg/mL caffeine for 24 hours respectively. During which their activity rest rhythm was recorded using DAM system.

Prolonged exposure to caffeine: Flies were treated in different caffeine concentrations for 10 consecutive days post which activity rest rhythm was recorded.

Sleep and activity analysis:

Sleep duration, sleep bout number and raw activity was analysed using SCAMP software. Free running period was analysed using Lombscargle algorithm using CLOCKLAB software. Fly rhythmicity was

analysed using autocorrelation method using VANESSA software. Gene transcription oscillation was analysed using JTK algorithm through MetaCycle software.

Food intake measurements:

Food intake was measured using CApillary FEeding assay (CAFE) post different treatments of caffeine.

RNA isolation and qPCR:

Time point based RNA isolation was performed using TRizol method. Upon quantification of RNA, cDNA was prepared and real time PCR was conducted on transcripts-
timeless, bruchpilot, synaptotagmin and homer.

Statistical Tests:

Statistical tests after analysing its distribution type was performed using PRISM software, R and Matlab.

Results (200 word limit)

Short exposure to caffeine affects sleep duration and causes fragmentation in flies:

When differently aged flies namely 1, 10, 20 and 30 day old flies were treated with above mentioned concentrations of caffeine for 24 hours sleep disturbance was observed in 10, 20 and 30 day old flies. With increased age caffeine caused a higher impact on sleep quality by increasing sleep fragmentation. Furthermore, this sleep decrease was not mediated by the homeostatic sleep circuit in flies. To understand the reason behind sleep decrease, we looked at their food intake. Caffeine intake indeed reduced feeding which resulted in decreased sleep.

Prolonged exposure to caffeine affects the circadian rhythm in flies:

Prolonged caffeine treatment for more than 10 days showed no impact on sleep duration in flies. But, decreased the morning and evening anticipatory activity indicating that it affects circadian rhythm. They also exhibited delay in the phase of clock gene *timeless* transcript oscillation and altered behavioral rhythm with either a longer free running period or arrhythmicity under constant darkness. Furthermore, prolonged caffeine treatment decreased life span in flies. Because, it reduced life span we also looked at if premature ageing caused circadian rhythm defects but that was not the case.

Conclusions (100 word limit)

Short exposure to caffeine alone decreases sleep. The decrease in sleep observed is not mediated through the sleep homeostat. But, causes sleep fragmentation. Furthermore, it causes reduced feeding. Reduced feeding leads to starvation like condition, which has already been shown to not affect the homeostatic sleep. But, more empirical evidence needs to be gathered to correlate this causation.

Prolonged effect of caffeine, causes resistance to sleep. But, affects the circadian clock. Our results show that prolonged treatment causes a phase delay in the core clock gene - *timeless* transcript oscillation. Although circadian rhythm is affected the molecular pathway needs to be elucidated.

Support

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54

Longitudinal changes in slow oscillation-spindle coupling in early childhood

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Full Name and Credentials

Sanna Lokhandwala

Introduction (100 word limit)

Naps are beneficial for memory in early childhood (3-5 years) even as children transition from biphasic to monophasic sleep. This memory benefit is thought to reflect sleep-dependent memory consolidation, a process orchestrated by three oscillations in the sleep EEG - neocortical slow oscillations (SO), thalamo-cortical sleep spindles (SP), and hippocampal sharp-wave ripples. Recent findings indicate that SO-SP temporal coupling in children's (5-6 years old) overnight sleep may be related to improved memory function. However, SO-SP coupling patterns in naps at this age have not been explored and whether these patterns change longitudinally is the focus of the present study.

Methods (200 word limit)

Data was collected as part of a larger study which included PSG-recorded naps, memory performance assessed before and after sleep and wake, and brain development assessment with MRI. Measures were collected at baseline (W1), ~6 months later (W2), and ~1 year after baseline (W3). Eight preschool-age children (5 female, M= 50 months, SD = .42) were included in the SO-SP coupling analyses. We identified the number of SOs (0.16-1.25 Hz) and SPs (10.8-13.8 Hz) in frontal, fronto-central, central and centro-occipital EEG regions. The number of SPs and SOs were further divided by the number of channels used for each target region. Due to differences in sleep duration across time points, we used SO and SP density (i.e., numbers per min) for comparison. The SO-SP coupling was calculated as a percentage of the ratio of the number of SPs that occurred during the ± 1.2 sec in the trough of SOs to the total number of SPs detected in each of target regions.

Results (200 word limit)

There was a significant increase in the SO-SP coupling in slow wave sleep (SWS) at W3 compared to W1 (mean $t(7)=-2.55$, all $ps<0.05$). SO density was marginally significantly greater at W3 relative to W1 (SO: $t(7)=-2.18$, $p=0.065$) while the density of SP was not significantly different at W3 compared to W1 (SP: $t(7)=-0.90$, $p=0.401$). However, SO and SP density showed a decreasing pattern at W2. Specifically, while SP density did not significantly differ between W1 and W2 ($t(7)= -0.90$, $p= 0.867$) and between W2 and W3 ($t(7)= -1.05$, $p= 0.327$), SO density was significantly lower at W2 than at W3 ($t(7)=-2.91$, $p=. 0.023$).

Conclusions (100 word limit)

Our findings indicate that SO-SP coupling in SWS of naps increases, irrespective of the change in SO and SP density, across development. The decrease at W2 may parallel the changes expected in the hippocampus across development (i.e., a decrease in hippocampal volume indicative of pruning of excess synapses). These results suggest that physiological markers of brain/memory development go through significant changes during this biphasic to monophasic sleep transition, and may further suggest the molding of a more efficient neural network.

Support

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55

Cross-species sleep-activity assessment confirms cis-regulatory function for insomnia GWAS variants

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Full Name and Credentials

Amber J. Zimmerman, PhD

Introduction (100 word limit)

Recent human genome-wide association studies (GWAS) identified hundreds of variants associated with insomnia revealing its polygenic nature; however, little is known of their relevance to sleep dysfunction. Most GWAS variants reside in non-coding regions, and their influence on gene regulation is poorly understood. Advances in genomics have revealed many non-coding variants act through cis-regulatory mechanisms, whereby distal genomic regions are brought in spatial proximity through 3D chromatin structure to tune gene regulation of an 'effector' gene. To identify causal effector genes associated with insomnia GWAS variants, we integrated advanced cell-based variant-mapping with high-throughput phenotyping in model organisms, *Drosophila* and zebrafish.

Methods (200 word limit)

Leveraging our cell-based library consisting of data from ATACseq, promoter-focused Capture C, and RNAseq, we first performed partitioned linkage disequilibrium (LD) score regression to identify a cell type showing significant enrichment for the cis-regulatory elements of interest (i.e., accessible promoter interactions with insomnia GWAS-associated variants). This analysis indicated neural progenitor cells (NPCs) were significantly enriched for the genomic elements of interest, and we proceeded to perform our variant-to-gene mapping approach in these cells to identify candidate effector genes. To do this, we took significant insomnia GWAS sentinel variants from Jansen et al., 2019 and mapped single nucleotide polymorphisms (SNPs) in strong LD (r^2>0.7 in Europeans) with sentinels to effector genes by combining chromatin conformation and accessibility data with epigenetic sequencing data. This approach revealed a list of candidate effector genes associated with insomnia GWAS SNPs. We then performed a large-scale genetic screen of these candidates in *Drosophila* using neuron-specific RNA interference. Sleep-wake behaviors were monitored across RNAi lines revealing multiple extreme short-and long-sleeping lines. The genes whose loss of function produced robust sleep phenotypes in *Drosophila* were next followed-up using CRISPR/Cas9 in F0 larval zebrafish to perform functional validation in a diurnal vertebrate model.

Results (200 word limit)

We identified 88 insomnia-associated effector genes through our variant-to-gene mapping approach in NPCs. Of these, 66 had moderate-to-strong orthologs in *Drosophila* and 54 had available RNAi lines. Neuron-specific knockdown of these effector genes in *Drosophila* revealed extreme long sleepers (*SKIV2L, GNB3, PIGQ,* and *ATP5G1*) as well as extreme short sleepers (*TCF12, CHADL, ARFGAP2, MEIS1,* and *CBX1*). Our follow-up screen of these extreme sleepers found significant sleep-activity phenotypes including altered sleep and activity duration, sleep fragmentation, and increased sleep latency for orthologs of *SKIV2L, PIGQ, MEIS1,* and *ARFGAP2* in zebrafish, suggesting a highly conserved regulatory role for these genes in sleep-activity maintenance. Interestingly, mutation of the zebrafish ortholog of *MEIS1* produced a night-specific increase in sleep bout number, suggesting a fragmented sleep phenotype consistent with its known role in restless leg syndrome, which predominantly occurs at night. This is the first study to perform variant-to-gene mapping for insomnia-associated GWAS signals and demonstrate a conserved role for implicated effector genes in sleep-activity regulation relevant to insomnia.

Conclusions (100 word limit)

Our work has created a pipeline for cross-species validation of significant GWAS signals that is broadly applicable to polygenic traits. Moreover, it has demonstrated the importance of performing cell-specific fine-mapping of GWAS-associated loci revealing that cis-regulatory architecture plays an important role in the maintenance of sleep-associated genes. Importantly, we confirmed a conserved role for MEIS1 in sleep fragmentation, and identified three novel genes, PIGQ, SKIV2L, and ARFGAP2, producing sleep-activity phenotypes not previously indicated through standard positional mapping practices.

Support

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56

Effects of a Sleep Extension Intervention on Multiple Dimensions of Sleep Health

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Full Name and Credentials

Michelle Kubicki

Introduction (100 word limit)

Short sleep duration and poor sleep quality are associated with increased risk of obesity. To better operationalize different components of sleep, dimensions of sleep health have been defined and include regularity, satisfaction, alertness, timing, efficiency, and duration. Disruptions in these various dimensions have been linked to adverse health outcomes, including risk of obesity. Increasing sleep duration by sleep extension is one intervention being tested to improve health outcomes. However, little is known about how sleep extension may impact the dimensions of sleep health in people with habitual short sleep duration; investigating this is the aim of our analysis.

Methods (200 word limit)

Data collection is ongoing. To date, 12 healthy adults (8M/4F), aged 20.7 ± 2.5 y (mean±SD), BMI 21.8 ± 2.3 kg/m^2 with self-reported insufficient sleep (<6.5h per night) have completed the study. The protocol comprises of 2 weeks of baseline home monitoring followed by a 4-week sleep extension intervention where participants are instructed to achieve ≥8 hours of time-in-bed. Sleep is monitored via self-report and wrist-actigraphy. Regularity is quantified as the standard deviation of actigraphy-derived sleep duration. Efficiency is measured by wrist-actigraphy. Satisfaction and alertness are reported in daily sleep diaries using the following questions: “How would you rate the quality of your sleep last night?” and “How would you rate your daytime alertness level today?”. Responses are based on a 5-point scale with 1 indicating “Very good” and 5 indicating “Very poor.” Sleep timing is measured as the midpoint of sleep calculated from participant self-reported daily sleep and wake times.

Results (200 word limit)

Sleep duration was 5.7±0.2 (mean±SEM) hours at baseline and significantly increased ($p<0.001$) by 38.4±5.4 (mean±SEM) minutes during sleep extension. Sleep midpoint was 4:58 am±17 minutes (mean±SEM) at baseline and shifted significantly earlier ($p<0.05$) to 4:38 am±7 minutes (mean±SEM) during sleep extension. No statistically significant differences were detected between baseline and sleep extension for sleep regularity, efficiency, alertness, or satisfaction.

Conclusions (100 word limit)

Preliminary findings indicate that sleep extension can increase sleep duration and shift timing of sleep earlier in the day without impacting other dimensions of sleep health including regularity, efficiency, satisfaction, and alertness. Although increased sleep duration and earlier sleep timing are considered positive changes to sleep health, it remains to be determined if such changes can help prevent chronic disease like obesity and diabetes in people with habitual short sleep duration. Further distinguishing which dimensions of sleep health are most linked with cardiometabolic disease could help improve efficacy of sleep-based interventions with the long-term goal of mitigating adverse cardiometabolic risk.

Support

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57

WITHDRAWN

58

WITHDRAWN

59

NF-κB Activation in the Central Nervous System Disrupts Circadian Rhythm and Sleep/Wake Behavior

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Full Name and Credentials

Mehari Endale, VMD, PhD

Introduction (100 word limit)

Introduction. Circadian regulation of sleep and immunity is essential to health. Chronic activation of the proinflammatory transcription factor, NF-kB is one of the primary causes of many human diseases, including immunological disorders, neurodegenerative diseases, the metabolic syndrome, and cancer. Recent studies (including ours) support the emerging view that NF-κB plays a vital role in the regulation of circadian functions and sleep homeostasis. For example, NF-κB perturbation affects circadian rhythms across cell and tissue types and in animal locomotor activity behavior. However, it is not well understood how NF-kB in the central nervous system (CNS) regulates sleep/wake functions.

Methods (200 word limit)

Methods. We generated CNS cell type-specific NF-κB activation mouse models including in neurons, microglia, and astrocytes. NF-kB activation was induced by tamoxifen-mediated Cre recombination and subsequent expression of a constitutively active form of IKK2 (Ikk2CA). We then examined the effect of its activation on circadian behavior and sleep/wake cycle. We also performed molecular, immunological and biochemical experiments to characterize and validate these mouse models.

Results (200 word limit)

Result. We show that neuron-, microglia-, and astrocyte-specific NF-κB activation altered sleep timing, duration, and quality, in a cell type-specific manner. Notably, these mice spent more time sleeping at the short sleep bout duration and less time at the long bout duration, characteristic of sleep fragmentation. NF-kB activation also changed the circadian locomotor activity rhythms, with lengthened period length and reduced activity levels. Further, the circadian and sleep deficits are correlated with compromised cognitive functions.

Conclusions (100 word limit)

Conclusion. The results support a significant role for NF-kB in the CNS in regulating circadian and sleep functions. Current studies aim to determine the mechanisms of how NF-kB in neurons, microglia and

astrocytes regulates the circadian clock and sleep at the molecular and network levels. For example, genome-wide studies will uncover NF-kB-impacted genes and pathways that are involved in circadian and sleep functions. Understanding of the interplay between the circadian and neuroimmune systems may offer new strategies to improve circadian function, sleep quality, and overall health.

Key word: circadian clock, sleep, NF-κB, inflammation, neuron, astrocytes, microglia

Support

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60

A Global Transcriptional Atlas of the Effect of Sleep Loss in the Mouse Cortex

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Full Name and Credentials

Kaitlyn Ford

Introduction (100 word limit)

It is well established that sleep deprivation (SD) has broad effects on brain gene expression that mediate both the adverse consequences of sleep loss and the molecular signature of sleep homeostasis. However, it is not known how SD influences isoform specific gene expression or how different cell-types are affected by sleep loss. To address this, we characterized the response to sleep loss in the adult mouse pre-frontal cortex (PFC) using bulk and single-nuclear (sn)RNA sequencing in parallel, while defining pipelines for reproducible data analysis of isoform-level and single-nuclear gene expression in the mouse brain.

Methods (200 word limit)

Pre-frontal cortex (PFC) tissue was collected from adult male WT mice following 5 hours of SD, and from time-matched controls allowed to sleep. For bulk RNA-seq, RNA was extracted from PFC (n=5 animals per group), sequenced using Illumina technology and transcripts quantified using Salmon. After correcting unwanted effects using RUVseq, differential gene/transcript expression and differential transcript usage was performed using Swish. For snRNA-seq, intact nuclei were purified from PFC (n=3 animals per group) using a sucrose gradient, individually bar-coded using 10X genomics v3 chemistry, and sequenced using Illumina technology. Transcript quantification was performed with Salmon-Alevin, and cell-type assignment was performed with SingleR/Azimuth using Allen Brain Institute data as reference. Differential-state analysis (effect of SD per cell-type) was performed using edgeR and muscat. Positive and negative controls obtained from a cross-study cross-brain tissue benchmark were used to evaluate the performance of our differential expression analysis pipelines.

Results (200 word limit)

We identified ~8000 genes differentially expressed after SD (qvalue < 0.05) and recovered ~80% of positive controls. At the transcript level, ~15000 transcripts are differentially expressed, with a majority of those downregulated, and ~4000 transcripts show differential isoform usage, including Homer1. At the cell-type specific level, SD has a large detectable effect in neurons with glutamatergic neurons preferentially affected. We also define cell-type specific patterns of expression regulation by SD.

Conclusions (100 word limit)

We report a 3-5 fold increase in detection of differentially expressed genes by SD, while showing a large down-regulation effect at the transcript level that cannot be detected at the gene level. We also report, for the first time, a large effect of SD of isoform switching, as well as cell-type specific effects of SD in gene expression regulation in the PFC.

Support

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61

Characterization of the distribution and phenotype of wakefulness-promoting NPAS1+ neurons in the basal forebrain

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Full Name and Credentials

Timothy Troppoli PHD

Introduction (100 word limit)

The basal forebrain (BF) is critical for the regulation of sleep and wakefulness, as well as motivation and emotional processing. It contains multiple nuclei, including the ventral pallidum (VP), horizontal diagonal band (HDB), and the magnocellular preoptic nucleus (MCPO). While the BF's cholinergic outputs have been of primary focus, it contains a range of neurons whose distribution and phenotype are poorly defined. Altered activity or numbers of BF neurons may contribute to psychiatric, sleep, and neurodegenerative disorders. Here, we describe the distribution and function of a population of BF cells expressing NPAS1, a transcription factor associated with neuropsychiatric illness.

Methods (200 word limit)

Immunohistochemistry was performed in the BF of mice expressing the red fluorescent protein, tdTomato (TdTom), as well as in vGlut1-cre-TdTom, vGlut2-cre-TdTom and GAD67-GFP knock-in mice. An AAV5 expressing ChR2-EYFP was infused into the BF of NPAS1-cre-TdTom mice to identify projections of NPAS1+ cells. Additionally, an AAV8-hM3Dq was bilaterally infused into the BF of NPAS1-cre-TdTom mice, then chemogenetically activated by systemic administration of clozapine-n-oxide (CNO). EEG activity was then recorded bilaterally over the frontal cortex of these mice to analyze sleep/wake behavior.

