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Sleep & Circadian SCIENCE

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Distressing dreams, cognitive decline, and risk of dementia: A prospective study of three population-based cohorts

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Full Name and Credentials

Abidemi Otaiku, MD

Introduction (100 word limit)

Distressing dreams are associated with faster cognitive decline and increased dementia risk in people with Parkinson's disease (PD). Whether distressing dreams are associated with cognitive decline and dementia in people without PD is unknown. This study investigated the association between self- reported distressing dream frequency and the risk of cognitive decline and incident dementia in community-dwelling men and women in the general population.

Methods (200 word limit)

Risk of cognitive decline was evaluated in 605 middle-aged adults from the Midlife in the United States (MIDUS) study who were followed-up over 13 years. Cognitive decline was defined as having an annual rate of decline in global cognitive function ≥1 standard deviation faster than the mean decline rate. Risk of incident all-cause dementia was evaluated in 2600 older adults from the Osteoporotic Fractures in Men Study (MrOS) and the Study of Osteoporotic Fractures (SOF), who were followed-up over 7 years. Distressing dream frequency was assessed in all cohorts at baseline using item 5h of the Pittsburgh Sleep Quality Index. The association between self-reported distressing dream frequency and later cognitive outcomes was evaluated using multivariable logistic regression.

Results (200 word limit)

Compared with middle-aged adults who reported having no distressing dreams at baseline, those who reported having weekly distressing dreams had a 4-fold risk of experiencing cognitive decline (adjusted odds ratio [aOR] = 3.99; 95% CI: 1.07, 14.85). Amongst older adults, the difference in dementia risk was 2.2-fold (aOR = 2.21; 95% CI: 1.35, 3.62).

Conclusions (100 word limit)

Distressing dreams predict cognitive decline and all-cause dementia in middle-aged and older adults in the general population.

Support

N/A

10

A genome-wide analysis of pleiotropy between morning circadian preference and BMI reveals the tissue-specific rhythmicity of ADCY3 in adipose tissue.

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Full Name and Credentials

Cynthia Tchio, PhD

Introduction (100 word limit)

Chronotype is the behavioral manifestation of our internal circadian clock, influencing whether individuals are early birds (morning people) or night owls (evening people). Chronotype significantly impacts complex diseases such as obesity. This study dissects the relationship between circadian preference and body mass index (BMI) using a genome-wide pleiotropy approach.

Methods (200 word limit)

We conducted a genome-wide pleiotropy analysis to explore the associations between morning circadian preference and BMI, focusing on the tissue-specific expression and rhythmicity of key genes.

Results (200 word limit)

We identified a lead locus, rs11676272 in *ADCY3*, associated with increased morning preference and decreased BMI. The rs11676272 (S107P) is a missense variant that destabilizes the ADCY3 protein structure, highlighting the gene's role in both circadian behavior and metabolic processes. In mouse models, *Adcy3* expression was rhythmic in white and brown adipose tissues during constant darkness, and it oscillated anti-phase to the canonical circadian genes Clock and Bmal1. The presence of the Bmal1-specific E-box motif CACGTG on the Adcy3 gene suggests Bmal1 regulation of this rhythmicity, indicating a novel mechanism of circadian control within these tissues.

Conclusions (100 word limit)

Findings from our genome-wide pleiotropy approach underscore a novel role of ADCY3 at the intersection of circadian regulation and metabolic health. This study offers new insights into the genetic architecture of these complex traits, highlighting the significance of tissue-specific rhythmicity in adipose tissue.

Support

N/A

12

Inhibition of Acetyl-CoA synthesis alters stress resilience and sleep homeostasis

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Full Name and Credentials

Ashton Arocho

Introduction (100 word limit)

In mammals, sleep is required for the brain to properly function. Sleep loss impairs the ability to overcome stressful conditions known as resilience. Non-rapid eye movement (NREM) sleep is necessary for resilience to occur, yet mechanisms underlying sleep's ability to alter resilience remain unclear. One potential mechanism is histone acetylation, specifically through the action of Acetyl-CoA synthetase 2(ACSS2). ACSS2 provides the substrate utilized for histone acetylation and is highly expressed in neurons. We hypothesize that ACSS2 inhibition both alters sleep homeostasis and reduces negative behavioral responses to social stress.

Methods (200 word limit)

Social defeat stress: A resident-intruder paradigm (social defeat stress) was used to socially stress groups of mice (n=10/group) for 10 consecutive days. Mice received either vehicle control or pharmacological inhibitor of ACSS2(ACSS2i) for the last 5 days of social defeat. We also were interested in how ACSS2i influenced behavior in a non-social defeat stress condition, so groups of mice (n=8/group) were exposed to novel cage conditions for 10 consecutive days, receiving either ACSS2i or vehicle control the last 5 days of novel cage exposure. Social interaction with a conspecific was then measured 1 day and 7 days after the last session of social defeat or novel cage exposure. We also analyzed another metric of stress resilience in the form of distance traveled. In a separate experiment, epidural electroencephalography (EEG) and electromyography (EMG) electrodes were implanted in mice. After recovery from surgical procedures, mice were kept awake for the first 6 hs of the light phase (ZT 0–6) once and allowed an 18 hr recovery opportunity. Mice were assigned to two cohorts (n=6/group) and received either vehicle control or pharmacological inhibitor of ACSS2 (ACSS2i) at ZT 5. EEG/EMG recordings were hand scored by a trained observer.

Results (200 word limit)

We found that inhibiting ACSS2 significantly promoted resiliency and anti-anxiety like effects to social defeat, as measured by social interaction time and distance traveled, up to 7 days following social defeat stress; compared to vehicle-treated controls. Interestingly, these similar trends were seen in ACSS2i treated novel cage control mice, implicating that ACSS2i changes behavior of mice in a non-social defeat context. Furthermore, inhibiting ACSS2 enhanced multiple measures of sleep quality after 6 hours of sleep deprivation. ACSS2i decreased awakenings, improved NREM sleep consolidation, and increased total NREM sleep during recovery when compared to vehicle-treatment.

Conclusions (100 word limit)

Inhibiting ACSS2 promoted resiliency to social defeat stress, with similar trends seen in novel cage exposed ACSS2i mice and enhanced multiple metrics of sleep quality after 6 hours of sleep deprivation. These findings implicate ACSS2 is a novel sleep regulatory target involved in resilience to stress and sleep homeostasis. Furthermore, it identifies a potential mechanism through which sleep promotes resilience to social stress

Support

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Dynamics of Gene Expression After Acute Sleep Deprivation and Subsequent Recovery Sleep in the Male Mouse Cortex

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Full Name and Credentials

Caitlin Ottaway, BS

Introduction (100 word limit)

Sleep is an essential, tightly regulated biological function. Sleep is also a homeostatic process, with the need to sleep (sleep pressure) increasing as a function of being awake. Acute sleep deprivation (SD) increases sleep need, and subsequent recovery sleep (RS) discharges it. SD alters brain gene expression in rodents, but it remains unclear which changes are linked to sleep homeostasis, SD-related impairments, or non-sleep-specific effects. We hypothesize molecular changes underlying sleep homeostasis will follow the dynamics of sleep need as known through brain electrical activity, which saturates at 5-6 hours of SD and discharges in 2-3 hours of RS.

Methods (200 word limit)

We integrated newly collected and publicly available RNA-seq data from the cortex or frontal cortex of adult male C57BL/6J mice (n = 3-5). Mice underwent 3 hours and 5-6 hours of SD, followed by 2 hours and 6 hours of RS. RNA was sequenced using Illumina technology, and data normalization was performed using the removal of unwanted variation (RUV) method to account for brain region and laboratory biases. Differential gene expression analysis was performed using the edgeR package with a false discovery rate (FDR) threshold of < 0.05. To investigate the functional implications of recovery patterns, we utilized the Database for Annotation, Visualization, and Integrated Discovery (DAVID) for functional annotation analysis, enabling insights into the biological processes associated with the identified differentially expressed genes (DEGs).

Results (200 word limit)

The analysis revealed that 5-6 hours of SD resulted in the most substantial effects on gene expression, identifying 7,493 DEGs that collectively impacted nearly half of the cortical transcriptome. Most DEGs were downregulated across all time points. DEGs uniquely expressed after 5-6 hours of SD and normalized by 2 hours of RS were associated with chromatin regulation, oxidative phosphorylation, glutathione metabolism, and RNA binding. In contrast, genes altered at both 3 and 5-6 hours of SD that returned to baseline after 2 hours of RS were linked to circadian rhythms and DNA damage/repair.

Conclusions (100 word limit)

Genes altered after SD that normalize quickly may underlie molecular processes involved in sleep homeostasis as they reflect the dynamics of sleep pressure. Associated functions altered by SD and recovered within 2 hours of RS include oxidative stress, DNA repair, DNA methylation, and chromatin regulation. While SD significantly alters cortical gene expression, many changes can be quickly reversed with adequate recovery sleep. The identification of specific pathways involved in sleep homeostasis and cognitive function offers potential targets for further research to understand the molecular substrate of sleep homeostasis.

Support

R35: GM147020-01

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Evaluating Predictors of Positive Airway Pressure Therapy Compliance in Patient with Sleep Apnea

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Full Name and Credentials

Min Young Seo

Introduction (100 word limit)

This study aimed to identify and analyze key factors that influence patient adherence with PAP (positive airway pressure) therapy in obstructive sleep apnea (OSA).

Methods (200 word limit)

This study involved 214 adult OSA patients, who were maintained PAP therapy for over 36 months. Each participant completed a self-administered questionnaire and cephalometric analysis.

Results (200 word limit)

Compliant group exhibited higher mean age compared to the non-compliant group at the 1-year (50.98 ± 10.78 vs. 47.44 ± 10.78, p = 0.019) and 3-year (50.87 ± 10.93 vs. 47.63 ± 10.50, p = 0.028) of PAP therapy. In short-term compliance, the apnea-hypopnea index (AHI; 47.36 ± 25.67 vs. 37.49 ± 22.92, p = 0.011), apnea index (AI; 34.36 ± 24.79 vs. 27.42 ± 23.69, p = 0.049), lowest oxygen saturation (70.44 ± 12.18 vs. 76.42 ± 9.22, p = 0.001), and oxygen desaturation index (ODI; 44.26 ± 26.35 vs. 32.53 ± 22.36, p = 0.003) was significantly different between two groups. Notably, for long-term compliance, ODI emerged as a significant predictor (44.38 ± 27.16 vs. 34.35 ± 20.790, p = 0.034). In multivariate logistic regression analysis, age and AHI were identified as significant predictors for adherence in short-term (OR = 1.030, p = 0.008; OR = 1.033, p = 0.021, respectively) and long-term contexts (OR = 1.038, p = 0.014; OR = 1.013, p = 0.039, respectively).

Conclusions (100 word limit)

Older age and OSA severity emerged as notable determinants correlated with favorable compliance of PAP therapy.

Support

N/A

16

Energetic Demands Regulate Sleep-Wake Rhythm Circuit Development

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Full Name and Credentials

Amy Poe, PhD

Introduction (100 word limit)

Normal sleep and circadian rhythms during early life are important for brain development. Although the molecular mechanisms encoding cellular rhythms are well understood, little is known about how rhythmic behaviors first emerge. We previously determined that sleep-wake rhythms are initiated in early 3rd instar *Drosophila* larvae (L3), coordinated through maturation of a circuit bridge connecting DN1a clock neurons and Dh44 arousal output neurons. Development of this circuit promotes deeper sleep, and related long-term memory (LTM) capabilities. The cues that trigger formation of this circadian sleep circuit are not known.

Methods (200 word limit)

Here, we use the LarvaLodge platform to examine early life sleep behaviors in Drosophila larvae. We also develop approaches for monitoring larval feeding across the day.

Results (200 word limit)

We demonstrate that developmentally dynamic changes in energetic demands drive maturation of behavioral patterns in sleep and feeding. During the 2nd instar (L2) period, sleep and feeding are spread across the day; these behaviors become organized into daily patterns by L3, with feeding consolidated to day and sleeping to night. Genetic and nutritional manipulations that force mature (L3) animals to adopt immature (L2) feeding strategies disrupt sleep-wake rhythms, leading to impaired sleep depth and deficient LTM capacity. We find that inducing deeper sleep stages in immature (L2) animals through pharmacological or genetic manipulations is energetically disadvantageous at this stage of life and does not improve LTM performance. Moreover, DN1a-Dh44 circuit formation itself is developmentally plastic: an insufficient nutritional environment prevents establishment of a functional connection, facilitating a more constant (strategic) feeding strategy that eschews deep sleep at the expense of LTM. Finally, Dh44 neurons act through glucose metabolic genes to sense an organism's nutritional environment and drive sleep-wake rhythm development.

Conclusions (100 word limit)

Together, our data demonstrate that the emergence of rhythmic behaviors in *Drosophila* is driven by developmental changes in energetic capacity.

Support

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Time-of-day patterns in CSF analytes across the lifespan

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Full Name and Credentials

Shelei Pan

Introduction (100 word limit)

While cerebrospinal fluid (CSF) analyte levels are critical for diagnosis and management of many neurological diseases, it is unknown how these analytes vary with time of day in patients across the lifespan. We sought to identify and compare time-of-day differences in two common CSF lab values, glucose and protein, in patients who underwent CSF collection as part of clinical care.

Methods (200 word limit)

Patients who had CSF collected between June 2018 and May 2023 at thirteen hospitals within the health system affiliated with our institution were identified. Clinical, demographic and laboratory results were recorded. CSF values were pooled by time-of-day at collection and divided into 1- and 4-hour intervals. Values were excluded from analysis if there was evidence of CSF infection, bleeding, as well as age criteria excluding neonates <3 (glucose) or 4 (protein) months. One-way ANOVA with post-hoc Tukey was used to analyze difference between the means.

Results (200 word limit)

15,272 patients underwent a total of 26,397 CSF collection encounters. After exclusion criteria, there were 8,210 unique CSF glucose and 10,103 CSF protein values for analysis. We observed time-of-day regulation of CSF glucose levels, with the CSF/blood glucose ratio being higher in the morning (00:00-08:00) and evening (16:00-00:00) than during the day (08:00-16:00) both across the overall cohort and specifically in adults. This pattern was also observed when dividing the time-of-day into 1-hour intervals, where CSF/blood glucose values peaked at 04:00-05:00 (0.698) with the nadir at 10:00-11:00 (0.582).We found that CSF glucose time-of-day regulation is conserved in children (3 months-18 years), but that the timing slightly varies where children have higher early morning (00:00-04:00) vs. late morning (04:00-08:00) and daytime (08:00-16:00) CSF/blood glucose ratios. Time-of-day fluctuations in CSF/blood glucose ratio were further conserved when patients were stratified into 10-year age sub-groups up to 80 years of age, above which time-of-day regulation was not observed. We also show that CSF protein is strongly associated with age (R^2 = 0.2182) but does not correlate with a time-of-day pattern.

Conclusions (100 word limit)

A higher CSF/blood glucose ratio in the morning and evening vs. daytime suggests diurnal fluctuations which may be driven by a circadian rhythm. As glucose enters the brain at the blood-brain-barrier (BBB), our findings may implicate circadian regulation of glucose transporter polarization in anticipation of waking. While the mean CSF/blood glucose ratio at all timepoints in this study were within normal range, time-of-day variations could

influence clinical interpretation, particularly for values near reference range limits. Our finding of increased CSF protein with age is consistent with previous studies and may be related to BBB breakdown and decreased clearance.

Support

N/A

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Distressing dreams and risk of premature mortality: A population-based multicohort study

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Full Name and Credentials

Abidemi Otaiku, MD

Introduction (100 word limit)

Distressing dreams are associated with an increased risk of developing age-related neurodegenerative diseases. Whether distressing dreams increase the risk of developing other age-related health outcomes is unknown. This study investigated the association between distressing dreams (bad dreams and nightmares) and the risk of premature mortality and accelerated biological ageing in community-dwelling adults.

Methods (200 word limit)

This longitudinal, multi-cohort study used pooled data from three US cohort studies (Midlife in the United States [MIDUS]; The Wisconsin Sleep Cohort [WSC]; The Osteoporotic Fractures in Men Study [MrOS]). Distressing dream frequency was self-reported at baseline in all cohorts. Premature all-cause mortality (before age 75 years) was defined using study records (compiled from 2000-2024). Cox regression was used to analyse the prospective association between distressing dreams and premature mortality. In MIDUS, the pace of biological aging was measured at baseline using the DunedinPACE epigenetic clock. Mediation analysis was used to assess whether accelerated biological ageing mediates the relationship between distressing dreams and premature mortality.

Results (200 word limit)

Among a total population of 4,196 participants (mean age = 60.6 years, age range = 26-74), 342 (8.2%) experienced frequent distressing dreams at baseline. During 18.3 years of follow-up, 227 premature mortality cases were documented. After adjusting for demographic characteristics, frequent distressing dreams were associated with a nearly 3-fold risk of premature death in the pooled cohort (HR = 2.57, P <0.001) and in each cohort separately (MIDUS: HR = 2.74; WSC: HR = 2.77; MrOS: HR = 2.25; P's <0.05). In MIDUS, individuals with frequent distressing dreams exhibited a faster pace of biological aging (P = 0.009). Accelerated biological ageing mediated 21% of the distressing dream-mortality association. These associations remained robust when adjusting for a wide range of possible confounders.

Conclusions (100 word limit)

Adults with frequent distressing dreams experience faster biological aging and die at younger ages. Future studies are needed to determine whether treating distressing dreams could slow biological ageing and reduce mortality risk in the general population.

Support

N/A

19

An iPhone App to Guide the Collection of the Dim Light Melatonin Onset at Home

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Full Name and Credentials

Helen Burgess, PhD

Introduction (100 word limit)

The dim light melatonin onset (DLMO) remains the gold standard circadian phase marker in humans. We previously developed a proof-of-concept home DLMO kit and demonstrated that valid DLMOs could be collected at home, with objective markers of light exposure and sample timing. Nonetheless, the proof-of-concept kit uses costly external technologies such as photosensors (to monitor light exposure) and MEMS TrackCaps (to monitor sample timing). Here we present a novel iPhone app designed to improve upon the original proof-of-concept kit.

Methods (200 word limit)

We have designed an iPhone app that: (1) streamlines the home DLMO collection procedure, (2) maintains the objective assessment of light exposure and sample timing with internal app features, (3) provides real-time feedback on errors in light exposure and sample timing to both participants and externally located researchers, and (4) provides some cost savings over the original proof-of-concept kit. Programming capability includes options to (1) sample saliva at half-hourly or hourly intervals, (2) end sampling at participants' habitual bedtime or up to 3 hours past habitual bedtime, and (3) set a light intensity threshold of choice to alert the research participant and externally located researcher in real-time that melatonin levels may be suppressed. At the conclusion of the home DLMO collection, the app generates a report of light levels and sample timing for the researcher. Saliva samples can then be sent to a qualified laboratory for assay processing.

Results (200 word limit)

Flow diagrams of the back-end programming capability available to researchers and the front-end step-by-step display screens guiding research participants will be shown. A demonstration model of the iPhone app will be available for hands on interaction.

Conclusions (100 word limit)

A novel iPhone app has been developed to better support the collection of DLMOs at home, and facilitate research examining circadian timing in human populations.

Support

N/A

20

Elevated embryonic kynurenine (EKyn) exposure impacts sleep, inflammation, and tryptophan metabolism during postnatal development

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Full Name and Credentials

Courtney Wright

Introduction (100 word limit)

Prenatal insults linked to neurodevelopmental disorders (NDDs) elevate tryptophan degradation via the kynurenine pathway and increase levels of the astrocytic metabolite kynurenic acid (KYNA). Elevations in KYNA are observed in adult patients with NDDs. As an antagonist of NMDA and a7nACh receptors, elevated KYNA may contribute to sleep and cognitive disturbances experienced by patients with NDDs. Rats exposed to elevated embryonic KYNA express sex-specific sleep and behavioral disturbances during young adulthood. To understand how enhanced gliotransmission of KYNA may contribute to these endophenotypes, we sought to elucidate the postnatal developmental time course of sleep dysfunction in these offspring.

Methods (200 word limit)

To model gestational elevations in KYNA that occur from prenatal insults, we employed the embryonic kynurenine (EKyn) paradigm, wherein pregnant Wistar rat dams are fed a control diet (ECon) or a diet laced with kynurenine (100 mg/day), the direct KYNA bio-precursor from embryonic day (ED) 15 to ED 22. We assessed sleep-wake patterns of EKyn and ECon offspring across development (N=4 per group), as poor adolescent sleep often precedes and contributes to adult behavioral disturbances in NDDs. Radio telemetry devices (HD-X02, DSI) were implanted on postnatal day (PD) 21 to acquire EEG/EMG polysomnography. We recorded 24-h of uninterrupted basal sleep weekly from PD 28 to PD 56. Given that brain KYNA levels are not basally elevated until young adulthood (PD 56) in EKyn offspring (Buck et al. 2020 *Neurobiol Learn Mem*), we acutely challenged offspring with 6-h sleep deprivation (SleepDep). Following each basal sleep recording described above, we evaluated recovery sleep after SleepDep. We also evaluated inflammation (plasma cytokines) and kynurenine pathway metabolism following SleepDep in separate animals (N=5-11 per group).

Results (200 word limit)

At young adulthood (PD 56), we confirmed previous findings that EKyn offspring exhibit reduced REM sleep duration in the light phase ($P<0.05$). When we assessed total sleep time between mid-adolescence (PD 42) and young adulthood (PD 56), control offspring slept more in the light phase ($P<0.05$) and less in the dark phase ($P<0.05$) at young adulthood. However, young adult EKyn offspring spent less total time asleep in the light phase than young adult ECon ($P<0.05$). In response to SleepDep, we saw significant sleep rebound in ECon offspring at mid-adolescence (PD42, $P<0.005$) and young adulthood (PD56, $P<0.001$). In EKyn offspring, however, rebound sleep was not observed until young adulthood (PD56, $P<0.0001$). The recovery sleep at young adulthood was lower in EKyn offspring compared to ECon ($P<0.05$). Biochemically, in pre-pubertal (PD 28) offspring, SleepDep significantly increased plasma kynurenine ($P<0.05$) and cytokines (IL-10 and IL-18) in EKyn females ($P<0.01$) compared to controls but not males.

Conclusions (100 word limit)

Our findings report, for the first time, developmental changes to sleep patterns in EKyn offspring, with impairment in recovery sleep in response to SleepDep challenge in EKyn offspring across late adolescence. We find a female-specific alteration in metabolic and inflammatory responses to SleepDep in pre-pubertal EKyn offspring. Taken together, our study highlights that prenatal exposure to elevated kynurenine pathway metabolism alters sleep homeostasis across adolescence. Future studies will assess novel pharmacological tools aimed at reducing kynurenic acid across neurodevelopment and improving sleep.

Support

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22

Postpartum maternal sleep disruption is associated with perception of infant temperament: Findings from a 6-month longitudinal study

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Full Name and Credentials

Rebecca Cox, PhD

Introduction (100 word limit)

Accumulating evidence links postpartum sleep disruption to adverse maternal and infant outcomes, including postpartum depression and anxiety and deficits in infant-maternal bonding. Infant temperament, or individual differences in infant reactivity and self-regulation, represents a combination of heredity, maturation, and context. For example, mothers with higher postpartum depression and anxiety perceive their infants as having more difficult temperaments. Maternal perception of infant temperament is likely also influenced by maternal sleep. We examined the associations between maternal postpartum sleep and maternal perception of infant temperament and the moderating effect of maternal depression and anxiety over 6 months following delivery.

Methods (200 word limit)

Postpartum women with a history of depression (*n*=166) completed self-report measures of their sleep (Pittsburgh Sleep Quality Index, PSQI), depression (Edinburgh Postnatal Depression Scale), and anxiety (Generalized Anxiety Disorder Scale-7), and a parent-report measure of infant temperament (Infant Behavior Questionnaire, IBQ) once per month for 6 months following delivery. The IBQ includes 3 temperament factors: surgency/extraversion (e.g., smiling, activity level), negative affectivity (e.g., sadness, recovery from distress), and orienting/regulation (e.g., duration of oriented attention, low intensity pleasure). The sleep duration and sleep efficiency components and PSQI total score were examined as predictors. Concurrent associations between maternal sleep and infant temperament and interactions with maternal anxiety and depression were tested via 2-level multilevel models. Predictors were person-mean centered at level 1 (month level) and grand-mean centered at level 2 (person level).

Results (200 word limit)

Maternal reports of infant surgency/extraversion, negative affectivity, and orienting/regulation significantly increased over time (*p's* < .001), and maternal self-reported sleep duration, efficiency, and quality significantly increased over time (*p's* < .001). Months with shorter sleep duration and lower sleep quality were significantly associated with higher maternal-reported infant negative affectivity (*p's* < .01), and months with shorter sleep duration, lower sleep quality, and lower sleep efficiency were associated with significantly lower maternal-reported infant orienting/regulation (*p's* < .05). Mothers who reported lower sleep efficiency and lower sleep quality on average also reported significantly higher infant negative affectivity (*p's* < .05). Depression symptoms moderated the effects of sleep efficiency and quality on maternal-reported infant negative affectivity at the person level, such that lower infant negative affectivity was reported by those with lower depression symptoms and higher sleep efficiency (*p* = .05) and quality (*p* < .05). Anxiety symptoms also moderated the effect of sleep

efficiency on maternal-reported negative affectivity at the person level, such that higher infant negative affectivity was reported by those with higher anxiety and lower sleep efficiency ($p < .05$).

Conclusions (100 word limit)

Maternal perception of infant negative affectivity and orienting/regulation was associated with postpartum maternal sleep disruption. Maternal perception of infant orienting/regulation may be specifically sensitive to acute disruptions in postpartum sleep, whereas maternal perception of infant negative affectivity is linked both acute and more trait-like maternal sleep disruption. Lower depression symptoms may buffer trait-like effects of maternal sleep disruption on perception of infant negative affectivity, whereas higher anxiety symptoms may be sensitizing. These findings highlight the importance of maternal sleep health for infant outcomes. Future research should replicate these findings with objective measures of maternal sleep and infant temperament.

Support

Happiest Baby, Inc.; NIH K23MH137376

23

Prevalence and Correlates of Obstructive Sleep Apnea Syndrome Among Older Adults Without a Sleep Apnea Diagnosis

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Full Name and Credentials

Erin-Leigh Gallop Ed.D., MS

Introduction (100 word limit)

Obstructive sleep apnea (OSA) is a risk factor for morbidity and mortality among older Black adults. Around 80% of older adults with OSA go undiagnosed, and we have limited knowledge about the factors that contribute to OSA risk among older Black adults. This study explored the prevalence of OSA risk among older Black adults using data from a nationally representative survey of older adults.

Methods (200 word limit)

A subgroup of 2,356 older Black adults from the 2016 Health and Retirement Study, a panel study of adults 50 and over, were studied. Our variable of interest, high OSA risk, is a binary measure (1=score of ≥ 3; 0=all else) based on survey questions that resemble elements of the STOP-Bang questionnaire, a validated screening tool for diagnosing OSA. Predictors included gender, age, doctor-diagnosed sleep disorder, health insurance status, region, education, and income.

Results (200 word limit)

Logistic regression models estimated the likelihood of high OSA risk. Participants were 66.7±10.7 years old, 59% were female, and approximately 75% had high OSA risk. Logistic regression analysis revealed that men had higher odds of high OSA risk compared to women, individuals with sleep apnea had higher odds of high OSA risk compared to those without any sleep disorder, and older age was associated with lower odds of high OSA risk.

Conclusions (100 word limit)

The study found significant prevalence of high-risk OSA among older Black participants with gender and prior sleep apnea diagnosis emerging as indicators of high OSA risk. Findings suggest that screening tools for OSA risk consider these indicators at different stages of life.

Support

N/A

25

Comparison of Sleep Features across Smartphone Sensors, Actigraphy, and Diaries in Young Adults: A Feasibility Study

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Full Name and Credentials

Jaclyn Kirshenbaum, PhD

Introduction (100 word limit)

Poor sleep health is pervasive and contributes to long-lasting physical and psychological problems. As traditional sleep measurement can be burdensome, testing scalable and accessible sleep measurements is important. Smartphone-sensor sleep measurement is a nascent research method that is highly scalable and has potential to reach populations such as those with limited means to purchase wearables, live in difficult-to-reach places, or for whom compliance with a wearable might be low (e.g., adolescents). The aim of this study was to test whether sleep features obtained through a smartphone app are comparable to other modes of sleep measurement (i.e., daily diary, wearable actigraphy).

Methods (200 word limit)

Healthy college students (n=29, 18-24-years-old) with no prior diagnosis of a sleep disorder consented to downloading a smartphone application, the Effortless Assessment of Research Systems (EARS). For one week, the EARS app collected data continuously using the phone's accelerometer, gyroscope, and exposed to light. Each morning, participants received a notification via EARS to complete a sleep diary, which asked participants what time they got into bed, fell asleep, woke up, and got out of bed. A random subset (n=13) of participants also consented to wear an ActiGraph wristwatch. All analyses examined bedtime (i.e., time going to bed), risetime (i.e., wake-up time), and time-in-bed (i.e., duration between time in- and out- of bed) as three measures of interest. For all analyses, diary data were considered the reference measurement, and analyses were repeated with ActiGraph as the reference measurement.

Results (200 word limit)

On average, EARS showed a high mean true positive rate (TPR; 86.6%) and low mean false positive rate (FPR; 4.0%) based on diary reported bedtime and risetime. Supplementary analyses comparing EARS to ActiGraph data showed a similar TPR (83.7%) and slightly higher FPR (8.5%). Although there were no significant differences in mean bedtime, rise time, and time-in-bed among sources (*Ps*≥.069), there was some misalignment. Compared to the diaries, EARS estimated bedtime to be later by an average of 20 minutes, risetime to be earlier by an average of 21.83 minutes, and time-in-bed to be shorter by an average of 41.82 minutes. Relative to ActiGraph estimates, EARS estimated bedtime to be earlier by an average of 21.87 minutes, but estimated risetime to be later by 2.5 minutes, and time-in-bed to be longer by 24.4 minutes. Day-to-day correlations showed that bedtimes, risetimes, and time-in-bed were positively correlated between diary and EARS

(0.52≤*rs*≥0.29, *ps*≤.002). Similarly, day-to-day bedtimes and time-in-bed were positively correlated between ActiGraph and EARS (0.55≤*rs*≥0.38, *ps*≤.013), and while trending in the expected direction, rise times were not correlated between ActiGraph and EARS (*r*=0.29, *p*=.067).

Conclusions (100 word limit)

Smartphone-based sleep sensors show acceptable alignment with more established methods and may provide a feasible alternative to measuring daily sleep patterns in a scalable way. Future studies will require larger and more diverse samples to corroborate our findings of concordance among EARS, diary, and actigraphy data. As noted above, given the feasibility of using smartphones in the general population, our findings show preliminary evidence for using mobile sensors as a scalable method to detect sleep health behaviors.

Support

N/A

28

Brain-specific elevations in kynurenic acid reduce REM and NREM sleep duration in rats

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University of South Carolina School of Medicine, Columbia, USA

Full Name and Credentials

Maria Virginia Piroli, B.S.

Introduction (100 word limit)

Individuals with neurocognitive disorders, like age-related dementias or schizophrenia, frequently experience sleep disturbances. Kynurenic acid (KYNA), a tryptophan metabolite of the kynurenine pathway, is implicated in the pathophysiology of these disorders. Modest increases in KYNA, which antagonizes N-methyl-D-aspartate (NMDA) and α7 nicotinic acetylcholine (α7nACh) receptors, result in cognitive impairments and altered sleep-wake behavior, specifically negatively impacted rapid eye-movement (REM) sleep and increased wakefulness (Pocivavsek et al. *Sleep* 2017). The lateral hypothalamus (LH) is implicated in sleep-wake behaviors through arousal-promoting orexinergic neurons. Systemic kynurenine administration, the bioprecursor of KYNA, increases activation of wake-promoting orexinergic neurons in the LH.

Methods (200 word limit)

To determine the impact of a dose response elevation of KYNA (0μM, 1μM, 3μM, 10μM) locally in the brain, Wistar rats (N=12 female, 9 male) were cannulated targeting the lateral ventricle and implanted with telemetry devices to record electroencephalogram (EEG) and electromyogram (EMG) polysomnography. After one week, a within animal design employed intracerebroventricular infusion to deliver each dose of KYNA at Zeitgeber time (ZT) 0 across different 24-hour recording days. Vigilance states—wake, REM sleep, and non-REM (NREM) sleep—were classified using an artificial intelligence neural network and validated by expert scoring.

To evaluate region-specific increases in KYNA, an astrocyte targeting (GFAP promoter), adeno-associated virus (AAV) was bilaterally injected into the LH of rats (N=6 males). This AAV5 stereotype was tagged with mCherry and contained the gene for kynurenine aminotransferase II (KAT II), the enzyme which converts kynurenine to KYNA. After 21 days, immunofluorescence confirmed viral expression and microdialysis was performed in the LH to evaluate extracellular KYNA. Briefly after basal evaluation (2 hours) rats received a kynurenine injection (25mg/kg, IP) and de novo KYNA synthesis was evaluated (4 hours) via high-performance liquid chromatography.

Results (200 word limit)

We presently evaluated vigilance state durations from ZT 0-4 and found a dose-dependent impact of KYNA on sleep duration. Notably, 10μM KYNA increased wake duration by 30% ($P<0.05$), reduced REM sleep duration by 48% ($P<0.05$), and reduced NREM sleep duration by 26% ($P<0.05$) between ZT 0-2 when compared to vehicle infusion. 3μM KYNA and 10μM KYNA had significantly more wake bouts that are longer than 15 minutes at ZT 0-2 compared to vehicle ($P<0.05$). Importantly, the impact of KYNA on sleep-wake behavior was transient, as

vigilance state durations returned to basal levels (vehicle infusion) from ZT 2-4, which also corresponds to the timeframe wherein exogenous application of KYNA was estimated from the brain.

We found that mCherry tagged virus spread throughout the LH, and was localized with GFAP indicating that the virus targeted astrocytes. KAT II was observed to be colocalized with GFAP and virus in active AAV rats. Kynurenine stimulation increased de novo KYNA synthesis in microdialysis.

Conclusions (100 word limit)

Our findings provide novel support for the hypothesis that brain-specific elevations in KYNA cause a significant decrease in NREM and REM sleep and an increase in wakefulness. Taken together, the implications of our study place further attention on the role of the kynurenine pathway, a pharmacologically targetable metabolic pathway, and KYNA, an astrocyte-derived metabolite, in regulating sleep behavior. Region specific elevations in KYNA are actively being explored with the LH being of primary interest due to its arousal-promoting orexinergic neurons. Future studies will evaluate behavioral impacts of elevating KYNA at other times along the circadian cycle, including the dark phase.

Support

R01 HL174802, P50 MH103222

30

Morning Chronotype is Associated with Improved Well-Being in Middle-Aged and Older Adults: Insights into Sleep, Internalizing Symptoms, and Alertness in Healthy Aging

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Full Name and Credentials

Xinran Niu

Introduction (100 word limit)

Misalignment between internal chronotype and societal demands (i.e., social jetlag) can disrupt circadian rhythms, particularly for evening chronotypes with morning obligations. While previous research has examined the negative consequences of evening chronotype in older adults —who often struggle to achieve restorative sleep following social jetlag —it is unclear whether these effects begin to manifest in middle age. Additionally, although morning chronotype appears protective against mental health issues, its influences on different types of internalizing psychopathology remains elusive. This study investigates how chronotype influences sleep, alertness, and common, depression-specific, and anxiety-specific dimensions of internalizing symptoms in middle-aged and older adults.

Methods (200 word limit)

The sample comprised 652 middle-aged and older adults (ages 35-98) free of sleep, psychiatric, or neurological disorders. Participants completed the Morningness-Eveningness Questionnaire to assess their chronotype. Among the 652 participant, 256 were morning chronotypes who preferred earlier sleep onset and offset (M_{age} = 56), 60 were evening chronotypes who preferred later sleep and rise times (M_{age} = 49), and 336 were intermediate chronotypes with neither morning nor evening preferences (M_{age} = 53). All participants completed a 3-minute version of the Psychomotor Vigilance Test (PVT), where they pressed the spacebar as quickly as possible whenever a red circle appeared on the screen. The PVT was completed once between 9-11 AM and again between 9-11 PM, with the order of the two testing times counterbalanced. Shorter median reaction times on the PVT indicated greater behavioral alertness. Additionally, participants filled out the Standard Sleepiness Scale for subjective alertness, the Pittsburgh Sleep Quality Index for sleep disturbance, and the Mood and Anxiety Symptom Questionnaire for assessing depression-specific anhedonia, anxiety-specific anxious arousal, and the common internalizing factor, general distress.

Results (200 word limit)

Compared to evening chronotypes, morning chronotypes reported significantly lower levels of sleep disturbance ($t(87) = -3.44$, $p_{Bonferroni} = .021$), general distress ($t(73) = -3.55$, $p_{Bonferroni} = .021$), and anhedonia ($t(85) = -5.47$, $p_{Bonferroni} < .001$). Similarly, when compared to intermediate chronotypes, morning chronotypes showed less severe general distress ($t(589) = -4.14$, $p_{Bonferroni} < .001$), anhedonia ($t(560) = -4.67$, $p_{Bonferroni} < .001$), and anxious arousal ($t(590) = -3.27$, $p_{Bonferroni} = .021$). Furthermore, chronotype interacted with time of day to predict subjective ($F(2, 649) = 14.99$, $p = .000$) and behavioral alertness ($F(2, 649) = 2.42$, $p = .090$). Specifically, morning

chronotypes felt more alert in the morning than intermediate ($t(578) = 4.90$, $p_{Bonferroni} < .001$) and evening chronotypes ($t(76) = 7.15$, $p_{Bonferroni} < .001$). Additionally, intermediate chronotypes felt more alert in the morning than evening chronotypes ($t(75) = 4.78$, $p_{Bonferroni} < .001$). Notably, when PVT was conducted in the evening, evening chronotypes showed higher behavioral alertness than intermediate ($t(111) = 03.26$, $p_{Bonferroni} = .012$) and morning chronotypes ($t(106) = -2.96$, $p_{Bonferron} = .048$). However, no significant differences were observed for subjective alertness in the evening or behavioral alertness in the morning ($ps_{Bonferroni} > .9$).

Conclusions (100 word limit)

Morning chronotype may provide a transdiagnostic protective mechanism against common, depression-specific, and anxiety-specific internalizing symptomatology, as well as sleep disturbance and daytime sleepiness. The benefits of a morning chronotype may begin to emerge in early middle age and extend into late adulthood, possibly due to its alignment of earlier sleep-wake timing with societal demands geared towards morning activities. Interestingly, evening chronotypes demonstrate higher behavioral alertness when tested during their optimal periods. Overall, these results highlight the importance of aligning daily schedules more closely with individual preferences to promote optimal cognitive and mental health outcomes in aging populations.

Support

This work was supported by the National Science Foundation under Grant BCS-2001025, awarded to J.D.P. as the Principal Investigator and E.A.K. as the co-Principal Investigator.

32

Higher training workload associates with longer sleep duration and higher nocturnal heart rate in collegiate football athletes

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Full Name and Credentials

Andrea Spaeth, PhD

Introduction (100 word limit)

The rigorous training schedules of athletes involve extensive exercise that purposefully damages muscle tissue to initiate a cascade of adaptive physiological responses during the post-exercise recovery period that enhance physical performance over time. Recovery is a critical element to gaining speed, strength and conditioning and much of this recovery occurs during nocturnal sleep. This study aimed to determine whether training workload (total session distance, high-speed running distance, practice session duration) alters subsequent nocturnal sleep and heart rate metrics.

Methods (200 word limit)

Male athletes (N=173) from a Division I football team contributed 16,089 instances of matched daytime training data with that night's sleep and heart rate data. Training metrics (total session distance, high-speed running distance, session duration) were tracked with GPS units, while nocturnal sleep (duration, sleep stages) and heart rate metrics (average heart rate and variability [RMSSD]) were recorded with the Oura ring on the non-dominant index finger. Training sessions were classified into low, medium, or high workload, with tertile cutoffs set by player position and season. Mixed-model ANOVAs analyzed the impact of training workload on sleep and heart rate metrics.

Results (200 word limit)

Across all nights, participants averaged 6.2±1.1h of nocturnal sleep. High workload, measured by total session distance and high-speed running distance, associated with longer nocturnal sleep duration and a higher rapid eye movement (REM) sleep percentage compared to lower workload ($ps<0.05$). Moderate workload, measured by practice session duration, associated with longer nocturnal sleep duration, higher REM sleep percentage, and lower nocturnal RMSSD ($ps<0.05$). High workload, measured by total session distance and practice session duration, associated with higher nocturnal heart rate average ($ps<0.05$).

Conclusions (100 word limit)

In NCAA Division I football athletes who were habitually sleeping less than the recommended amount for young adults (7h), higher physical workload associated with longer sleep duration and a higher nocturnal heart rate. Workload did not affect deep sleep duration or heart rate variability. Data suggests that these athletes may not have been able to recovery optimally during sleep due to the accrual of sleep debt and highlight the need for athletic departments to place greater emphasis on sleep health.

Support

N/A

33

Understanding the Debilitating Nature of Narcolepsy in Patients' Own Words: A Social Listening Analysis

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Full Name and Credentials

Jennifer Gudeman, PharmD

Introduction (100 word limit)

Real-world information and experiences from people with narcolepsy (PWN) may be better captured within a closed patient community. To further characterize the many struggles and unmet needs of PWN, passive social listening was used to explore how PWN describe the condition using their own words.

Methods (200 word limit)

MyNarcolepsyTeam is a social network where >10,000 members can organically share their experiences living with narcolepsy with one another. 3959 individuals from the MyNarcolepsyTeam community were invited to participate in a 27-question online survey. Following the survey, organic posts, comments, questions, and answers posted from January 2022 to October 2023 were analyzed to add more dimension to how PWN experience narcolepsy. All data were analyzed descriptively.

Results (200 word limit)

Of 110 survey respondents (age ≥50 years, 53%; female, 84%), 48% had narcolepsy type 1, 32% had narcolepsy type 2, and 20% were unsure. Almost half (44%) of survey respondents reported that a correct narcolepsy diagnosis took >5 years. Social listening revealed stories of diagnostic delays. Prior to a narcolepsy diagnosis, PWN noted misdiagnoses of mental health disorders (eg, depression) and "missed" diagnoses (eg, sleep apnea). 43% of survey respondents reported comorbid pain. Painful comorbidities (eg, fibromyalgia, migraines, neuropathy) often added to existing medication burden and furthered sleep disruption. The most troubling symptoms included excessive daytime sleepiness (90%), fatigue (84%), sleep disturbances (81%), and memory/cognitive issues (80%). PWN described frequent sleep disruptions, including fragmented sleep, vivid dreams, hallucinations, insomnia, sleep paralysis, and abnormal REM cycles. Both the survey and social listening highlighted a range of cataplexy experiences. The most common emotional triggers of cataplexy were fatigue (70%), anger (48%), startlement (46%), laughter (46%), fear (44%), excitement (38%), and crying (33%). Most PWN (65%)

reported taking ≥2 medications to treat daytime and/or nighttime symptoms. PWN often struggled with complex treatment regimens while trying to address the full 24-hour spectrum of symptoms.

Conclusions (100 word limit)

Organic conversations highlighted the challenges PWN experience managing complex treatment regimens to cope with the full spectrum of narcolepsy symptoms. Insight into how people experience narcolepsy and try to manage their symptoms will help sleep specialists better recognize the needs of PWN. These findings also underscore the importance of education to help PWN understand if they have narcolepsy type 1 or type 2, as 1 in 5 survey respondents were uncertain.

Support

Avadel Pharmaceuticals

34

Consistent Efficacy of Once-Nightly Sodium Oxybate Regardless of Patient Demographic and Baseline Disease Characteristics

Michael J. Thorpy MD[1], Thomas Roth PhD[2], Clete A. Kushida PhD[3], Anne Marie Morse DO[4], John Harsh MD[5], Luis E. Ortiz MD[6], Jennifer Gudeman PharmD[7], Yves Dauvilliers PhD[8]

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Full Name and Credentials

Jennifer Gudeman, PharmD

Introduction (100 word limit)

In the phase 3 REST-ON trial, efficacy and safety of once-nightly sodium oxybate (ON-SXB; LUMRYZ™) was evaluated in patients with narcolepsy type 1 (NT1) or 2 (NT2). At all tested doses, ON-SXB was associated with significant improvements vs placebo (all $P<0.001$) on all coprimary endpoints, including change from baseline (CFB) in mean sleep latency on the Maintenance of Wakefulness Test (MWT), Clinical Global Impression-Improvement (CGI-I) rating, and weekly number of cataplexy episodes (NCA), and secondary endpoint Epworth Sleepiness Scale (ESS) score. This post hoc analysis assessed efficacy of ON-SXB vs placebo in various demographic and clinical subgroups of REST-ON participants.

Methods (200 word limit)

REST-ON (NCT02720744) participants aged ≥16 years with NT1 or NT2 were randomized 1:1 to ON-SXB (4.5 g, 1 week; 6 g, 2 weeks; 7.5 g, 5 weeks; 9 g, 5 weeks) or placebo for 13 weeks. Least squares mean differences (LSMDs) in CFB for ON-SXB vs placebo for mean sleep latency on MWT, NCA (NT1 only), and ESS, and odds ratios (ORs) for "much" or "very much" improved on CGI-I, were compared among baseline demographic (age, sex, race, body mass index [BMI] category) and narcolepsy disease characteristic (NT1/NT2; concomitant alerting agent use) subgroups. Efficacy was evaluated in the modified intent-to-treat (mITT) population, which included all randomized participants with ≥1 efficacy measurement after receiving the 6-g dose of ON-SXB or placebo (n=190; ON-SXB, n=97; placebo, n=93).

Results (200 word limit)

LSMDs for ON-SXB vs placebo demonstrated significant improvements ($P<0.05$) in CFB on the MWT at week 13 (9-g) for age <35 years (7.3), sex (female: 6.8; male: 5.3), race (white: 7.0; non-white: 4.8), BMI (low: 10.0; high: 4.0), narcolepsy type (NT1: 6.0; NT2: 6.3), and alerting agent/no alerting agent use

(6.0, 6.3) subgroups. ORs at week 13 were significant (P<0.05) with ON-SXB 9g vs placebo for “much” or “very much” improved on CGI-I for age (<35 years: 7.1; ≥35: 3.6), female (4.2), white/non-white (5.5, 5.9), high BMI (4.2), NT1 (5.5), and alerting agent/no alerting agent use (7.6, 3.3) subgroups. For CFB in NCA, LSMDs were significant (P<0.05) for ON-SXB 9g vs placebo for age (<35 years: –7.6; ≥35: –5.0), female (–7.5), white (–7.0), BMI (low: –6.4; high: –6.5), and alerting agent/no alerting agent use (–7.2, –5.5) subgroups. LSMDs with ON-SXB 9g vs placebo in CFB on ESS were significant (P<0.05) for age (<35 years: –3.9; ≥35: –3.8), sex (female: –3.4; male: –3.9), race (white: –3.6; non-white: –4.9), BMI (low: –6.3; high: –2.6), NT1 (–4.3), and alerting agent/no alerting agent use (–2.6, –6.0) subgroups. Comparable differences were observed at weeks 3 (6g) and 8 (7.5g).

Conclusions (100 word limit)

Post hoc subgroup analyses demonstrate the robust and consistent efficacy of ON-SXB in the treatment of narcolepsy symptoms in patients with a variety of demographic and clinical characteristics. These findings may aid treatment recommendations for specific populations of people with narcolepsy.

Support

Avadel Pharmaceuticals

35

Stability of Once-Nightly Sodium Oxybate in Alternative Liquid Reconstitution Vehicles

Maggie Lavender MSN, RN, FNP-C[1], Ellen Wermter FNP, DBSM[2], Anne Marie Morse DO[3], Matthew Horsnell BS[4], Jason Vaughn PhD[5], Frederik Ascencion[6], Jennifer Gudeman PharmD[5]

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Full Name and Credentials

Jennifer Gudeman, PharmD

Introduction (100 word limit)

LUMRYZ™ is a once-nightly formulation of sodium oxybate (ON-SXB) approved for the treatment of cataplexy or excessive daytime sleepiness in adults with narcolepsy. ON-SXB contains both immediate-release and pH-dependent, controlled-release granules and is designed to be reconstituted in water and administered orally. Individuals with narcolepsy receiving treatment with ON-SXB may prefer to use alternative liquids for reconstitution over water. Experiments were conducted to determine the dissolution profile and pH of ON-SXB at prescribed doses after reconstitution in alternative liquids.

Methods (200 word limit)

Dissolution and pH testing was conducted with ON-SXB 4.5-g and 9-g single-dose packs reconstituted in Milli-Q® water (control), Crystal Light© Raspberry Lemonade (CL), MiO© Fruit Punch Concentrate (Mio), and alkaline water (AW). CL and Mio were prepared with 500 mL and 475 mL of tap water, respectively. Dissolution (percentage of exact ON-SXB label claim) was measured at 15 minutes and 1, 2, 2.5, 3, 3.5, 4, 5, 6, and 8 hours after 5-minute (AW only) and 30-minute (CL, Mio, and AW) rest periods (6 samples tested per liquid). Prior product specification experiments of ON-SXB in 0.1 N HCl and pH 6.0 buffer demonstrated dissolution of 40%–60%, 42%–62%, and ≥80% at 15 minutes, 2 hours, and 4 hours, respectively. The delivered dose was measured for 4.5- and 9-g reconstituted samples after 2 separate trials of 60 seconds and 10 seconds of shaking within the mixing container, then again after the mixing container was rinsed with diluent. The pH of each reconstitution solution was measured prior to and then 5 and 30 minutes after addition of ON-SXB (1 sample tested per liquid).