Results (200 word limit)

Most NPAS1+ cells of the BF are GABAergic, as in other forebrain regions and consistent with NPAS1 expression in parts of the developing brain which generate forebrain GABAergic neurons. There was minimal colocalization of BF NPAS1+ cells with either cholinergic or parvalbumin (PV)-expressing neurons (n=3). A large majority of stained BF NPAS1+ nuclei colocalized with GFP in GAD67-GFP mice (n=3), while only minimal colocalization was observed in vGlut1/2 glutamatergic populations (<2%, n=3-5), Additionally, the majority of BF NPAS1-cre-TdTom cells continue to express NPAS1 into adulthood (assessed at 8 months), positively staining between 70-90% of NPAS1-TdTom nuclei across the VP, HDB, and MCPO respectively (n=3).

Anterograde tracing indicated prominent BF NPAS1+ projections to frontal cortex, shell of the nucleus accumbens, lateral hypothalamus, supramammillary nucleus and ventral tegmental area. Chemogenetic activation of BF NPAS1+ cells strongly promote wakefulness (n=3).

Conclusions (100 word limit)

Taken together, our results indicate that NPAS1+ neurons are a novel GABAergic population of cells widely present throughout the BF. Their wakefulness-promoting properties, projections to sleep and mood-regulating regions of the brain, and expression of the psychiatric disease-regulating transcription factor NPAS1 makes their characterization critical to advancing our understanding of the neurobiology of sleep while presenting a putative target for the treatment of psychiatric disease and sleep disorders.

Support

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62

IMPACT OF COVID 19 INFECTION ON PATIENTS WITH OBSTRUCTIVE SLEEP APNEA: A LONGITUDINAL STUDY

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Full Name and Credentials

SAMARJIT DAS

Introduction (100 word limit)

The COVID 19 pandemic made a huge impact globally. OSA runs in the forefront or background of many comorbid conditions. COVID patients having OSA as their comorbidity are supposed to be at risk of having severe disease due to many factors such as sharing of common pro-inflammatory markers and many more. This study aims to evaluate this impact of COVID infection on OSA patients, with comparison to pre and post COVID data.

Methods (200 word limit)

This is a 11months analytical longitudinal study of 12 OSA and 15 non OSA patients. 53 patients underwent diagnostic PSG for evaluation of OSA from JAN to March 2021. They were on follow up through online or offline OPD visit. 37 patients suffered from COVID 19 infection between March to Aug, 2021.After screening through inclusion and exclusion criteria and few patients couldn't bring medical records, few died during COVID, few lost to follow up. Lastly, 12 OSA patients and 15 non OSA patients underwent follow up PSG 3 months after discharge from COVID 19 related hospitalization or home care. 12 OSA subjects had mean age of 52.92 (SD: 6.88) and majority were male 8 (66.7%) and mean BMI was 27.72(SD: 3.60). Subjects were assessed with COVID 19 infection medical record, repeat Epworth Sleepiness Score, present chief complaints and Polysomnography parameters including sleep architecture. This data set were analysed and compared between Pre and Post COVID data of OSA and Non OSA patients.

Results (200 word limit)

Chief complaints of anxiety, mean of awakening, non refreshing sleep, headache were more in OSA patients in post COVID period. Most of the OSA patients (66.7%) suffered from Severe COVID 19 infection than non OSA patients with Odd Ratio of 3.25. and majority were male (62.5%). Severity of COVID correlated significantly with BMI and AHI ($r=0.774$; p 0.003 and $r=0.907$; $p<0.001$ respectively) in OSA patients. There is more use of high flow oxygen device and noninvasive ventilation with mean hospital stay of 27.33d (SD: 10.7) more than non OSA patients of 2.13d (SD:0.91). Mean difference of increased AHI and ESS scores in post covid period were statistically significant ($r=0.907$; $p<0.001$ and $r=0.893$; $p<0.001$ respectively) in OSA patients. Mean of maximum desaturation dropped significantly from 83.33 (SD:4.83) to79.50(SD:4.81) ($r=-0.727$; $p=0.007$). There is statistically significant reduction in Sleep

efficiency, N3 stage and increased Arousal Index, Sleep latency, WASO among OSA patients. PAP adherence increased from mean of 5.375 (SD:0.85) hr to 5.6 (SD: 1.04) hr per night.

Conclusions (100 word limit)

This study reveals that OSA patients suffered more from severe COVID 19 infection than non OSA patients, requiring more healthcare support. Symptoms related to inadequate and disturbed sleep were more in post COVID period among OSA patients. Sleep architecture in OSA patients deteriorated significantly in post COVID period. This study reveals OSA as a risk factor for severe COVID 19 infection and may put forward some understanding regarding persistent post COVID symptoms observed in OSA patients

Support

N/A

63

Impact of Sleep Extension on the Timing and Duration of Food Intake in People with Habitual Insufficient Sleep

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Full Name and Credentials

Audrey Stegman

Introduction (100 word limit)

Chronic insufficient sleep is associated with an increased risk of cardiometabolic diseases including diabetes and obesity. In laboratory experiments, sleep restriction leads to increased and later timing of food intake, which contributes to adverse cardiometabolic risk. Alternatively, compressing the eating window with early time-restricted eating is associated with increased weight loss compared to a greater than 12-hour eating window. However, outside the laboratory, little is known about the impact of increased sleep duration on the timing of energy intake. Thus, we investigated the impact of sleep extension on the timing of energy intake in people with chronic insufficient sleep.

Methods (200 word limit)

Data collection is ongoing. To date, 12 healthy participants (4 female; aged 20.7±2.5yr; BMI 21.8±2.3kg/m2 [mean±SD]) with self-reported insufficient sleep (<6.5h per night) have completed data collection. The protocol consists of a two-week baseline segment where participants maintain their habitual sleep schedules, and then a four-week sleep extension intervention targeting ≥8 hours of time-in-bed. Sleep was monitored by wrist-actigraphy. During the final week of baseline and sleep extension, timing of food intake was tracked using time stamped photographic food diaries over three consecutive days, including one weekend day. The daily eating interval was defined as the duration from first calorie consumed to last calorie consumed. The regularity of the eating interval was measured as the standard deviation of the eating interval within each participant for each study segment.

Results (200 word limit)

Sleep duration was 5.7±0.2 (±SEM) hours at baseline and significantly increased ($p<0.001$) by ~38.4±5.4 minutes during sleep extension. The daily eating interval was 10.1±0.7 hours at baseline and significantly ($p<0.05$) decreased by 1.5±0.7 hours during sleep extension. Additionally, the timing of first calorie consumed was significantly ($p<0.05$) later by 1.0±0.5 hours during sleep extension versus baseline. The timing of last calorie consumed and the regularity of the eating interval were not statistically different between study segments.

Conclusions (100 word limit)

Preliminary findings from this ongoing study suggest that sleep extension may decrease the duration of the eating interval in people with habitual insufficient sleep. A later timing of the first calorie consumed appears to be a key factor driving this shorter eating interval. Further understanding the potential interactions between sleep duration and timing of energy intake in free-living conditions, outside the laboratory, could help improve interventions designed to mitigate the adverse cardiometabolic health consequences of habitual insufficient sleep.

Support

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64

Brain dynamics during wakefulness before sleep: understanding wake-sleep physiology by complexity-based and conventional spectral measures

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Full Name and Credentials

Yan Ma, MD, MPH

Introduction (100 word limit)

Sleep EEG signals are typically nonlinear and non-stationary, yet complexity-based entropy measures have not been widely used to inform the neurophysiological basis of wake and sleep. Despite the relatively common use of multiscale entropy to analyze sleep data, there is a lack of understanding regarding the choice of scale factors and the interpretation of entropies on different scales. As the first step of a series of secondary analyses, we aimed to explore the associations (1) between novel entropy-based measures and conventional spectral measures and (2) between EEG dynamics during the sleep onset period and the quality of subsequent overnight sleep.

Methods (200 word limit)

We conducted a secondary analysis using PSG data from the Sleep Heart Health Study (SHHS) dataset of the National Sleep Research Resource (NSRR). In this analysis, we included 169 healthy subjects with no sleep disorders (e.g., no insomnia or moderate/severe sleep apnea). EEG signals (sampled at 125 Hz) were extracted from PSG recordings, along with epoch information for lights-out, sleep onset, and all technician-scored sleep stages. We applied standard techniques to remove noise and artifacts mainly utilizing the automated cleaning process. Full EEG spectra included slow oscillations (0.5-1Hz), delta (1-4 Hz), theta (4-8 Hz), alpha (8-12 Hz), sigma (12-16Hz), beta (16-30 Hz), and gamma power (30-55 Hz). For complexity indices (CI), we used multiscale entropy on both short/small (Scales 1-4, CI_{1-4}) and long/large (Scales 1-30, CI_{1-30}) scales.

Results (200 word limit)

In the first five minutes after lights-off, complexity of small scales (CI_{1-4}) was significantly associated with slow oscillations ($r=-0.658$, $p<0.001$), delta power ($r=-0.702$, $p<0.001$), and theta power ($r=-0.379$, $p<0.001$). Complexity of large scales (CI_{1-30}) was significantly associated with all spectral powers although the associations were moderate ($r=-0.4$~-0.3, $p<0.001$). CI_{1-4} was significantly and negatively associated with slow oscillations and beta power during deep sleep. All associations remained, although

less significant (about 15-20% decrease in correlation coefficient), when we looked at the entire sleep onset process.

Conclusions (100 word limit)

Our results support the concept that lower complexity of brain dynamics during sleep onset is associated with increased slow-wave sleep. Non-linear, complexity measures may provide complementary tools for informing wake-sleep physiology. Future studies are encouraged to better understand the implications of different scale factors when interpreting complexity measures, to further explore the differences in EEG dynamics by comparing subjects with and without insomnia, and to evaluate whether complexity measures can serve as a marker for treatment effects.

Support

This research project was made possible by a grant from the American Academy of Sleep Medicine Foundation (PI: Yan Ma). The Sleep Heart Health Study (SHHS) was supported by National Heart, Lung, and Blood Institute cooperative agreements U01HL53916 (University of California, Davis), U01HL53931 (New York University), U01HL53934 (University of Minnesota), U01HL53937 and U01HL64360 (Johns Hopkins University), U01HL53938 (University of Arizona), U01HL53940 (University of Washington), U01HL53941 (Boston University), and U01HL63463 (Case Western Reserve University). The National Sleep Research Resource was supported by the National Heart, Lung, and Blood Institute (R24 HL114473, 75N92019R002).

65

Circadian and diet contributions to anxious and depressive like behavior in mice

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Full Name and Credentials

Athena Rivera

Introduction (100 word limit)

Obesity has detrimental effects on mental health. While this association is commonly observed in humans, animal models have been understudied and have yielded mixed results. We hypothesized that both an animal's diet and the timing of the assessment would affect the presentation of anxious- and depressive-like behaviors.

Methods (200 word limit)

Mice on an FVN background were placed on either 45% high fat diet or a standard chow immediately after birth and maintained for 15 weeks. To measure anxious-like behaviors, the open field test was used. The number of times that the mouse entered the center field with all four paws was scored as a center visit and indicative of less anxious-like behavior. The tail suspension test was used to determine depressive-like behavior and scored based on the percentage of time that the mouse showed movement. Each mouse was tested both in the morning (ZT 0-2) and at night (ZT 12-14). All mice were recorded during the tests and results were manually scored.

Results (200 word limit)

Lean mice show increased center field visits in the open field test during the night than in the daytime. On the other hand, obese mice did not differ in center field visits between night and day trials. During the night trial, the lean mice had increased center field visits compared to the obese mice indicating that lean mice express less anxious like behaviors than obese mice. The day trial between obese and lean mice did not significantly differ. Obese mice demonstrated less time spent moving (time spent struggling) on the tail suspension test while lean mice spent significantly more time moving, indicating that obese mice showed an increase in helplessness when compared to the lean mice.

Conclusions (100 word limit)

Diet can alter diurnal the variation of anxious- and depressive-like behaviors in mice. Moreover, the time of day investigators may be studying these behaviors may be driving increased anxious and depressive-like behaviors in mice. Together, these results implicate that both diet and time of day are essential considerations when measuring anxious- and depressive-like behaviors in mice.

Support

N/A

66

Effects of sleep deprivation on the frequency and complexity of sleepwalking episodes.

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Full Name and Credentials

Cloé Blanchette-Carrière, PhD Candidate, Psychology

Introduction (100 word limit)

Laboratory studies have shown that adult sleepwalkers experience more frequent and complex forms of sleepwalking during their recovery sleep following sleep deprivation than during normal polysomnographic (PSG) recordings. While this suggests that sleep deprivation can be used to establish a PSG-based diagnosis of sleepwalking in predisposed patients, samples sizes in these studies remain limited. The aim of the present study was to investigate the effects of 25hrs of sleep deprivation on the frequency and the complexity of somnambulistic behaviors recorded in the laboratory in a large cohort of adult sleepwalkers.

Methods (200 word limit)

We retrospectively analysed data collected from 124 adult sleepwalkers (45 men, 79 women; mean age = 32.0 ± 10.2 years) with an ICSD-based diagnosis of sleepwalking and who underwent one night of continuous PSG recording followed by a standard 25hr sleep deprivation protocol under video supervision. Recovery sleep was initiated the subsequent morning after 21 to 28 hours of continuous wakefulness. For each of the two sleep periods (baseline and recovery sleep), we analyzed the overall mean frequency of recorded somnambulistic episodes, the number of patients having experienced at least one episode, and the number of episodes arising from N2 and N3. We also used a 3-point scale to assess the number of patients experiencing episodes with various levels of complexity (type 1 = simple behaviors such as playing with the bed sheets; 2 = more complex such as chasing away imaginary animals/objects; 3 = getting out of bed).

Results (200 word limit)

A greater mean number of somnambulistic episodes were recorded during patients' recovery sleep (1.83 ± 2.06) as compared to baseline (1.15 ± 1.57) ($t_{(123)} = -4.29$, $P < 0.01$). Seventy-eight of the 124 patients (63%) experienced at least one episode during their recovery sleep compared to 60 patients (48%) during baseline PSG ($\chi^2_1 = 5.29$, $P = 0.02$). During baseline as well as recovery sleep, approximately 80% of all recorded episodes occurred out of N3 sleep with the remaining 20% arising out of N2 sleep.

Regarding episode complexity, a greater proportion of patients experienced at least one simple (type 1) somnambulistic event during recovery sleep than at baseline (χ^2_1 = 15.34, P < 0.01) but no pre-to post-sleep deprivation differences were found in the number of patients experiencing more complex forms (type 2 and 3) of somnambulistic events or in the overall mean frequency of these behavioral events (all *Ps* > 0.13).

Conclusions (100 word limit)

Our results indicate that sleep deprivation is effective in facilitating the occurrence of somnambulistic episodes in predisposed adults. Although the effect of sleep deprivation on episode occurrence was significant, the magnitude of the effect observed in the present study (approximate 15% increase in patients experiencing at least one episode) was considerably lower than the almost 40% increase found in a smaller sample studied by our group. Hence, the possible costs and benefits of using sleep deprivation in adult sleepwalkers need to be weighed depending on study goals, patient profiles, and available resources.

Support

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67

Elucidating Influence of Underlying Pulmonary Physiology in the Association of Sleep-Related Hypoxemia and Incident Atrial Fibrillation

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Full Name and Credentials

Catherine M. Heinzinger, DO

Introduction (100 word limit)

Sleep-related hypoxemia has been implicated in atrial fibrillation (AF) in population-based studies and our clinic-based preliminary findings adjusted for pulmonary comorbidities and tobacco use. However, due to shared risk factors and residual confounding of obesity via restrictive pulmonary physiology from visceral adiposity, the extent to which this hypoxemia is specific to sleep disordered breathing (SDB) remains uncertain. We hypothesize greater risk of 5-year incident AF with sleep-related hypoxemia adjusted for pulmonary physiologic measures in a clinic-based cohort.

Methods (200 word limit)

Cleveland Clinic patients (age>18) who underwent sleep studies from 1/2/2000-12/30/2015 with available spirometry data were examined. Predictors, continuous and reported in 10 unit increments, included apnea hypopnea index (AHI; hypopneas defined by 3% desaturation or arousal), % sleep time oxygen saturation<90% (T90), minimum and mean oxygen saturation ($minSaO_2$ and $meanSaO_2$, respectively), and maximum end-tidal carbon dioxide ($maxETCO_2$). Cox proportional hazards models, adjusted for each spirometry measure independently, were fit with time from sleep study to AF diagnosis as the dependent variable. Covariates included age, sex, race, body mass index (BMI), cardiovascular risk factors (hypertension, diabetes mellitus, hyperlipidemia), heart failure, coronary artery disease, myocardial infarction, history of coronary artery bypass grafting, chronic obstructive pulmonary disease, tobacco use, use of anti-arrhythmic drugs, and one of: forced expiratory volume (FEV1), forced vital capacity (FVC), or FEV1/FVC ratio. Data were censored at date of last follow up or at 5-years.

Results (200 word limit)

The sample included 10,958 patients: age 56.3±13.7, 55.5% female, 71.5% White, median BMI 34 (IQR 29, 40), and 1,275 (11.6%) with AF. Of those without AF, 847 (8.7%) developed 5-year incident AF. Accounting for restrictive physiology, i.e. FVC, higher AHI increased AF by 3% (HR=1.03, 95%CI=1.01-1.06), greater T90 increased AF by 4% (HR=1.04, 95%CI=1.01-1.07), and lower $meanSaO_2$ increased AF

by 19% (HR=1.19, 95%CI=1.01-1.40). Accounting for obstructive physiology, i.e. FEV1/FVC ratio, higher AHI increased AF by 4% (HR=1.04, 95%CI=1.01-1.06) and in severe sleep apnea (AHI>30) by 47% (HR=1.47, 95%CI=1.01-2.14), greater T90 increased AF by 5% (HR=1.05, 95%CI=1.02-1.07), lower minSaO_2 increased AF by 9% (HR=1.09, 95%CI=1.01-1.19), and lower meanSaO_2 increased AF by 22% (HR=1.22, 95%CI=1.04-1.43). Accounting for degree of obstruction, i.e. FEV1, higher AHI increased AF by 3% (HR=1.03, 95%CI=1.01-1.06) and greater T90 increased AF by 4% (HR=1.04, 95%CI=1.01-1.07). Max$ETCO_2$ did not demonstrate a meaningful association.