Results (200 word limit)

After 15 minutes, ON-SXB 4.5 g was 50% dissolved in control, 51% in CL and Mio, and 49% in AW, while the 9-g dose was 50% dissolved in control and CL, and 51% in Mio and AW after both rest periods. At 8 hours, the 4.5-g dose was 98% dissolved in control, 101% in CL and Mio, and 99% in AW, while the 9-g dose was 98% dissolved in control, 101% in CL and Mio, and 97% in AW after the 5-minute rest period, and 98% in AW after the 30-minute rest period. After shaking, 97.9%–100.8% of the ON-SXB dose is delivered, with 1.0%–1.6% of the label claim remaining in the mixing container if the rinsing step is omitted. Initial pH values of control, CL, Mio, and AW were 7.03, 3.02, 2.95, and 9.53, respectively. pH values of ON-SXB 4.5 g in control, CL, Mio, and AW were 5.6, 5.3, 5.4, and 5.6 at 5 minutes and 5.6, 5.2, 5.3, and 5.5 at 30 minutes, respectively. For ON-SXB 9 g in control, CL, Mio, and AW, the pH was 5.7, 5.5, 5.6, and 5.7 at 5 minutes and 5.7, 5.6, 5.6, and 5.7 at 30 minutes, respectively.

Conclusions (100 word limit)

CL, Mio, and AW met all acceptance criteria as a vehicle for reconstitution of ON-SXB, with nearly superimposable results compared to the control. Across alternative vehicles tested, pH of ON-SXB at both doses remained well below the trigger pH. These results demonstrate consistent and acceptable dissolution of ON-SXB in various liquids, which may be preferred by some patients as alternatives to water.

Support

Avadel Pharmaceuticals

36

Effects of Solriamfetol on Cognition in Obstructive Sleep Apnea With Excessive Daytime Sleepiness and Impaired Cognition in the SHARP Clinical Trial

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Full Name and Credentials

Kwame Brown, PhD

Introduction (100 word limit)

This analysis evaluated the effect of solriamfetol (Sunosi®), approved to treat excessive daytime sleepiness (EDS) associated with obstructive sleep apnea (OSA; 37.5–150 mg/day), on subjective cognitive function by examining overall scores and individual items of the British Columbia-Cognitive Complaints Inventory (BC-CCI).

Methods (200 word limit)

SHARP was a randomized, double-blind, placebo-controlled, crossover trial in participants with impaired cognition associated with OSA and EDS. Participants received solriamfetol (75 mg for 3 days, then 150 mg/day), and placebo, each for 2 weeks, with a 1-week washout. BC-CCI items included forgetfulness/memory problems, slow thinking speed, trouble expressing thoughts, trouble finding the right word, poor concentration, trouble figuring things out, and vocational, family/friends, and social/recreational functioning. Changes from baseline were assessed using mixed models with repeated measures.

Results (200 word limit)

SHARP enrolled 59 participants (mean±SD age 52.2±10.7y; 36% female). Baseline overall BC-CCI scores were 11.4±2.5; scores were comparable between solriamfetol/placebo (n=30; mean=11.4) and placebo/solriamfetol (n=29; mean=11.4) sequences. Overall BC-CCI scores improved with solriamfetol versus placebo (P=0.002; Cohen's d=0.45). Baseline individual BC-CCI items scores were generally similar between sequences. Solriamfetol led to greater improvements compared with placebo in poor concentration (P=0.007; d=0.37), slow thinking speed (P=0.009; d=0.36), trouble finding the right word (P=0.042; d=0.28), trouble figuring things out (P=0.030; d=0.30), and forgetfulness/memory problems (P=0.013; d=0.34). No significant differences were found for functional items.

Conclusions (100 word limit)

Consistent with previously observed improvement on objective cognition, solriamfetol led to significant subjective improvements overall, and particularly in domains related to memory, executive functioning, and processing speed.

Support

Axsome Therapeutics.

37

Characterizing the Time-Course of Sleep-Wake Abnormalities in the MCI-Park Mouse Model of Parkinson's Disease

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Full Name and Credentials

Jasmine Benitez

Introduction (100 word limit)

Sleep disturbances affect as many as 90% of Parkinson's Disease (PD) patients, often presenting years before the characteristic motor symptoms of the disease. Interactions between sleep changes with other non-motor and motor changes in disease progression remain poorly understood. The mitochondrial complex I (MCI) Park mouse model of PD exhibits progressive symptom worsening, including both motor and many sleep changes characteristic of PD. Using MCI-Park mice, we examined the time-course of sleep disturbances relative to other PD-like changes. The findings shed light on the complex relationship between mitochondrial dysfunction, sleep, motor- and non-motor symptoms in PD.

Methods (200 word limit)

The MCI-Park mouse has a conditional deletion of the mitochondrial complex I gene, Ndufs2, in cells expressing the Cre recombinase under the control of the dopamine transporter promoter. Dat-cre−/−; Ndufs2fl/fl mice served as controls, while Dat-cre+/−; Ndufs2fl/fl mice were designated as cNdufs2−/− (MCI-Park) mice. Multiple cohorts of animals of both genotypes were monitored in parallel for changes in sleep and behavior from 60-110 days of age. In one cohort, one group received continuous heat support via a heat pad placed beneath one side of the cage, while the other cohorts did not.

Sleep EEG and EMG was recorded using Pinnacle Technology implants. Automated machine learning-assisted scoring, supplemented by manual inspection, categorized 10-sec epochs into wake, NREM, or REM.

Motor function was assessed via the righting reflex, where mice were placed in a supine position, and the time taken to right themselves was recorded. Sensorimotor deficits were measured through the adhesive removal test. Adhesive strips were placed on each paw, and the time taken to remove them, up to 300 seconds, was recorded.

Results (200 word limit)

Mice without heat support showed typical disease progression, with early sleep fragmentation and altered REM/NREM patterns. REM sleep was elevated early but declined by day 80. Disrupted sleep-wake cycles were evident, with increased activity during the light phase and reduced activity during the dark phase, aligning with prior lab data.
In mice with heat support, sleep disturbances appeared later and were less severe. They maintained more regular REM/NREM patterns, and sleep fragmentation progressed more slowly. Total sleep duration was similar

across groups, but mice with heat support showed fewer disruptions in their sleep-wake cycles as the disease progressed.
By day 60, MCI-Park mice without heat support displayed significant motor impairments. Locomotor activity decreased in the late disease stages, and mice preferred familiar environments. Despite motor decline, they consistently maintained the righting reflex throughout the study.

Mice with heat support exhibited slower motor decline. Motor impairments appeared later and were less severe compared to those without heat support, with more stable motor function maintained over time.

Conclusions (100 word limit)

In the cohort without heat support, disease progression was comparable to previous findings in our lab. Unlike prior studies that identified age-related sleep deterioration, we observed no progressive worsening in sleep or behavior in MCI-Park mice who received heat support during later stages. We propose that heat support may have contributed to slowing disease progression. Literature suggests ambient temperature affects sleep, oxidative stress, and circadian rhythms in PD. These findings highlight temperature's potential as a therapeutic tool, warranting further exploration of its effects to enhance sleep and quality of life for PD patients. Future research should focus on temperature-based treatments.

Support

N/A

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Prevalence of Central Disorders of Hypersomnia in Patients diagnosed with Major Depressive Disorder: A Meta Analysis

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Full Name and Credentials

Yamna Waseem, MD

Introduction (100 word limit)

The present study is aimed to review the prevalence of central hypersomnia disorders in patients with major depressive disorder. Further analysis was done to assess for factors associated with increased prevalence of hypersomnia.

Methods (200 word limit)

Meta analysis was done following the PRISMA guidelines. We searched for articles published listed in the following databases: PubMed, Embase, CINAHL, Cochrane Library, PsycINFO. The search terms included key words related to depression and central hypersomnia: 'major depressive disorder', 'depression', 'central disorders of hypersomnia', 'idiopathic hypersomnia', 'narcolepsy', and 'prevalence'. RevMan software was used to perform the meta analysis. Random effects model was used to account for heterogeneity. Subgroup analysis was performed based on study design, diagnostic criteria, and population characteristics.

Results (200 word limit)

Our search strategy yielded 2692 papers, after which duplicates were removed and eligible articles were assessed to be 84, out of which 12 were chosen for the meta-analysis. The pooled prevalence of central hypersomnia was found to be 49% in patients with major depressive disorder. Further subgroup analysis was done based on diagnostic criteria for major depressive disorder: DSM IV, DSM V and others. Subgroup analysis based on gender showed higher prevalence in female focused studies. Findings were significant (p= 0.009) when comparing age groups with older adults having a higher prevalence of central hypersomnia versus younger age groups. Subgroup analysis was done by publication year: Before 2010, 2010-2015, and after 2015. Higher prevalence was noted in studies done earlier.

Conclusions (100 word limit)

The results of this meta analysis of observational studies show a significant prevalence of 49% of central hypersomnia disorders in patients with major depressive disorder. Further subgroup analysis show increase prevalence in older age groups and females. Understanding the patterns related to hypersomnia can help clinicians and researchers develop targeted interventions for people with both hypersomnia and depression, with improving care and treatment outcomes.

Support
N/A

39

The associations between sleep duration, subjective sleep quality, and mental health among Chinese population: a population-based cross-sectional study

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Full Name and Credentials

Chenglin Hong, PhD, MSW, MPH

Introduction (100 word limit)

Sleep is a critical factor influencing physical and mental well-being, with both inadequate and excessive sleep associated with adverse health outcomes. In China, rapid urbanization, lifestyle changes, and increasing stress levels may be contributing to widespread sleep disturbances. While sleep duration and subjective sleep quality have been studied in other populations, limited research has explored how these sleep variables impact mental health outcomes, such as psychological distress, within the Chinese population. Understanding these associations is crucial for informing public health interventions aimed at improving sleep and mental well-being in this demographic.

Methods (200 word limit)

This study analyzed baseline data from the Cohort Study on the General Population in the Beijing-Tianjin-Hebei Region, part of China's National Key R&D Program. We conducted a population-based cross-sectional study from January 2018 to January 2020, enrolling attendees at eight medical examination centers. Participants were eligible if they were 18 years or older, voluntarily participated, and signed informed consent. Exclusion criteria included cognitive or hearing impairments, severe mental illness, or articulation issues. Sleep duration and subjective sleep quality were measured using an adapted Pittsburgh Sleep Quality Index (PSQI). Self-reported sleep duration was analyzed categorically (<7 hours, 7 to <8 hours, 8 to <9hours and ≥9hours) based on the distribution and suggestions in the literature, and subjective sleep quality was asked by the following question: 'What do you think of your sleep quality in the prior month?'(excellent, good, bad and awful). Psychological distress was measured using the Kessler Psychological Distress Scale (K10). Multivariate regression models were used to examine associations between sleep duration, subjective sleep quality, and mental health, adjusting for potential confounders such as age, gender, and socioeconomic characteristics.

Results (200 word limit)

A total of n=41,209 participants who reported key measures were included in the analysis. The median age was 44 years (IQR: 35-56), and 51.4% were male. Most participants (84.0%) were married, and 3.8% identified as belonging to an ethnic minority group. Sleep duration was distributed as follows: 11.1% reported <7 hours, 35.5% slept 7–8 hours, 40.5% slept 8–9 hours, and 12.9% slept ≥9 hours. Regarding subjective sleep quality, 40.1% rated their sleep as excellent, while 11.3% reported bad sleep and 1.0% described it as awful. Based on K10, 4.5% of participants were screened for mild to severe psychological distress. Women, and individuals who

were single or divorced/widowed, were more likely to screen positive for psychological distress ($p<0.001$ for all). In multivariate models, individuals who reported sleeping for <7 hours and 7–8 hours were significantly more likely to be screened for psychological distress compared to those who slept ≥9 hours (adjusted odds ratio [aOR] = 1.63, 95% CI: 1.36-1.96; aOR = 1.33, 95% CI: 1.14-1.56, respectively). Similarly, individuals who reported bad and awful sleep quality were more likely to screen positive for psychological distress (aOR = 7.64, 95% CI: 6.62-8.84; aOR = 18.27, 95% CI: 14.24-23.31).

Conclusions (100 word limit)

This large, population-based study highlights the significant associations between sleep duration, subjective sleep quality, and psychological distress among adults in Northern China. Findings indicate that both insufficient sleep and poor sleep quality are linked to increased psychological distress, particularly among women and those who are single or divorced/widowed. Given the rising prevalence of sleep disturbances due to urbanization and lifestyle changes, these results underscore the need for public health interventions focused on improving sleep health as a vital component of mental well-being in this population. Targeted strategies could mitigate mental health issues associated with sleep disturbances.

Support

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Sex Differences in the Association Between Circadian Preference and Alcohol-Related Problems

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Full Name and Credentials

Justin Verlinden, MS

Introduction (100 word limit)

Rates of Alcohol Use Disorder (AUD) among women are rising dramatically, emphasizing the need for the identification of sex-specific risk factors for AUD. Although recent work has highlighted poor sleep as an important risk factor for women, little attention has been given to circadian factors. Eveningness is strongly associated with hazardous drinking, and findings from one study suggest that women with an evening preference are at a higher risk for future binge drinking. Here, we evaluated how sex differences in circadian preference are related to alcohol consumption and related problems.

Methods (200 word limit)

Heavy drinking women (n=204) and men (n=89) with clinically relevant symptoms of insomnia (Insomnia Severity Index scores > 14) completed measures assessing circadian preference and alcohol consumption during the baseline phase of a clinical trial testing the efficacy of an online insomnia intervention. Circadian preference was assessed using the Composite Scale of Morningness (CSM) and alcohol consumption and alcohol-related problems (e.g. alcohol induced blackouts) were assed using the Alcohol Use Disorders Identification Test Consumption (AUDIT-C) and Problems (AUDIT-P) subscales, respectively. Hierarchical regression analyses were conducted to test interactions between sex and circadian preference (entered as a continuous predictor) on 1) alcohol consumption and 2) alcohol-related problems. Age (sample range of 18-50), ethnicity, depression symptom severity (Center for Epidemiologic Studies Depression Scale revised 10-item version; CESDR), and perceived stress levels (Perceived Stress Scale; PSS) were controlled for in both models. All significant interactions were further probed by analyzing simple slopes and examining bivariate correlations separately by sex.

Results (200 word limit)

Women reported higher levels of depression ($p = 0.02$) and stress ($p = 0.003$) whereas men had higher scores on the AUDIT-C ($p < 0.001$). There was also a higher proportion of Hispanic/Latino men compared to women ($p = 0.04$). The distribution of circadian preference did not differ by sex and consisted primarily of intermediate and evening types. Sex and circadian preference significantly interacted to predict alcohol-related problems (AUDIT-P subscale; $p = 0.005$). After probing the simple slopes, men showed a significant, positive relationship between circadian preference and AUDIT-P scores, $B = 0.175$, $t = 2.089$, $p = 0.038$, suggesting that shifts toward morning preference were associated with more alcohol-related problems. In contrast, women showed a trend level, negative relationship between circadian preference and AUDIT-P scores, $B = -0.108$, $t = 1.818$, $p = 0.070$, such that shifts towards eveningness were associated with greater alcohol related problems. Indeed, bivariate correlations revealed a significant negative association between CSM and AUDIT-P scores among women ($r = -$

0.19, p = 0.006) and a significant positive association among men (r = 0.22, p = 0.041). No main effects or interactions emerged in the model predicting alcohol consumption (AUDIT-C; p = 0.269).

Conclusions (100 word limit)

These results suggest evening preference may be a particularly salient risk factor for AUD among women. It will be important for future studies to further investigate this possibility using longitudinal data and more generalizable samples.

Support

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Persistent short sleep duration from pregnancy to 2-7 years after delivery and metabolic health

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Full Name and Credentials

Minjee Kim MD

Introduction (100 word limit)

Short sleep duration during pregnancy and the peri-menopausal period has been linked to adverse cardiometabolic outcomes. However, how sleep duration changes after delivery and whether such changes affect the cardiometabolic health of birthing people remain unclear. Thus, the objectives of this study were to characterize patterns of self-reported sleep duration from pregnancy to 2-7 years after delivery and determine whether persistently short sleep during pregnancy and after delivery is associated with incident metabolic syndrome.

Methods (200 word limit)

This secondary analysis utilized data from the Nulliparous Pregnancy Outcomes Study: Monitoring Mothers-to-be (nuMoM2b) and the ongoing nuMoM2b-Heart Health Study (nuMoM2b-HHS). Between 2010 and 2013, 10,038 nulliparous individuals in their first trimesters were recruited at 8 U.S. academic centers, where they completed sleep questionnaires and provided anthropometric and biological measures. A subset of these participants (n=4,509) were invited to repeat these measures 2 to 7 years post-delivery. Based on self-reported sleep duration during pregnancy and at follow-up, participants were categorized into four sleep patterns: 'persistent short sleep (<7 hours),' 'resolved short sleep,' 'new short sleep,' and 'never short sleep.' The primary outcome, incident metabolic syndrome (MetS) at follow-up, was defined by the presence of three or more of the following: abdominal obesity, high blood pressure (≥130/85 mmHg), impaired fasting glucose, high triglyceride, and low high-density lipoprotein levels. Participants under 18 and those with baseline MetS were excluded. Relative risks of MetS associated with each sleep pattern were estimated using regression models, adjusted for baseline age and time from delivery to follow-up. We also tested an alternative definition of short sleep (<6 hours) in sensitivity analyses.

Results (200 word limit)

This analysis included 3,373 birthing people (age 27.3 ± 5.4; 64.8% non-Hispanic White, 12.4% non-Hispanic Black, 15.2% Hispanic; 64.3% married at baseline). Persistent short sleep was found in 475 (14.1%), new short sleep in 806 (23.9%), resolved short sleep in 383 (11.4%), and never short sleep in 1,709 (50.7%) of participants.

Overall, the median sleep duration decreased from 8.0 (IQR 7.3-8.6) hours during the index pregnancy to 7.0 (6.5-8.0) hours at follow-up. Non-Hispanic Black (aOR, 2.17; 95% CI, 1.59-2.97) and unmarried (aOR, 1.68, 95% CI, 1.29-2.19) participants were significantly more likely to experience persistent short sleep compared to their non-Hispanic White and married counterparts, respectively. Sleep duration patterns were not associated with educational attainment or commercial insurance. At follow-up (3.1±0.9 years post-delivery), 447 (13.3%) participants developed MetS. Persistent short sleep (< 7 hours) was associated with higher odds of incident MetS (aOR, 1.60; 95% CI, 1.21-2.11), after adjusting for covariates. In the sensitivity analysis where short sleep was defined as < 6 hours, persistent (aOR, 2.31; 95% CI, 1.36-3.93), new (aOR, 2.12; 95% CI, 1.55-2.91), and resolved (aOR, 1.73; 95% CI, 1.16-2.57) short sleep were all associated with greater odds of incident MetS, compared to never short sleep.

Conclusions (100 word limit)

In this cohort study of nulliparous birthing people, short sleep duration that persisted from pregnancy to 2-7 years after delivery was associated with a higher risk of adverse cardiometabolic outcomes. These findings highlight pregnancy and early parenthood as critical windows to address sleep insufficiency as a potential strategy to mitigate long-term health risks. Study limitations include self-reported sleep duration, relatively short follow-up periods, and uncertain temporality between cumulative exposure to short sleep and the onset of MetS. Future research should investigate whether sleep-targeted interventions during and after pregnancy can improve cardiometabolic health, particularly among vulnerable populations.

Support

Support for the NuMoM2b study was provided by grant funding from the National Heart, Lung, and Blood Institute (R01-HL105549; U10-HL119991; U10-HL119989; U10-HL120034; U10-HL119990; U10-HL120006; U10-HL119992; U10-HL120019; U10-HL119993; U10-HL120018, and U01-HL145358) and the Eunice Kennedy Shriver National Institute of Child Health and Human Development (U10-HD063036; U10-HD063072; U10-HD063047; U10-HD063037; U10-HD063041; U10-HD063020; U10-HD063046; U10-HD063048; and U10-HD063053). In addition, support was provided by Clinical and Translational Science Institutes (UL1-TR001108 and UL1-TR000153), the Claude D. Pepper Older Americans Independence Center at Northwestern University Feinberg School of Medicine (P30-AG059988), and the National Institute on Aging (K23-AG088497-01), with supplemental support to U10-HL119991 from the Office of Research on Women's Health and the Office of Disease Prevention.

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Impact of a Circadian Intervention on Sleep in People with Habitual Short Sleep Duration

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Full Name and Credentials

Audrey Stegman, PhD Student

Introduction (100 word limit)

Habitual short sleep duration (HSSD) is associated with cardiometabolic disease risk. We have shown sleep loss increases late-night exposure to electronic light, thereby inducing circadian misalignment. Moreover, worse circadian misalignment is associated with greater impairments in insulin sensitivity during sleep loss, suggesting circadian misalignment is a potential mechanism contributing to the adverse cardiometabolic risk of HSSD. However, there is limited research on how circadian-based lighting interventions may influence sleep and insulin sensitivity in people with HSSD living outside the laboratory. Thus, we investigated the impact of a randomized controlled circadian intervention in people with overweight/obesity and HSSD.

Methods (200 word limit)

Data collection is ongoing. To date, 8 participants (7 male; aged 31.9±8.7yr; BMI 29.5±2.3kg/m^2 [mean±SD]) with habitual sleep <6.5h/night have completed data collection. The protocol consists of a 1-week baseline segment where participants maintain their HSSD followed by an 8-week intervention segment in which they are randomized (1:1) to healthy lifestyle control or circadian intervention groups. The circadian intervention consists of using a light-box and/or light emitting glasses for 1 hour in the morning immediately after waking and wearing blue-light blocking glasses for 4 hours prior to bedtime. Additionally, participants in the circadian intervention are instructed to stop all food consumption 4 hours prior to bedtime. Matsuda Index insulin sensitivity is measured by oral glucose tolerance testing (OGTT) at the end of baseline and intervention segments. Sleep is continuously monitored by wrist-actigraphy. Dim-light melatonin onset (DLMO) and offset (DLMOff) are quantified during 24-hour overnight laboratory visits at the end of baseline and intervention segments.

Results (200 word limit)

Data collection is ongoing. Thus far, 5 participants were randomized to the healthy lifestyle control group and 3 participants were randomized to the circadian intervention group. Preliminary analyses show the change in total sleep time (TST), bedtime, waketime, regularity, and midpoint of sleep are not significantly different between groups. Changes for the intervention group between baseline and the final two weeks of intervention are as follows: TST = +41 min *(P=0.27)*, bedtime = 19 min later *(P=0.074)*, waketime = 66 min later *(P=0.14)*, regularity [Standard Deviation of TST] = + 13 min *(P=0.59)*, and midpoint of sleep = 42.5 min later *(P=0.10)*. Data analyses for insulin sensitivity and DLMO and DLMOff are in progress.

Conclusions (100 word limit)

Preliminary analyses using 8 of 22 planned participants from our ongoing study indicate no significant changes in sleep. However, if the directional changes for currently observed trends in the intervention group are sustained with our full sample, it could suggest the circadian intervention impacts sleep timing and duration. Additional analyses we will report in the full sample include insulin sensitivity, DLMO and DLMOff, and food intake timing. Our sample mostly includes males at this time though we will be recruiting equal numbers of men and women. Due to these factors, definitive conclusions cannot be made at this time.

Support

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Integrating Sleep Diaries and Actigraphy Data to Characterise Sleep and Rest-Activity Rhythms in 22q11.2 Deletion Syndrome

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Full Name and Credentials

Abiola Saka

Introduction (100 word limit)

Our goal is to develop longitudinal metrics of sleep and circadian rhythms able to inform precision psychiatry. 22q11.2 Deletion Syndrome (22q11.2DS) is strongly associated with neurodevelopmental and psychiatric outcomes, including schizophrenia. Sleep disruption is common in children and young people with 22q11.2DS and may serve as an early prognostic indicator and/or therapeutic target. However, the acceptability, validity and interrelationships between standard sleep and circadian measures have not been characterized in this at-risk population. We therefore compared actigraphy and sleep diary-based inferences of sleep timing, quantity and quality in young people with 22q11.2DS and their unaffected siblings.

Methods (200 word limit)

8-20 year old participants from the “Experiences of CHildren with cOpy number variants (ECHO)” cohort included 29 probands with 22q11.2DS and 22 unaffected siblings (Probands: 17F/12M, Siblings: 10F/12M). All participants wore Philips ActiWatches and kept sleep diaries for 14 days/nights. Only participants with <5h of missing actigraphy data per day/night and at least seven days of consecutive sleep records (actigraphy and sleep diary entries) were included.

Time in Bed (TiB), Sleep Onset Latency (SOL), and Sleep Efficiency/Quality (SE/Q) were derived from both actigraphy and diaries, and their concordance evaluated using modified Bland Altman plots and Linear Mixed Models (LMM). Total Sleep Time (TST) and Wake After Sleep Onset (WASO) were equally derived from Actigraphy alone.

Singular Spectrum Analysis of actigraphy was used to derive circadian proxies based on Rest-Activity Rhythms (RAR), including MESOR, acrophase and amplitude. LMM and Random Forest Regressor assessed associations between RAR and sleep measures, validated using SHapley Additive exPlanations (SHAP) analysis. Finally, K-means clustering identified clusters of participants - based on their sleep patterns and RAR metrics signatures. Between-group differences were based on Mann-Whitney U tests and the False Discovery Rate (FDR) technique used to correct for multiple testing.

Results (200 word limit)

Actigraphy/diaries showed moderate concordance between global measures of TiB – with consistent underestimation by actigraphy and higher variance in probands. Non-significant proportional bias indicated relative stability across groups and methods. SOL had the least reliability/concordance due to high variability (up to 103% deviation from mean SOL) and the SQ showed poor consistency as SE/SQ values increased - especially among probands (proportional bias slope: -1.59).

Acrophase correlated positively with TiB (r = 0.22) and TST (r = 0.21), while MESOR negatively correlated with TiB (r = 0.13). Age and MESOR contributed negatively to TiB and TST, while Acrophase had a positive effect. Interaction effects showed that siblings were less impacted by MESOR's and Acrophase's influences on TiB. WASO, TIB, Acrophase, TST, and age were the key predictors of SQ. (R^2 = 0.26).

K-means identified two clusters (Silhouette score: 0.56): Cluster 0 had better SE, WASO, and SOL, while Cluster 1 showed better values for TiB, TST, MESOR, and Acrophase. Cluster 0 participants were older (13-20 years) compared to Cluster 1 (7-15 years). Eight (age: 13.2 ± 3.2) of the 14 families, with both proband and sibling(s), were assigned to the same cluster.

Conclusions (100 word limit)

Our findings show moderate agreement between actigraphy and sleep diaries only for TiB. Therefore, caution should be taken when comparing actigraphy/diary metrics, particularly in probands. Significant interactions between RAR metrics (acrophase & MESOR) and sleep outcomes highlight the importance of setting sleep measures in their circadian context. Age proved an important covariate of sleep quality and patterns in this population. This emphasises the need for longitudinal studies to distinguish between the developmental and genetic influences of sleep in young adolescents with 22q11.2DS – a pivotal step towards precision psychiatry.

Support

N/A

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TDP-43 sleep disturbances are driven by peripheral metabolic dysfunction and modified by Sik3 knockdown in Drosophila

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University of Pennsylvania, Philadelphia, USA

Full Name and Credentials

Anyara Rodriguez, B.A

Introduction (100 word limit)

Sleep disturbances are associated with neurodegenerative disorders, but underlying mechanisms are largely unknown. We previously identified sleep disruptions connected to brain metabolic signaling in a *Drosophila* model of trans-activation response element (TAR) DNA-binding protein 43 (TDP43) proteinopathy. Knockdown of *Atx2* attenuated the sleep phenotype and rescued deficits in brain glycogen metabolism. These findings suggest sleep disturbances in TDP-43 might result from metabolic impairments.

Methods (200 word limit)

Building on these initial findings, we characterized the TDP43 body metabolic profile by measuring glucose, glycogen, and triglyceride levels in the fly bodies, assessing metabolic rate, and are performing RNA sequencing. Additionally, we performed a large-scale RNAi screen (~1000 candidates) for genetic modifiers of TDP43 toxicity, in which we used sleep as a behavioral output.

Results (200 word limit)

We observe a dramatic reduction in body glucose, glycogen and triglyceride levels in TDP43 flies, in contrast to the glycogen increase in brain. Respirometry experiments confirm deficits in carbohydrate metabolism. These findings suggest alterations in glucose metabolism, leading us to test modulation of dietary glucose. Consistent with previous work showing lifespan and locomotor rescue through a high-glucose diet, our results indicate that a carbohydrate-rich diet restores sleep deficits. To test directionality of the sleep-metabolic relationship, we induced sleep and found this was insufficient to normalize metabolic phenotypes, suggesting metabolic alterations drive sleep disruption. An RNAi-based genetic modifier screen in TDP43-expressing flies identified SIK3 as a candidate for regulating sleep and metabolic dysfunction in the setting of TDP43. SIK3 knockdown restored sleep deficits and rescued body metabolic measures. Ongoing efforts aim identify the specific pathways of carbohydrate metabolism upon which SIK3 acts to improve sleep.

Conclusions (100 word limit)

TDP-43 pathology has been linked to dysregulation of glycogen metabolism but how this connects to disease pathology remains unclear. Our findings suggest peripheral metabolic disturbances are a key factor in sleep abnormalities associated with TDP-43, and that metabolic targets represent a novel therapeutic avenue.

Unexpectedly, SIK3 – a conserved sleep regulatory gene – is a modifier of sleep alterations in TDP-43, implicating convergent glycogen signaling pathways as a bridge between sleep and neurodegenerative disease.

Support

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Sleep and diurnal alternative polyadenylation sites associated with human APA-linked brain disorders

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Full Name and Credentials

Jason Gerstner, PhD

Introduction (100 word limit)

Sleep and circadian rhythm disruption are comorbid in many pathologies, and can negatively influence health conditions, including neurodegenerative disease, metabolic illness, cancer, and various neurological disorders. Genetic association studies linking sleep and circadian disturbances with disease susceptibility have mainly focused on changes in gene expression due to mutations, such as single-nucleotide polymorphisms. The interaction between sleep and/or circadian rhythms with the use of Alternative Polyadenylation (APA) has been largely undescribed, particularly in the context of other disorders. APA is a process that generates various transcript isoforms of the same gene affecting its mRNA translation, stability, localization, and subsequent function.

Methods (200 word limit)

Male Long Evans rats (7–9 weeks old, n = 5/group) were housed in pairs at 22 ± 2°C on a 12:12 h light-dark cycle with water and chow *ad libitum,* and sampled every 4 h, beginning 2 h after light onset (zeitgeber time (ZT)) (i.e., ZT2, 6, 10, 14, 18, and ZT22), following 6 h sleep deprivation (SD) from ZT0–6, (automated stir bar, Pinnacle), and recovery: 2 h (R2), 4 h (R4), or 8 h (R8) along with time-matched controls. Brain tissue was collected following guillotine under dim red light, flash frozen in 2-methylbutane suspended in dry ice, and then stored at -80°C until homogenization for RNA extraction. Whole transcriptome termini site (WTTS)-seq libraries were prepared, processed reads were aligned to the Rattus norvegicus genome (mRatBN7.2/rn7) by the torrent mapping program (TMAP, v3.4.1). Poly(A) sites (PAS) within 25 nucleotides from one another were grouped in one PAS cluster (PAC) using GetPolyaSiteCluster. MetaCycle R package (meta2d) identified circadian oscillations ($p<0.05$), and WebGestalt was used for Gene ontology (GO) and Pathway analysis ($p<0.05$). DESeq-2 with "Apeglm" Shrinkage and Wald Test were used to generate test statistics (R), FDRtool was used to determine the Local FDR.

Results (200 word limit)

We identified 31,757 PAS clusters, with 26,635 PASs that mapped to named gene loci, and 45% mapped to genes with ≥ 2 APA sites. 2,011 PASs were diurnal, of which 1,173 were in genes with ≥ 2 total APA sites, including known diurnal transcripts, such as *Dbp*, *Nr1d2*, *Per2*, and *Ntrk2*. GO and pathway analysis of each significant circadian group identified phase 18 with the most over-representation of multiple signaling pathways,

including 'neuron to neuron synapse' and 'post-synaptic specialization.' Of the 1,502 PASs that cycled with a 12 h ultradian period, 1,198 were in genes, with 827 having ≥ 2 APAs, representing 778 unique genes. Pathway analysis showed enrichment of CREB phosphorylation and circadian entrainment, while GO analysis of this data set resulted in 16 GO terms related to the synapse. Following sleep deprivation, the most significant differences in APA usage were seen immediately after (R0) and following 4 hours of recovery (R4). A *Homer1a* APA isoform was the most abundant at R0, R4 and ZT6, whereas a full-length isoform was dominant at ZT10. We also identified 54 APAs representing 46 genes that were observed in common with genes having recently described APA-linked human brain disorder susceptibility, including neurodegenerative disease and neurological disorders.

Conclusions (100 word limit)

This is the first unbiased discovery-based report of novel APA usage over time-of-day or changes in sleep pressure. Our data leverage a call to action to elucidate the core mechanisms of PAS usage in mammalian brain and to examine the capacity of APA to affect the transcriptomes and proteomes that regulate central nervous system processes altered by circadian rhythms and sleep/wake homeostasis. As PAS usage varies across brain region and cell types, these hypothesis-generating data provide an impetus for future research to delineate the influence of sleep and clock mechanisms on mental health and neurodegenerative disease.

Support

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48

Variant-to-function analyses define highly conserved regulatory elements at the *MEIS1* locus for insomnia and identify a role for *MEIS1* in sleep maintenance.

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Full Name and Credentials

Amber Zimmerman, PhD

Introduction (100 word limit)

The *MEIS1* locus is one of the strongest genetic associations to insomnia. However, there is debate to whether the *MEIS1* association with insomnia is independent of the association with restless leg syndrome (RLS) and/or periodic leg movements during sleep (PLMS) in the same genomic region. Much focus is directed toward the association signal within intron 8 of *MEIS1* harboring an enhancer driving expression during early development of the ganglionic eminence. We therefore sought to fully dissect the *MEIS1* locus through a human cell-based variant-to-gene mapping strategy paired with cross-species phenotyping and single cell analysis to understand this genetic association.

Methods (200 word limit)

We first queried genome-wide significant SNPs identified from the Genome Wide Association Study for insomnia performed by Jansen *et. al.* in 2019, and then analyzed linkage disequilibrium to define insomnia SNPs that were independent ($r^2 < 0.1$) from those of the RLS or PLMS associations. We applied our human cell-based datasets consisting of ATAC-seq, promoter-focused Capture C, and RNA-seq, to implicate putatively causal insomnia variants and corresponding effector genes at the *MEIS1* locus. Using CRISPR/Cas9, we selectively mutated critical functional sequences of the orthologous *MEIS1* genes in zebrafish, *meis1a* and *meis1b*, and measured sleep-wake behaviors with automated video tracking. We analyzed public single cell data gene expression data from human and zebrafish brain across different developmental stages to identify conserved patterns of gene expression and then applied *in situ* hybridization chain reaction (HCR) on wholemount larval zebrafish to examine *meis1* expression during brain development.

Results (200 word limit)

We executed an extensive characterization of the *MEIS1* locus, identifying an independent, insomnia-only, putatively causal variant in the promoter region of *MEIS1* harbored within accessible chromatin and forming a chromatin contact loop with multiple highly conserved distal regulatory elements. This variant also colocalizes with an expression quantitative trait locus impacting expression in the cerebellum. Loss of *meis1b* in zebrafish produced a phenotype highly similar to human insomnia, marked by an increase in sleep bouts during the night and an increase in latency to sleep onset, with no alterations in motor function or arousal. Single cell expression

analysis paired with HCR in larval zebrafish brains revealed *meis1b* is expressed in cerebellar granule cells and their progenitors during early development in a pattern highly similar to the human *MEIS1* gene.

Conclusions (100 word limit)

Taken together, our data has defined an insomnia association at the *MEIS1* locus that is independent of underlying genetic risk for RLS or PLMS. While the intron 8 association involves an enhancer expressed in the ganglionic eminence that gives rise to the basal ganglia, our data suggest that the insomnia-only association is acting in the developing cerebellum and controls sleep-wake transitions during the night. Thus, we propose a novel role for the *MEIS1* locus in its contribution to insomnia pathogenesis.

Support

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Disruption of circadian rhythms in Myotonic Dystrophy Type I (DM1), a multi-systemic microsatellite repeat expansion disease

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Full Name and Credentials

Belinda Pinto

Introduction (100 word limit)

Myotonic Dystrophy Type 1 (DM1) is caused by the expansion of CTG repeats in the 3' UTR of the *DMPK* gene. Expression of these expanded repeats sequesters the Muscleblind-like (MBNL) splicing factor into nuclear foci resulting in global splicing misregulation caused by a loss of MBNL function. While DM1 was identified as a muscular dystrophy, it is actually a neuromuscular disease with patients exhibiting a range of CNS symptoms including hypersomnolence and sleep dysregulation. As defects in the circadian system could contribute to these symptoms, we are investigating whether circadian rhythms are affected in the context of the DM1 mutation.

Methods (200 word limit)

We are examining the effect of the DM1 causing expanded CTG repeats on the circadian system through studies in Drosophila and mouse models of the disease. To study the effects of the DM1 mutation on circadian activity rhythms we have performed behavioral analyses in both model systems. We are extending these analyses to examine how the circadian system is affected at cellular and molecular levels through a combination of western and immunostaining analyses, bioluminescence imaging of tissues expressing a circadian reporter and transcriptomic analyses.

Results (200 word limit)

The Drosophila DM1 model, where expanded CTG repeats are expressed specifically in the pacemaker neurons, displays weaker activity rhythms with an ~1 hour longer period. Western and immunostaining analyses of key clock proteins show that this phenotype is associated with delayed decrease in PERIOD (PER) levels. Genetic studies with PER phosphorylation mutants suggest that repeat expression affects PER degradation kinetics through effects on the stabilization and degradation of PER. Interestingly, these effects are not mediated through loss of MUSCLEBLIND (MBL) function, suggesting that expanded CTG repeats affect the circadian clock through a novel mechanism independent of the loss of function of MBL.

Similar to the Drosophila model, the *DMPK 480 CTG knockin* mouse model of DM1 also displays a change in the period of circadian activity. To determine the basis of this phenotype, we will examine whether PER2:LUCIFERASE reporter rhythms are affected in the central pacemaker, the suprachiasmatic nucleus. In addition, we will examine whether molecular oscillations are affected in the peripheral choroid plexus tissue clock as nuclear foci are most prominent in this tissue in the *DMPK knockin* CNS. We will extend these studies to examine how the transcriptomes of the tissue clocks are affected in this DM1 model.

Conclusions (100 word limit)

Taken together, our data show that circadian rhythms are disrupted in DM1 through direct effects on the circadian clock. Future studies will provide key insights into how circadian disruption contributes to disease pathology in DM and other repeat expansion diseases. In addition, these systems can potentially serve as a platform for the development of drugs targeting DM-linked hypersomnia.

Support

MDF Pilot grant

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Effects of Sleep Deprivation, Recovery, and Time-of-Day on the Astrocyte Proteome

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Full Name and Credentials

Andrew Brown

Introduction (100 word limit)

Sleep is regulated by circadian and homeostatic processes. Though circadian regulatory elements are comparatively well understood, the cellular and molecular mechanisms that homeostatically balance sleep and wakefulness are less defined. We recently showed astrocytes respond dynamically to sleep, wake, and sleep loss, and disrupting astrocyte intracellular signaling impairs the brain's ability to encode and accumulate sleep need. To better define the relationship between astrocyte signaling and sleep homeostasis, we conducted proteomic analyses of cortical astrocytes from rested, sleep deprived, and recovered mice.

Methods (200 word limit)

Male mice (8-weeks-old) were split into sleep deprived (SD; n=36) and homecage control (HC; n=33) groups. SD mice were sleep deprived for 6 hours starting at light onset (i.e., Zeitgeber time (ZT) 0) using gentle handling and then either sacrificed immediately (SD6, n=18) without opportunity for sleep or after 3 hours of recovery sleep (RS3, n=18) and brains collected. HC mice were left undisturbed but similarly sacrificed at ZT6 (HC6; n=18) or ZT9 (HC9; n=15) to serve as circadian controls. Samples were pooled, producing 5-6 biological replicates per condition, and cortical astrocytes were isolated from each sample using a magnetic bead-based approach. Samples were then subjected to untargeted ultra performance liquid chromatography tandem mass spectrometry to quantify astroglial protein expression. We used removal of unwanted variation (RUV) analysis (k=8) to normalize data and identify proteins differentially expressed between groups (adj. p-value=0.1). Pathway analysis was then used to group differentially expressed proteins (DEPs) by function using gene ontology terms from the NIH's DAVID database.

Results (200 word limit)

Mass spectrometry detected 6,011 total astroglial proteins that we filtered to 2,552 proteins that were detected in all samples with a minimum intensity threshold of 50,000. Only 7 proteins were differentially expressed immediately after sleep deprivation (i.e., SD6) relative to HC6. These proteins were upregulated after sleep deprivation, and most were associated with transcription and protein binding. After recovery sleep (i.e., RS3), however, we identified 289 and 999 DEPs compared HC9 and SD6, respectively. We found that recovery sleep upregulated proteins involved in mRNA processing and chromatin composition, but downregulated proteins involved in cell structure, trafficking, and metabolism. By contrast, time-of-day had an opposite effect on astroglial metabolism, upregulating metabolic proteins at ZT9 (i.e., HC9) compared to ZT6 (i.e., HC6), and proteins associated with translation and protein degradation were downregulated as determined by 89 identified DEPs.

Conclusions (100 word limit)

We showed recovery from sleep deprivation has a greater impact on the astroglial proteome than sleep deprivation itself. This finding suggests astrocytes contribute to the homeostatic processes that rebalance sleep and wakefulness, and astroglial proteins associated with transcription, trafficking, cell structure, and metabolism are associated with this homeostatic recovery. Circadian processes impact astroglial metabolism as well, but in an opposite direction from homeostatic processes. Now, we are defining a role for the astroglial phosphoproteome in these homeostatic and circadian processes, and we identified high-confidence candidate proteins to further uncover a mechanistic role for astrocytes in sleep homeostasis.

Support

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Afternoon exercise attenuates impaired insulin sensitivity associated with insufficient sleep

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Full Name and Credentials

Grissy Simé-Mora

Introduction (100 word limit)

1 in 3 US adults report insufficient sleep, which is associated with negative metabolic health outcomes such as impaired insulin sensitivity, resulting in an increased risk for type 2 diabetes. Physical activity can improve insulin sensitivity although, it is unknown whether it can attenuate the effects of insufficient sleep on metabolic impairments. Therefore, the aim of this study was to test the hypothesis that prior moderate physical activity status will attenuate the impairment of insulin sensitivity due to insufficient sleep that occurs in sedentary individuals.

Methods (200 word limit)

Eleven active participants (6F, 23.5±1.0y, BMI:22.1±0.7 kg/m^2, VO_2max:48.2±2.3 ml/kg/min, mean±SD) and 11 sedentary participants (6F, 24.9 ± 1.3y, BMI:22.3±0.5 kg/m^2, VO_2max:41.5±6.6 ml/kg/min) were recruited to participate in a controlled 5-day inpatient protocol. Activity status was defined by ACSM physical activity guidelines. Participants were admitted to the Sleep and Chronobiology Laboratory at the University of Colorado Boulder and were given one night of baseline sleep (9h time in bed, BL) followed by 4 nights of insufficient sleep (IS). IS was achieved by delaying bedtime by 4 hours without a change to habitual wake-time, allowing for 5 hours of sleep opportunity. Oral glucose tolerance tests (OGTT) were performed on day-2 (BL) and day-5 (IS). Active participants continued to exercise 8.5 hours after wake each day during IS by conducting 60 minutes of treadmill running at 65% of maximum heart rate (HR), as assessed by a VO_2max during screening and confirmed by HR monitors during exercise. Sedentary participants performed sedentary activities (e.g. reading). Statistical analyses included linear mixed models to assess changes in insulin sensitivity, glucose, and insulin from BL to IS, with group (active vs. sedentary) as fixed and subjects as random effects.

Results (200 word limit)

Insulin sensitivity, as assessed by the Matsuda Insulin Sensitivity Index, was significantly reduced during IS compared to BL in sedentary participants (14.4±2.6 (BL) vs. 9.7±1.3 (IS); p=0.005), whereas no differences were observed in active participants between conditions (11.8±1.6 (BL) vs. 10.0±1.2 (IS); p=0.332). Glucose area under the curve (AUC) was not significantly different between conditions in either group (p>0.05 for both). IS led to an increase in insulin AUC in sedentary participants as compared to BL (p=0.0081); whereas no differences were detected in active participants (p= 0.09).

Conclusions (100 word limit)

Consistent with previous studies, insufficient sleep results in decreased insulin sensitivity in sedentary participants. In contrast, active participants did not experience a significant reduction in insulin sensitivity as a result of insufficient sleep. As 33% of the US population reports insufficient sleep, it is critical to identify effective strategies to mitigate the risks of metabolic diseases associated with sleep restriction. Our results indicate that physical activity may mitigate some of the metabolic consequences of insufficient sleep.

Support

This work was supported by the Sleep Research Society Foundation Early Career Development Award, the National Institute of Heart, Lung and Blood (NHLBI) R01HL132150 to KPW, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) K01DK110138 to JLB, Society in Science—The Branco Weiss Fellowship, administered by the ETH Zürich.

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Moon effect on human sleep patterns, evidence in rural and highly urbanized populations

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Full Name and Credentials

Guadalupe Rodríguez Ferrante

Introduction (100 word limit)

Although popular belief associates moon phases with changes in human behavior, scientific evidence of this influence has been elusive and contradictory. Recently, we established an association between moon phases and sleep timing/duration in Toba/Qom communities (Formosa, Argentina) with restricted access to electric lighting. Participants start sleeping later and sleep less during the days before the full moon, when moonlight is available during the first hours of the night. Here, we longitudinally study lunar influence on human sleep in both Toba/Qom communities and residents of Seattle, a highly urbanized area.

Methods (200 word limit)

Participants from Toba/Qom communities (n=101) and Seattle (n=112) wore an actigraphy watch and completed sleep diaries for 24-60 days (at least 80% of a lunar month). Data collection was carried out throughout different years (2016-2024 for Toba/Qoms and 2020-2024 for Seattle) and seasons. Daily moon phase data was calculated using the R package 'suncalc'.

We studied the association between sleep onset and duration with the synodic lunar and semilunar cycles. We used the GLMMcosinor R package, which allowed us to fit a mixed (including random effects) cosinor model. We fitted two types of cosinor models: with a period of either 30 days or 30+15 days. The models included 'type of day' (weekend or weekday) 'time of data collection' (defined by year and season) as predictors, and 'participant id' as a random factor. At the individual level, we used non-linear models to fit a sinusoid with a 30-day period or combination of sinusoids (30-day plus 15-day period).

Results (200 word limit)

We observed that sleep onset and duration presented an approximately 30-day period rhythmicity at the individual levels in both groups. For most individuals (>85%), the oscillations in sleep variables were better explained by a model that considers not one, but two periods: of 30 and 15 days. The variation in sleep onset and duration throughout the lunar month ranged between a few minutes and more than 90 minutes depending on the individual.

At the population level, this association was present in most years and seasons (i.e., the amplitude of the fitted cosinor is significantly different from zero), but not in all of them. Sleep duration varied ~35 min for Toba/Qoms and 20-15min for Seattle. Sleep onset changed between 6 and more than 35min depending on when data was collected. Interestingly, the phase of these rhythms changed with the time (year and season) of data collection,

but the acrophase was always close to either the full or the new moon. Moreover, when the time of data collection coincided for the two communities (e.g. during the spring of 2024), the phases of the sinusoids were similar in both of them.

Conclusions (100 word limit)

Although limited by the correlational nature of our approach, our results further support the idea that the moon cycle affects human sleep patterns. It remains unclear whether this is associated with the luminance of the moon or other geophysical variables associated with the moon cycle. However, the fact that we also observed this association between the moon and sleep in a highly urbanized community suggests that other variables other than moonlight are involved in this phenomenon. We are currently working to further characterize this association, both at the behavioral and physiological levels, using sleep lab studies.

Support

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Tryptophan Effects on Down Syndrome: Sleep, Behavior, Learning, and Memory in the Ts65Dn mouse model

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Stanford, Stanford, USA

Full Name and Credentials

Jessie Ong

Introduction (100 word limit)

Down syndrome (DS) is the current most common chromosomal condition that affects 6 million people worldwide (1). DS is characterized by the triplication of chromosome 21 which causes sleep, learning, memory, and behavior impairments. DS disrupts tryptophan (TRP) metabolism and results in TRP deficiency (2). We hypothesized that feeding DS mice (Ts65Dn) with a TRP diet would restore TRP deficiency and rescue associated deficits. Our results show that a TRP-enriched diet restored the sleep deficits, long and short-term memory impairments, and depressive-like behaviors of the Ts65Dn mouse. TRP supplementation is a potential therapeutic to rescue impairments associated with DS.

Methods (200 word limit)

Ts65Dn, a DS mouse model, and a disomic control (2N) were used for this study. Mice were instrumented with EEG/EMG electrodes to record sleep duration and quality. Prior to diet administration, mice were subjected to a battery of cognitive and behavioral tests to assess learning, memory, social behaviors, and depressive-like behaviors. These specific tests were selected because deficiencies in serotonin and melatonin in DS directly impact mood, cognition, and sleep.

Following baseline testing, mice were subjected to a TRP-enriched or controlled diet. Four weeks into the diet, mice underwent the same battery of tests and sleep recording to determine if the diet improved cognition, behavior, and/or sleep. Following this, we wanted to assess if TRP enrichment improved decision-making by subjecting mice to a Mouse Gambling Task. After testing, mice were euthanized, and samples were extracted for further TRP metabolites analysis.

Results (200 word limit)

Pre-Diet Results

TS = DS mouse, 2N = non-DS control

Figure 1. DS mice display decreased exploratory and social behaviors and increased depressive-like behaviors. (Left) TS show impaired exploration than 2N. **(Middle)** TS show impaired sociability and increased anxiety than 2N. (Right) TS show more depressive-like behavior than 2N.

Figure 2. DS mice display deficits in short-term spatial memory. TS show impaired short-term memory when than 2N.

Pre-vs-Post TRP Diet Results

Our preliminary data indicates that the TRP diet improves sleep quality and quantity in TS compared to the controlled diet (figures unfinished).