Conclusions (100 word limit)

SDB, defined by AHI, and sleep-related hypoxemia demonstrated associations with incident AF in this clinic-based cohort. These findings strongly implicate sleep-related hypoxemia as an impetus in AF development, independent of the restrictive pathophysiology resulting from obesity-related visceral adiposity. Although all three hypoxemia predictors (T90, minSaO_2, meanSaO_2) were associated with AF when adjusted for FEV1/FVC, of these, only T90 was associated with AF when adjusted for FEV1, suggesting that the arrhythmogenic effects of hypoxemia in the context of obstructive pathophysiology may not depend on the degree of obstruction.

Support

Cleveland Clinic Neurological Institute Center for Outcomes Research & Education Pilot Grant, Neuroscience Transformative Research Resource Development Award

68

Sleep and Explicit Memory Impairments In Naïve Mice Induced By Fecal Microbiota Transplantation From Mice Exposed To Chronic Intermittent Hypoxia

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Full Name and Credentials

Clementine Puech, PhD

Introduction (100 word limit)

Obstructive sleep apnea (OSA) is a highly prevalent and chronic disease characterized by intermittent hypoxia (IH) during sleep and associated with a large spectrum of morbidities including excessive sleep propensity (EDS) and cognitive deficits. Fecal microbiota transfer (FMT) from mice exposed to chronic IH mimicking moderate-severe OSA into naïve mice recapitulates the sleep disturbances induced by IH. Furthermore, IH-induced cognitive declines may be mediated by alterations in the Blood Brain Barrier (BBB) permeability, which is increased by IH. We hypothesized that sleep perturbation induced by FMT-IH will result in explicit memory dysfunction via BBB dysfunction.

Methods (200 word limit)

C57Bl/6J mice were exposed to chronic IH (cycles of FiO2 21% 90s-6% 90s or room air (RA:21%) for 12 hours / day during the light period) for 6 weeks, then fecal samples were collected and frozen. C57Bl/6J naïve mice (male and female) were randomly assigned to a validated FMT protocol by gavage for 6 weeks with either IH or RA fecal slurry. Sleep activity was recorded using a non-invasive, high throughput piezoelectric system, and sleep states were automatically scored by validated AI-derived computer algorithms. Cognitive function was evaluated using the novel object recognition (NOR) test, which is based on the spontaneous behavior of mice to explore novelty and provides reliable assessments of explicit memory. To evaluate the BBB permeability, 4KDA-dextran-FITC was injected in the tail vein, and FITC brain immunofluorescence was quantified.

Results (200 word limit)

The 24-h total sleep time percentage significantly increased in the FMT-IH group (43 ± 13% vs. 33 ± 10 % in FMT-RA group; p=0.043; n=13/group). Dark phase sleep percentage was significantly increased in FMT-IH mice during the dark phase (29 ± 10 %) when compared to FMT-RA treated mice (20 ± 10 %, p= 0.034) but no differences emerged in the light phase. Sleep bout lengths were significantly increased in FMT-IH mice (1254 ± 471 sec) vs. FMT-RA mice (731 ± 321 sec, p= 0.029), and such effect was prominent in the light phase (FMT-IH: 1815 ± 907 sec vs. FMT-RA: 842 ± 484 sec, $p<0.0001$). FMT-IH mice exhibited reduced interest for novelty in NOR tests compared to FMT-RA mice (preference scores: FMT-IH: 59 ±

20% vs FMT-RA: 75 ± 21% p =0.031, n=20/group). However, in contradistinction with BBB increased permeability following actual IH exposures, BBB permeability values in FMT-IH and FMT-RA were similar.

Conclusions (100 word limit)

GM transfer from mice exposed to IH induces sleep disturbances and deficits in explicit memory functioning in naïve recipient mice that recapitulate the effects of IH. However, these adverse effects do not seem to be associated with alteration of the BBB and may be potentially mediated by direct effects on the gut-brain network.

Support

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69

Kynurenic Acid Modulates Sleep during Pregnancy: Implications for Neurodevelopmental Disorders

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Full Name and Credentials

Courtney Wright

Introduction (100 word limit)

Sleep is critical for overall health, yet sleep disturbances are common in patients with neurodevelopmental disorders (NDDs) and during pregnancy, a period crucial for neurodevelopment and sensitive to homeostatic imbalances like disturbed sleep. Kynurenic acid (KYNA) is an endogenous antagonist of NMDA and a7nACh receptors. Acute KYNA elevations disturb sleep, while neurodevelopmental KYNA elevations disrupt KYNA levels and sleep in adult offspring, mimicking the symptoms of individuals with NDDs. We hypothesize that gestational elevations in KYNA also impair maternal sleep during the peripartum period, contributing to long-term homeostatic disruptions that may influence the etiology of NDD endophenotypes in the offspring.

Methods (200 word limit)

To record sleep in freely moving female Wistar rats (N = 3-4 per group), radiotelemetry devices were surgically implanted to acquire continuous cortical electroencephalography and muscle electromyography. Following one week of recovery, females in proestrus were mated overnight with males, and embryonic day (ED) 0 was recorded after evidence of successful mating the next morning. Sleep was recorded from ED 15 to postnatal day (PD) 14 to encompass the gestational treatment and subsequent postnatal period. To elevate KYNA levels during gestation, female rats were fed control (ECon) or kynurenine-laced chow (EKyn; 100mg/day) during the last week of pregnancy (ED 15-22). As kynurenine can cross the placental and blood-brain barriers, this method elevates KYNA levels in the mothers and fetuses, evaluated biochemically by HPLC. Sleep was scored automatically using an artificial neural network and verified by hand-scoring. Vigilance states were classified in 10-s epochs as wake, rapid eye movement (REM) sleep, and non-REM (NREM) sleep.

Results (200 word limit)

Our studies revealed that pregnancy significantly altered sleep homeostasis, as female rats spent more time asleep and less time awake than nulliparous controls by the end of the gestational period (NREM: $P<0.05$; wake: $P<0.05$). Biochemical analyses revealed maternal brain KYNA levels are also elevated following EKyn treatment ($P<0.05$). At ED 20, we determined a significant phase x treatment interaction for total sleep duration ($P<0.01$) whereby EKyn mothers spent more time asleep in the light phase and less time asleep in the dark phase compared to ECon, yet no difference existed between total sleep or

wake durations across 24 hours. EKyn mothers also had significantly lower core body temperatures in the light phase than ECon ($P<0.05$). EKyn dams had normalized body temperatures that were accompanied by an increase in relative cage activity in the dark phase ($P<0.05$).

Conclusions (100 word limit)

Our data indicate that pregnancy and EKyn treatment significantly alter sleep in rats and support our hypothesis that elevated KYNA affects sleep. Importantly, EKyn treatment significantly altered sleep timing across 24 hours, indicating potential circadian misalignment that may contribute to the changes we observe in adult offspring. Our novel findings concerning the role of sleep during gestation serve to better our understanding of the etiology of NDDs and inform future work on a novel therapeutic target, to reduce KYNA, for preventative treatments. Future work will investigate behavioral endophenotypes in juvenile offspring and test therapies aimed to improve sleep during pregnancy.

Support

This work was funded by R01 NS102209 and a Pilot Award from the Carolina Autism and Neurodevelopment Research Center at the University of South Carolina.

70

Early to Bed and Early to Rise: Associations between Objective Measures of Sleep Midpoint, Sleep Quality (Odds Ratio Product), and Performance across 18-31 Nights of In-Home Polysomnography

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Full Name and Credentials

Amy M. Bender, PhD, MS

Introduction (100 word limit)

Going to bed later and having more variable sleep-wake rhythms have been associated with poorer outcomes. Sleep midpoint is typically measured with subjective measures or actigraphy but not normally with polysomnography (PSG) across multiple nights in the home. The aim of the present study was to investigate the associations between PSG sleep midpoint, sleep quality (Odds Ratio Product; ORP), and psychomotor vigilance performance across 18-31 nights of in-home PSG.

Methods (200 word limit)

19 participants (age 41.1±10.9; 9 females) recorded their sleep with in-home PSG using the Cerebra Sleep System for 18 to 31 nights (21.5±3.4). Sleep quality was measured using ORP derived from micro-analyzing frontal EEG channels during wake and sleep across the sleep recording. Sleep midpoint was assessed by determining the midpoint between the first and last epoch of sleep. In the morning, participants completed a 2-minute psychomotor vigilance test (PVT) with lapses of attention defined as >355ms. All correlations controlled for total sleep time and results did not differ based on chronotype.

Results (200 word limit)

On average, the sleep midpoint occurred at 3:22 a.m.±1.47 hours. Individual variation in sleep midpoint across the 18-31 nights was 52.2 (±0.26) minutes. When the sleep midpoint was later there was poorer sleep quality as indicated by higher ORP during NREM sleep ($r=0.206$, $p<.001$), REM sleep ($r=0.360$, $p<.001$), and shallower sleep after an EEG arousal with ORP-9 ($r=0.257$, $p<.001$). A later sleep midpoint was also associated with more lapses of attention on the reaction time test the following morning ($r=0.272$, $p<.001$). Participant standard deviation in sleep midpoint was a significant predictor of ORP REM ($R2=0.029$, $F(1,327)=9.66$, $p=0.002$; $\beta=0.169$, $t=3.11$, $p=0.002$). Thus, a greater range in sleep midpoint was associated with a higher ORP during REM.

Conclusions (100 word limit)

Having an earlier sleep midpoint was associated with better sleep quality across NREM, REM, and after an EEG arousal. Having an earlier sleep midpoint was also associated with better performance on the PVT. Additionally, having more variability in sleep midpoint was associated with poorer sleep quality during REM sleep. Thus, going to bed earlier, waking up earlier, and being more consistent across nights were associated with better objective sleep quality and next-day performance. Results were based on associations, so further research could focus on controlled experiments with earlier bedtime interventions to see if there are definitive benefits for all chronotypes.

Support

N/A

71

Factors influencing the likelihood to take controlled rest on the flight deck

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Full Name and Credentials

Cassie J. Hilditch, PhD

Introduction (100 word limit)

Fatigue is a known issue in aviation due to long and irregular working hours. To mitigate inflight sleepiness, some regions allow pilots to sleep during a controlled rest (CR) period on the flight deck. Few studies have investigated the factors influencing a pilot's decision to take CR. We aimed to determine the relative influence of prior sleep-wake history and circadian timing on the likelihood to take CR.

Methods (200 word limit)

Data from 122 long (> 6 h flight duration), non-augmented (two flight crewmembers) flights were analyzed (n = 31 pilots). Mixed-effects logistic regression was used to assess the likelihood of CR based on the following predictors measured at flight departure: (1) total sleep time in prior 24 h; (2) total sleep time in prior 48 h; (3) number of hours of continuous wakefulness; and (4) timing of flight (night: 17:00-05:00 vs. day: 05:00-17:00).

Results (200 word limit)

Controlled rest was taken on 68.85% (n = 84) of the analyzed flights. Pilots were more likely to take CR on night flights ($b \pm SE$: 2.23 ± 0.98; *Odds Ratio*, OR: 9.28; p = .023), and flights with less sleep obtained in the 48 hours prior to departure ($b \pm SE$: -0.44 ± 0.22; OR: 0.64; p = .049; $R^2_{Marginal}$: .20, $R^2_{Conditional}$: .57). Total sleep time in the 24 h prior to departure ($b \pm SE$: 0.38 ± 0.33; OR: 1.46; p = .25) and hours of wakefulness prior to departure ($b \pm SE$: 0.04 ± 0.12; OR: 1.04; p = .70) did not significantly predict CR.

Conclusions (100 word limit)

Our results suggest that the both the circadian and homeostatic component of sleep need influence the likelihood of taking controlled rest on the flight deck. Consideration of other factors – operational, physiological, and qualitative – are needed in order to better understand the decision to take CR.

Support

NASA Airspace Operations and Safety Program, System-Wide Safety Project.

72

Association between chronotype and melatonin onset in typical home and dim-light home lighting environments

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Full Name and Credentials

Katrina Rodheim

Introduction (100 word limit)

Circadian rhythms are important for the timing of physiology and behavior, including the timing of sleep and wakefulness. Chronotype describes the temporal organization of behavior (e.g., sleep timing) and represents an interaction between biological properties of the circadian clock (e.g., circadian period) and environmental input (e.g., light exposure). Modern electrical lighting conditions, including exposure to electrical lighting after sunset, have altered the timing of the internal circadian clock. Here we examined associations between chronotype and melatonin onset under typical self-selected electrical lighting conditions and under controlled dim-light conditions in the home environment.

Methods (200 word limit)

Thirteen young healthy adults [4 males (24.5±6.1 yr; mean±SD)] were included in this analysis. Participants reported habitual bed and wake times on work and free days during screening and these values were used to calculate mid-sleep on free days according to the Munich Chronotype Questionnaire (MCTQ) as an indicator of chronotype. Participants sleep and light exposure were monitored via wrist-worn actigraphs (Actiwatch Spectrum, Philips) and a daily sleep diary. After two baseline days in the participant's habitual lighting environment, saliva samples were collected for melatonin assessment every hour in their homes from 6pm until bedtime first under typical self-selected electric lighting conditions and on the next night under controlled dim-candlelight conditions. The study took place during October in Boulder Colorado, United States, at a latitude of 40°N with average sunset of ~1806h and sunrise of ~0724h during the study. Saliva samples were immediately stored in the home freezer until returned to laboratory. Melatonin onset was defined as the linear interpolated point in time at which melatonin levels rose above a 3 pg/ml threshold. One participant did not reach the melatonin onset threshold on the typical self-selected electric lighting night and was excluded from analyses.

Results (200 word limit)

On average, after 1800 and until habitual bedtime, participants were exposed to ~18 lux under typical home electric lighting and ~3.2 lux on the candlelight night. The timing of the melatonin onset was not statically different under typical self-selected electric lighting (20.69h ± 2.0) versus under controlled dim-candlelight night (20.24h ± 1.9; mean ± SD) in the home environment ($p>0.1$). Chronotype was more

strongly associated with circadian phase under the candlelight ($r=0.77$ $p<0.01$) than typical self-selected electric lighting ($r=0.56$, $p=0.06$) night, though the difference between the correlations was not statistically significant ($z=1.59$, $p=0.11$).

Conclusions (100 word limit)

The strength of the association between melatonin onset and chronotype was somewhat reduced under typical self-selected lighting conditions compared to controlled dim candlelight conditions in the home environment. Findings are consistent with the notion that evening exposure to electrical lighting in the home environment impacts circadian rhythms in humans and highlights the need for additional research of circadian rhythms under real world lighting conditions, especially the impact of new dimmable and tunable lighting technologies.

Support

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73

Frequent nightmares are associated with lower waking health and performance during longitudinal military training

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Full Name and Credentials

Remington Mallett, PhD

Introduction (100 word limit)

Sleep is a major contributor to waking health and performance. However, sleep is a broad category that encompasses many features, and the mental contents of sleep (i.e., dreams) are often overlooked when evaluating sleep's consequences. Here, we offer a comprehensive profile of nightmares (i.e., extremely distressing dreams) and their covariates in a US military population before and during a longitudinal training period.

Methods (200 word limit)

Data come from a sample of sailors in a large operational shipboard setting (i.e., U.S. Navy warship). Data collection procedures included objective sleep data gathered by wearable sleep tracking devices (i.e., Oura Ring) and individual responses solicited through questionnaires delivered during an operational underway.

Results (200 word limit)

Before training, baseline nightmare frequency was higher in sailors relative to the general population. Both nightmare frequency and distress increased further during subsequent training. Critically, increases in nightmare traits during training were associated with reduced waking performance above and beyond traditional measures of sleep quality.

Conclusions (100 word limit)

Altogether, these results suggest that nightmare distress is abnormally high surrounding military training and tied to waking performance. More generally, these results support a view of psychological sleep quality, where the mental contents of sleep make predictable contributions to waking health. Sleep education programs aiming to promote healthy sleep in service members might begin to incorporate information about the prevalence and treatment options for recurring nightmares.

Support

 This work was supported by the Military Operational Medicine Research Program under work unit no. N2010. The study protocol was approved by the Naval Health Research Center Institutional Review Board in compliance with all applicable Federal regulations governing the protection of human subjects. Research data were derived from an approved Naval Health Research Center Institutional Review Board protocol, number NHRC.2017.0008 and NHRC.2021.0003.

74

Correlates of Chronotype in Urban Adolescents and Young Adults

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Full Name and Credentials

Susan Lin, DrPH

Introduction (100 word limit)

Chronotype refers to one's tendency towards morningness versus eveningness for activity and function. Chronotype has both genetic and environmental components, and has been linked to a variety of physical and mental health outcomes. Therefore, identifying potentially modifiable environmental correlates of chronotype in adolescents may inform interventions to improve health.

Methods (200 word limit)

Data were obtained from the Stress & Justice Sleep Study, a cohort investigation designed to examine the household and neighborhood environment, sleep, and mental health in urban youth and young adults disproportionately exposed to parental criminal justice system involvement (CJSI). Participants included 308 youth (53% female; 30% African-American and 60% Hispanic; median age=20, IQR=18-21). Chronotype was examined as a continuous measure using the Composite Scale of Morningness-Eveningness Questionnaire (MEQ), with higher scores indicating morning type. Subjects' demographic and personal (age, sex, race/ethnicity, household income, parental CJSI, religiosity, history of adverse life events); household/family (parenting style, number of persons in the household, inside and outside household conditions, youth/young adult rated family satisfaction); and neighborhood (neighborhood safety, exposure to community violence) characteristics were ascertained through youth and parent in-person interview. The associations of individual, family, and neighborhood characteristics with youth MEQ score were assessed using linear regression.

Results (200 word limit)

MEQ score was approximately normally distributed, with mean=42.1 (SD=6.4; range=28-69). MEQ scores were higher in subjects in the highest quartile of religiosity (p=0.05), and lower among those of mixed or other races versus Hispanic and African-American participants (p=0.05). In models adjusted for personal characteristics, more persons living in the household was associated with lower MEQ (more evening type, beta=-0.43 per additional household member; p=0.01) as was increased family dissatisfaction (beta=-0.31 per scale unit; p=0.02). Exposure to community violence (binary) was associated with higher MEQ (beta=1.34; p=0.07).

Conclusions (100 word limit)

Personal, household and family factors may be associated with sleep chronotype in urban youth/young adults.

Support

National Heart, Lung, and Blood Institute (R01HL134856, PI Christina Hoven).

75

The ventilatory response to hypoxia exhibits a circadian rhythm that is driven in by the molecular clock within respiratory, Phox2b-expressing cells in a sex-dependent manner.

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Full Name and Credentials

Allison Spears

Introduction (100 word limit)

The superchiasmatic nucleus (SCN) controls daily rhythms in resting breathing – including the 24-hr pattern in the minute ventilation (measured in mL of gas exchange per min). However, the extent to which the SCN influences other aspects of breathing, such as the ventilatory response to low oxygen (hypoxia) or high carbon dioxide levels (hypercapnia), is unknown.

Methods (200 word limit)

We used whole-body plethysmography to assess day-night variation in the hypercapnic (HCVR) and hypoxic ventilatory responses (HVR) of male and female mice. Morning ventilation was measured at ZT 6 and evening ventilation was measured at ZT 14. To determine the role of the molecular clock in the day-night variation of HCVR and HVR, we tested global BMAL1-KO mice alongside their wildtype littermate controls. Additionally, we tested a BMAL1$^{fl/fl}$Phox2b$^{Cre/+}$ mouse model in which **B**MAL1 was selectively **k**nocked **o**ut of **P**hox2b cells (hereafter called BKOP) alongside their wildtype controls to determine the extent to which the molecular clock within key chemoreceptive cells affected the daily variation in HCVR and HVR.

Results (200 word limit)

We observed that, unlike their wildtype littermates, BMAL1-KO mice lacked a day-night variation in HVR. Absence of a functioning molecular clock also resulted in an overall decrease in HCVR and a sex-dependent absence of HCVR day-night variation. Male BKOP lacked day-night variation in HVR consistent with the findings in the global BMAL1-KO mice. However, unlike the BMAL1-KO mice, BKOP mice continued to exhibit day-night variation in HCVR in a sex-dependent manner.

Conclusions (100 word limit)

These data indicate that a functional circadian clock is necessary for daily variation in ventilatory chemoreflex. More specifically, the molecular clock of Phox2b cells is sufficient to drive the circadian variation in the ventilatory response to hypoxia, but not hypercapnia, in male mice. To our knowledge,

these data demonstrate for the first time that the molecular clock within a specific subset of O_2-sensing cells can drive daily variations in how an animal breathes in response to hypoxia.

Support

N/A

76

Pathogenic MTOR mutations in Smith-Kingsmore syndrome affect circadian rhythms and sleep

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Full Name and Credentials

Yang Shen

Introduction (100 word limit)

Smith-Kingsmore syndrome (SKS) is a rare autosomal dominant condition. MTOR functions to coordinate cell metabolism and growth with energy and nutrient signals in the cell. Thus far, a few dozen individuals with SKS have been reported. The core clinical features of SKS include macrocephaly/megalencephaly, developmental delay, intellectual disability, and seizures. Our recent studies also show dysregulation of homeostatic functions, including sleep disorders and hyperphagia. Most of these SKS alleles are considered variants of uncertain significance (VUSs). This line of research aims to determine and characterize how these VUSs contribute to pathogenesis and underlie the diverse range of clinical manifestations.

Methods (200 word limit)

To characterize these MTOR variants, we used the U2OS cells harboring the Per2-dLuc reporter which allows measurements of MTOR activity and circadian rhythms. We used lentiviral vectors to generate stable cell lines ectopically expressing a select panel of the genetic variants. We also used CRISPR-Cas9 to generate site-specific knock-in mutations at the endogenous locus in these cells. Then, we used these cells to measure the mTOR activity with Western blot and to monitor the cellular circadian rhythms using real time bioluminescence assay. SKS patients were treated with rapamycin off label.

Results (200 word limit)

First, we show that under conditions of glucose-, serum-, or amino acid-starvation, the basal activities of all mutants were higher than WT. MTOR Δ(R1480-C1483) and the classic SKS variant C1483F were 3-4 times more active than WT, whereas G2464V is only slightly more active than WT. Second, when treated with the MTOR inhibitor, rapamycin, C1483F and G2464V displayed a similar inhibitory response as WT, whereas Δ(R1480-C1483), S2215Y, and V2406M were more resistant to rapamycin. Third, we generated Δ(R1480-C1483) CRISPR knock-in cells, namely +/+, +/Δ, and Δ/Δ. We show that MTOR+/+ activity was the lowest and Δ/Δ the highest under basal conditions. Further, cells of all three genotypes displayed dose-dependent but differential sensitivity to rapamycin. MTORΔ/Δ and MTOR+/Δ cells cultured in a low

amino acid condition had significantly lower rhythm amplitude than +/+ controls. Importantly, however, low dose rapamycin was able to restore the rhythm amplitude and decrease the damping rate in these mutant cells. Finally, this information was used to guide treatment options in SKS patients. We show that low dose rapamycin use was able to restore the sleep/wake behavior in patients and improve higher-levels functions including cognition.

Conclusions (100 word limit)

Our results suggest that pathogenic SKS variants cause hyperactivation of MTOR and alter the balance between the basal MTOR activity and the cell's energy state, leading disruption of cellular circadian homeostasis and sleep/wake behavior.

Support

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77

Correlations between subjective sleep onset and factors related to social isolation during the pandemic.

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Full Name and Credentials

Nir Eilon

Introduction (100 word limit)

Worry, stress, and exercise have known impacts on sleep health. Social isolation likely has a negative impact on sleep health, but social isolation can be difficult to quantify. More data on this topic are needed. During pandemic lock-downs, there were many changes to people's routines and lifestyle, allowing for the examination of downstream effects of staying at home and other social limitations. Our research was based on survey results and examined independent effects of leaving the house, socializing, worry, stress, feelings of isolation, and exercise on sleep quality. Our analysis attempted to isolate the influence of these key factors.

Methods (200 word limit)

To quantify the impact of social isolation and related factors on sleep quality, we used publicly available data from the Boston College Daily Sleep and Well-Being Survey. The dataset includes 1,518 participants, aged 18 to 90 years, including 37,882 survey responses collected during COVID lockdowns from March 20 to June 23, 2020. Sleep quality was measured as self-reported sleep-onset latency (SOL). Because worry is known to increase SOL, other factors such as leaving the house were analyzed in relation to levels of worry. Similarly, because physical exercise is known to decrease SOL, other factors were analyzed in relation to exercise. Correlations and linear regressions were used to examine the independent influence of each variable and the statistical significance of the results.

Results (200 word limit)

As predicted, we found that leaving the house was a significant predictor of lower SOL ($p < .001$). When not leaving the house, virtual socializing was a significant predictor of lower SOL ($p < .001$). These results were significant even after controlling for known effects of exercise, worry, and stress (all $ps < 0.001$). The length of socializing time when not leaving the house did not show a specific influence on SOL.

Conclusions (100 word limit)

Average SOL was improved when leaving the house that day. This result was consistent for different levels of worry, socializing, isolation, and physical exercise. Leaving the house had an effect in addition to the socializing and physical movement positively associated with leaving the house. The exact factors

contributing to this effect require additional research; however, we suggest that leaving the house may provide a means to improve SOL. When not leaving the house, socializing (virtually) that day was associated with reduced SOL. As expected, higher levels of worry, stress, and feelings of isolation increased SOL, while physical exercise decreased SOL.

Support

Our results show that changes in behavior during the pandemic within a 24-hour cycle influenced sleep quality as indicated by SOL ratings given by individuals.

78

Toward Precision Medicine and Better Sleep in Smith-Kingsmore Syndrome (SKS)

Kristen Groseclose[1], Susan Dando[1], David Smith[2,3], Yang Shen[4], Carolyn Serbinski[5], Thomas Dye[3], Darcy Krueger[3], John Hogenesch[3], Andrew Liu[4], Carlos Prada[5]

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Full Name and Credentials

Andrew Liu

Introduction (100 word limit)

Smith-Kingsmore syndrome (SKS) is a newly discovered rare genetic disorder caused by gain-of-function mutations in the MTOR gene. The core clinical features of SKS are neurodevelopmental, including variable degrees of macrocephaly or megalencephaly, developmental delay, intellectual disability, and seizures or epilepsy. MTOR functions to sense cell energy states and coordinate metabolism with cell growth. MTOR dysregulation is implicated in various neuropathological conditions. Recent studies show that MTOR signaling and the circadian clock are also functionally intertwined. MTOR dysregulation is associated with altered circadian rhythms and sleep/wake behavior.

Methods (200 word limit)

Our team recruited a cohort of 28 individuals with SKS that represent 9 new MTOR pathogenic variants. We conducted a detailed natural history study on these patients. We also performed molecular, cell and biochemical experiments to characterize the functional consequence of these genetic variants.

Results (200 word limit)

In addition to the core neurodevelopmental symptoms, our studies also uncovered new disease manifestations, including sleep-wake disturbance, hyperphagia, and hyperactivity, all indicative of homeostatic imbalance. Functional assays show that these SKS alleles had differently biochemical activities and responded to rapamycin (an MTOR inhibitor) differently. Of relevance, 77% of all SKS patients report disordered sleep with varying levels of severity, such as irregular sleep/wake and self-harm during sleeping-phase wakings. Many have not received dedicated clinical attention for these issues. Sleep issues are often seen as “just part of life” for children with disabilities and their families. This under-appreciation of sleep as both a physical and psychological issue for SKS patients, as well as their families, mirrors the clinical experience of the families in the rare disease community. Importantly, we used rapamycin off-label to treat several patients and show that optimal dosing of rapamycin and

adjunct hypnotic restored the circadian sleep/wake cycle and improved other cognitive functions in an SKS patient.

Conclusions (100 word limit)

While the primary concern remains neurodevelopmental, sleep/wake disturbance is a significant challenge that SKS patients and caregivers face daily, impacting other behaviors and overall health. Long-term disruption can exacerbate neuropathology and is a significant stressor for caregivers and siblings. Future research will explore allele-specific drug regimens to normalize MTOR and improve functional outcomes, using sleep/wake behavior as a neurophysiological biomarker and health intervention. The SKS Foundation (the leading SKS patient organization) has sought out circadian/sleep researchers to pioneer research into how SKS affects MTOR to drive precision medicine.

Support

NIH-NINDS R01NS054794 to JBH and ACL and R01NS117457 to ACL

79

Characterizing the output network of a sleep homeostat in Drosophila

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Full Name and Credentials

Abigail Aleman

Introduction (100 word limit)

Sleep is a universally conserved behavior essential for the maintenance of various aspects of health. Many of the genes and neural circuits that regulate sleep have been uncovered. Particularly, recent studies have implicated the central complex as a crucial center for the regulation of sleep in Drosophila. However, it remains unclear how local activity within specific neuropils, such as the dorsal fan-shaped body (dFB) neurons of the central complex, effectuate systemic sleep.

Methods (200 word limit)

Here we take an unbiased genetic approach to identify and activate dFB post-synaptic targets. First, we used the genetically-encoded anterograde synaptic tracing tool trans-Tango to reveal downstream targets of sleep homeostatic dFB neurons. We also employed the trans-Tango system to express the thermosensitive TrpA1 channel to acutely activate dFB post-synaptic neurons. Based on the trans-Tango data we searched the Janelia FlyLight repository to obtain Gal4 drivers with anatomically similar characteristics. Eighteen Gal4 drivers were used to conduct a Gal4 thermogenetic activation screen to target subpopulations of neurons within the fan-shaped body, allowing for more precise anatomical and behavioral classification. Sleep was measured in female flies using Drosophila Activity Monitors; day and night heat shift protocols were used to uncover sleep and wake promoting drivers respectively. We generated split-Gal4 reagents to refine the expression pattern of the cells of interest. Where appropriate, we performed two- or three-color genetic labeling to identify overlap between sleep-modulatory Gal4 drivers and dFB post-synaptic targets labelled with trans-Tango. Additionally, we performed a Gal80 screen to identify neurotransmitter types expressed by these neurons. Lastly, we performed loss of function experiments to catalog environmentally specific contexts in which the identified neurons may be active to influence sleep regulation.

Results (200 word limit)

Trans-Tango showed Pontine interneurons to be likely downstream targets of dFB neurons. The net effect of acutely activating this downstream population suppressed sleep, suggesting that the labeled neurons include sleep-regulatory cells. The Gal4 thermogenetic screen also revealed an anatomically similar, but functionally distinct population of cells with opposing effects on sleep. One population promoted wakefulness upon stimulation and inhibiting neurotransmission via tetanus toxin dampened their wake-inducing effect. Exposing flies to constant light or starvation, conditions that normally suppress sleep in the fly, resulted in an atypical response in animals when wake-promoting Pontine

neurons were silenced. We also uncovered a population of Pontine neurons that promote sleep, and deletion of glutamatergic cells from our sleep promoting Gal4 driver resulted in an even stronger sleep promoting effect. Dual labeling experiments showed partial overlap between wake and sleep promoting drivers; while three color labeling between wake promoting drivers and trans-Tango showed that wake-promoting Pontine neuron drivers overlap with post-synaptic targets of dFB neurons.

Conclusions (100 word limit)

Our Gal4 screen uncovered drivers with bidirectional effects on sleep, and anatomy experiments showed overlap between sleep and wake drivers. This suggests that the Pontine neurons downstream of dFB represent a mixed population of cells. Depending on sleep history and experience, different dFB postsynaptic populations may become activated to generate the appropriate response. Blocking synaptic release from wake-promoting pontine cells suppressed the typical wake-inducing effects of food deprivation and constant light, indicating that these neurons could integrate circadian and physiological drives to influence sleep/wake regulation.

Support

This project was supported by an Early Career Development Award from the Sleep Research Society Foundation to JD, a Career Development Award from the Human Frontiers Science Program to JD (CDA00026-2017-C) a Neuroscience Fellowship from the Klingenstein and Simons Foundations, NIH grant NS105967 to JD

80

Bmal1 overexpression in skeletal muscle alters behavioral responses to stress

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Full Name and Credentials

Ketema Paul, PhD

Introduction (100 word limit)

There is a reciprocal relationship between sleep and stress; stress often produces sleep loss which, in turn, can worsen stress responses. Research in mice has shown that whole body knockout of the circadian clock gene, Bmal1 affects several aspects of sleep. Surprisingly, restoring Bmal1 exclusively in skeletal muscle rescues many sleep phenotypes. Moreover, overexpression of Bmal1 in skeletal muscle alters sleep homeostasis and reduces the recovery response to sleep loss. Due to the role of Bmal1 expression in skeletal muscle in sleep regulation, we hypothesized that this gene may also play a role in stress regulatory mechanisms and stress resilience.

Methods (200 word limit)

In this study, the anxiety-like behavior of eight Bmal1 muscle overexpression mice (4 males, 4 females) was compared to their wildtype littermates (4 males, 4 females) using the Open Field Test (OFT). After evaluating the baseline open field behavior of all mice at PNW12 at ZT16 for 15 minutes, mice underwent 1 hour of restraint stress at PNW14 at ZT14 in a semi-cylindrical, plastic restraint device. After the 1-hour stress episode, all mice were released back to their homecage for 1 hour before their open field behavior was evaluated again using the OFT at ZT 16. During the 15 minute OFT, video recording and automatic behavior scoring assessed anxiety by measuring the amount of time each mouse spent in the exposed center of the open field along with other measures such as overall distance traveled and clear signs of freezing.

Results (200 word limit)

Analysis of the open field behavior, specifically freezing and exploratory behavior supports the hypothesis that Bmal1 muscle overexpression mice show resistance to stress and are more explorative

compared to their wildtype littermates in the open field. Our results show that Bmal1 muscle overexpression mice have significantly less freezing time and freezing episodes compared to their wildtype littermates both at baseline OFT and after restraint stress. Additionally, our findings indicate that Bmal1 muscle overexpression mice travel a longer distance in the OFT, enter the center of the open field faster, and explore the center longer after restraint stress compared to wildtype mice.

Conclusions (100 word limit)

The behavioral data of this study suggest that expression of Bmal1 gene in skeletal muscle may play a role in stress regulation in addition to sleep regulation which further confirms the reciprocal relationship between sleep and stress and provides insight into potential stress regulatory mechanisms that occur in muscle.

Support

N/A

81

The Pupillary Light Reflex in Response to Evening Red and Blue Light in Children and Adolescents

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Full Name and Credentials

Lauren Hartstein, PhD

Introduction (100 word limit)

Stimulation of the eye's intrinsically-photosensitive retinal ganglion cells (ipRGCs) influence both circadian timing and the pupillary light reflex (PLR) which controls pupil diameter in response to a light stimulus. The ipRGCs have a peak sensitivity to blue light at ~480 nm, and in adults, pupil constriction is significantly greater and more sustained after exposure to blue light compared with red light. The PLR has been identified as a marker of the melanopsin-driven response to light. We examined how the PLR differs in response to blue vs. red evening light in children and adolescents, and whether age-related differences are present.

Methods (200 word limit)

39 healthy good-sleeping participants aged 8-9 years (n = 21) or 15-16 years (n = 18) completed a 6-day protocol. For 5 days, participants maintained a stable sleep schedule, verified through actigraphy and a sleep diary. On the 6th day, participants remained indoors wearing a pair of dark glasses to limit bright light exposure and reduce variability in light history. They then completed an in-lab pupillary assessment in the hour before their habitual bedtime. After a dim-light adaptation (<1 lux), we measured their pupil diameter during a 30 s baseline, 10 s light exposure to either red light (627 nm) or blue light (459 nm) presented at the same photon flux (3.0×10^{13} photons/cm^2/s), and 40 s recovery. Following a 7-min dim-light re-adaptation, the procedure was repeated for the other light condition, the order of which was counterbalanced across participants. We examined the impact of lighting condition and age group on: (1) percent pupil constriction 500 ms after light onset, (2) maximum pupil constriction during the light exposure, (3) slope of constriction during the light exposure, and (4) percent pupil constriction 6 s after light offset (PIPR 6s).

Results (200 word limit)

Within each age group, the maximum pupil constriction was significantly larger during exposure to blue compared with red light (children: blue = 57.8%, red = 55.6%; adolescents: blue = 53.4%, red = 50.8%). The constriction response was also more sustained during the blue light exposure than the red ($p < 0.01$, $\eta_p^2 = 0.46$). For adolescents, the PIPR 6s was significantly larger after blue (13.9%) compared with red

light (11.5%). Finally, across both light colors, children had a faster initial response ($p < 0.01$, $\eta_p^2 = 0.22$) and greater maximum constriction ($p < 0.01$, $\eta_p^2 = 0.20$) than adolescents.