Figure 3. TRP enrichment improves long-term recognition and short-term spatial memory in DS mice. (Left) Long-term recognition memory in TS mice improve after 4 weeks of TRP Diet than pre-diet. **(Right)** Short-term memory in TS mice improved after 4 weeks of TRP Diet than pre-diet.

Figure 4. Tryptophan enrichment increases social and exploratory behaviors and decreases depressive-like behaviors in DS mice. **(Left)** Increased social behaviors and decreased anxiety of TS TRP mice than TS CTRL. (Middle) Less depressive-like behavior of TS mice after 4 weeks of TRP Diet than 2N. **(Right)** Less depressive-like behavior of TS mice after 4 weeks of TRP Diet than 2N.

Conclusions (100 word limit)

Ts65Dn mice demonstrate decreased sociability and short-term memory as well as increased depressive-like behavior. The TRP diet improves sleep quality and quantity, long & short-term memory, and depressive-like behaviors in TS. TRP supplementation is a potential non-invasive avenue to restore the sleep, cognitive, and behavioral deficits of DS.

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Support

The Heller Lab

Stanford BioX

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Chronic CRYPTOCHROME deficiency enhances cell-intrinsic antiviral defences.

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Full Name and Credentials

Dr Christine Major-Styles

Introduction (100 word limit)

The within-host environment changes over circadian time, influencing virus replication and severity. Cell-intrinsic antiviral responses are predominantly driven by type I/III interferon (IFN) signalling of >300 interferon stimulated genes (ISG), shaping subsequent inflammatory and adaptive immunity. Many IFN signalling components are IFN-inducible; constitutive secretion of IFN-b ensures homeostatic ISG expression that mediate viral recognition and signal transduction. Additionally, the integrated stress response (ISR) limits viral protein synthesis, restoring proteostasis. Genetic ablation of the circadian 'clock genes' CRYPTOCHROME 1/2 ($cry^{1-/-}cry2^{-/-}$;CKO) disrupts protein homeostasis leading to chronic ISR activation. Loss of proteostasis and stress could therefore impact upon cellular antiviral defences.

Methods (200 word limit)

We investigated the hypothesis that loss of proteostasis and accompanying stress in cells with genetic ablation of CRY (CKO) could impact upon cellular antiviral defence using *ex vivo* lung fibroblasts derived from WT and CKO mice. Analysis of differential gene expression of CKO and WT lung fibroblasts was undertaken using quantitative mass spectrometry. In addition, quantitative mass spectrometry was also used to analyse differential gene expression in cells with chronic CRY deficiency (CKO) compared to cells with transient CRY deficiency, achieved using a small molecule CRY degrader. This was further investigated in the context of influenza A infection through viral growth curve analysis in WT and CKO cells, alongside western blot analysis of antiviral protein expression. Pharmacological inhibition of components of pathways that underpin IFN and ISR signalling were investigated in both WT and CKO cells and protein expression analysed via western blotting.

Results (200 word limit)

We reveal that many viral recognition proteins and type I interferon effectors are significantly upregulated in primary lung fibroblast cells from CKO mice compared to wild type (WT) mice. This basal 'antiviral state' restricts the growth of influenza A virus (IAV) and is not governed by the loss of CRY proteins, but by interactions between the chronically activated proteotoxic stress response pathway and altered constitutive type I interferon phenotype identified in these chronically CRY-deficient cells. Transient CRY depletion showed CKO proteome composition and type I interferon signature was modestly partially phenocopied, but the magnitude of changes in antiviral protein expression was lower than observed in chronically CRY deficient CKO and was not sufficient to restrict IAV growth.

Conclusions (100 word limit)

Lung fibroblasts with genetic ablation of CRY (CKO) show upregulation of type I IFN effectors and antiviral proteins compared to WT cells, this phenotype requires constitutive type I IFN signalling via canonical TBK1 and JAK/STAT pathways. In addition to constitutive IFN signalling, the difference in the antiviral state of CKO compared to WT cells is underpinned by altered proteostasis and stress response pathways. Our results highlight the crosstalk between circadian rhythms, cell-intrinsic antiviral defences and protein homeostasis, providing a tractable *in vitro* model to investigate the interface of these key contributors to human health and disease.

Support

N/A

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Circadian alignment and sex-specific differences in inflammatory markers

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Full Name and Credentials

Brooke Shafer, PhD

Introduction (100 word limit)

The misalignment between behaviors (i.e., eating/sleeping) and the circadian timing system affects inflammatory mechanisms and increases the risk of cardiovascular disease (CVD). Given sex differences in circadian biology, however, it is unclear if circadian alignment influences inflammatory pathways in a sex-specific manner, particularly in individuals with overweight/obesity who are more vulnerable to adverse health outcomes. Therefore, we sought to identify sex-specific associations between circadian alignment and markers of inflammation in females and males with overweight/obesity.

Methods (200 word limit)

Plasma C-reactive protein (CRP) and tumor necrosis factor-α (TNF-α) were assessed in 19 individuals (9 female) with overweight/obesity (body mass index [BMI] ≥25kg/m^2). Circadian alignment was determined via phase angle of entrainment, defined as the time difference between dim-light melatonin onset (DLMO; 3pg/ml threshold) and diary-determined sleep onset over 7-days. Narrow or wide phase angle groups (less or more time between DLMO and sleep onset, respectively) were based on the median split within this group. Within sex differences between phase angle groups were assessed using linear models. Pearson correlation analyses evaluated the relationships between phase angle and inflammatory markers.

Results (200 word limit)

No sex differences were observed (all p>0.26) for age (mean±SD, females vs. males, respectively; 33.8±7.1y vs. 38.0±10.6y), BMI (31.2±4.7kg/m^2 vs. 29.1±3.0kg/m^2), DLMO timing (19:41±1:05 vs. 19:18±1:06), or phase angle (3.2±1.5h vs. 2.9±1.2h). Within sex differences showed that CRP was higher in the narrow compared to wide phase angle group in females (5.2±2.2mg/L vs. 1.6±1.5mg/L; p=0.02) and TNF-α was higher in the narrow compared to high phase angle group in males (0.83±0.19pg/mL vs. 0.54±0.08pg/mL; p=0.03). Phase angle was negatively correlated with TNF-α in males (r=-0.67; p=0.04) and there was a trend for a negative association between phase angle and CRP in females (r=-0.62; p=0.07).

Conclusions (100 word limit)

Alignment of the circadian system (i.e., smaller phase angle of entrainment) may be one contributing factor to inflammatory mechanisms involved in circadian disruption-related CVD pathology, particularly in individuals with overweight/obesity. The mechanistic role of circadian alignment on inflammation may be differentially affected by sex and obesity, which could have implications for targeted interventions to prevent CVD.

Support

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A role for NREM sleep in cortical response to stress

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Full Name and Credentials

Eva-Jenee Andrews, BA

Introduction (100 word limit)

Interactions with the environment and sleep-wake history influence both the quality and quantity of sleep. Negative social encounters are especially capable of having major influences on sleep and behavior. Animals, including humans, can exhibit resilience to these negative social encounters. Recent evidence from our lab demonstrates that non-rapid eye movement (NREM) sleep in the ventromedial prefrontal cortex (vmPFC) is necessary and sufficient to confer resilience to the negative effects of social-defeat stress. Based on this evidence, we hypothesize that resilience to social defeat stress is determined by neuronal dynamics during NREM sleep within the vmPFC.

Methods (200 word limit)

We characterized the behavior of vmPFC neurons in socially defeated mice using deep, multichannel electrodes. We performed an established social defeat paradigm on mice with channelrhodopsin-2/EYFP fusion protein expressed in excitatory cortical neurons (CaMKIIα-Cre x Ai32). Daily, 5 – minute, defeats were repeated for five consecutive days. We recorded multi-unit activity (MUA), local field potentials and EEG and EMG recordings, in vmPFC neurons continuously throughout the defeat paradigm.

Results (200 word limit)

We found significant differences in the firing patterns of vmPFC neurons between mice resilient or susceptible to social stress. Resilient mice had more off-periods (periods with no neuronal firing) during post-stress NREM sleep, when compared to susceptible mice. Neurons of resilient mice also fired at lower frequencies during NREM sleep, before social defeat stress. In contrast, neurons of susceptible mice fired at a significantly higher rate than resilient mice; this was at frequencies much higher than typically observed in the EEG during NREM sleep. Furthermore, firing rates varied little in resilient mice, whereas susceptible mice showed high variations in firing rate and two distinct neuronal populations. In addition, the percentage of cells contributing to NREM frequencies was higher in resilient mice. These results identify a relationship between the firing of vmPFC neurons during NREM sleep and resilience to stress.

Conclusions (100 word limit)

Higher synchrony in neuronal firing after social defeat stress suggests that cortical synchrony during NREM sleep may be important for resilience. Furthermore, before stress most cortical neurons in resilient mice were firing at low frequencies during NREM sleep; at frequencies typical for NREM sleep in EEG. In contrast, the neurons of susceptible mice fired at higher, and a large range of, frequencies. These findings suggest that during NREM

sleep the number of cortical neurons firing synchronously, and low frequencies, can be highly variable in the vmPFC. Furthermore, higher synchrony and lower firing rates within cortical cells is associated with resilience.

Support

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Association of Objective Sleep Measures and White Matter Limbic System Integrity within and between Community-Dwelling Cognitively Unimpaired Older Black and White Adults

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Full Name and Credentials

Joshua L. Gills, Ph.D.

Introduction (100 word limit)

Microstructure impairments of the limbic tracts are seen in early stages of the Alzheimer's disease (AD) continuum. Subjective sleep disruptions have been linked with reduction in white matter structure. Moreover, Black/African Americans experience are often more sleep deprived and experience reduced sleep times compared to non-Hispanic White Americans. Additionally, sleep disruptions differ between sex and have been linked with AD. However, specific objective sleep mechanisms contributing to white matter microstructure decline has not been investigated within and between Black/African Americans and Non-Hispanic White Americans.

Methods (200 word limit)

This cross-sectional study included 170 community-dwelling cognitively unimpaired older adults (mean±SD: age=67.2±5.2y) participating in NYU studies of sleep, aging, and memory. Subjects completed polysomnography (NPSG) and brain magnetic resonance imaging (MRI). Sleep measures of interest included total sleep time (TST), NREM stages 2, 3 and REM sleep duration, fragmentation, and AHI4% and REM AHI4%. Microstructural properties of the cingulum, uncinate fasciculus (UF), and fornix were estimated using diffusional tensor imaging (DTI) metrics including radial kurtosis (RK), radial diffusivity (RD), and fractional anisotropy (FA). Subjects were relatively matched on AHI4%, age, BMI and educational level. Linear mixed-effects regression models examined associations between sex*sleep variables and DTI metrics. Models were adjusted for age, race, education, BMI, time between NPSG and MRI.

Results (200 word limit)

Participants were 71.2% female, 44.1% Black/African-Americans(B/AA). In the cingulum, increased fragmentation was associated with higher RD(β[Left]=0.0026, *p*=0.022), in B/AA. In the UF, increased fragmentation was associated with higher RD(β[Left] =0.0052, *p*=0.019) in B/AA; increased AHI4%REM was associated with lower RK (β[AHI4%, Left] =-0.0029, *p*=0.001) in B/AA. In the cingulum of white participants, higher AHI4%REM was associated with reduced FA β[right] =-0.0006, *p*=0.013), reduced RK β[Right]=-0.00198, *p*=0.008). In the UF, increased fragmentation was associated with reduced RK β[Left] =-0.017, *p*=0.047) in Whites. In the cingulum, females showed increased fragmentation associated with higher RD(β[fragmentation

Right] =0.0021[0.00087], *p*=0.016. Females demonstrated higher AHI4% being associated with higher RD (β [AHI4% left] =0.0006[0.00036], *p*=0.029); and, reduced REM duration was associated with higher RD (β [REM left]=-0.0016[0.00077], *p*=0.042). In the UF, females showed greater fragmentation being related to higher RD (β[Fragmentation Left]=0.0045[0.00173], *p*=0.0097). Higher AHI4% was related to higher RD (β[AHI4% Right]=0.00072[0.00030], *p*=0.015) in women; but in men lower RK β[AHI4% Right]=-0.00161[0.00080],*p*=0.046).

Conclusions (100 word limit)

Cognitively unimpaired Black and White older-adults showed sleep disruption associations with limbic white matter integrity, with race-specific associations observed across various microstructural properties of the cingulum, UF, and fornix. Moreover, sex-specific sleep disruption associations were observed with limbic white matter tract alterations within the limbic system. Thus, highlighting possible sleep related underpinnings of racial and sex differences in Alzheimer's disease risk.

Support

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61

A reverse genetic screen in dopaminergic neurons to identify molecular mediators of sleep maturation in Drosophila melanogaster

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Full Name and Credentials

Hayle Kim

Introduction (100 word limit)

Across the animal kingdom, young animals sleep more, and more deeply, than adults, suggesting a privileged role for sleep in early life. Indeed, early life sleep is essential for the dramatic brain and behavior development that occurs during this time. However, the molecular mechanisms that regulate juvenile sleep, and the maturation of sleep characteristics over time, remain almost entirely unknown. A juvenile sleep state is conserved in the genetically tractable *Drosophila melanogaster*, affording us the opportunity to identify the molecular and circuit-level control of juvenile sleep.

Methods (200 word limit)

The arousal-promoting neuromodulator dopamine (DA) plays a conserved role in regulating the juvenile sleep state. In *Drosophila*, high sleep pressure in juvenile adults (1 day after eclosion) is maintained by low DA neuron activity. During the first week of adult life, DA tone increases, leading to reduced sleep duration in mature adults (~7 days after eclosion). Importantly, the endogenous molecular regulators of DA neuron activity in early life remain entirely unknown. In order to identify the genes that regulate early-life sleep by acting in DA neurons, we identified differentially expressed genes (DEGs) between juvenile and mature DA neurons. DA neurons from 0-1 day-old and 6-9 day-old flies were fluorescently labeled, sorted from mechanically dissociated brains using fluorescence activated cell sorting, and subjected to bulk RNA sequencing. To complement this approach, we utilized published single-cell RNA sequencing data from adult brains to identify DA neuron clusters at these two timepoints. Using a 2-fold difference cut-off, and focusing on the most highly expressed genes, we identified >400 DEGs. Using high throughput activity monitors, we used DA neuron-specific transgenic RNA interference to screen for abnormal sleep in mature and juvenile adults.

Results (200 word limit)

To enrich for genes regulating DA neuron activity and not general health, we focused on RNAi conditions that caused short sleep. The initial high-throughput screen in mature adults identified hits in two assembly factors for mitochondrial complexes (MC) I and II, *ndufAF4* and *sdhAF4,* respectively. Both genes are essential for assembling MCI and MCII, entry points for electrons into the OXPHOS pathway of ATP synthesis. Higher sensitivity sleep assays indicated a robust role for MCI in regulating sleep in DA neurons. Depletion of other MCI subunits phenocopied Th>*ndufAF4* IR flies. Disruption of MCI function did not cause locomotor defects at these early timepoints, arguing against premature neurodegeneration. To test if the short sleep phenotype is caused by loss of ATP, we depleted a component of ATP synthase in DA neurons and did not observe the same phenotype. MCI dysfunction is associated with reactive oxygen species (ROS) production, leading to oxidative damage of dopaminergic neurons which drives DA neurodegeneration. Interestingly, we also identified loss of

superoxide dismutase 2—a ROS scavenger in mitochondria—as a sleep-regulating gene in DA neurons. These results suggest that DA neurons maintain control over ROS production to limit their activity and promote normal sleep.

Conclusions (100 word limit)

Understanding the molecular control of dopaminergic functional maturation in sleep has immense potential for treating neuropsychiatric disorders associated with early-life sleep disruptions. Evidence in humans and other vertebrates support a role for MCI in maintaining DA neuron function during aging, but our findings suggest an early—possibly developmental—requirement for MCI activity in *restraining* DA neuron output, perhaps by limiting ROS production through electron leak. A strong short-sleeping phenotype, in the absence of locomotor defects, could suggest that MCI dysfunction in DA neurons first manifests as a sleep disorder before degenerative processes emerge.

Support

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Feasibility of Closed-Loop Thermoregulatory Sleep Enhancement in Mice

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Full Name and Credentials

Diane Iradukunda

Introduction (100 word limit)

There is great interest in sleep enhancement based on noninvasive sensory stimulation in humans and animal models. Exposing mice to thermoneutral temperatures significantly enhances both NREM and REM sleep, which offers a simple means for titrating sleep and pathophysiology. However, mouse sleep is polyphasic with bouts of activity, in which they prefer cooler temperatures, even during their primary sleep period. Most previous thermal assays of mouse sleep were conducted at static temperatures. Here, we investigate the feasibility of thermoneutral exposure exclusively during bouts of sleep and investigate the effects on sleep architecture and composition in normal and epileptic mice.

Methods (200 word limit)

With institutional approval, C57BL/6 mice (10 female, 4 with verified epilepsy; 4 males) all 7-9 months were surgically instrumented for EEG/EMG monitoring and subjected to thermal sleep manipulation in a chamber equipped with infrared ceramic heating lamps. In a week-long experiment, each mouse underwent two days of acclimation, a two-day baseline recording, and three days of closed-loop (CL) treatment, during which cage temperature was elevated from ambient (22°C) to thermoneutral (30°C) only when sleep was detected for over a minute using a piezoelectric motion sensor; sustained wakefulness for over a minute returned the temperature to 22°C. A yoked control (YC) animal experienced temperature changes in sync with the CL animal, but without regard to sleep-wake state, while a SHAM control remained at ambient temperature the entire week. Each animal underwent SHAM, CL, and YC treatment in separate weeks. Sleep metrics, including percent time and mean bout duration (MBD) of Total Sleep, NREM, and REM states, were computed based on automated EEG/EMG sleep scoring. Changes (Δ) in these metrics were calculated by subtracting the baseline day means from treatment days. Repeated measures ANOVA was conducted to compare the CL, YC, and SHAM conditions.

Results (200 word limit)

A repeated measures ANOVA showed that selectively increasing cage temperature at sleep onset led to significant differences between the three groups in overall ΔSleep% ($p<0.001$), ΔNREM% ($p<0.001$), ΔSleep MBD ($p<0.001$), and ΔNREM MBD ($p=0.002$), primarily during the light period (LP), for all mice combined.

Specifically, CL had a significantly greater ΔSleep% and ΔNREM% compared to both YC ($p<0.001$) and SHAM ($p<0.001$) in the LP. There were no significant differences in ΔREM%. For MBD, CL showed significantly greater ΔSleep MBD compared to YC ($p=0.002$) and SHAM ($p<0.001$), as well as in ΔNREM MBD compared to YC ($p=0.009$) and SHAM ($p=0.003$), all during the LP. In contrast, the only significant change observed in the dark period (DP) was a decrease in ΔREM MBD in the CL group compared to SHAM ($p=0.013$).

Unlike the differences seen above for Δ (each animal relative to its own pre-treatment baseline), no significant differences were seen in the raw metrics between the groups except for a significantly greater NREM% for CL compared to YC ($p=0.046$) in the LP. The positive effects on Sleep and NREM were mirrored in the epileptic mice, but due to the small sample size ($n=4$) further experiments are needed to determine significance.

Conclusions (100 word limit)

This study demonstrates that a simple CL thermoregulatory protocol can be used to manipulate and enhance sleep in mice. CL treatment increased total sleep and NREM sleep duration, with less fragmented sleep bouts and more stable REM-NREM cycles during the LP, an indication of improved sleep quality. The greater effect in CL compared to YC indicates that accounting for state-dependent thermal preferences is critical for optimizing sleep. This protocol holds promise as a preclinical assay of the effects of sleep titration in neurodegenerative disorders such as epilepsy.

Support

N/A

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Investigation of EEG slow wave recovery following sleep disruption in a mouse model of Alzheimer's disease

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Gabriella Morillo Segovia

Introduction (100 word limit)

Sleep is vital for brain health, but is often disrupted in Alzheimer's disease (AD) by sleep fragmentation (SF)—multiple interruptions during sleep—which accelerates amyloid beta (Aβ) accumulation in the brain and reduces slow-wave activity (SWA) during non-rapid-eye-movement (NREM) sleep. While previous studies show that SF similarly increases hippocampal Aβ in AD mouse models, the effects on sleep architecture—particularly the consolidation of SWA in the EEG—are poorly characterized. Here, we employ electroencephalography (EEG) and electromyography (EMG) to investigate these effects using an SF protocol that mimics the sleep disruptions frequently observed in AD patients.

Methods (200 word limit)

A study was conducted on ten 8-month-old male APP/PS1-KI mice, genetically predisposed to amyloid plaque formation, by surgically instrumenting them for EEG and EMG monitoring. After recovery, mice were acclimated to cages equipped with piezoelectric motion sensors to supplement EEG/EMG recordings.

After a one-week baseline recording, the mice were divided evenly into two groups, one exposed to an SF protocol and the other to Undisturbed Sleep (US) as a control for four weeks. The SF protocol involved four one-hour blocks of gentle sensory stimulation during the 12-hour light phase (the inactive period), each separated by undisturbed intervals of 1.5 hours. EEG/EMG recordings continued throughout the study.

Power spectral analysis was performed to extract delta (0.5–4 Hz), theta (6-9 Hz), and broadband (0.5-80 Hz) EEG power. Sleep stages (wakefulness, NREM, and REM sleep) were scored from these spectral features using Gaussian Mixture Models. The effects of SF on SWA were compared for SF and US groups in terms of delta band EEG power during NREM within SF blocks and the subsequent undisturbed post-SF intervals. After the five-week study, mice were euthanized, and their brains harvested for future immunohistochemical analysis of amyloid pathology.

Results (200 word limit)

Trends in NREM sleep were monitored over the five-week period across the 12h:12h light:dark cycle. As expected, NREM percent decreased during SF blocks but rebounded immediately afterward. Regression analysis showed that SF mice had increasing levels of post-SF NREM recovery from weeks 2 to 5 of treatment. Moreover, SF mice had consistently higher NREM during the dark phase throughout treatment compared to US.

To assess the effect of SF on SWA, we measured sleep efficiency (SE), defined as the ratio of SWA power to time spent in NREM in a 30-minute interval, during SF blocks and the post-SF recovery periods. SE was consistently higher for SF mice during SF blocks. Despite a decreasing trend in SE over treatment weeks, the SF group sustained higher SE levels than the US group over the course of each day. No significant differences between groups were found in SE during the post-SF recovery periods.

Conclusions (100 word limit)

This study demonstrates that SF in an AD mouse model leads to immediate reductions in NREM sleep during disruption periods, followed by significant rebound during subsequent undisturbed intervals. SF mice showed increasing levels of post-SF NREM recovery over four treatment weeks. Notably, despite reduced NREM during SF blocks, elevated SE led to the finding that mice managed “catnaps” with enhanced SWA. Thus, mice adapt to SF by consolidating SWA during brief sleep bouts, potentially mitigating some negative effects of SF on disease progression. Ongoing IHC analyses may provide further insight into the relationship between SWA, sleep efficiency, and amyloid pathology.

Support

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The effects of chronic sleep restriction on the hypothalamic-pituitary-adrenal (HPA) axis with and without a history of exposure to opioids

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Full Name and Credentials

Carol A. Everson, PhD

Introduction (100 word limit)

The hypothalamic-pituitary-adrenal (HPA) axis is an essential homeostatic system controlling a wide array of life processes (e.g., fuel management and adaptive responses to both physiological and psychogenic stresses). Chronic sleep restriction results in abnormalities of the HPA axis that are poorly understood. We employed dynamic tests of pituitary and adrenal function in rats to determine the effects of chronic sleep restriction, studied alone (as a state of disrupted homeostasis) and as a potential cause of co-morbid HPA axis abnormalities during abstinence from opioid drug use, both of which are implicated in relapse.

Methods (200 word limit)

Male and female rats underwent chronic sleep restriction or control conditions, and prior opioid vs saline treatments (2x2x2 design; 8 rats per group). Rats were trained by operant conditioning for a sucrose reward and then self-administered either oxycodone or saline for two weeks after which they were abstinent for the remainder of the study. During abstinence, the rats were either chronically sleep restricted (SR) or allowed to sleep *ad libitum* (ambulation controls, AC) for five weeks to permit phenotypes to manifest. Chronic SR was produced by restricting sleep by 35% for 5-day cycles with 2-day periods of sleep *ad libitum* between cycles to model repeated exposure. Pituitary and adrenal function were evaluated at the end of the five-week abstinence period by CRH and by dexamethasone-ACTH (DEX-ACTH) stimulation testing. The hypothalamic peptide, CRH, stimulates pituitary ACTH release. Dexamethasone suppresses endogenous ACTH and corticosterone. Exogenous ACTH during dexamethasone suppression is used to measure adrenal sensitivity to ACTH. Blood samples were obtained before and at 15, 30 and 60 min after CRH or ACTH administration. Food and water intake, and body weight were recorded for seven days at the time of hormone testing. Adrenal weights were measured at the end of the study.