Conclusions (100 word limit)

In school-aged children and adolescents, blue light elicited a greater and more sustained pupillary response than red light in the hour before habitual bedtime. Furthermore, the overall amplitude of the response was greater in children than adolescents. This aligns with previous work demonstrating a heightened circadian sensitivity to light in early vs. late adolescence. Future research will examine the association between the PLR and circadian response to light (i.e., melatonin suppression and circadian phase shift) in children.

Support

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83

Time of Light Exposure and Light Intensity Predominantly Predict Circadian Phase in Free-Living Individuals

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Full Name and Credentials

Hash Brown Taha

Introduction (100 word limit)

Light is the strongest environmental time cue entraining the mammalian circadian clock to the 24-hour day. Light dimensions including timing, intensity, duration, and spectrum have been shown to influence circadian phase-shifting responses. These light dimensions are integrated by the biological clock and have been mainly studied separately in controlled-laboratory settings. Here we examined the associations between multiple light dimensions and circadian phase in free-living individuals.

Methods (200 word limit)

Free-living individuals (n=28; Aged 25.8±6.1, 10m, 18f) were studied. Participants were instructed to wear an f.luxometer (f.lux), a pendant-worn device capable of measuring light in gaze direction, for 14 days. The f.luxometer recorded light every 2-min during wakefulness using a digital red, green, and blue color light sensor with an infrared-blocking filter. Melanopic lux values obtained were transformed to Melanopic equivalent daylight illuminance (mEDI) for mean timing of low light exposure (Mlit) < 25 mEDI lux (Mlit 2 < mEDI lux < 25), of intermediate light exposure (Mlit 25 < mEDI lux < 250), and of bright light exposure >250 mEDI lux (Mlit > 250 lux), as well as average wakefulness light intensity, and duration of light exposure > 100 and > 500 mEDI lux. Saliva samples were obtained from participants under controlled laboratory settings in dim light and assayed for melatonin levels (Buhlmann). DLMO was calculated as the linear interpolated point in time at which melatonin levels rose above a 3 pg/ml threshold. Pearson's correlations tested associations between light dimensions. Associations between the light dimensions and DLMO were conducted using univariate and multivariate logistic regression models.

Results (200 word limit)

Average mEDI lux correlated with the duration > 100 ($r=0.52$, $p<0.05$) and > 500 ($r=0.75$, $p<0.05$) mEDI lux. Similarly, the duration > 100 mEDI lux and > 500 mEDI lux strongly correlated with one another ($r=0.84$, $p<0.05$). Univariate regression models revealed that Mlit > 250 mEDI lux, average wakefulness light intensity and duration > 500 mEDI lux were significantly associated with DLMO such that the earlier the timing of Mlit, the brighter the light, and the longer duration above threshold respectively, the

earlier the DLMO. When considering light exposure variables concurrently using a multivariate regression model, only Mlit > 250 mEDI lux and average wakefulness mEDI lux intensity remained significant predictors of DLMO ($p<0.05$).

Conclusions (100 word limit)

We demonstrate here using a pendant-worn device able to measure light in the gaze direction that the mean timing of light exposure (Mlit > 250 mEDI lux) and daily average light exposure intensity during wakefulness are the strongest predictors of circadian phase in a small cohort of healthy free-living individuals. Findings suggest that light wearables may be viable technology to predict circadian phase in free-living humans. Additional testing is needed in larger cohorts.

Support

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84

Dim light melatonin offset (DLMOff) in healthy adults and associations with chronotype

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Full Name and Credentials

Rebecca Cox, PhD

Introduction (100 word limit)

Dim light melatonin onset (DLMO), or the rise in melatonin secretion representing biological night beginning, is the gold standard indicator of circadian phase. Considerably less is known about dim light melatonin offset (DLMOff), or the decrease in melatonin secretion representing biological night end. Morning circadian misalignment, or energy intake after wake time but before DLMOff, is linked to impaired insulin sensitivity, suggesting the need to characterize DLMOff and morning circadian misalignment risk. We examined the distributions of DLMOff clock hour and the phase relationship between DLMOff and wake time, and associations between DLMOff, phase relationship, eveningness, chronotype, and social jetlag.

Methods (200 word limit)

Data were examined from a sample of healthy adults who completed one of four in-laboratory protocols (N=100) that included salivary melatonin collected in dim light (<10 lux maximum, ~1.9 lux, ~0.6 W/m2 in the angle of gaze), self-reported habitual sleep timing on work days and free days, and the Morningness-Eveningness Questionnaire. DLMOff was defined as the linear interpolated point in time at which melatonin levels fell below 25% of the fitted peak-to-trough amplitude of the 3-harmonic of each individual's data. Chronotype (i.e., mid-sleep on free days corrected) and social jetlag were calculated according to the Munich Chronotype Questionnaire using self-reported bedtime and waketime on work days and free days at screening. Participants maintained a consistent sleep schedule for 1-2 weeks prior to melatonin assessment in the laboratory. The phase relationship between DLMOff and wake time was calculated by subtracting wake time (from the in-laboratory protocols) from DLMOff, such that a larger, positive phase relationship indicates that DLMOff is later than wake time.

Results (200 word limit)

The mean DLMOff clock time was 8.55h (SD=2.08), and the mean phase relationship between DLMOff and wake time was 0.63h (SD=1.63). The percentage of the sample with a phase relationship between DLMOff and wake time greater than or equal to 0h, 1h, 1.5h, and 2h was 86.8%, 26.5%, 22.1%, and 14.9%, respectively. Later DLMOff was significantly associated with eveningness ($r=-.50$, $p<.001$) and later chronotype ($r=.47$, $p<.001$) but was not significantly associated with social jetlag ($r=-.06$, $p=.38$). Phase relationship between DLMOff and wake time was not significantly associated with eveningness ($r=-.22$, $p=.13$), chronotype ($r=.12$, $p=.41$), or social jetlag ($r=-.03$, $p=.84$).

Conclusions (100 word limit)

These findings characterize DLMOff and phase relationship between DLMOff and wake time in healthy adults. Even among healthy adults, a notable percentage demonstrated high melatonin levels after wake time suggesting a substantial risk of morning circadian misalignment. Evidence for associations between DLMOff and chronotype, but not phase relationship and chronotype is consistent with prior research on DLMO. Future research is needed to replicate these findings in larger samples, more racially diverse samples, samples across the lifespan, and in clinical populations.

Support

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85

Temporal stability of circadian misalignment: do night shift workers really rock around the clock?

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Full Name and Credentials

Philip Cheng

Introduction (100 word limit)

Despite having important clinical implications, the stability of circadian phase (and thus circadian misalignment) has not been well characterized in night shift workers. The stability of circadian phase is particularly important for implementation of light therapy to reduce circadian misalignment. Indeed, if night shift workers are "rocking around the clock", phase assessments (e.g., dim light melatonin onset) may only be valid for a short period of time.

Methods (200 word limit)

To characterize the stability of circadian phase in night shift workers, we conducted in-lab assessments of dim light melatonin onset (DLMO) twice. The first and second DLMO were separate by four weeks.

Results (200 word limit)

Results indicated that the intraclass correlation between the repeated DLMOs was 0.79 (good reliability), $F(44,45) = 8.71$, $p < .001$. On average, the mean absolute error between the two DLMOs was 2.78 hours ± 0.42 SE.

Conclusions (100 word limit)

These results suggest that circadian phase in night shift workers are quite reliable over a four week period, with an average change of just under 3 hours. Moderating factors will also be discussed. These results provide evidence that while the circadian phase in night shift worker are spread out across the 24 hour period, the temporal stability of circadian phase is quite high suggesting minimal "rocking around the clock". The clinical implication is that DLMO assessments in night shift workers can be reliable over a period of 4 weeks, and thus has high feasibility for the purpose of light treatment.

Support

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86

The protective role of sleep in the cortical response to stress

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Full Name and Credentials

Eva-Jeneé Andrews

Introduction (100 word limit)

Environment and sleep-wake history influence the quality and quantity of sleep. Specifically, negative social encounters can alter sleep and behavior. Some individuals are resistant to these negative effects of stress, however, the mechanism of this resilience is unknown. Our lab has demonstrated that non-rapid eye movement (NREM) sleep confers resilience to social-defeat stress. The medial prefrontal cortex (mPFC) suppresses limbic circuits responsible for negative behavioral responses to stress and predicts resilience. Thus, we hypothesize that NREM sleep within the mPFC determines resilience. These studies characterize the mPFC single-unit activity in socially-defeated mice to assess the role of sleep in resilience.

Methods (200 word limit)

Mice, 8-10 weeks old, expressing (CaMKIIα-Cre X Ai32) channelrhodopsin-2/EYFP exclusively in CaMKIIα neurons were used in this study. After identifying CaMKIIα-expressing neurons using blue-light stimulation, we recorded multi-unit activity (MUA) and local field potential changes in mPFC neurons. This was before, during, and after social-defeat stress administered using a resident intruder paradigm.

Results (200 word limit)

Preliminary findings suggest that firing patterns in mPFC CaMKIIα neurons differ in mice resilient to the effects of social defeat stress. Furthermore, these results suggest that resilience is predicted by sleep-related local firing patterns in the mPFC. These findings suggest that sleep may serve to enhance the ability of mPFC cells to promote resilience to social-defeat stress.

Conclusions (100 word limit)

Sleep has a protective role in the resilient behavioral response based on the relationship between the firing patterns of vmPFC neurons in response to social-defeat stress and prior sleep-wake history.

Support

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87

Restoration of proteostasis with chaperone therapy increases XBP1s and ADAM10, rescuing cognitive performance in a mouse model of Alzheimer's disease.

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Full Name and Credentials

Nirinjini Naidoo

Introduction (100 word limit)

Perturbation of proteostasis in the endoplasmic reticulum (ER) leads to ER stress. Activation of a signal transduction pathway, the unfolded protein response (UPR) acts to reduce ER stress and restore proteostasis. However, with protein misfolding diseases, such as Alzheimer's disease (AD) with aberrant Aβ42 production, ER stress is chronic and UPR activation can become maladaptive. In addition to this disruption in proteostasis, cognitive deficits are a debilitating symptom of AD. As protein synthesis is required for memory, we tested the hypothesis that restoring proteostasis by reducing ER stress in AD could improve pathology and cognition.

Methods (200 word limit)

Transgenic APP knock-in mice were used as a model for AD. Two groups of mice and their wildtype littermates, (treatment starting early at 1-2 mos old and late at 10-12 mos old) were administered the chemical 4-phenyl butyrate (PBA) by weekly I.P. for 10 weeks. Controls were given I.P. injections of sterile saline. After 10 weeks of PBA or saline treatment, mice were subjected to the Spatial Object Recognition (SOR) cognitive tasks. A third group of APP knock-in mice and wildtype littermates were given stereotaxic local hippocampal AAV-BiP overexpression or AAV-mCherry control injections. Following recovery and viral expression, mice were subjected to the SOR test. All mice were perfused, and tissue was collected for both immunohistochemistry and biochemical analyses.

Results (200 word limit)

We report that PBA treatment improved performance in the Spatial Object Recognition test both with early ($p < 0.001$) and late-stage ($p < 0.05$) PBA intervention. AAV-BiP overexpression recapitulated those results. Histological data indicates that chaperone treatment, both with PBA injections and AAV-BiP overexpression, reduced hippocampal ER stress and was correlated with increased p-CREB. Chronic PERK activation which leads to inhibition of protein translation was reduced, while the pro-survival transcription factor XBP1s was increased with chaperone treatment. This was coupled with increased ADAM10, which is associated with non-amyloidogenic cleavage of APP.

Conclusions (100 word limit)

Our results suggest that reducing ER stress improves cognition in a mouse model of Alzheimer's disease. The implications of these results could have an impact on the development of therapies to inform the development of potential treatments for Alzheimer's disease.

Support

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NIA: R01 AG064231 Cellular and Molecular Basis of Sleep Loss Neural Injury in Alzheimer Disease

88

X chromosome dosage contributes to sex differences in sleep regulation.

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Full Name and Credentials

India Nichols-Obande

Introduction (100 word limit)

There are pronounced sex differences in sleep that have been conserved across species. From studies in fruit flies, mice, and humans, evidence shows that females sleep less than males in a twenty-four-hour period, males sleep more during the active period, and females have more consolidated sleep during the rest period. In mice, it has been reported that sex hormones are responsible for some of these differences. However, sex differences during the rest phase were eliminated in the absence of circulating hormones while active phase sex differences remained.

Methods (200 word limit)

This observation led to the investigation of the role of sex chromosomes and sex genes in sleep regulation to which it was found that sex chromosome complement and SRY gene were responsible for phenotypes associated with sleep homeostasis. In order to determine the effect of X chromosome, Y chromosome, and chromosome dosage on various sleep phenotypes, this study uses EEG and EMG to measure spontaneous sleep and recovery sleep in a mouse model with four sex chromosome complement genotypes. The XY* mouse line allows for investigation of the role of two X chromosomes vs one (XXY, XX vs XO, XY) and Y presence vs absence (XY, XXY vs XO, XX). To eliminate the role of circulating hormones, the mice are gonadectomized before the EEG cap is implanted. Spontaneous sleep is defined as undisturbed behavior over a 24-hour period. Recovery sleep is defined as 18 hours of undisturbed behavior immediately following 6 hours of forced wake.

Results (200 word limit)

Two-way ANOVA measuring Y presence vs X dosage shows that in spontaneous and recovery sleep during the active phase, there is an effect of Y chromosome presence on NREM and total sleep where mice with a Y chromosome had more total sleep (p-value =0.04) and NREM (p-value =0.01) than mice without a Y chromosome. Two X chromosomes regulate spontaneous REM sleep during the active phase (p-value= 0.004) by increasing REM sleep in XXY and XX mice. Two-way ANOVA did not reveal an effect of Y presence nor X dosage on sleep during the rest phase in baseline nor recovery sleep.

Conclusions (100 word limit)

Taken together these results show that sex differences in sleep are the sum of sleep phenotypes regulated by either X dosage or Y presence.

Support

R01 NS078410.

89

The Effect of BMAL1 Expression on Autophagy Activity in Astrocytes

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Full Name and Credentials

Connor Cortez Campbell

Introduction (100 word limit)

Circadian rhythm disruption is an established symptom of neurodegenerative disease, however, there is mounting evidence that circadian systems drive early pathogenesis. The core circadian clock gene, BMAL1, mediates daily oscillations in transcription for homeostatic processes. Furthermore, the deletion of BMAL1 renders tissue transcriptionally arrhythmic and influences non-circadian genes, which causes reactive astrogliosis in the brain. We have recently observed in-vitro that BMAL1 knockout astrocytes exhibit increases in the pH-sensitive lysosomal dye lysotracker. This suggests that the clock potentially modulates an even broader array of cellular processes, like autophagy, which maintains cellular homeostasis by delivering proteins to lysosomes for degradation.

Methods (200 word limit)

Given that deficits in the clearance of stable protein aggregates is characteristic of many neurodegenerative disorders, the manipulation of autophagic machinery displays therapeutic potential. Therefore, the purpose of this project is to evaluate whether autophagy activity in astrocytes is regulated rhythmically by circadian clocks or through non-rhythmic genes linked to BMAL1 expression. By using autophagy reporter mice expressing fluorescent (RFP)-EGFP-LC3 proteins, we can detect differential organelle pH values to monitor dynamics of autophagosomes. In this model, both the RFP (pKa 4.5) and the EGFP (pKa 5.9) will be fluorescent in the early autophagic vacuole, however, the EGFP signal will be quenched upon the autophagosome fusing with the acidic conditions of the lysosome (pH 4-5). With the RFP fluorescence remaining in this low pH environment, the ratio of these fluorescent reporters can model the progression of autophagosomes from vacuoles to machinery for protein degradation.

Results (200 word limit)

When comparing the astrocyte-specific BMAL1 knockout mice to wildtypes with this reporter, there are significant increases in both EGFP and RFP channels within white matter regions, suggesting greater endolysosomal and autophagy activity. Moreover, this outcome that BMAL1 deletion augments autophagy activity in white matter provides the basis for evaluating whether these changes arise from dysfunctional circadian clocks, which requires establishing the wildtype baseline fluorescence across the 24-hour circadian cycle. Although both the EGFP and RFP reporters suggest potential rhythmic activity in the white matter for mice harvested across different times of day for light-stimulated cohorts, this trend

vanishes for dark-only counterparts. Thus, this result reduces the likelihood that autophagy activity is controlled by the rhythmicity of clock genes. When attempting to locate the sources of EGFP and RFP signal in the white matter, there did not appear to be localization with S100B-stained astrocytes.

Conclusions (100 word limit)

Consequently, the effect of BMAL1 deletion on autophagosomes in white matter tissue likely originates from other glial cell types that are uniquely susceptible to dysfunction in non-rhythmic pathways related to BMAL1 expression in astrocytes. Through identifying the processes for how BMAL1 protects against neurodegeneration, additional therapeutic approaches can be designed to target the mechanisms related to clearance of stable aggregates by autophagy.

Support

N/A

90

Assessment of homeostatic and circadian timing of REM and deep sleep in young healthy adults, a longitudinal approach under real life conditions

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Full Name and Credentials

Charlotte von Gall, PhD

Introduction (100 word limit)

Sleep problems, which are highly prevalent in modern society, are associated with multiple adverse health consequences. Sleep timing involves a circadian and a homeostatic component. Individuals differ in their preferred midpoint of sleep (MS), defining the intrinsically determined chronotype. In modern society, MS on workdays is mainly defined by the alarm clock in the morning. Thus, early work schedules result in a discrepancy of MS between workdays and free days, thus a misalignment between social and biological time. This misalignment of sleep timing results in reduced sleep quality on workdays and has various health consequences including mood related symptoms.

Methods (200 word limit)

Understanding how sleep quality, sleep composition, and sleep stage timing differs between workdays/social timing and free days/biological timing is crucial. So far very little is known about timing and interactions of sleep stages under real life conditions. We show individual longitudinal sleep stage time patterns based on Fitbit Inspire HR sleep data in combination with a questionnaire survey in young healthy adults. The cohort consists of first-year medical students who have been able to attend mostly online classes on their own schedule due to the COVID-19 pandemic. We analysed sleep timing and interconnections among sleep stages separately for workdays and free days to account for social/external and biological/internal sleep timing, respectively. In addition, sleep stage percentiles were related to minutes after sleep onset and clock time for homeostatic and circadian component, respectively.

Results (200 word limit)

Sleeping in on free days and more flexible sleep durations ameliorate subjective impact of psychosocial stress as well as anxiety and depression, respectively. Tiredness on free days and sleep disturbance are associated with lower proportion and higher fragmentation of REM sleep as well as a higher proportion of light sleep. Thus, REM proportion and REM sleep fragmentation (RFI) are promising digital markers for poor sleep quality/sleep disturbance. Higher subjective workload is associated with higher intraindividual variability of REM fragmentation of workdays. Thus, RFI variability might be a promising

digital marker for psychosocial stress. Deep sleep supports robustness of the circadian rhythm in REM sleep. While early deep sleep seems to be stronger controlled by the circadian component, late REM and deep sleep seem to be stronger controlled by the homeostatic component of sleep timing. Biological sleep timing conditions on free days promote deep sleep timing. Intraindividual variability in sleep timing and sleep composition, in particular of REM sleep, are related to better homeostatic and circadian sleep stage timing. Mood-related factors and biological sex interfere with homeostatic sleep stage timing while sleep quality is more related to circadian sleep stage timing.

Conclusions (100 word limit)

Our study provides insight into homeostatic and circadian timing of REM- and deep sleep as well as sleep composition under social and biological sleep timing conditions, and indicates promising digital markers. We found interconnections among amount, proportion, circadian rhythm stability and fragmentation of REM sleep as well as relations with sleep quality/disturbances, psychosocial stress, and biological sex. Differences in sleep stage timing as well as composition and sleep quality between workdays and free days indicate a strong impact of external demands on intrinsic sleep regulation. More flexible sleep schedules are supportive for mental health and coherent sleep stage timing.

Support

N/A

91

WITHDRAWN

92

Pediatric OSA and neurocognition

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Full Name and Credentials

Arvind Chandrakantan, MD

Introduction (100 word limit)

Pediatric OSA and its associated sleep disorders occur in up to 7% of children. Whereas the sequelae of the disease are well characterized, the underpinnings of its phenotypes are poorly understood. This is mainly because of a lack of comprehensive model of the disease in the young. Here I present the neurobehavioral phenotype of pediatric OSA utilizing a preclinical murine model, which demonstrates ablation of key learning and memory tasks with greater exposure times to the OSA stimulus, which in this case is modeled through intermittent hypoxia. We are working on drilling down the phenotype for further study.

Methods (200 word limit)

This proposal represents a departure from prior models and is embedded in the sculpting of the IH model.

1) Human polysomnographic data: We examined polysomnographic data available from 200 cases (2-8 years of age) at TCH who had comorbid neurocognitive deficits utilizing parametric statistics and have published this data. This informed us of the number of times, depth of desaturation, and desaturation time in these children.

2) Murine oximetry data: p14 mice (corresponding to approximately 18 months of age in humans) were exposed to 10% O2 utilizing a specialized rodent anesthesia circuit with isoflurane exposure . We then calculated SpO2 curves in a time dependent fashion using six mice (n=3 male, n=3 female).

D3) Cross Species Curve Fitting: We then adjusted this to human SpO2 utilizing human-mouse SpO2 curves using a validated cross species methodology.

D4) Optimization of hypoxic gas throughputs: We calculated required time to drop to 10% O2 within our Biospherix intermittent hypoxia chamber and to the desaturation level determined from the human SpO2 nadir. We created a 9-minute sequence followed by scheduled normoxia which was repeated ~7 times per hour following the apnea hypopnea index (AHI) observed in pediatric OSA.

Results (200 word limit)

We examined 4 behavioral tasks over a number of time periods to establish the temporality needed for hippocampal task ablation, as the hippocampus is the center of learning and memory in mammals. 2 tasks were hippocampal dependent: a) Novel object recognition: the ability to recognize novelty between two objects (a familiar v novel) b) Object place recognition: utilizes encoded spatiotemporal function of the hippocampus in order to ascertain object placement in space. 2 tasks were hippocampal independent: 1) Hot plate: tests dorsal root nocioceptive properties. 2) OFA: tests locomotion and cortically based exploration. We utilized 3 time periods initially: exposure from p14 to p23 (9 days acute), to p35 (21 days intermediate), and p70 (56 days, chronic). There was no deterioration in hippocampal tasks from p14 to p35, however at p70 we noted task ablation. We therefore systematically worked backwards, until we discovered the 'tipping point' where hippocampal task ablation is observed is p49 (35 days of exposure). Prior to this, we note no task ablation, however after this time point, hippocampal task ablation is preserved.

there were no noted differences between groups or genders with regards to non-hippocampally mediated tasks.

Conclusions (100 word limit)

We have demonstrated a tractable, temporal model for pediatric OSA over a systematic set of time points to show when hippocampal deficits from the OSA stimulus manifest phenotypically. We have further isolated these deficits to the hippocampus of developing mice, which is the mammalian center of learning, memory, and retrieval. Utilizing this data, we are currently drilling down the phenotype to study synaptic (circuit), cellular, and molecular underpinnings to understand which cells or groups of cells underlie this phenotype.

Support

Arvind has previously received support from AASM and BCM OOR. He is currently supported by 1K08HL161263-01 from NHLBI.

93

A Mixed Methods Examination of Sleep and Cannabis Use in Young Adults

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Full Name and Credentials

Allison Marie Yurasek, PhD

Introduction (100 word limit)

Cannabis use and insomnia are common among young adults and college students. College students report using cannabis to help with sleep, however using cannabis for coping and sleep-related reasons is linked to more cannabis-related consequences. Further, poor sleep quality is a commonly endorsed phenomenon following a cannabis quit attempt with participants indicating using cannabis to cope with this sleep difficulty. The aim of the current study was to qualitatively and quantitatively explore the relationship between cannabis use and sleep in young adults, including comparisons between those who are and are not experiencing sleep related withdrawal symptoms.

Methods (200 word limit)

Participants were 65 cannabis using young adults (55.4% female; 96.9% college students) who completed a series of cannabis related measures including cannabis use frequency, cannabis use disorder (CUD) symptoms, and reasons for using cannabis (medical, recreational, both), followed by participation in focus groups (N = 9 groups). Focus groups were conducted as part of a larger study aimed at adapting a novel cannabis activity assessment. The current analyses focused on themes and content relating specifically to cannabis and sleep. All focus groups were recorded and later transcribed verbatim. Transcripts were free coded by the first author.

Results (200 word limit)

Approximately half of participants (N = 29; 44.6%) reported sleep difficulty after stopping or reducing their cannabis use. ANCOVA analyses demonstrated that despite similarities in frequency of cannabis use, those who indicated sleep related withdrawal symptoms reported significantly more CUD symptoms. Most participants reported using cannabis for recreational-only purposes (64.6%), followed by both medical and recreational purposes (30.8%), and finally medical-only purposes (4.6%). This did not differ by sleep-related withdrawal experience.

Five themes emerged from the qualitative data analysis: “Sleep-related cannabis motives”, Cannabis-related consequences”, “Method of use”, “Co-use of cannabis and alcohol” and “Improving cannabis activity measures.” Using cannabis for sleep was mentioned in all groups. A smaller number of young adults however, negatively viewed their tendency to fall asleep after using cannabis as it interfered with important activities (e.g., schoolwork). Others indicated cannabis inhibited their ability to fall asleep or reported experiencing negative residual effects after using cannabis for sleep including headaches,

grogginess, and feeling hungover. Regardless, many participants indicated the importance of including sleep on cannabis-related assessments. Discussions also indicated a need to examine the influence of using both alcohol and cannabis on sleep behaviors as well as methods of use (e.g., dabbing) and cannabis strains (e.g., indica).

Conclusions (100 word limit)

Results suggest that sleep difficulties are a primary motive for using cannabis among young adults but may have unintended consequences (e.g., difficulty staying asleep, headaches). Hence, future research should prospectively examine the influence of cannabis on sleep patterns and circadian rhythms. With sleep difficulties being a common symptom of cannabis withdrawal as evidenced in the current study, cannabis using young adults may benefit from intervention programs that target both cannabis and sleep.

Support

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94

Sex differences in sleep exist prior to exposure to social defeat stress

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Full Name and Credentials

Brittany J Bush

Introduction (100 word limit)

Poor sleep quality is linked to neuropsychiatric disorders in men and women. Social defeat stress is frequently used to model features of neuropsychiatric conditions. Evidence from this model shows that sleep differences predict stress-induced maladaptive behavioral outcomes and that NREM sleep plays a causal role in such behaviors. Despite this, the lack of an effective female model prevented similar investigations in females. An animal model of social stress in female rodents may provide the opportunity to conduct these investigations. In the present study, we test the hypothesis that sleep differences predict behavioral responses to social defeat stress in female mice.

Methods (200 word limit)

We recorded electroencephalographic (EEG) data in a cohort of female and male mice at baseline and after exposure to a six-hour sleep restriction. This was done both before and after exposure to social defeat stress. The defeat model used a resident-intruder paradigm where pairs of one female and one male mouse were maintained in the same cage, separated by a perforated barrier, throughout the duration of the study. Pairs were then exposed to social defeat stress simultaneously. Twenty-four hours after 10 days of social defeat stress, social avoidance was tested against a caged novel mouse. Sleep was score in ten second epochs and comparisons were made between sexes and animals susceptible and resilient to social defeat stress.

Results (200 word limit)

We observed significant sex differences between mice that displayed resilience or susceptibility to social defeat stress, prior to social stress exposure. In baseline sleep, resilient female mice displayed increased NREM sleep during the active period compared to males. Conversely, susceptible males displayed increased NREM sleep, and REM sleep-time when compared to females. During recovery from sleep deprivation, both resilient and susceptible female mice displayed increased recovery of NREM and REM sleep immediately following sleep restriction. This difference was not observed in the males. However, resilient and susceptible males had increased REM sleep-time during the active period, when compared to females. No differences in slow wave activity (SWA) were observed in susceptible males or females; however, there were significant differences in NREM SWA in both resilient male and female mice following sleep restriction. Resilient males displayed an increase in NREM SWA following sleep recovery

during the inactive period, while resilient females displayed a decrease in NREM SWA during the active period.

Conclusions (100 word limit)

Our results show that pre-existing differences in sleep regulation predicts the behavioral responses to social defeat stress in both males and females. Furthermore, there are sex differences in sleep patterns that predict these outcomes. Our findings suggest that sex plays a significant role in the interaction of sleep, stress and behavior. Furthermore, our results suggest that sex differences in behavioral responses to stress may be partially due to differences in sleep and sleep regulation.

Support

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95

The G-protein Coupled Signaling of APP-C terminal Fragment in the Suprachiasmatic Nucleus Impacts Cognition in Tau and Amyloidogenic Alzheimer's Disease Mouse Models.

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Full Name and Credentials

Emily Sandefur

Introduction (100 word limit)

Alzheimer's disease (AD) patients develop sleep disruptions before memory deficit, which exacerbate cognitive impairment. Aβ accumulation, generated through amyloid precursor protein (APP) cleavages, and increased tau phosphorylation are AD hallmarks. We previously reported that G-protein signaling associated with APP C-terminal fragment (APP-CTF) favors GSK3β inhibition, a downstream effector of tau phosphorylation, and rescues memory impairment in AD mouse model. Noteworthy, Aβ and tau fluctuate during wake-sleep cycles, and wakefulness increase Aβ accumulation. Accordingly, we targeted the suprachiasmatic nucleus (SCN), the brain master clock, to explore APP-CTF accumulation's impact on circadian biology and cognition in tauopathy and AD mouse models.

Methods (200 word limit)

In light of our previous findings, we used stereotaxic delivery of Adeno-Associated Virus (AAV) into the SCN of 4-6 months-old mice to express membrane tethered (mAICD) and mAICDmutAAA variant that lacks the GalphaS interaction site. We expressed a control construct (mCtl) to compare groups under study. We employed the PS19 transgenic mouse model that carries the P301S mutation of the human tau protein mimicking tauopathy associated with AD and the 5XFAD mice as an aggressive amyloidogenic mouse model. 2-3 months after the AAV injection, we conducted spontaneous alternation and fear conditioning behavioral tests to determine the effect on cognition. We also injected neonate PS19 mice into the ventricles (ICV), which gave us a broad expression of the plasmids, and examined the cognitive behavior around 6-8 months of age.

Results (200 word limit)

We confirmed that PS19 mice exhibit cognitive impairment compared to their non-transgenic littermates (NTg). Fear conditioning data indicate that PS19 mice injected into the SCN with mAICDmutAAA exhibit a decrease in freezing time compared to the NTg and mAICD injected mice, which is consistent with an impairment of memory performance. PS19 mice injected ICV since birth did not show any difference between groups. We also observed a significant reduction in freezing time in the 5XFAD mouse model expressing mAICDmutAAA in the SCN. Expression of mAICD/mAICDmutAAA did not affect spontaneous alternation behavior in all cohorts.

Conclusions (100 word limit)

Our results support that the accumulation of membrane APP-CTF in the circadian control center can impact cognitive function in tau and amyloidogenic mouse models. Our findings suggest inhibiting the cAMP/PKA pathway associated with mAICD expression might contribute to the observed disparities.

Support

N/A

96

Understanding the Patient Experience With Sodium Oxybate Therapy for Narcolepsy

Matthew Horsnell[1], Enming Zhang[1], Rachelle Cook[1], Lauren Dougherty[1], Allison Foley Shenk[1], Frederik Ascencion[2], Anne Marie Morse[3], Luis E. Ortiz[4], Wei Li[1], E. Robert Wassman[1], Christopher DeFelice[1], Maria Picone[1]

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Full Name and Credentials

Maria Picone, CEO

Introduction (100 word limit)

Narcolepsy is a chronic sleep disorder defined by excessive daytime sleepiness, impaired rapid eye movement sleep, disrupted nighttime sleep with frequent waking, and several molecular biomarkers; it may also be accompanied by cataplexy. Narcolepsy has no cure and affects both men and women. The purpose of this research was to harness the power of natural language processing (NLP) with social listening to better understand patient experiences in the narcolepsy community with taking sodium oxybate (SO) therapy.

Methods (200 word limit)

Using a proprietary analytics engine that incorporates artificial intelligence and NLP to quickly analyze conversations, we analyzed 25,018 posts/comments which occurred from August 2011 to October 2022 and contributed by 15,280 participants in 2 narcolepsy communities: the Reddit thread r/Narcolepsy and a private Facebook group. A clinical entity recognition tagger leveraging medicine ontology was used to build the co-occurrence network and identify relationships between entities. We filtered conversations that mentioned (1) second dosage (e.g., second dose, 2nd) and (2) SO (e.g., Xyrem, SO) to build a unique co-occurrence network for all conversations discussing second doses of SO. Patient experiences with SO were then documented by surveying and interviewing community members and analyzing the stories and experiences they shared on social media.

Results (200 word limit)

A total of 4275 of the subreddit users mentioned SO, with 398 (9.31%) users communicating challenges with taking a second SO dose. The co-occurrence network revealed that second SO dose was co-mentioned with physical conditions (e.g., nausea, headache) and mental conditions (e.g., anxiety, depression). A group of 87 users from the private Facebook group was then surveyed (n = 85 patients, n = 2 caregivers). Missing the second dose was reported by 75% of patients (65% at least monthly). The most common reported impacts of missing doses were poor sleep quality, increased daytime sleepiness,

work/school absences, and brain fog affecting next-day functioning. Regarding whether they suffered injuries resulting from waking to take a second dose of SO therapy, 32% responded yes (one-third at least once a month). Delayed dosing (>4 hours after) was another issue reported by 59% (74% at least once a month). Impacts of this delayed dosing led to school/work tardiness and missed responsibilities. Patients reported adverse effects with SO therapy, including mental health issues (especially depression), racing heart, muscle spasms, acid reflux, bedwetting, and eating problems. Seventy-six percent of the respondents strongly agreed or agreed that a single bedtime dose of SO would be safer.

Conclusions (100 word limit)

There is converging evidence from both the social media and survey results that show that the need to take the second dose of SO is associated with various sleep-related issues and disruption for people with narcolepsy and their caregivers. Daily functioning, physical and mental health, injuries, and quality of life were affected. These impacts are present both for missed second doses and doses taken more than 4 hours after the first dose.

Support

Avadel Pharmaceuticals.

97

Sleep-promoting neurons of the dFB suppress aggression following sleep loss in Drosophila

Benjamin Mainwaring[1], Christine Dubowy[1], Jose Duhart[2], Kyunghee Koh[2], Matthew Kayser[1]

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Full Name and Credentials

Ben Mainwaring

Introduction (100 word limit)

Sleep deprivation impairs a wide range of essential processes such as cognition, alertness, and metabolism. In addition, insufficient sleep has been shown to influence emotional processing and aggression, though mechanisms linking sleep loss to changes in affective state are largely unknown. Recent work in Drosophilahas demonstrated that acute sleep deprivation transiently suppresses motivated behaviors such as courtship and aggression. Here, we provide evidence that an area of the brain previously implicated in sleep homeostasis, the dorsal fan-shaped body (dFB), regulates both sleep and aggression following sleep deprivation.

Methods (200 word limit)

Acute inhibition of 23e10-GAL4 neurons of the dFB, by blocking vesicle endocytosis using UAS-Shibire, has no effect on baseline daily sleep. In contrast, sleep rebound following sleep deprivation is attenuated with dFB inhibition, and the suppression of aggression following sleep loss is likewise relieved. We examined known aggression centers and found that following sleep deprivation, acute activation of P1 neurons using the heat sensitive cation channel Trpa1, rescues aggression to normal levels despite sleep loss. Next we confirmed functional connectivity between these two cell populations by stimulating the dFSB using the ATP-gated channel P2X2 and quantifying P1 activity using GCaMP. We observed a reduction in activity in P1 neurons upon dFB activation. These results suggest that the dFB mediates the suppression of aggression following sleep deprivation via action on P1 neurons. To probe for the signaling molecule that is released by the dFB to modulate downstream aggression loci, we conducted a genetic screen. We found that knocking down Allatostatin-C (AstC) in 23e10 cells relieves the suppression of aggression normally observed after sleep deprivation.

Results (200 word limit)

Combined Methods + Results above.