Results (200 word limit)

Adrenal weights did not differ within sex by treatment or by opioid history. In male rats, chronic SR decreased basal corticosterone concentrations to <70% of male AC rats ($P \leq 0.006$), an observation not found in female SR rats, and regardless of opioid history. In male SR rats, the ACTH response to CRH was augmented compared to male AC rats [assessed by peak ACTH concentrations and area under the curve (both $P<0.002$)]. Suppression of ACTH by dexamethasone was attenuated in SR rats of both sexes. In SR females, the corticosterone peak response to CRH-stimulated ACTH occurred earlier compared to AC females, regardless of opioid history (both $P<0.05$). DEX-ACTH testing demonstrated sexual dimorphism: 2.3-fold higher corticosterone response in females compared with males ($P<0.001$). There was no effect of SR on the corticosterone response to exogenous ACTH during dexamethasone suppression There was no effect of prior opioid exposure in any of the major findings.

Male and female SR rats increased food consumption relative to body weight compared to same-sex groups, regardless of opioid history.

Conclusions (100 word limit)

In SR males, pituitary sensitivities to both glucocorticoid negative feedback and hypothalamic stimulation were increased. In SR females, adrenal sensitivity, which is a marker of stress reactivity, was increased. Changes in the HPA axis occurred in opioid exposed rats only if sleep restricted, indicating that SR can cause HPA axis abnormalities observed during abstinence. The results indicate that chronic SR modifies the HPA axis in sexually dimorphic ways that are expected to mediate responses to both disrupted homeostasis and psychogenic stress. The extent to which chronic SR impacts sexual dimorphism in drug taking or risk for relapse is yet undetermined.

Support

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Reversible assemblies of CLOCK and circadian resilience to stress

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Full Name and Credentials

Sarah Ferraro, Ph.D.

Introduction (100 word limit)

Circadian dysfunction strongly contributes to cardiovascular, metabolic, and neuropsychiatric disorders, suggesting that the circadian timekeeper plays a critical role in the maintenance of cellular homeostasis. While circadian clocks anticipate physiological need, they also buffer unpredictable environmental perturbations, pointing to compensation as a central feature of the circadian system. However, the mechanistic underpinnings underlying this environmental compensation remain unknown.

Methods (200 word limit)

In response to various environmental stressors, certain proteins can engage in the formation of biomolecular condensates (BMCs, e.g., stress granules and P bodies). These BMCs are highly enriched for proteins with intrinsically disordered regions (IDRs). We utilized *in silico* prediction tools that identify that the C-terminus of CLOCK is largely disordered, and encompasses an evolutionarily conserved prion-like domain (PrD) and coiled-coil domain (CCD). We hypothesized that CLOCK'S IDR acts as an environmental sensor by providing the structural framework to form BMCs in response to cellular stressors. We synchronized U2OS cells (100uM dexamethasone) and exposed them to baseline or heat-shock conditions (a classic stressor known to promote BMC formation) (42C for 2 hours) at ZT 12 and 24. These cells were fixed, immunostained for endogenous CLOCK, and imaged using confocal microscopy to determine a time-of-day response to BMC formation with and without stress. Similarly, we engineered HaloTag-CLOCK variants with and without it's predicted IDR, PrD, or CCD for visualization and biochemistry purposes, and were subsequently expressed in U2OS cells. These cells were also exposed to baseline or heat-shock conditions, fixed, and imaged to determine the contribution of CLOCK's structural domains to its biophysical response to environmental stressors.

Results (200 word limit)

We have found that the core circadian protein CLOCK forms ***reversible, rhythmic BMCs*** in the cytoplasm that demonstrate time-of-day responsivity to stress. We have mapped this behavior to the large, evolutionarily conserved IDR, which encloses the coiled-coil domain required for timekeeping. This domain is essential for CLOCK's condensation. On the other hand, an abutting prion-like domain is required for the resolution of these structures and is essential for the recovery of circadian timekeeping after stress. Thus, we have identified a biochemical and biophysical mechanism intrinsic to a core circadian regulator that senses and compensates for environmental perturbation.

Conclusions (100 word limit)

Remarkably, both the functions and protein structure of CLOCK are similar to many disease-associated neurodegenerative proteins like huntingin, TDP-43, and FUS. We propose that loss of prion-like resilience is a key mediator for the loss of circadian function that accompanies physiological aging and neurodegenerative disease.

Support

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Parafacial GABAergic Neurons Modulate Anaesthetic-Induced Hypnosis but Not Sleep-Wake State

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Full Name and Credentials

Toshihiro Imamura, MD

Introduction (100 word limit)

General anesthesia is a cornerstone of modern medicine, yet the mechanisms by which anesthetics induce unconsciousness remain unclear. While some anesthetics are known to activate neural circuits associated with non-rapid-eye-movement (NREM) sleep, distinctions between natural sleep and anesthetic-induced unconsciousness persist. This study investigates the role of GABAergic neurons in the parafacial zone (PZ^{GABA}) of the brainstem, a region implicated in NREM sleep, to assess their role in modulating anesthetic sensitivity and explore whether PZ^{GABA} contributes differently to anesthesia compared to natural sleep-wake regulation.

Methods (200 word limit)

We used Vgat-IRES-Cre;Ai6 mice, which express a fluorescent reporter protein in a Cre-dependent manner. To evaluate if a hypnotic dose of anesthetics activates PZ^{GABA} neurons, mice were exposed to either 1.2% isoflurane or 100% oxygen. C-Fos immunohistochemistry was performed to assess neuronal activation. The righting reflex assessment was performed to study anesthetic sensitivity. Mice were transfected with an AAV to drive either fluorescent reporter protein expression or caspase-mediated irreversible lesioning of PZ^{GABA} neurons. In a separate cohort, EEG and EMG recordings were used to evaluate sleep-wake architecture and recovery sleep following 6-hour sleep deprivation to assess homeostatic sleep response. Statistical analyses included unpaired t-tests for comparing neuron density and sleep architecture, as well as two-way ANOVA for assessing anesthetic sensitivity over time. Pearson's correlation was used to analyze the relationship between PZ^{GABA} neuron density and anesthetic resistance.

Results (200 word limit)

Isoflurane exposure significantly increased c-Fos expression in PZ^{GABA} neurons by 3.6-fold compared to controls ($P=0.002$), indicating activation during anesthetic-induced hypnosis. Partial lesioning of PZ^{GABA} neurons, resulting in a 27% remaining neuron population, produced a robust increase in resistance to isoflurane ($P=0.0021$) and sevoflurane ($P=0.04$). Notably, the degree of resistance to isoflurane was inversely proportional to PZ^{GABA} neuron density ($R^2=0.4114$, $P<0.0001$), supporting the role of these neurons in anesthetic sensitivity. Contrary to expectations, PZ^{GABA} lesioning did not significantly alter sleep-wake states: lesioned mice showed comparable durations of NREM, REM sleep, and wakefulness to controls, with no changes in state transitions or bout structure. Furthermore, the homeostatic response to sleep deprivation, as indicated by delta power during recovery sleep, was unaffected in lesioned mice ($P=0.59$). These findings suggest that PZ^{GABA} neurons contribute specifically to anesthetic sensitivity without influencing natural sleep-wake regulation or homeostatic responses.

Conclusions (100 word limit)

Our study reveals an unexpected dissociation between mechanisms regulating anesthesia and sleep. While PZ^{GABA} neurons play a crucial role in modulating anesthetic sensitivity, they appear dispensable for sleep-wake architecture and homeostatic regulation. This finding challenges the prevailing assumption that sleep-promoting neural circuits are uniformly engaged by anesthetics to induce unconsciousness, highlighting a distinct role for PZ^{GABA} neurons in pharmacologically induced hypnosis. These insights enhance our understanding of the neural circuitry involved in unconsciousness regulation, emphasizing the need to distinguish between the neurophysiological underpinnings of anesthesia and natural sleep.

Support

This work was supported by funding from the National Institutes of Health, R35 GM1511166 (TI), T32 HL007713 (TI), T32 GM112596 (AZW), P01 HL160471 (AIP), R01 GM088156 (MBK), R01 GM151556 (MBK) as well as from the American Thoracic Society, ASPIRE Fellowship (TI).

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Exploring the Association Between Sleep Metrics and Nocturnal Blood Pressure in Individuals with Hypertension

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Full Name and Credentials

Joshua Landvatter

Introduction (100 word limit)

Sleep disturbances are common in hypertensive individuals, worsening cardiovascular outcomes. However, less research exists on how objective and self-reported sleep measures interact with blood pressure (BP) variability and asleep BP, such as BP drops within hypertensive populations. This study investigates associations between sleep metrics and sleep BP phenotypes and variability in individuals with hypertension. Research on sleep disturbances, sleep BP, and sleep BP variability is essential because these factors significantly impact cardiovascular health and mortality in this at-risk population.

Methods (200 word limit)

This cross-sectional study examined how sleep metrics relate to sleep BP phenotypes in 119 adults with elevated BP, assessed through office BP readings (SBP>120 and or a DBP>80). Participants completed 7 days of actigraphy and one night of 24-hour ambulatory BP monitoring. Sleep variables from Actiware included sleep efficiency (SE), total sleep time, wake after sleep onset (WASO), and sleep onset latency (SOL), alongside self-reported insomnia severity (ISI), PROMIS sleep disturbance, and impairment. BP metrics from 24-hour monitoring included asleep systolic and diastolic BP, BP drop percentage calculated as (daytimeBP-nightimeBP/daytimeBP)100, and BP variability, defined as the average squared deviation of BP. Sleep hypertension was defined as SBP ≥ 120 mmHg or DBP ≥ 70 mmHg during sleep, and non-dipping BP as sleep BP reduction under 10% compared to awake. Simple linear regression was used to regress sleep metrics onto BP measures to assess associations between sleep metrics and asleep BP phenotypes, with and without covariates (age, race, sex). BP variability, calculated over 24 hours, was tested as a predictor of sleep outcomes to explore how BP fluctuations may disrupt sleep. This study aims to clarify the impact of BP variability and nocturnal patterns on sleep disturbances in this high-risk population.

Results (200 word limit)

Significant associations were found between ISI, SE, SOL, and asleep BP phenotypes. Higher ISI scores (indicating worse sleep) were associated with lower asleep systolic BP (b = -0.50, 95%CI[-0.98, -0.02], t(118) = -2.03, p = .044), suggesting a potential inverse relationship between sleep quality and nighttime SBP levels. Participants with higher sleep efficiency had attenuated drops in their asleep BP (b = -0.36, 95%CI[-0.70, -0.02] t(106) = -2.10, p = .038), while longer sleep onset latency was associated with lower asleep diastolic BP (b = 0.15, 95%CI[-0.29, -0.01], t(106) = -2.05, p = .043). Additionally, diastolic BP variability, calculated over a 24-hour period, was strongly associated with lower sleep efficiency (b = -0.02, 95%CI[-0.03, -0.003], t(113) = -2.33, p = .02), reinforcing the idea that BP variability, rather than nighttime BP drops, may interfere with sleep quality. Notably, ISI was the only subjective sleep metric that showed a significant relationship with BP phenotypes, while

PROMIS measures (sleep disturbance and impairment) were not associated with any BP patterns. These findings provide new insights into potential physiological mechanisms linking BP variability and sleep disturbances, highlighting the importance of monitoring BP variability in hypertensive populations.

Conclusions (100 word limit)

This study demonstrates associations between sleep metrics and nocturnal BP phenotypes in hypertensive individuals. Diastolic BP variability was linked to decreased sleep efficiency, highlighting the potential role of autonomic dysregulation in sleep disturbances. These findings suggest that BP variability, may impair sleep quality through disrupted autonomic regulation. Given the established link between sleep disturbances and cardiovascular risk, managing BP variability may be critical for improving both sleep and cardiovascular outcomes in hypertensive individuals. Future research may benefit by exploring interventions targeting autonomic regulation to reduce BP variability and its impact on sleep quality.

Support

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Linking DHEAS Diurnal Rhythms and Sleep: Evidence of Associations with Sleep Disturbances and Subjective Sleep Ratings

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Full Name and Credentials

Abigail Marne, MS

Introduction (100 word limit)

The hormone Dehydroepiandrosterone (DHEA), and its sulfate, DHEAS, has been proposed as a biomarker of cognition, cardiometabolic health, and life expectancy (Rutkowski et al., 2014). DHEAS declines in older age, and studies report that supplementary DHEA might improve overall sleep quality, and other metrics of well-being (Maninger et al., 2009). While other hormones, such as cortisol, have been linked with specific sleep quality outcomes, there is comparatively less work linking sleep quality to DHEAS levels and circadian variation. This study's aim was to investigate the pattern of association between total DHEAS and its diurnal fluctuations and specific sleep quality measures.

Methods (200 word limit)

Participants were recruited from the metro-Atlanta area (M_{age} = 50.3 years, SD = 19.33, range = 20-80, N = 183, 104 females) for a study investigating everyday stressors. Upon study entry, participants completed the Pittsburg Sleep Quality Index (PSQI); participants subsequently completed a modified PSQI immediately after waking for 10 consecutive days, which allowed for a Daily Subjective Sleep Quality rating that was then averaged across all days. Additionally, participants provided saliva samples seven times a day for 10 consecutive days: immediately after waking, 30 minutes after waking, then approximately every three hours until 21:00, for a total of 70 saliva measurements for each participant. The saliva samples were then assayed for Cortisol and DHEAS. Daily DHEAS levels were averaged across the 10 days to provide a Mean DHEAS value. Diurnal fluctuation of DHEAS, operationalized as the daily Peak-Nadir Difference averaged across all 10 days, was also extracted from the DHEAS data. Because Cortisol is known to interact with DHEAS synthesis, it was included in the analyses as a covariate.

Results (200 word limit)

Mean DHEAS and Component 5 of the PSQI (Sleep Disturbance) were significantly correlated ($p < 0.01$), such that fewer sleep disturbances were correlated with higher mean DHEAS levels. Hierarchical linear regression showed that Sleep Disturbances was a significant predictor of Mean DHEAS, and Model Comparison Approach and Likelihood Ratio Test further supported this (MCA: $p < 0.01$, LRT: $p < 0.01$). Additionally, Daily Subjective Sleep Quality and DHEAS Peak-Nadir Difference were positively correlated ($p < 0.05$) such that higher Daily Subjective Sleep Quality was associated with greater DHEAS Peak-Nadir Difference. The use of hierarchical regression in which DHEAS Peak-Nadir Difference predicted Daily Subjective Sleep Quality was found to be significant and Model Comparison Approach and Likelihood Ratio Test supported this finding (MCA: $p < 0.01$, LRT: $p < 0.01$).

Conclusions (100 word limit)

These results support previous literature that sleep quality is associated with mean/total DHEAS output as well as DHEAS diurnal rhythm. We have identified specific attributes of sleep quality (i.e. Sleep Disturbances) which predict Mean DHEAS and we have classified new diurnal dynamics (i.e. Diurnal Fluctuation) which parallel Daily Subjective Sleep Quality. These results offer more specific insight into the relationship between DHEAS and sleep quality and may inform the clinical implementation of DHEA.

Support

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Pre-sleep digital media use and its perceived impact on sleep quality in adults.

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Full Name and Credentials

Antonio Zadra, PhD

Introduction (100 word limit)

Digital media are becoming more interactive and ubiquitous in people's everyday lives. Given this increasingly complex media landscape, up-to-date assessments of pre-sleep media use habits are needed. Most studies in the field have focused on children and adolescents and typically on one type of media use (e.g., social media), without differentiating between different activities and devices. The present study aimed to document a wide range of pre-sleep media habits in a diverse international adult population while examining how these various pre-sleep media use profiles are associated with self-reported sleep quality and duration.

Methods (200 word limit)

Participants aged 18 years and older recruited nationally and internationally from the general population were invited to complete an online questionnaire comprising 42 items assessing demographic information, various media usages in the hour before bedtime over the past month, as well as items related to sleep quality and quantity.

Results (200 word limit)

A total of 722 participants (73% female, 54% Canadian, mean age = 37 years) provided completed surveys. The overwhelming majority of the sample (99%) reported using a digital device and engaging in media activities within an hour before going to sleep. The most commonly used device were smartphones, with 91% of participants reporting their usage, and among these, 56% reporting consistent usage. Browsing social media (83%) and watching movies or shows (79%) were the most popular media activities within an hour prior to going to sleep. Moreover, 74% of participants reported using media for longer than intended before going to sleep while 64% reported usually engaging with media while in bed. Approximately one third of participants reported that their pre-sleep media usage had strong negative effects on their sleep onset latency (34%) and/or their sleep quality (36%). Study findings highlight differences in media use habits across age groups and gender while hierarchical ordinal regressions reveal that specific media activities (e.g., interactive engagement) best predict sleep quality, sleep onset latency, and sleep duration, beyond mere device usage.

Conclusions (100 word limit)

This study provides a detailed and up-to-date picture of the frequency and range of pre-sleep media use habits among adults. Our results highlight how pre-sleep engagement in specific media activities explains some of the variance in people's self reported sleep quality and sleep duration beyond device usage alone while bringing nuances to previously reported relationships between digital media use and sleep.

Support

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72

The influence of APP-mediated intracellular signaling on sleep, cognition and the blood-brain barrier in Alzheimer's disease mouse model.

Clementine Puech PhD[1], Anjana Sadanand PhD[2], Neil Coleman[2], Mohammad Badran PhD[1], Rong Wang[2], David Gozal MD[3], Angele Parent PhD[2]

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Full Name and Credentials

Angele Parent, PhD

Introduction (100 word limit)

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive cognitive decline frequently associated with sleep disturbances. A bidirectional relationship between sleep disturbance and AD pathology has been proposed, but the underlying mechanisms are not fully understood. It has been suggested that the blood-brain barrier (BBB) breakdown may precede the emergence of cognitive decline and neurodegeneration. BBB dysfunction can reduce β-amyloid peptide (Aβ) clearance, a pathological hallmark of AD. We follow up on our previous studies to determine if brain expression of APP C-terminal fragment (APP-CTF) could modify the sleep pattern and the BBB integrity in an AD mouse model.

Methods (200 word limit)

Aβ is produced through the successive cleavage of APP and its membrane-bound APP-CTF. We previously reported that retention of APP-CTF at the membrane favored intracellular signaling that prevented Aβ generation and improved cognitive function in the AD mouse model. We investigated if the membrane-tethered APP intracellular domain (mAICD) and its $G\alpha_S$ associated signaling could modify the sleep pattern and the BBB integrity in the amyloidogenic 5XFAD mouse model. We expressed mAICD and an mAICD variant lacking the $G\alpha_S$ interacting site (mAICDmutAAA) constructs in the brain of 5XFAD mice through intracerebroventricular AAV delivery in neonates. The random aa membrane-tethered mCtl served as a control protein. Sleep patterns, cognitive behaviors, and BBB integrity were examined in 6-8 months male and female mice. The sleep/wake activity was monitored using a computerized piezoelectric system. The behavioral assessments encompassed the novel object recognition (NOR) test and the elevated-plus maze test (EPM). The BBB permeability was determined through fluorescence assessment in the brains of mice injected with the conjugated 3-5kDa dextran–FITC in the tail. GFAP immunostaining was performed in brain sections to examine changes in astrogliosis.

Results (200 word limit)

We found that sustained mAICD expression initiating APP signaling in the brain rescued the sleep impairment and the BBB leakage observed in the 5XFAD mouse model. A robust correlation was observed between the level of sleep and BBB permeability, paralleling changes in spatial memory performance. We observed that mAICD expression correlates with improvement of sleep and cognition. These positive outcomes were not detected in mice expressing mutAAA variant, suggesting a cAMP/PKA-dependent contribution of APP-CTF to that process.

5XFAD mice expressing mAICD had lower dextran accumulation in their brains than 5XFAD-mCtl ($p<0.0001$), similar to the level seen in NTg-mCtl. Expression of mutAAA did not reduce the dextran level in 5XFAD mice but produced significant augmentation in NTg mice, therefore suggesting that the $G\alpha_s$ interaction with mAICD might contribute to preserving the BBB integrity. We also observed that the sleep score correlated strongly with the BBB permeability in either male or female mice ($r=-0.671$, and $r=-0.665$, respectively). Further analysis between the sleep and the NOR scores indicated that female performances are strongly linked to a better sleep score ($r=0.600$, $p=0.0002$), an effect that was not observed in males ($r=-0.125$, $p=0.551$). We also found that APP-mediated signaling reduced astrogliosis in 5XFAD mice.

Conclusions (100 word limit)

Altogether, we established a critical role of APP-mediated signaling in regulating sleep and BBB integrity, which can impact memory function. Our results also demonstrated the significant contribution of APP-CTF in restoring sleep and memory loss in an AD mouse model.

Support

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Investigating the contribution of circadian rhythm disruption to hypersomnia in myotonic dystrophy type 1 (DM1)

Emily Davey[1], Belinda Pinto PhD[1], Valeria Sansone MD, PhD[2], Sub Subramony MD[3], Eric Wang PhD[1]

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Full Name and Credentials

Emily Davey

Introduction (100 word limit)

Myotonic dystrophy type 1 (DM1) is a multisystemic disease caused by a $(CTG)_n$ repeat expansion in the 3'UTR of *DMPK*. While DM1 is known as a form of muscular dystrophy, it is a neuromuscular disease characterized by numerous central nervous system symptoms, one of the most prevalent being hypersomnolence. One possible cause of hypersomnia is disrupted circadian rhythms and interestingly, circadian activity analyses of DM1 patients have shown that these individuals display a low amplitude rest-wake rhythm. However, the molecular mechanisms driving dysregulated activity rhythms are unknown.

Methods (200 word limit)

We aim to investigate the basis of circadian activity disruption in DM1 patients at a cellular and whole organism level. To determine whether the DM1 expanded CTG repeats affect the circadian clock itself, we will conduct cell-culture based assays where we examine how expression of 480 CTG repeats affects the oscillations of a PERIOD2::LUCIFERASE (PER2::LUC) fusion protein in U2OS cells. We will examine how the clock oscillations are affected using real-time bioluminescence recordings. We are extending these studies by similarly analyzing how circadian rhythms are affected in DM1 patient myoblasts expressing the PER2::LUC reporter. To understand the basis of the weak circadian activity rhythms in DM1 patients, we are investigating whether abnormal melatonin rhythms, a biomarker of human circadian rhythms, contribute to the defective activity rhythms. To address this, we are conducting an actigraphy based study of DM1 patients combined with the measurement of levels of the melatonin metabolite, 6-sulfatoxy melatonin (aMT6s), in urine of patients collected at 4-hour intervals over a 48-hour period. aMT6s levels will be measured with an ELISA assay.

Results (200 word limit)

We have generated a stable cell line by introducing 480 or 0 CTG repeats into a U2OS cell line expressing a PER2::LUC reporter. We have subsequently used real-time bioluminescence imaging to track circadian rhythms in these cell lines to test how the presence of this repeat expansion in DM1 patients may affect circadian activity rhythms. We have successfully measured circadian rhythms over the course of seven days and preliminary results suggest that cells expressing 480 CTG repeats exhibit a shorter period than those expressing 0 repeats. We have also introduced the PER2::LUC reporter into DM1 patient and unaffected control myoblasts and are presently measuring circadian rhythms in these stable cell lines to determine whether differences in period or amplitude are observed in DM1 patient cells as compared to cells from unaffected individuals. Furthermore, we

will also differentiate PER2::LUC DM1 patient and unaffected control myoblasts into myotubes and measure circadian rhythms, as DM1 patient myotubes generally exhibit more severe disease signatures than myoblasts. For the patient study, we are currently recruiting DM1 patients and unaffected individuals as controls at the University of Florida and in Milan through our collaborators.

Conclusions (100 word limit)

These studies will help to uncover whether circadian rhythms are disrupted in DM1 patients and whether this dysregulation is a direct result of the effect of the CTG repeat expansion on the core circadian clock mechanism. Our findings will be an important first step in understanding whether circadian disruption contributes to the hypersomnolence associated with DM1 and potentially other neuromuscular disorders.

Support

N/A

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Proteomic biomarkers for acute and chronic sleep debts in healthy adults

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Full Name and Credentials

Adrien Specht, PhD Student

Introduction (100 word limit)

Sleep is crucial for maintaining biological homeostasis, and sleep deficiency impairs cognitive functions as well as physiology. Despite extensive research on the health impacts of sleep loss, its effects on the human proteome remain underexplored. This study aims to investigate the proteomic changes associated with acute and chronic sleep loss using a model based on ligand-receptor interactation (taking adenosine, a sleep promoting agent, as example), and to explore if these changes can be used as an index of sleep debt. Understanding these proteomic alterations could reveal systemic effects of sleep loss and inform novel diagnostic and therapeutic strategies.

Methods (200 word limit)

We analyzed up to 7,000 proteins from more than 1,000 plasma samples collected in 56 adults who participated in controlled sleep studies, including total sleep deprivation, chronic sleep restriction, and constant routine conditions. Proteomic profiling was performed using SomaScan, enabling high-throughput quantification of proteins in plasma. Acute and chronic sleep loss were quantified from sleep-wake history using a receptor-ligand model, originally validated against psychomotor vigilance test (PVT) performance data. This model captures cognitive performance variations following sleep loss and recovery by accounting for changes in adenosine and A1 receptor concentrations; parameter values were sourced from existing literature. Proteomic data were analyzed using a linear mixed model with subject-specific random effects accounting for inter-individual variability, identifying proteins significantly associated with acute and/or chronic sleep loss. We performed pathway analysis on the significant proteins to determine the impacted biological processes. Additionally, a multivariate soft-DTW (Dynamic Time Warping) technique was used to visualize protein expression patterns relative to acute and chronic sleep loss, assessing temporal dynamics in proteomic changes. This approach identified potential biomarkers for sleep deficiency from single blood samples.

Results (200 word limit)

Our analysis found that 1,434 proteins (20% of the tested proteome) were significantly associated with acute, and 1,080 proteins (15%) with chronic sleep loss. Only 422 proteins were significant across both conditions, highlighting distinct proteomic profiles following acute and chronic sleep loss. Acute sleep loss primarily influenced extracellular matrix organization and anatomical structure development. In contrast, chronic sleep loss predominantly affected metabolic pathways, including digestion, and regulation of insulin-like growth factor (IGF) transport and uptake by IGF binding proteins. Comparing sleep and wake states as an on/off feature, our analysis further revealed 1,867 proteins with altered sleep-wake expression, with pathways involving organ development and multicellular organismal processes. Importantly, inter-individual variability was the largest

source of proteomic variation rather than sleep loss itself. This substantial subject-specific variability underscores the challenge of predicting fatigue directly from a single blood sample, emphasizing the need for personalized approaches in assessing sleep debt through proteomic profiling.

Conclusions (100 word limit)

Proteomic alterations associated with both acute and chronic sleep loss affect key biological processes, particularly in extracellular matrix organization, metabolic pathways, and hormone regulation. The limited overlap of impacted proteins between acute and chronic sleep loss highlights the importance of distinguishing between these two processes. The identified pathways provide insights into the systemic effects of sleep loss and underscore the importance of maintaining adequate sleep for physiological health.

Support

N/A

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Sleep exacerbates sepsis in mice, via a TLR2-dependent mechanism

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Full Name and Credentials

Wendy Walker

Introduction (100 word limit)

Sleep is frequently disrupted in the hospital setting, particularly within intensive care units where patients are at high risk of developing sepsis. Poor sleep in the hospital is also associated with worse outcomes. We undertook this study in mice to determine how sleep affects sepsis and elucidate the mechanism.

Methods (200 word limit)

We interrupted sleep in mice by housing them on an intermittent orbital shaker, with a binder clip attached to their cage for auditory stimulus. The apparatus was set to cycle on for 30 seconds, then off for 90 seconds, to repeatedly interrupt their sleep over 48h. Control animals were allowed to sleep normally. A third group of mice underwent a control procedure: they were housed on the intermittent orbital shaker during the nighttime only, when mice are normally awake. Subsequently, sepsis was induced via cecal ligation and puncture (CLP) and animal survival and disease score were measured. Blood samples were obtained to prepare serum and quantify leukocytes. Cytokines were measured by Legendplex bead assay and ELISA, and leukocytes were stained and analyzed via FACS. We utilized male and female C57BL/6 mice in our study. We also utilized TLR2-KO and TLR2-KIGFP animals to dissect the effects of Toll-like receptor 2.

Results (200 word limit)

We found that sleep interruption exacerbated sepsis in C57BL/6 mice. Animal subject to sleep interruption showed increased sepsis mortality rates and a higher disease score, in comparison to mice that slept normally or underwent the control procedure. This effect was significant in both males and females. Sleep interruption also altered the inflammatory network, resulting in higher levels of IL-23 before sepsis, and higher levels of TNF-a, MCP-1 and IL-10 after sepsis. Furthermore, sleep interruption amplified the increase in circulating blood T cell percentage in response to sepsis. Regarding the mechanism, ex vivo experiments suggest that peritoneal macrophages produced more cytokines in response to sepsis-causing bacteria after sleep interruption. In contrast to the C57BL/6 mice, TLR2-KO mice exhibited similar sepsis mortality rates after sleep interruption vs normal sleep, suggesting TLR2-dependent effects. Analysis of TLR2-KI-GFP mice revealed differential changes in TLR2 expression in peritoneal macrophages and B cells.

Conclusions (100 word limit)

We conclude that poor sleep exacerbates sepsis in mice, via a TLR2-dependent effect. Further research is needed to reveal the mechanisms at play, and how this translates to human sepsis patients. Ultimately, people

could benefit from strategies to optimize their sleep in the ICU, or therapies to mitigate the effects of poor sleep.

Support

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Characterizing EEG Spectral Dynamics Across Sleep Stage Transitions: Implications for Enhancing Sleep Quality and Stability

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Full Name and Credentials

Antony Passaro

Introduction (100 word limit)

Sleep involves distinct stages characterized by specific neural oscillations that reflect different levels of brain activity and consciousness. Understanding the spectral changes that occur during transitions between these stages is crucial for elucidating the mechanisms underlying sleep regulation and improving interventions targeting sleep disorders. This study focused on the spectral dynamics of EEG activity during transitions from wakefulness to N1, N1 to N2, N2 to N3, and N2 to REM sleep. The goal was to characterize the frequency-specific power changes associated with each transition and identify distinct patterns that could inform approaches to enhancing sleep quality and stability.

Methods (200 word limit)

Data were obtained from 1,137 participants in the Stanford Technology Analytics and Genomics in Sleep (STAGES) study. EEG recordings were down-sampled to 100 Hz for uniformity, and contiguous five-minute segments surrounding each sleep stage transition (wakefulness to N1, N1 to N2, N2 to N3, and N2 to REM) were selected for analysis. A fast Fourier transform (FFT) was applied to ten-second epochs every 10 milliseconds over the ten-minute segments (five minutes before and after the transition) for each participant, generating time-frequency power spectra from 0.1 to 50 Hz. The power spectra were normalized by dividing by the mean power across frequencies for each time step and subject. The resulting time-frequency plots were averaged across participants to visualize group-level spectral changes during each transition. Statistical analyses were performed to assess the significance of power changes across different frequency bands, and linear regressions were used to identify consistent spectral shifts during each sleep transition.

Results (200 word limit)

Significant spectral power changes were observed during each sleep stage transition. For the wakefulness to N1 transition, there was a marked decrease in beta (16-30 Hz) and gamma (30-50 Hz) power, indicating reduced high-frequency neural activity as sleep began. Concurrent increases in delta (0.1-4 Hz), theta (4-7 Hz), and sigma (13-15 Hz) power were observed, reflecting the initiation of sleep-related oscillatory activity. During the N1 to N2 transition, further decreases in beta and gamma power occurred, accompanied by growth in delta (0.1-4 Hz), theta (4-8 Hz), and more pronounced sigma activity, suggesting increased sleep stability. The N2 to N3 transition showed a slight reduction in high alpha (10-13 Hz) and sigma power, while low-frequency activity (0.1-0.4 Hz) increased, indicative of slow-wave activity characteristic of deep sleep. The N2 to REM transition exhibited relatively smaller power changes, with some increases in delta (0.7-6 Hz) and reductions in higher frequencies, indicating a nuanced reorganization of neural networks as the brain entered REM.

Conclusions (100 word limit)

The results provide a detailed characterization of spectral changes during sleep stage transitions, highlighting distinct frequency-specific patterns associated with each shift. Earlier transitions, such as wakefulness to N1 and N1 to N2, showed more pronounced spectral shifts compared to the subtle changes observed in transitions to deep sleep and REM. These findings suggest that different sleep stage transitions may involve distinct neural mechanisms. The identified spectral patterns offer potential targets for interventions, such as brain-computer interfaces or neuromodulation techniques, to enhance sleep quality and stability, emphasizing the importance of frequency-specific modulation in sleep-related therapeutic approaches.

Support

N/A

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Effects of a Physical Activity Intervention on Sleep Characteristics in Colorectal Cancer Survivors

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Colorado State University, Fort Collins, USA

Full Name and Credentials

Erin Kishman, PhD

Introduction (100 word limit)

Sleep disturbance is one of the most commonly reported side effects of cancer treatment. Results from previous studies suggest that physical activity may improve aspects of sleep in cancer patients, but findings have been mixed, possibly due to varied lengths of physical activity interventions or variability in when sleep was assessed during cancer treatment. Additionally, many of the previous studies have been in breast cancer patients and only measured subjective sleep quality. Therefore, the purpose of this study was to examine changes in subjectively and objectively measured sleep characteristics following a 12-week physical activity intervention in colorectal cancer survivors.

Methods (200 word limit)

This study is a secondary data analysis from a pilot randomized controlled trial. Twenty-six participants (16F, 61±11y, 28.6±5.7 kg/m^2; mean±SD) with non-metastatic colon or rectal cancer who had completed curative therapy within the previous 5 years participated in the study. The intervention group participated in twice weekly videoconference-delivered aerobic and resistance exercise sessions and physical activity behavior change discussion sessions. The control group received standard physical activity recommendations for cancer survivors. Sleep was measured at baseline and at 12-weeks by the Pittsburgh Sleep Quality Index (PSQI) and 7 days of actigraphy. Valid baseline data were available in 13 participants in each group, and in 10 and 9 participants at 12-weeks for exercise and control, respectively. Independent t-tests were used to examine baseline differences between groups. Linear mixed effects models were performed to examine changes in sleep characteristics.

Results (200 word limit)

At baseline, participants spent an average of 508.1 ± 49.4 minutes in bed (mean±SD), resulting in 432.7 ± 55.9 minutes of sleep per day, 85.5% ± 5.1% sleep efficiency, 14.6 ± 8.2 minutes of sleep onset latency, and 46.0 ± 22.8 minutes wake after sleep onset. There were no significant differences in objective sleep measures between groups. At baseline, PSQI global score was significantly higher in the intervention group (8.5 ± 4.5 vs 4.9 ± 3.6, p=0.028), suggesting worse subjective sleep quality.

The physical activity intervention increased daily light and moderate to vigorous physical activity levels by 57.4 and 5.1 minutes, respectively (p>0.05 for both) and resulted in a significant decrease in total sleep time (-26.6 ± 10.4 minutes; p=0.038 compared to baseline) without changes in wake after sleep onset or sleep efficiency. No significant changes in total sleep time were detected in the control group (+14.5 ± 7.8 minutes; p=0.10). There were no significant changes in PSQI global score for either group.

Conclusions (100 word limit)

Overall, participants slept more than the recommended 7 hours per night minimum and had >85% sleep efficiency. In contrast, wake after sleep onset and PSQI scores were indicative of poor sleep quality. Following a 12-week physical activity intervention, there was a significant decrease in total sleep time, but no significant changes in objective or subjective sleep quality. It is not clear if participants curtailed sleep to allow for physical activity to occur. Regardless, strategies are needed to improve sleep quality in cancer survivors.

Support

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Unveiling the Influence of Tau pathology on Circadian Activity

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University of South Florida, Tampa, USA

Full Name and Credentials

Emily Sandefur

Introduction (100 word limit)

Alzheimer's Disease (AD) patients are reported to suffer from circadian disruption, often before cognitive symptoms manifest. Additionally, phosphorylated tau and β-amyloid peptide (Aβ) accumulation, two pathological hallmarks of AD, display cyclical changes in the CSF depending on the time of the day. Hyperphosphorylated tau drives tau pathology progression. GSK3β, a major kinase associated with tau phosphorylation, also plays a role in photic entrainment in the SCN. Our laboratory reported that the amyloid precursor protein (APP) has a direct GαS protein interaction site, leading to downstream adenylate cyclase and PKA signaling that reduced GSK3β activation.

Methods (200 word limit)

We explored the effect of manipulating this signaling capability using AAV-delivered constructs equipped with amino-acid sequences for targeting proteins in the lipid raft, therefore allowing sustained signaling. We used a membrane-tethered APP intracellular domain (mAICD) construct, a mutated membrane-tethered APP intracellular domain that can no longer partake in the GαS signaling (mAICDmutAAA), and the membrane-tethered control protein (mCtl) to identify the effect of APP-mediated signaling in a tauopathy PS19 transgenic mouse model overexpressing human tau. To examine the impact of manipulating this signaling on circadian function, we injected the AAV into the suprachiasmatic nucleus of the hypothalamus (SCN), the brain's circadian control hub. We utilized actimetry behavior testing to evaluate activity over time in different lighting conditions to determine circadian rhythm functionality. In the first week of the experimentation, we exposed the mice to a normal 12h light period from 6AM to 6PM followed by 12h of darkness and measured the running wheel activity. The inter-daily stability and intra-daily variability were analyzed. In the second week, we adjusted the light cycle to begin 7h earlier to assess photic entrainment. We examined the internal clock activity measured in total darkness for the final week.

Results (200 word limit)

Our actimetry results detailing circadian rhythm function in normal 12h of light followed by 12h of darkness indicated hyperactivity in PS19 mice only during the dark period as compared to non-transgenic mice (NTg, P=0.001), which suggests a reduction in sleep during their active period. PS19 mice exhibited more inter-daily stability, improving consistency of their rhythm between days, and decreased intra-daily variability, therefore better sustained rhythmicity in their activity when compared to NTg littermates. We did not observe any significant changes in the total running wheel activity associated with mAICD or mAICDmutAAA variant expression in the SCN. During the phase advance, the PS19 mice showed more rapid photic entrainment, as they required fewer days to adjust to the new light cycle than the NTg littermates (P=0.009). Analysis of the daily

activity in total darkness revealed that the PS19 mice possessed a longer circadian period (P=0.027). Noteworthy, we observed that mAICD expression in the SCN further enhanced the length of the daily period in PS19 mice, suggesting that APP and its C-terminal fragment (APP-CTF) might contribute to extending the length period.

Conclusions (100 word limit)

The results suggest reduced sleep in a tauopathy mouse model during the active dark period. This period of hyperactivity was not affected by APP-CTF expression in the SCN, suggesting that the hyperactivity outcome is not mediated throughout an APP-circadian pathway. However, in constant darkness, we observed a longer circadian period in the PS19 mice. Expression of mAICD in the SCN enhanced this effect, which supports the idea that APP-CTF might participate in that process. In a phase-advanced condition, photic entrainment was also faster in the PS19 mice, suggesting downstream events associated with hyperphosphorylated tau might be contributing factors.

Support

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Slow-wave-sleep enhancement reduces seizure duration and shifts seizure timing in a mouse model of medial temporal lobe epilepsy

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University of California, Davis, Davis, USA

Full Name and Credentials

Danny Lasky, BS

Introduction (100 word limit)

Epilepsy has a well-established bidirectional relationship with sleep, whereby epileptiform activity disrupts sleep, and sleep deprivation precipitates seizures. Notably, the treatment of sleep apnea in epileptic patients improves seizure control. To date, no study has investigated the specific role of slow-wave-sleep (SWS) in epilepsy. Prolonged and robust SWS can be induced through chemogenetic activation of the GABAergic neurons in the medullary parafacial zone. For the first time, we will apply this novel SWS enhancement method in a mouse model of medial temporal lobe epilepsy and examine the impacts on sleep architecture, epileptiform activity, and cognition.

Methods (200 word limit)

Vgat-IRES-Cre C57BL/6J mice were injected with an AAV bilaterally in the parafacial zone to express excitatory hM3Dq receptors in the GABAergic neurons. After a two-week recovery, mice were implanted with a headplate to measure electrocorticography, hippocampal field potentials, and electromyography. Mice were singly housed and habituated to recording cables for a week and then recorded with continuous video-EEG and a 12-hour light/dark cycle. Temporal lobe epilepsy was induced using the intra-amygdala kainic acid model, associated with fragmented sleep, after one week of baseline recording.

Voluntary oral administration of jelly was used to deliver the hM3Dq receptor agonists. Mice were randomized to drug or vehicle jellies for a three-week treatment period before cross-over. Mice were presented with jelly daily at ZT 2, and the drug treatment cycled between the hM3Dq agonists of CNO, C21, and DCZ to reduce the non-specific effects of metabolite build-up.

Spike2 was used to score seizures and sleep using video/EEG recordings. Sleep was scored into 20-second epochs of wakefulness, SWS, REM sleep, seizure, the post-ictal state, and artifact. Preliminary statistical analysis was performed using Mann-Whitney U tests.

Results (200 word limit)

As observed in healthy animals, we have found that activating the GABAergic parafacial zone neurons in an epileptic mouse induces sustained and intense SWS, providing the first means to enhance SWS in an epileptic animal.

We next assessed how SWS enhancement affects epileptiform activity. The intensity, duration, and circadian timing of seizures were quantified across the recorded 22 days of epileptic baseline, 18 days of drug treatment,

and 18 days of vehicle treatment. The mouse had 1 seizure during epileptic baseline, 6 seizures during drug treatment, and 13 seizures during vehicle treatment, with the final Racine 5 seizure killing the mouse.

The intensity of the seizures was measured using Racine scoring and was not significantly different between drug and vehicle treatments (p=0.9631). Seizures were significantly shorter during drug treatment compared to vehicle treatment (p=0.0014). The circadian timing of seizures shifted significantly between drug and vehicle treatments (p = 0.0365). During drug treatment, only 1 of 6 seizures were during the lights-on period when sleep enhancement occurred, but during vehicle treatment, 9 of 13 seizures were during the lights-on period.

Conclusions (100 word limit)

This preliminary work in a single mouse demonstrates the first successful methodology for enhancing SWS in an epileptic animal. After ceasing SWS enhancement, seizure count and duration increased. Seizures also shifted to occur more during the lights-on period, in which SWS enhancement previously occurred, which did not appear to be fully accounted for by increased sleep during the lights-on period. Together, these results suggest that SWS improves seizure control, most notably in the immediately following hours. This novel method of sleep manipulation has further application in examining how SWS can reduce the burden of other comorbidities of epilepsy.

Support

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81

mTORC drives daily rhythms in mammalian physiology

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Full Name and Credentials

Aiwei Zeng

Introduction (100 word limit)

Circadian rhythms in physiology and behaviour exist at all levels of life, and are proposed to be driven by transcriptional-translational feedback loops involving a handful of 'clock genes/proteins'. How these gene expression loops result in temporal compartmentalisation of physiology is poorly understood. We asked whether daily organisation of physiology may instead be largely driven by post-translational regulatory mechanisms, such as the mTORC pathway.

Methods (200 word limit)

We used an oral pharmacological inhibitor of the mTORC pathway, INK128, to evaluate the extent of regulation of daily mouse physiology by the mTORC pathway. In all experiments, mice were entrained for at least 7 days, followed by at least 3 days of vehicle or 1 mg/kg INK128 in drinking water. Experiments were carried out under light-dark cycles.

First, we used quantitative mass spectrometry to explore the role of the mTORC pathway in regulating daily variation in the proteome and phosphoproteome of mouse brain and liver. Mice treated with vehicle or INK128 were culled every 6 hours (N=4) across one day, and mouse and liver were analysed using 18-plex tandem mass spectrometry. For rhythmicity analysis, BioDare eJTK Cycle was used with a cutoff of $q \leq 0.05$, and subsequent downstream bioinformatic analysis was carried out using R. Next, we used indirect calorimetry to measure respiration rate under mTORC inhibition (N=4). Finally, we measured drug uptake and metabolism by injecting mice at two timepoints with a drug cocktail. After 30 minutes, blood was drawn by cardiac puncture, and blood plasma and livers were subjected to quantitative mass spectrometry to measure levels of drugs and their respective metabolites.

Results (200 word limit)

In mouse brain, mTORC inhibition abolished almost all daily variation in protein abundance apart from clock protein PER1, and accounted for most of the variation in phosphosite abundance. In liver, mTORC inhibition abolished most daily variation in protein abundance, and ~40% of variation in phosphosite abundance. In addition, mTORC inhibition attenuated rhythms in respiratory exchange ratio. Finally, mTORC inhibition abolished time-of-day variation in drug metabolism.

This occurred with only partial mTORC inhibition (~25% reduction in S6K phosphorylation at Thr389 in brain and ~40% reduction in liver). Furthermore, mTORC inhibition did not affect core clock protein rhythms, nor locomotor activity.

Conclusions (100 word limit)

We show that mTORC is required for rhythmic regulation of diverse physiological processes in mice, such as proteome and phosphoproteome composition of brain and liver, respiration, and drug uptake in the liver. Partial mTORC pathway inhibition does not affect daily clock protein rhythms, suggesting it acts downstream of the core clock, as an output. Therefore, daily cycles of the mTORC pathway may be sufficient to account for rhythmic regulation of many aspects of cellular biology. This may be generalisable to other eukaryotes.

Support

N/A

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Investigating rhythmicity of circadian biomarkers using an electrochemical sweat based wearable device.

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Full Name and Credentials

Annapoorna Ramasubramanya

Introduction (100 word limit)

With advancements in wearable technologies like smartwatches, there has been growing interest in developing noninvasive sensors that can integrate with wearables for real-time health monitoring. The focus of this research is the detection of melatonin through sweat, which contains similar biomarkers found in blood at measurable concentrations and develop a sensor algorithm for real-time melatonin monitoring in sweat. The research aims to analyze melatonin's circadian patterns and understand how light exposure impacts melatonin levels and explore whether sweat can serve as a reliable surrogate for saliva in tracking melatonin.

Methods (200 word limit)

Features were extracted from raw sensor data, and the model was trained using the interpolated melatonin levels as the output variable. For the training and validation of the model, strict protocols were followed by 23 participants to control variables affecting melatonin levels. Participants avoided caffeine, stimulants, and alcohol after 6 p.m., stayed in dim light from 10 p.m. to 4 a.m., and wore watches to measure light exposure and activity levels.

Results (200 word limit)

Circacompare for melatonin and cortisol showed distinct peaks and troughs over the 24-hour period, aligning with the known circadian pattern. The influence of light exposure on melatonin expression was examined using hedge's G and had a Pearsons R -0.15 showing inverse relation between light and melatonin levels. Results showed that melatonin levels are highest at night when light exposure and cortisol were low, then drop during the daytime when light exposure is higher, and cortisol levels were higher. The p-value for the melatonin levels across age groups was <0.0001 showing a significant difference between the age groups. The Pearsons R between sweat and saliva was 0.84.

Conclusions (100 word limit)

The research demonstrates the efficacy of a non-invasive sensor to monitor melatonin in sweat continuously. Utilizing statistical tools and assessing circadian rhythm, significant patterns were identified that aligned with established circadian rhythms and showed how melatonin is affected by environmental factors like light. The rhythmicity data from Circacompare validates that the sensor algorithm effectively captures melatonin's natural cycle.

Support
N/A

83

Obstructive Sleep Apnea in a Teen: Nasal Congestion and Anatomical Variations Without Typical Risk Factors

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Full Name and Credentials

Ahmed Saleh, MD

Introduction (100 word limit)

Obstructive Sleep Apnea (OSA) is a common sleep disorder characterized by repeated airway collapse during sleep, typically linked to obesity, older age, or enlarged tonsils. Adolescents without these risk factors may develop OSA due to anatomical issues, such as nasal obstruction. This case demonstrates the need for evaluating OSA in adolescents with chronic nasal congestion and daytime fatigue, regardless of body habitus.

Methods (200 word limit)

A 17-year-old female (BMI 21) presented with chronic fatigue, non-restorative sleep, and difficulty concentrating. Her mother reported loud snoring and observed apneas during sleep. The patient also experienced chronic nasal congestion and frequent nighttime mouth breathing. Physical examination revealed turbinate hypertrophy and a deviated septum, without tonsillar enlargement. Polysomnography revealed an apnea-hypopnea index (AHI) of 18 events/hour, consistent with moderate OSA.

Results (200 word limit)

The patient was treated with nasal steroids and saline rinses to alleviate nasal inflammation. CPAP therapy was initiated, leading to significant improvement in sleep quality, daytime alertness, and concentration. Referral to an ENT specialist resulted in a successful septoplasty and turbinate reduction. Post-surgical follow-up polysomnography showed further reduction in AHI and improved CPAP tolerance with lower pressure settings.

Conclusions (100 word limit)

This case highlights that OSA can occur in adolescents due to anatomical airway obstruction rather than traditional risk factors like obesity or advanced age. Chronic nasal congestion can exacerbate airway collapse, contributing to OSA. Early recognition and treatment, including addressing nasal obstruction and implementing CPAP therapy, can significantly improve outcomes and prevent long-term complications in this population. OSA should be considered in adolescents with fatigue and chronic nasal obstruction to ensure timely diagnosis and effective management.

Support

N/A

86

Tonic-clonic seizures induce hypersomnia and suppress REM sleep in mouse models of epilepsy

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Full Name and Credentials

Yueqing Peng

Introduction (100 word limit)

The reciprocal relationship between sleep and epilepsy has been reported by numerous clinical studies. However, the underlying neural mechanisms are poorly understood. Animal models of epilepsy are powerful tools to tackle this question. A lagging research area is the understudied sleep in epilepsy models.

Methods (200 word limit)

Here, we performed EEG/EMG recording in two mouse models of epilepsy. Then, we characterized sleep architecture, tonic-clonic seizures, and their correlation.

Results (200 word limit)

We demonstrated that nocturnal tonic-clonic seizures induce more NREM sleep but suppress REM sleep, resulting in altered sleep architecture in the KCNT1 mouse model. Importantly, the seizure number is quantitatively anti-correlated with the amount of REM sleep. Strikingly, this modulation of NREM and REM sleep states can be repeated in another mouse model of epilepsy with diurnal tonic-clonic seizures.

Conclusions (100 word limit)

Together, our findings provide evidence from rodent models to substantiate the close interplay between sleep and epilepsy, which lays the ground for mechanistic studies.