Conclusions (100 word limit)

Together, our findings further elucidate mechanisms governing the interaction between two phylogenetically conserved behaviors, sleep and aggression, and support a circuit-based model for how sleep loss can impact behavior.

Support

N/A

98

Perils of the nighttime: impact of behavioral timing and preference on mental and physical health in 74,000 community-dwelling adults

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Full Name and Credentials

Renske Lok, PhD

Introduction (100 word limit)

Humans often express a preference for going to sleep later ('owl') or earlier ('lark'). While humans may prefer a specific time at which to sleep, life may interfere with such plans, causing people to go to sleep later or earlier than they might otherwise prefer. Both morningness-eveningness and going to sleep later and earlier have been associated with susceptibility to a variety of mental and physical disorders. The goal of this study is to examine the impact of morningness-eveningness, actual sleep timing, and the alignment between the two on a variety of mental and physical health outcomes.

Methods (200 word limit)

We analyzed data from participants in the UK Biobank (n=73,888), a community-dwelling sample of adults. Preference for morningness or eveningness was determined by a single chronotype question and parsed into three categories: morning, intermediate, or evening preference. The actual timing of behavior was derived from non-parametric analysis of accelerometry data (start time of least activity, L5), which was also parsed in three categories (early, intermediate, and late). Health status was derived from the International Classification of Diseases-10 codes. X^2-tests and odds ratios (corrected for demographics including self-reported sleep duration) were used to determine statistical significance.

Results (200 word limit)

As compared to morning or intermediate, having either a preference for the evening or actually exhibiting late behavior increased the prevalence and likelihood of mental and physical health disorders, including Generalized Anxiety Disorder, depression, metabolic disorder, diabetes, obesity, hypertension, circulatory disorder, digestive disorder, respiratory disorder, and all-cause cancer. For morning-types, having an earlier behavioral timing was beneficial (aligned). For evening-types, however, alignment was detrimental as going to sleep later was associated with worse mental and physical health than going to sleep early (misaligned).

Conclusions (100 word limit)

Both mental and physical health outcomes are worse in individuals who rise later. To age healthily, individuals should time their rest earlier, despite their chronobiological preferences.

Support

N/A

99

Slumber neurons in *Drosophila* dissipate sleep drive via the memory gene *Radish*

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Full Name and Credentials

Clark Rosensweig, PhD

Introduction (100 word limit)

Sleep is thought to be regulated by both the circadian clock, which is well understood, and a poorly understood homeostatic process. Although several populations of sleep promoting neurons have been identified in *Drosophila*, there are few reports of neurons whose activity results in decreased sleep following activation (antirebound). Because there are many reports of neurons whose activity increases sleep drive (as evidenced by elevated sleep following activation), we hypothesized that there must be a set of neurons whose activity is sufficient to decrease sleep drive as evidenced by antirebound.

Methods (200 word limit)

500 GAL4 fly driver lines were used to express TrpA1, a temperature sensitive ion channel. Progeny expressing TrpA1 were exposed to a 12 hr temperature pulse during the daytime to exogenously activate various sets of neurons. To identify markers of a key sleep dissipating driver, Slumber-GAL4, this driver was used in downstream experiments to express GFP for fluorescence activated cell sorting and single cell RNA-sequencing experiments. Additionally, the driver was used to express RNAi for neurotransmitters/neuropeptides and synaptic regulators in small follow up screens to understand the mechanism underlying the dissipation of sleep drive observed here.

Results (200 word limit)

We identified a single line out of a screen of 500 drivers that displayed a reproducible antirebound phenotype. The line, which we have named Slumber-Gal4, expresses in roughly 3000 neurons, robustly promotes sleep behavior, and following activation reduces nighttime sleep by more than 200 minutes. Single cell RNA-sequencing of Slumber neurons reveals that the expression pattern is composed of 17 distinct cell types. Several clusters, including a mushroom body subset, are marked by expression of a previously reported sleep promoting neuropeptide, short neuropeptide F (sNPF). Knockdown of sNPF in Slumber neurons significantly decreases baseline sleep and the antirebound phenotype. We hypothesized that homeostatic potentiation of Slumber synapses might underlie the dissipation of sleep drive. To test this idea, we ran a small screen to knock down genes with a role in synaptic regulation or plasticity identified in our single cell dataset. Knockdown of *radish* in Slumber neurons, a predicted Rap-

like GTPase activating protein with a major role in anesthesia-resistant memory, significantly decreased the antirebound phenotype. We also find that activation of Slumber neurons after training enhances long term memory further supporting a role in sleep-dependent plasticity.

Conclusions (100 word limit)

Here we identify a novel driver marking a set of sleep promoting neurons with a robust antirebound phenotype. Knockdown of sNPF and *radish* both block the antirebound phenotype suggesting that the homeostatic process is modulated by both specific signaling modalities and small GTPase signaling cascades.

Support

F32 NS110183 (CR), R01NS106955 (RA)

100

An exploratory study of Sleep Quality and Quantity in Children with SYNGAP1-ID

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Full Name and Credentials

Constance Smith-Hicks, MD PhD

Introduction (100 word limit)

Sleep disturbances are commonly reported in neurodevelopmental disorders such as autism and epilepsy and are reported in 62% of children with SYNGAP1-Related Intellectual Disability (SYNGAP1-ID). SYNGAP1-ID is a rare neurodevelopmental disorder characterized by intellectual disability, epilepsy, sensory processing challenges, autism, and behavioral challenges. Although Children's Sleep Habits Questionnaire (CSHQ) scores are elevated in children with SYNGAP1-ID relative to typical developed peers, factors that predict sleep disturbance are not well understood. The goals of this study are to evaluate sleep quality and quantity in this population of individuals with epilepsy, intellectual disability, and autism and identify predictors of sleep problems.

Methods (200 word limit)

CSHQ and Social Responsiveness Scale-2 (SRS2) questionnaires were completed by the parents of 21 individuals with SYNGAP1-ID, and continuous 14-day accelerometry /actigraphy data was collected for 6 of these individuals. Non-parametric analysis of psychometric scales, and analysis of psychometric scales with actigraphy data were performed. We also compared sleep parameters; latency, sleep efficiency, time in bed (TIB), total sleep time (TST) and wake after sleep onset (WASO) in children with SYNGAP1 to actigraphy data from a community control sample of children.

Results (200 word limit)

CSHQ total sleep disturbance scores in children with SYNGAP1 and ASD were not different from children with SYNGAP1 without ASD ($p = 0.$). Multiple linear regression analysis indicates that sleep anxiety and parasomnias are strong predictors of bedtime resistance [$F(5,15) = 9.88$, $p = 0.0002$, $R2 = .77$]. The sedentary to active transition probability and the number of minutes spent active during the 12-18h epoch was strongly correlated with total sleep disturbance [$F(1,4) = 23.45$, $p = 0.008$, $R2 = .85$] while mean duration of the active bout during the 18-24h epoch predicts sleep disturbance [$F(1,4) = 11.24$, $p = 0.029$, $R2 = .74$].

Conclusions (100 word limit)

Children's Sleep Habit Questionnaire may be a reliable measure of sleep difficulties in children with SYNGAP1-ID, and sleep disturbance in this population is independent of the presence of an autism

spectrum diagnosis. Sleep anxiety and parasomnias are significant contributors to sleep disturbances in children with SYNGAP1-ID. Medications that affect sleep are commonly administered in this population of children with SYNGAP1 and may explain the lower sleep latency and WASO scores in this cohort.

Support

n/a

102

Influence of APP expression on cognitive behaviors in Alzheimer's Disease mouse models subjected to sleep fragmentation

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University of South Florida, Tampa, USA

Full Name and Credentials

Anjana Sadanand

Introduction (100 word limit)

Alzheimer's disease (AD) is associated with progressive memory loss and Aβ accumulation, and AD is also accompanied by sleep disturbance that impacts Aβ clearance and cognitive function. While the cerebral Aβ burden and sleep fragmentation are critical issues in AD pathogenesis, the underlying mechanisms are poorly understood. We explored if sleep disruptions and the accumulation of intracellular fragments of the amyloid precursor protein (APP) influence cognitive behavior using knock-in mice expressing the endogenous level of APP and the familial AD-linked presenilin 1 M146V variant (PS1KI), an AD mouse model that does not exhibit amyloidogenic pathology.

Methods (200 word limit)

We employed sleep cages that contain a bottom sweeping rod device to induce sleep fragmentation in mice for six hours every day for 4-6 weeks. We assessed the anxiolytic behavior using the zero maze, open field, and light/dark transition chamber. Spatial memory was evaluated using the spontaneous alternation in Y-maze, Morris water radial tread maze, and contextual fear conditioning. We examined cognitive behavior in PS1KI and compared these mice to their wild-type littermates (Wt) and cohorts lacking the expression of APP (APPKO) and in PS1KI background (APPKO-PS1KI).

Results (200 word limit)

We observed that the spontaneous alternation in Y-maze was not significantly changed between cohorts before and after sleep fragmentation. On the other hand, we identified that the PS1KI mice performed poorly in the contextual fear conditioning test, as seen by a profound decrease in freezing time after exposure to the shock. This effect was not observed in mice lacking APP expression (APPKO-PS1KI). Sleep-perturbed mice did not affect the freezing patterns. Spatial memory was also examined using the radial tread maze. We observed that all cohorts exhibited better recognition of the targeted arena five days after the beginning of the training if subjected to sleep fragmentation. Interestingly, 12 days after training, the PS1KI performed better, which is consistent with superior memory retention, whereas the sleep fragmented PS1KI did not recognize as well the targeted zone. This effect was not seen in APPKO-PS1KI mice, suggesting that APP expression contributes to this divergence. All cohorts did not show any overall differences in anxiolytic behaviors.

Conclusions (100 word limit)

Thereby, our results show distinct consequences on cognitive behavior and associated memory depending on the changes in the sleep cycle in an AD mouse model before amyloid pathogenesis takes place. We demonstrated the implication of APP expression as a critical contributor to cognitive behavior in sleep-perturbed conditions.

Support

NIH

103

Effect of sensor resolution on estimation of artificial light at night and sleep outcomes in Los Angeles County

Charlie Zhong[1,2], Jennifer Benbow[3], Sophia S Wang[3], James V Lacey Jr[3], Meredith Franklin[1,4], Travis Longcore[5]

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Full Name and Credentials

Charlie Zhong, PhD MPH

Introduction (100 word limit)

Several studies have highlighted associations between artificial light at night (ALAN) and greenspace on sleep. These studies rely on satellite-based estimates, which enable study of large geographic areas, but specificity of exposure assessment is limited by sensor resolution. A concern is that correlation between outcomes and environmental exposures at coarse resolutions cause bias. We evaluated effects on sleep utilizing ALAN data at various resolutions including new high-resolution imagery from the International Space Station (ISS).

Methods (200 word limit)

The California Teachers Study (CTS) is a prospective cohort of 133,477 current and former female public-school professionals recruited in California and given a baseline questionnaire in 1995-1996. A follow-up questionnaire in 2012-2015 assessed self-reported measures of sleep. We previously assigned satellite derived environmental exposures of ALAN (750-meter New World Atlas and VIIRS), greenspace (250-meter enhanced vegetation index, EVI), noise (270-meter US Parks noise map), and air pollution (1-kilometer Harvard ensemble-based particulate matter <2.5 microns, PM2.5) to participants and evaluated associations with sleep duration (<7 hours vs 7+ hours) and latency (<15 minutes vs 15+ minutes). A high-resolution color photo of Los Angeles (LA) County taken onboard the ISS closest to the follow-up questionnaire (August 2016) was processed to estimate ALAN at 80-meter resolution. Color photos also allowed for calculation of the melatonin suppression index (MSI), to estimate exposure to wavelengths of light more associated with sleep disruption. We then evaluated sleep outcomes of 14,738 CTS participants residing in LA County at the time and compared them to previously assigned 750-meter ALAN estimates. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated.

Results (200 word limit)

More participants were identified as residing in locations of greater levels of ALAN through ISS estimates compared to the New World Atlas. Compared to the New World Atlas (OR per 5 mcd/m2 1.15, 95% CI 1.04-1.27), a log increase in ALAN estimated from ISS imagery (nW/cm2/sr/Å) was associated with 1.06

greater odds (95% CI 1.01-1.11) of sleeping less than 7 hours a night. Correlations between 80-meter ISS ALAN and 270-meter noise were similar, but the correlation with 250-meter greenspace and ISS imagery was lower (Spearman correlation (ρ) = -0.29) compared to the New World Atlas (ρ = -0.41). Correlation with PM2.5 was lower as well (ρ 0.18 vs 0.28). Greenspace appeared to be more protective in the ISS model (OR 0.75, 95% CI 0.33-1.70) than the New World Atlas model (OR 0.95, 95% CI 0.40-2.23). Compared to the New World Atlas (OR 1.01, 95% CI 0.92-1.11), MSI produced a more precise estimate with sleep latency (OR 1.02, 95% CI 0.98, 1.06).

Conclusions (100 word limit)

High-resolution, photo-based ALAN imagery yielded similar results to coarser satellite-based data when evaluating ALAN and sleep duration. Use of higher resolution ALAN imagery did appear to reduce bias in greenspace associations. When estimating outdoor environmental light exposures and associations with sleep, estimated skyglow brightness from the World Atlas may have advantages over spectrum specific light radiance that can be obtained from ISS photos. Further work is necessary to better understand how different estimates of ALAN affect associations between the environment and sleep.

Support

Support for this research was provided by a grant from the National Institute of Environmental Health Sciences, National Institutes of Health under award number T32-ES013678 and the Sleep Research Society Small Research Grant 07-SRG-21. The California Teachers Study is supported by the National Cancer Institute of the National Institutes of Health under award numbers U01-CA199277, P30-CA033572, P30-CA023100, UM1-CA164917, and R01-CA077398.

104

Daytime and nighttime profiles of ghrelin and growth hormone concentrations differ between individuals with and without obesity

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Full Name and Credentials

Erin C. Hanlon, PhD

Introduction (100 word limit)

Ghrelin, an orexigenic hormone, plays a key role in modulating food intake and energy balance. Plasma ghrelin levels rise preceding a meal and fall shortly after consumption. Moreover, ghrelin is known to stimulate pituitary release of growth hormone (GH), a hormone stimulated during sleep and known to promote lipolysis. Potential differences in concomitant ghrelin and GH secretion across the entire 24-h period in individuals with and without obesity have not previously been reported, despite epidemiologic evidence indicating that adults with obesity are more likely to shift majority of caloric intake to later in day and suffer from poor sleep quality.

Methods (200 word limit)

We examined plasma ghrelin and growth hormone in group matched healthy individuals with and without obesity, in a laboratory setting with controlled sleep, energy expenditure, and caloric intake. A total of 27 individuals participated; 14 with normal weight (3 women) and 11 with obesity (7 women). Only individuals without obstructive sleep apnea were included (AHI < 5 events/h). Mean age between the groups was similar (23.4 ± 0.7yrs vs 26.6 ± 1.9yrs). Body mass index (BMI) was 39.0 ± 2.3 kg/m2 versus 23.6 ± 0.7 kg/m2 in the participants with versus without obesity ($p < 0.0001$). Following two experimental nights of normal sleep (23h00-07h00/07h30) in the laboratory, blood sampling was performed continuously at 15- to 30-min intervals. Total ghrelin was measured by radioimmunoassay (Linco Research, St. Charles, MO) and GH was measured by a chemiluminescence assay (Immulite, Siemens Healthcare Diagnostics Inc., Erlangen, Germany). Participants ingested isocaloric identical high carbohydrate meals at 0900, 1400, and 1900 and each meal was 33% of total caloric intake.

Results (200 word limit)

Consistent with previous reports, ghrelin and GH levels were decreased across the entire 24-h period in those with obesity (ghrelin, lean 731.7 ± 76.1 pG/mL vs obese 614.2 ± 85.9 pG/mL, $p = 0.32$; growth hormone lean 2.2 ng/mL ± 0.3 vs obese 1.1 ng/mL ± 0.3, $p = 0.02$). Each individual profile was expressed as a percentage of the individual 24-h mean concentration to examine the waveshape of the profile independently of individual differences in mean ghrelin and GH levels. Relative levels of ghrelin were significantly higher in the obese during waking (and eating) than in the lean (lean 91.1 ± 1.0 vs obese 100.1 ± 1.1, $p < 0.0001$) and lower during the sleep period (lean 117.8 ± 2.1 vs obese 99.8 ± 2.3, $p <$

0.0001). Similarly, relative GH levels were significantly higher in obese during waking (lean 37.0.1 ± 8.5 vs obese 71.2 ± 9.6, $p = 0.014$) and lower during sleep (lean 234.2 ± 14.9 vs obese 166.5 ± 16.9, $p < 0.006$).

Conclusions (100 word limit)

Interestingly, while overnight secretion of ghrelin and GH was markedly diminished in participants with versus without obesity, levels of ghrelin and GH were actually higher throughout waking, when activity and food intake occur. The results suggest that the presence of obesity is associated with a major shift of ghrelin and GH secretion from nighttime to daytime.

Support

This study was supported by Contract W81XWH-07-2-0071 from the Department of Defense Peer Reviewed Medical Research Program and Grant P50 HD057796 from the National Institutes of Health

105

Phenotypic differences in sleep and circadian rhythms in delayed sleep wake-phase disorder patients with and without comorbid depression

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Full Name and Credentials

Cátia Reis

Introduction (100 word limit)

Delayed Sleep Wake-Phase Disorder (DSWPD) is one of the most common circadian rhythm sleep-wake disorders. Is characterized by chronic (i.e., ≥ 3 months) inability to fall asleep and wake up at conventional or socially acceptable times (e.g., to meet work or school obligations). As a result, individuals with DSWPD often report sleep loss, disturbed sleep, excessive daytime sleepiness, impaired waking function, and in some cases develop depression. The aim of this study was to compare circadian and sleep behavioral variables in DSWPD patients with and without a diagnosis of depression.

Methods (200 word limit)

76 DSWPD patients from a Portuguese sleep medicine center (CENC) (2012-2017). Patients performed salivary dim light melatonin onset (DLMO) measurement for internal time phase assessment and had a diagnosis of depression performed by a physician. Sleep-wake behavior (sleep onset - SO; sleep end; sleep duration - SD) for work (w) and work-free days (f) and sociodemographic data were present in the clinical records, allowing us to calculate the mid-points of sleep (MSW and MSF), social jetlag (SJL), chronotype derived by the mid-point of sleep on free-days sleep corrected (MSFsc) and the average weekly sleep duration (SDweek). The phase relationship between DLMO and the sleep-wake behavior variables was also calculated.

For comparisons of patients with and without depression, we used the t-student, Mann-Whitney, or Qui-square tests according to the type of data and distribution. Statistical analysis was performed with SPSSv28, and test significance was set for $p < 0.05$.

Results (200 word limit)

The sample was composed of 50 (65.8%) DSWPD patients without depression (DSWPD-ND) and 26 (34.2%) with depression (DSWPD-D). Of the DSWPD-ND patients, 31(62%) were male; with a median age of 35 [24-47], and 42(84%) were employed. Of the DSWPD-D patients, 11 (42.3%) were male with a median age of 35 [27-41], and 18 (69.2%) were employed.

From all group comparisons, differences were found for Sleep endw (DSWPD-ND=8.75 [8-11]; DSWPD-D=11[9.5-12]; p=0.003), MSW (DSWPD-ND=5.63[5-7.13]; DSWPD-D=7.04[6.17-8.50]; p=0.010), SDw(DSWPD-ND=6.33(1.75); DSWPD-D=7.50(1.12); p=0.002), SDweek(DSWPD=6.74 (1.41); DSWPD-D=7.80(0.99); p=0.001, SJL (DSWPD-D=1.77 [0.5-2.84]; DSWPD-D=0.49 [0.0-1.17]; p=0.006), and Sleep endw-DLMO phase relationship (DSWPD-ND=8.16(2.21); DSWPD-D=9.16(1.55); p=0.044).

Conclusions (100 word limit)

DSWPD patients with and without comorbid depression differ in some sleep and circadian-relevant variables (i.e., sleep timing during work days, sleep duration, and social jetlag) but not others (i.e., chronotype, DLMO, and sleep timing during work-free-days). Further research is needed to determine the stability of these phenotypic differences and how they may contribute to the etiology and effective treatment of DWSPD.

Support

The presenting author received a travel sponsorship for the conference attendance from Gasoxmed.

107

Circadian Contributions to Blood Pressure Dipping in African American Adults

S. Justin Thomas, Gabrielle Gloston, Rebecca Williams, Mackenzie Hogue, Kristen Hays, Karen Gamble, Courtney Peterson

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Full Name and Credentials

S. Justin Thomas, PhD, FSBSM

Introduction (100 word limit)

Non-dipping blood pressure (BP), defined as systolic BP that decreases ≤10% during sleep, is more prevalent among African American than white adults and is associated with increased risk for cardiovascular morbidity and mortality. While studies have demonstrated a circadian rhythm in BP, the contribution of circadian versus behavioral factors to BP dipping is unknown, particularly in African American adults.

Methods (200 word limit)

To examine circadian and potential sleep contributions to BP dipping, we enrolled 29 African American adults (17 women, 12 men) to complete self-report questionnaires, actigraphy, and 24-hour ambulatory BP measurements (ABPM) at screening followed by a 30-hour constant routine (CR) protocol. We used ABPM under ambulatory conditions to determine BP dipping status. During the CR protocol, participants remained awake in dim light (<10 lux) in a semi-recumbent posture and were provided with iso-caloric snacks over a 30-hour period. Saliva samples for melatonin and BP measurements were obtained every 60 and 30 minutes, respectively, while core body temperature was recorded continuously. Cosinor analyses were performed to assess circadian rhythmicity of melatonin, core body temperature, and BP. ANOVA was used to determine whether these rhythms were statistically significant.

Results (200 word limit)

At screening, 10 participants (4 women and 6 men) were dippers, and 19 participants (13 women and 6 men) were non-dippers. Under constant conditions, 6 dippers (60.0%) and 16 non-dippers (84.2%) did not have a significant rhythm in BP (individual BP rhythm profiles will be presented). Nine dippers (90.0%) and 17 non-dippers (89.5%) had a significant rhythm in core body temperature ($p<.001$). There were no significant differences in mesor, amplitude, or phase in melatonin or core body temperature in dippers compared with non-dippers (all p=ns).

Conclusions (100 word limit)

These preliminary results need to be verified but suggest that the circadian system may not play a major role in BP dipping in African American adults. Rather, behavioral factors (e.g., sleep, eating behaviors,

and activity levels at night) may play an important role in BP dipping and may serve as intervention targets for lowering BP and restoring BP dipping during sleep. Future research will include white and Latine/LatinX adults to examine racial/ethnic differences in sleep and circadian contributions to BP dipping, as well as specifically examine the contributions of eating and sleep behaviors to BP dipping.

Support

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109

The Interrelationships Between Insomnia, Sleep Apnea and Nightmares in Veterans with Psychological Trauma

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Full Name and Credentials

Anne Richards

Introduction (100 word limit)

Observational research demonstrates a strong association between nightmares, insomnia, and sleep apnea in veterans with posttraumatic stress disorder (PTSD), supportive of the notion of a complex sleep disturbance in this population. For example, sleep apnea has been shown to be highly prevalent in veterans with PTSD, insomnia has been shown to reduce adherence to CPAP in veterans with PTSD, and treatment of sleep apnea has been shown to improve PTSD and reduce nightmares. We aimed to examine relationships between nightmares, respiratory events, adherence to CPAP use, and insomnia in trauma-exposed veterans with frequent nightmares using self-report and at-home physiological measurements.

Methods (200 word limit)

Forty-one (41) male and female veterans with a history of psychological trauma and frequent nightmares were enrolled as part of an ongoing DOD-funded study of trauma nightmares and sleep disturbance. Participants completed three weeks of daily sleep diaries on a mobile application designed for the study, including standard sleep-related behaviors as well as number of prior-night nightmares, and prior-night use of their CPAP device and number of hours used. They also completed the insomnia severity index (ISI) and PTSD symptom reports (PTSD-8) at baseline and at the end of each week (4 timepoints total). Of the 41 participants, a subset of 15 participants completed 6-9 nights of at-home objective sleep measurements using a single-channel frontopolar EEG device (Sleep Profiler) integrated with measurement of airflow, respiratory effort and oxygen saturation. Concurrently, participants wore a wristband actigraph with event marker to report awakenings from distressing dreams in real time. Using sleep diary data, the relationships between weekly nightmares (total number), CPAP use, sleep efficiency, and ISI and PTSD-8 scores were analyzed using linear regression with standard errors adjusted for clustering of weeks within participants. Sleep physiology data were examined to identify the proportion of nightmare awakenings preceded by apnea or hypopnea events.

Results (200 word limit)

41 veterans (mean age 48 (SD=16), 30% female) participated. Higher prior week ISI score predicted reduced CPAP adherence (β=-.53, p=.005) in the subsequent week, although PTSD symptom score did not. In turn, increased frequency and average nightly duration of CPAP use was associated with a significant increase in average sleep efficiency the same week (β=.53, p=.018 and β=.56, p=.026, respectively). Analysis of respiratory events indicates that 20 of the 64 nightmare awakenings (31%) were preceded (i.e., within seconds of awakening) by apnea or hypopnea events. Of these 20, 11 events were reported by participants with a diagnosis of OSA and a CPAP device. Amongst these 11 events, 7 (64%) occurred on nights during which the participant reported use of their CPAP device.

Conclusions (100 word limit)

Analyses demonstrated statistically significant and clinically meaningful relationships among insomnia, nightmares, respiratory events and CPAP adherence in our sample, highlighting their interrelationships in trauma subjects. These data support the hypothesis that OSA contributes to nightmare experiences by demonstrating an immediate temporal relationship between apnea/hypopnea events and nightmare awakenings, and they underscore the importance of detecting and adequately treating sleep apnea for the treatment of nightmares in veterans.

Support

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110

Lack of association between excessive daytime sleepiness, depressive symptoms, and social isolation in older adults living in long-term care.

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Full Name and Credentials

Suzanne Hood, PhD

Introduction (100 word limit)

Excessive daytime sleepiness (EDS) is linked with poorer outcomes for both physical and mental health. In community-dwelling older adults, EDS has been associated with greater depressive symptoms (Spira et al., 2012; Vashun et al., 2015; Lai et al., 2020). Multiple factors may underlie this association; however, recent evidence that EDS leads to withdrawal from social contact in community-dwelling adults suggests that social isolation and loneliness could contribute (Holding et al., 2020). Here, we examined whether similar associations between EDS, depressive symptoms, and social isolation were present in a sample of older adults living in communal residential care.

Methods (200 word limit)

A cross-sectional sample of older adults (N=53, age range 69–102 years old, 81% female) living in long-term residential care completed self-report questionnaires including the Epworth Sleepiness Scale (ESS), the Center for Epidemiologic Studies – Depression Scale (CESD), the UCLA Loneliness Scale, and the Satisfaction With Life scale (SWLS).

Results (200 word limit)

Approximately 20% of participants met ESS scoring criteria for EDS (score > 10). Measures of depressed mood (CESD), social isolation (UCLA Loneliness scale), and satisfaction with life correlated significantly with each other but did not predict ESS score.

Conclusions (100 word limit)

Contrary to findings reported in community-dwelling older adults, we did not find any associations between EDS, depressive symptoms, and social isolation in our sample of adults living in long-term residential care. These results could indicate that contextual factors unique to long-term residential care settings influence how EDS may interplay with mood states and social engagement.

Support

N/A

111

Drosophila photoreceptors converge in circadian/arousal neurons as a possible coincidence detector system

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David Au

Introduction (100 word limit)

Cryptochrome (CRY) is classically associated with its role in regulating the circadian molecular clock via light-induced degradation of the Timeless (TIM) clock protein in flies. Recent discoveries in our lab have identified additional processes occurring as a result of CRY phototransduction, such as light-evoked excitation of the ventral lateral subset in the circadian/arousal neural circuit. CRY is primarily a blue (450 nm) and UV (365 nm) photoreceptor, but light-evoked excitation using red light (635 nm) also elicits an acute response in the ventral lateral neurons. We explored other opsin-based photoreceptors as potential red inputs to this circuit.

Methods (200 word limit)

Previously established protocols from (Baik et al., 2019) were modified to run our light-evoked potential electrophysiology experiments. Adult male fly brains were dissected in external recording solution and patched with internal recording solution. Custom multichannel LED source fitted to the Olympus BX51 WI microscope was used as the primary light source for our electrophysiology experiments. LED peak wavelengths are as follows: UV (365 nm), violet (405 nm), blue (450 nm), and red (635 nm), and all exposures were set to an intensity of 200 $\mu W/cm^2$ by use of a Newport 842-PE Power/Energy meter. Each LED was triggered on and off for each sweep with TTL pulses programmed by pClamp (Molecular Dynamics) data acquisition software. The light-evoked potential protocol is as follows: 50 seconds of dark for baseline recording, 5 seconds of colored-light stimulation, then 95 seconds of inter-pulse darkness for recovery back to baseline. The protocol repeats five times per recording. For analysis, sweeps are averaged, and baseline adjusted to pre-pulse signal, then low-pass noise filtered using Gaussian and Butterworth filters in the ClampFit 10 software (Molecular Dynamics). Our light-evoked potential protocol captures averaged light-evoked changes in membrane potential, thus providing a kinetically robust light-evoked potential.

Results (200 word limit)

Fly groups that were tested consisted of externally blind gl60j, cry-null, rh7-null, and double mutant gl60j-cry-null. Our electrophysiology experiments show CRY and Rh7 contribute the greatest light-excitatory effect with blue and UV light. Rh7 is the predominant sensor for violet light-induced electrical excitability. Gl60j flies show the greatest attenuation of red light-induced electrical excitability in l-LNvs.

Further, these neurons are critical for light-arousal and phototaxis responses. We employed a light-pulse arousal assay to measure the awakening arousal response of flies during subjective nighttime. Our results show that at lower intensities of blue light (10 $\mu W/cm^2$) gl60j and rh7-null mutant flies have the greatest loss of light-pulse arousal, whereas at high intensities (400 $\mu W/cm^2$) cry-null and rh7-null mutant flies exhibit the greatest phenotype. These results extend to violet light as well, with gl60j mutant flies exhibiting the greatest loss of light arousal for lower intensities, and rh7-null having the greatest loss at higher intensities. Interestingly, gl60j mutant flies exhibit the greatest loss of arousal for both higher and lower intensities for red light pulses.

Conclusions (100 word limit)

These results suggest different photoreceptor systems converge input to the ventral lateral neurons and provide UV to red sensation in flies for non-image forming processes. Each photoreceptor system provides photoelectrical input to the circadian/arousal circuit in different manners depending on intensity and wavelength. Removal of any one photoreceptor system will typically lead to an overall attenuation of photoexcitability, depending on the color stimuli. Altogether, external opsin-based photoreceptors and internal CRY and Rh7 seem to function as a coincidence detector within the fly circadian/arousal circuit.

Support

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112

Sleep Disruption Alters Cellular Immune Response to Endotoxin-Mediated Sepsis

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Full Name and Credentials

Michael T Lam, M.D., Ph.D.

Introduction (100 word limit)

The mechanistic role of sleep in immune homeostasis has yet to be fully understood. Animals subjected to acute sleep disruption exhibit higher mortality in pathogen-based sepsis models; however, it is unknown whether the mortality is due to the initial exuberant inflammatory response (i.e., cytokine storm) or a suppressed immune response leading to inadequate pathogen clearance. This study dissects this question by applying a pathogen-free, endotoxin model focusing on the impact of sleep on the early inflammatory response of septic shock.

Methods (200 word limit)

C57BL/6 mice were subjected to seven days of sleep disruption by tactile stimulations every two minutes. We tested whether sleep disruption alters the survival of endotoxin-mediated sepsis by intraperitoneal injection of lipopolysaccharide (LPS, 5mg/kg) at zeitgeber time 0-1 hour (Control = 32, Disrupted Sleep = 27). After LPS injection, mice were given uninterrupted sleep opportunities and monitored for survival. For mechanistic insights, we assessed serum cytokines measurement, flow cytometry immune subtype profiling, and single-cell immune cell transcriptomic analysis 24 hours after LPS administration comparing sleep-disrupted and normal sleep controls.

Results (200 word limit)

Sleep-disrupted mice have higher sepsis survival than normal sleep control after LPS injection (85% vs. 53%, Log Rank p-value = 0.007). LPS-induced serum cytokine is globally decreased in the sleep-disrupted group, with significantly lower levels of IFN-g, MIP-1a, and MCP-1 24 hours after LPS injection. Interestingly, we observed increased recruitment of Ly6C+Cxcr4+ monocyte into the peritoneal cavity in the sleep-disrupted group after sepsis onset. Among the diverse peritoneal myeloid population, the Ly6C+ Cxcr4+ monocytic subgroup specifically enriches in gene functional terms in phagocytosis, antigen presentation, and degranulation. Sleep disruption led to decrease gene expression in these inflammatory pathways.

Conclusions (100 word limit)

We demonstrated that sleep-disrupted mice have higher survival and reduced global inflammatory cytokine production in endotoxin-mediated sepsis. Integrating this data in the sleep-immune literature, sleep disruption suppresses early immune responses in sepsis. Our experiment suggests that the Ly6C+/Cxcr4+ monocytes are preferentially susceptible to sleep disturbance, with increased cell recruitment in the primary inflammatory sites and decreased gene programs essential for acute infection and inflammatory responses. This work is implicated in the higher prevalence of infection in poor sleepers. It may also apply to hospitalized patients, where poor sleep potentially increases the risk of nosocomial infection.

Support

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113

The glial-enriched *Fabp7* gene regulates circadian electroshock seizure threshold and activity-dependent mRNA expression in mouse brain

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Full Name and Credentials

Jason R Gerstner

Introduction (100 word limit)

Epilepsy patients often experience seizures that follow time-of-day and/or sleep/wake rhythms. Brain-type fatty acid binding protein gene (Fabp7) mRNA expression cycles in a circadian rhythm, is driven by core-clock components, and is necessary for sleep regulation. Fabp7 is expressed in glial cells, regulates fatty acid signal transduction and mediates gene transcription. Glial cells modulate sleep, circadian rhythms, and neuronal excitability, so here we hypothesized that circadian changes in seizure threshold are influenced by Fabp7. In the current study we examined if Fabp7 influences seizure threshold upon electrical stimulation, and whether there are associated differences in gene expression.

Methods (200 word limit)

Male C57/BL6N wild type (WT) and Fabp7 knockout (KO) mice were maintained on a 12:12 hour light/dark cycle. Seizure thresholds were measured by administering a once daily, regularly increasing electroshock stimulus stepwise paradigm during the light-phase, zeitgeber time (ZT)4-ZT8, or dark-phase ZT16-ZT20. Generalized and Maximal electroshock seizure threshold (GEST and MEST, respectively) were documented. SHAM control mice following the MEST condition from each genotype were handled identically without electroshocks. Brains were dissected and harvested for hemisections of frontal-cortex and hippocampus (Bregma 0 to -4 and 2mm deep from dorsal cerebral cortex). RNA was isolated and libraries were prepared for RNA-sequencing on an Illumina HISEQ 2500 for paired-end reads of 100bp per sample. Differential gene expression analysis was done using DE-Seq2 and BioJupies-Jupyter platforms.

Results (200 word limit)

Fabp7 KO mice required a higher current to elicit both GEST and MEST compared to WT mice during the dark phase but not during the light phase (Kaplan-Meier log rank test). Differential gene expression of immediate early genes (IEGs) was observed between the WT MEST and WT SHAM condition, and included Fos, Junb, Npas4, Egr1, Egr2, Nr4a1, and Arc. None of these IEGs were found to be altered between in the Fabp7 KO MEST and Fabp7 KO SHAM condition. A comparison between WT SHAM and Fabp7 KO SHAM showed significant differential expression of many genes, including Npas4 and Arc mRNA, suggesting baseline differences in IEGs exist in the absence of Fabp7.

Conclusions (100 word limit)

The glial expressed Fabp7 plays an integral role in modulating neuronal excitability and activity-dependent gene expression. Fabp7 KO mice have an increased resistance to normal levels of electrical current that elicit MEST compared to WT mice during the dark phase but not during the light phase. This effect is accompanied by a lack of differential IEG expression associated with seizures. Together, these results suggest that the glial Fabp7 gene is required for normal neuronal function and differences in activity-dependent gene expression. Future studies will be needed to determine the neural-glial pathways by which Fabp7 alters seizure threshold.

Support

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