Support

This work was supported by NIH/NINDS R21NS120027.

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Exploring Long-term Impact of Psilocybin Administration on Sleep-Continuity via Wearable Sensor Time-series: Preliminary-results in Post-Treatment Lyme Disease

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Full Name and Credentials

Matthew J Reid., BMedSci DPhil

Introduction (100 word limit)

Post-treatment Lyme Disease (PTLD) is a post-infectious syndrome characterized by fatigue, sleep disturbance, musculoskeletal pain and/or cognitive difficulties. We conducted a pilot study of psilocybin-assisted treatment for PTLD exploring its potential to remedy sleep-related disturbances, including impacts on short-term and long-term sleep outcomes. Intensive sleep monitoring was performed using an actigraphy-based wearable device, assessing nightly sleep continuity, continuously throughout the baseline, treatment and post-treatment phases. In this report, we describe the time-course of these high-dimensional sleep outcomes during the six-month open-label study of structured psilocybin administration amongst participants with PTLD.

Methods (200 word limit)

The parent study recruited 20 participants to complete an open-label clinical trial of psilocybin-assisted treatment for PTLD, of which 12 consented to undergo continuous sleep monitoring throughout this period using a non-intrusive consumer smart-ring. Participants completed an 8-week course of study-treatment including two psilocybin sessions (15mg and 25mg at weeks 4 and 6, respectively), with follow-up assessments 1, 3, and 6 months after the final psilocybin session. Sleep-wearable data were captured continuously throughout the entire study period, beginning at approximately three weeks prior to the first dosing session. During this period, we obtained nightly estimates of total sleep time (TST in mins), sleep efficiency (SE in %), and Wake after Sleep Onset (WASO in mins). To better reflect the temporal dynamics captured in long-term time-series data, we elected to conduct Bayesian structural time-series models using Bayesian Causal Impact Analysis (bCIA). The pointwise and cumulative effects of the two observed time-series [*Time-series 1*): Treatment-session 1 to Treatment-session 2; *Time-series 2):* Treatment-session 2 onwards] were inferenced against a counterfactual Bayesian prediction of the time-series that predicted to have occurred during the same period in the absence of an intervention, based on pre-treatment time-series data.

Results (200 word limit)

bCIA Structural time-series models demonstrated a cumulative decline in the trajectory of the total sleep time (TST: in mins) time-series following the first (-613 mins, 95% Credible Interval = [-1216 mins to -33.4 mins]) and second psilocybin sessions (-153 mins, 95% Credible Interval = [-458mins to 165mins]), relative to the counterfactually predicted time-series. However, the probability (84.5%) of an average causal effect following psilocybin session two (-16.99 mins, 95% Credible Interval = [-51 mins to +18 mins]), was substantially lower than the probability (97.95%) of an average causal effect (-32.4 mins. 95% Credible Interval = [-64 mins to -1.8 mins]) after psilocybin session one.

Likewise, Sleep Efficiency (%) demonstrated a cumulative decline in its trajectory following the first (-90.1%, 95% Credible Interval = [-173.9 % to -9,98 %]) and second psilocybin sessions (-40.3%, 95% Credible Interval = [-79.6% to -1.27 %]), relative to the counterfactual predicted time series. The probability (98.6%) of an average causal effect following psilocybin session one (-4.6%, 95% Credible Interval = [-9.2% to -0.59%]), was higher following treatment session one than the probability (97.76%) of an average causal effect (-4.5%. 95% Credible Interval = [-8.8% to -0.14%]) following psilocybin session two.

Conclusions (100 word limit)

Findings suggest a long-term reduction in actigraphy-TST following psilocybin administration that is maximal following the first dose, as well as a sustained decrease in sleep efficiency following both treatment sessions. The tendency of psilocybin to decrease both actigraphy-TST and actigraphy-SE over time could possibly linked to a normalization of hypersomnia and excessive sleepiness, potentially manifesting in excessive TST and SE prior to the intervention. Future work is required to further align these findings within the conceptual framework of PTLD symptomology by examining whether these behavioral assessments of sleep continuity align with participant-reports of sleep quality, and daytime somnolence

Support

This work was supported through the Johns Hopkins Center for Psychedelic and Consciousness Research with funding provided by Tim Ferriss, Matt Mullenweg, Blake Mycoskie, Craig Nerenberg, and the Steven and Alexandra Cohen Foundation.

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The Orexin 2 Receptor Agonist ALKS 2680 in Patients With Narcolepsy Type 2: An Initial Proof of Concept Phase 1b Study

Ron Grunstein[1], Brendon Yee[1], Julia Chapman[1], Jian Eu Tai[1], Sheila Sivam[1], Craig Hopkinson[2], Jandira Ramos[2], Shifang Liu[2], Daniel Smith[2], Sergey Yagoda[2], Bhaskar Rege[2]

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Full Name and Credentials

Anthony Hutchinson

Introduction (100 word limit)

ALKS 2680 is a highly potent, orally bioavailable, and selective orexin 2 receptor agonist being developed for the once-daily treatment of narcolepsy and idiopathic hypersomnia. Safety and pharmacodynamic results from a phase 1b study in patients with narcolepsy type 2 (NT2) are presented.

Methods (200 word limit)

Patients with NT2 received single doses of 5, 12, and 25 mg ALKS 2680 and matching placebo in a 4-way randomized crossover design. Safety assessments included adverse events (AEs), vital signs, clinical laboratory assessments, and electrocardiograms (ECG). Efficacy assessment was mean sleep latency on the Maintenance of Wakefulness Test (MWT).

Results (200 word limit)

In patients with NT2 (N=9), there were no serious AEs; no patient discontinued due to any AE. AEs related to study drug (>1 patient) were pollakiuria, insomnia, and dizziness. All AEs were mild except 1 case of moderate pollakiuria. No treatment-emergent, clinically meaningful changes from baseline were identified in laboratory values, vital signs, or ECGs. On the MWT, ALKS 2680 increased mean sleep latency versus placebo over 8 hours post-dose, as assessed by estimated least square mean difference in change from baseline, by 11.6 minutes (5 mg, $p<0.05$), 18.6 minutes (12 mg, $p<0.001$), and 21.0 minutes (25 mg, $p<0.001$). Observed mean sleep latencies at 12 and 25 mg were >30 minutes over 8 hours.

Conclusions (100 word limit)

ALKS 2680 was generally well-tolerated among patients with NT2. Single doses of ALKS 2680 at 5, 12, and 25 mg led to statistically significant, clinically meaningful sleep latency improvements and support further clinical evaluation of ALKS 2680 in phase 2.

Support

Alkermes, Inc.

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Safety and Pharmacodynamic Effects of the Orexin 2 Receptor Agonist ALKS 2680 in Patients With Narcolepsy Type 1: A First-in Human Phase 1 Study

Ron Grunstein[1], Brendon Yee[1], Julia Chapman[1], Angela D'Rozario[1], Craig Hopkinson[2], Jandira Ramos[2], Daniel Smith[2], Sergey Yagoda[2], Bhaskar Rege[2]

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Full Name and Credentials

Anthony Hutchinson

Introduction (100 word limit)

ALKS 2680 is a highly potent, orally bioavailable, and selective orexin 2 receptor agonist being developed for the once-daily treatment of narcolepsy and idiopathic hypersomnia. Here we present results from a double-blind, phase 1 study assessing ALKS 2680 safety, tolerability, and pharmacodynamics.

Methods (200 word limit)

Patients with NT1 received single doses of 1, 3, and 8 mg ALKS 2680 and matching placebo in a 4-way randomized crossover design. Safety assessments included adverse events (AEs), vital signs, clinical laboratory assessments, and electrocardiograms (ECG). Pharmacodynamic efficacy assessments included the Maintenance of Wakefulness Test (MWT) and the Karolinska Sleepiness Scale (KSS).

Results (200 word limit)

In patients with NT1 (N=10), there were no serious or severe AEs; no patient discontinued due to any AE. Drug-related AEs (>1 patient) included insomnia, pollakiuria, salivary hypersecretion, decreased appetite, dizziness, and nausea. No drug-related, treatment-emergent, clinically meaningful changes from baseline were identified in laboratory values, vital signs, or ECGs. On the MWT, ALKS 2680 increased mean sleep latency, demonstrating placebo-corrected changes from baseline of 18.4 minutes (1 mg), 22.6 minutes (3 mg), and 34.0 minutes (8 mg) through 8 hours post-dose ($p<0.001$ for each dose vs placebo). On the KSS, ALKS 2680 showed clinically meaningful, dose-dependent improvements of 2-3 points in self-reported alertness between 1 and 8 hours ($p<0.001$ for each dose vs placebo).

Conclusions (100 word limit)

ALKS 2680 was generally well-tolerated. Single doses up to 8-mg led to statistically significant, clinically meaningful improvements in sleep latency and patient-reported alertness and support further clinical evaluation of ALKS 2680 in phase 2.

Support

Alkermes, Inc.

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The Orexin 2 Receptor Agonist ALKS 2680 in Patients with Idiopathic Hypersomnia: An Initial Proof of Concept Phase 1b Study

Brendon Yee[1], Ron Grunstein[1], Julia Chapman[1], Jian Eu Tai[1], Sheila Sivam[1], Craig Hopkinson[2], Jandira Ramos[2], Shifang Liu[2], Daniel Smith[2], Sergey Yagoda[2], Bhaskar Rege[2]

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Full Name and Credentials

Anthony Hutchinson

Introduction (100 word limit)

ALKS 2680 is a highly potent, orally bioavailable, and selective orexin 2 receptor (OX2R) agonist being developed as a once-daily treatment for narcolepsy and idiopathic hypersomnia (IH). Results from a randomized, double-blind, phase 1b study that assessed the safety/tolerability and pharmacodynamics of ALKS 2680 in patients with IH are presented.

Methods (200 word limit)

Patients with IH received single doses of 5, 12, and 25 mg ALKS 2680 and matching placebo in a 4-way randomized crossover design following 2-week washout from their current IH medications. Safety assessments included adverse events (AEs), vital signs, clinical laboratory assessments, and electrocardiograms (ECGs). The key pharmacodynamic assessment was mean sleep latency on the Maintenance of Wakefulness Test.

Results (200 word limit)

In patients with IH (N=8), there were no serious AEs and no patient discontinued due to any AE. All AEs were mild except 1 moderate case of pollakiuria. AEs related to study drug and occurring in >1 patient were pollakiuria, insomnia, and dizziness. No clinically meaningful changes from baseline were identified in laboratory values. No cardiovascular safety signals were identified in vital signs or ECGs. ALKS 2680 increased mean sleep latency versus placebo over 8 hours post-dose, as assessed by estimated least squares mean difference in change from baseline, by 8.1 minutes (5 mg, $p<0.05$), 11.1 minutes (12 mg, $p<0.01$), and 17.7 minutes (25 mg, $p<0.001$). Observed mean sleep latencies for 12 and 25 mg were >30 minutes over 8 hours post-dose.

Conclusions (100 word limit)

ALKS 2680 was generally well-tolerated. Single doses of ALKS 2680 at 5, 12, and 25 mg led to statistically significant, clinically meaningful improvements in sleep latency. At the 12- and 25-mg doses, mean sleep latencies were within the reported range for healthy individuals. These data are the first to support the use of an oral OX2R agonist in the treatment of IH.

Support
Alkermes, Inc.

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Implementing an Ecological Momentary Assessment (EMA) Study on Daily Sleep and Smoking among Individuals with Lower Socioeconomic Status who Want to Quit Smoking

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Full Name and Credentials

Chaelin Karen Ra, PhD, MPH

Introduction (100 word limit)

Cigarette smoking is the leading preventable cause of morbidity and mortality in the United States, and is highly concentrated among individuals with lower socioeconomic status (SES). Previous studies have shown that lower SES is associated with higher rates of poor sleep. Moreover, poor sleep is associated with lower likelihood of smoking cessation. However, the temporal and daily associations between sleep and smoking remain poorly understood. Therefore, the current study aimed to assess the feasibility and compliance of a real-time data capture approach of sleep and smoking among lower SES smokers who are attempting to quit.

Methods (200 word limit)

Participants were lower SES smokers visiting the 11 state-funded tobacco quit centers across New Jersey. Eligibility criteria included: household income <200% of the federal poverty line; a score ≥ 4 on the Rapid Estimate of Adult Literacy in Medicine-Short Form; willingness to quit smoking 14 days after the baseline visit; ≥ 18 years of age; an expired CO level ≥ 7 ppm; currently smoking ≥ 5 cigarettes per day; no overnight shiftwork; and not currently using potentially sedating medications. This study was conducted 100% remotely. Study participation lasted 6 weeks (2-week pre-quit and 4-week post-quit) and included 4 virtual visits (i.e., baseline, quit date, end of study, and 1-month follow-up). Participants were provided with a study phone (if they did not own a compatible Android phone), Actiwatch and Bluetooth breathalyzer with detailed instructions regarding the use of the phone, Actiwatch, and EMA procedures via mail. Participants completed questionnaires at the baseline visit, including assessment of sleep, other tobacco use, past/current medical history. Subjects wore an Actiwatch and used the study app and Bluetooth breathalyzer to record daily sleep and smoking-related behaviors for the entire 6-week study period. At the end of the study, participants returned the Actiwatch and study phone via mail.

Results (200 word limit)

Since April 2024, 10 participants have completed the 6-week EMA study. They were 70% White, 30% Black, and 60% female, with mean age of 53.2 years. The average EMA compliance rate was 82% during pre-quit and 74% during post-quit periods. Biochemically confirmed abstinence rates were high (i.e., 40% on the quit date and

20% at the end of the study). Most participants (80%) wore the Actiwatch more than 90% of the study days. Participants provided positive feedback about the study (e.g., “It made me more mindful of possible triggers”). The limited negative feedback primarily stemmed from a lack of knowledge, with one participant stating, “Trying to link smoking with emotional problems is ridiculous.” In response to this feedback, a tailored report was provided to the participants who completed the 6-week EMA study, offering them a clearer understanding of how their sleep, mood, and smoking behaviors can be interrelated.

Conclusions (100 word limit)

Collecting real-time data via devices (i.e., smartphone app, wrist-worn sleep sensor, and Bluetooth breathalyzer) over 6 weeks was feasible and acceptable among individuals with lower SES who want to quit smoking. The role of sleep health and circadian rhythms has been understudied in smoking cessation interventions. This is the first known study to elucidate associations between daily sleep dysregulation and smoking cessation using mHealth technology. Findings from this project will inform the development of future tailored sleep interventions in smoking cessation programs for this underserved population.

Support

This research was supported by NIDA K99DA058711 and used the NCI designated Stephenson Cancer Center’s mHealth Shared Resource (P30CA225520).

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Circadian Phenotyping of Drosophila DAT mutants

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University of Alabama at Birmingham, Birmingham, USA

Full Name and Credentials

Jonathan Reid Black, MD

Introduction (100 word limit)

While core circadian (~24-hour) rhythms are well-characterized, secondary intrinsic rhythms remain less understood. One such rhythm, the methamphetamine-sensitive circadian oscillator (MASCO), was identified through chronic methamphetamine administration, which extended the period of circadian locomotor rhythms in rodents. Similarly, lengthened locomotor rhythms have been observed in dopamine transporter (DAT) knockout mice. However, this has not been demonstrated in *Drosophila*. Here, we examine the endogenous locomotor rhythms of the *Drosophila* mutant *fumin*, which lacks a functional DAT gene, and *Drosophila* flies containing human DAT (hDAT) in a *fumin* background.

Methods (200 word limit)

Canton S (n = 32), *fumin* (n = 32), and hDAT (n = 24) flies were grown and maintained on standard cornmeal-molasses media at 25 °C under a 12:12 h light-dark (LD) schedule. Virgin male flies ages 3-6 days were transferred individually to activity tubes containing standard cornmeal-molasses media. TriKinetics *Drosophila* Activity Monitoring system (Waltham, MA) was used to measure locomotion. Flies were placed in locomotion tubes on day 0 and exposed to 2-3 days of 12:12h LD schedule. Flies were then exposed to constant darkness (DD) for 7 days. ClockLab (Actimetrics, Wilmette, IL) was used to generate actograms and associated Lomb-Scargle periodograms for days under DD. Flies were excluded from final analysis if they lacked a statistically significant circadian rhythm (defined as no consistent periodicity in locomotor activity) or demonstrated locomotor periods more than two standard deviations from the group mean.

Results (200 word limit)

Fumin flies (n = 27, M = 23.86h, SD = 0.89), demonstrated significantly lengthened circadian periods ($p = 0.002$) compared to Canton S flies (n = 29, M = 23.30h, SD = 0.30). hDAT flies (n = 18, M = 23.96h, SD = 1.86) did not differ significantly from either *fumin* ($p = 0.83$) or Canton S ($p = 0.06$) with respect to circadian period. 9.4% of *fumin* flies were arrhythmic versus 13.6% of hDAT flies and 0.0% of Canton S flies.

Conclusions (100 word limit)

These findings mirror results observed in mice lacking functional DAT genes, which also have lengthened locomotor rhythms. By establishing this effect in *Drosophila*, we demonstrate a crucial genetic tractable model for investigating the molecular mechanisms of MASCO. Additionally, it is significant that hDAT flies do not have a significantly different circadian period from that of Canton S wildtypes. This sets the stage for future studies involving hDAT transporter genetic variations associated with neuropsychiatric disorders with disrupted

rhythms. These results hold significant implications for understanding psychiatric disorders characterized by dopaminergic network dysregulation.

Support

This study was supported by the National Institute of Drug Abuse grant R01DA038058 and R01DA035263.

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Smoking Disrupts Actigraphy-Measured Sleep, Circadian Rhythms, and Melatonin Profiles in a Racially Diverse Sample from the UAB CRAVESS Study

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Introduction (100 word limit)

Tobacco use is the leading preventable cause of death, with an estimated 8 million annual deaths worldwide by 2030. Socioeconomic disadvantage and poor sleep quality increase relapse risks in smoking cessation, yet racial disparities remain underexplored. Black individuals are underrepresented in studies, creating critical gaps in knowledge and limiting tailored interventions. The CRAVESS study aims to address these disparities by investigating racial differences in sleep and circadian rhythms, focusing on mechanisms sustaining smoking behavior and dependence in Black versus White populations. These insights can inform more equitable, effective smoking cessation strategies.

Methods (200 word limit)

Here, we present data from the UAB Circadian Rhythms and Variability in Everyday Sleep and Smoking (CRAVESS) study, focusing on the impact of racial disparities in sleep and circadian markers. Over a 10-day period, Black and White Smokers (N= 75) and Non-smokers (N=70) completed Ecological Momentary Assessments (EMA) with simultaneous monitoring of sleep-wake rhythmicity via actigraphy (MotionWatch 8, CamNTech™). After adjusting for age, race, and gender, smokers and nonsmokers were compared on the following actigraphy measures: sleep onset, midsleep, sleep offset, sleep duration, sleep efficiency, activity during sleep, fragmentation index, light exposure during sleep, central phase, relative amplitude (RA), inter-daily stability (IS), and intra-daily variability (IV). On Day 10, Dim Light Melatonin Onset (DLMO) was measured via radioimmunoassay (RIA) using the 3 pg/mL threshold method from saliva samples collected between 6:00pm to 2:00am. In addition, blinded raters scored the melatonin profiles as one of six patterns: a typical sigmoidal shape (pattern 1), typical linear shape and stays <10 pg/mL (pattern 2), melatonin fails to exceed 3 pg/mL (pattern 3), melatonin levels chronically above 3 pg/mL (pattern 4), typical sigmoidal shape but declines at the end (pattern 5), and highly variable melatonin levels (pattern 6).

Results (200 word limit)

Analysis of DLMO levels showed no significant effects of smoking status, age, race, or gender. However, pattern analysis revealed that 25% of smokers exhibited low melatonin levels (pattern 3) vs. 10% of nonsmokers, whereas fewer smokers showed a typical melatonin pattern that declined at the end (pattern 5) (9.5% of smokers vs 28% of non-smokers; *p=0.005*). We also examined whether various actigraphy measures were associated with certain melatonin pattern types. We found that pattern 5 exhibited significantly higher IV

compared to pattern 1 and pattern 2 (ANOVA, *p=0.018; Tukey p < 0.05*). Regarding actigraphy measures, smokers (vs nonsmokers) demonstrated later sleep onset (*p=0.009*) and midsleep time (*p<0.05*), shorter sleep duration ($p<0.001$), reduced sleep efficiency (*p=0.002*), increased activity during sleep (*p<0.05*), greater sleep fragmentation (*p=0.003*), and increased light exposure at bedtime (*p<0.001*). Black participants had significantly shorter sleep duration ($p<0.001$), lower RA ($p<0.001$) and IS ($p<0.05$), and increased light exposure ($p=0.006$) than White participants, independent of smoking status. Similarly, smokers had lower RA ($p<0.001$) and IS ($p=0.005$) compared to non-smokers ($p<0.05$), regardless of racial background

Conclusions (100 word limit)

Tobacco use impacted melatonin profiles and levels, suggesting future measures beyond the standard DLMO. Smokers went to sleep later but woke up at a similar time as nonsmokers, resulting in delayed midsleep and shorter sleep. Given no difference in DLMO, our results suggest that the delayed sleep start is not due to a delayed central circadian clock. Smoking resulted in poor sleep quality and reduced circadian amplitude, which are also more likely to occur in Blacks compared to Whites. Thus, treatments to improve sleep duration, timing, and quality may increase successful quit attempts, especially in Black smokers.

Support

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Irregularity of sleep onset time and sleep duration are not associated with differences in endogenous circadian rhythmicity parameters of core body temperature in Black adults

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Full Name and Credentials

Brittanny Polanka, PhD

Introduction (100 word limit)

Sleep irregularity in timing and duration is thought to serve as a behavioral marker of circadian disruption; however, few studies have examined this assumption under conditions that control for exogenous influences on the circadian system. Thus, we examined potential differences in core body temperature (CBT) rhythmicity under constant conditions between those with high versus low sleep irregularity under free-living conditions.

Methods (200 word limit)

Participants completed a 7-day at-home sleep diary followed by a 30-hour laboratory Constant Routine protocol, conducted with constant dim-light, prolonged wakefulness, and evenly distributed iso-caloric snacks while in a semi-recumbent position. Within-person standard deviation of sleep onset time and total sleep time were used to identify high (> 60 minutes) versus low (≤60 minutes) sleep irregularity in timing and duration, respectively. Linearized cosinor analyses with a 12-hour harmonic component were performed to confirm circadian rhythmicity of CBT and to estimate parameters of mesor, amplitude, and phase. T-tests were used to examine differences in parameters between high versus low sleep irregularity groups.

Results (200 word limit)

Our analytic sample of 13 participants (100% Black, 69.2% female, mean age 42.3 years) had 38.5% with high irregularity in timing and 69.2% with high irregularity in duration. Under constant conditions, those with high versus low sleep onset irregularity under free-living conditions did not differ on CBT mean mesor (36.9 versus 37.0, p=0.857), amplitude (-0.06 versus -0.03, p=0.630), or phase (0.34 versus -0.87, p=0.536). Similarly, those with high versus low sleep duration irregularity under free-living conditions did not differ on CBT mean mesor (36.9 versus 37.1, p=0.294), amplitude (-0.06 versus 0.0008, p=0.228), or phase (-0.77 versus 0.42, p=0.564).

Conclusions (100 word limit)

Irregularity in sleep timing and duration do not appear to individually result in circadian disruption of CBT in this small sample of Black adults. Future research is needed to examine other circadian markers (e.g., melatonin), sleep irregularity over longer periods of time, and the overlap between types of sleep irregularities to delineate whether results are due to a lack of circadian consequences of social jetlag (high irregularity in timing) or

whether this could be due to a protective effect of catch-up sleep (high irregularity in timing within the context of high irregularity in duration).

Support

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Circadian Regulation of Hippocampal Excitatory Neurons: Implications for Synaptic Plasticity and Alzheimer's Disease Pathology

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Introduction (100 word limit)

The circadian clock plays a fundamental role in regulating neuronal and synaptic function, with BMAL1, a core clock protein, orchestrating rhythmic gene expression crucial for maintaining cellular homeostasis. Deletion of BMAL1 in forebrain excitatory neurons has been linked to significant memory impairments, though anxiety and depression-like behaviors remained unaffected. These findings suggest a specialized role for the excitatory neuronal clock for cognitive processes. Here, we investigate how loss of rhythmicity of hippocampal excitatory neurons impacts synaptic plasticity mechanisms and with potential contributions to memory deficits and link to Alzheimer's disease (AD).

Methods (200 word limit)

Using hippocampal slices of *Camk2a*$^{cre+}$:*Bmal1*$^{fl/fl}$ animals (CBKO) and littermate *Bmal1*$^{fl/fl}$ controls, we performed a nighttime field potential and plasticity assay to observe input/output curves (I/O), as well as pair-pulse ratio (PPR) and long-term plasticity (LTP) on the Shaffer Collateral pathway after tetanic stimulation. We also performed a BAC-TRAP method to isolate actively translating transcripts from *Camk2a*$^{cre+}$:Rpl10a hippocampal neurons at circadian time (CT) 10 and CT 22 for real-time qPCR.

Results (200 word limit)

The I/O curves revealed a decrease stimuli-response from CBKO mice compared to controls ($F(1.221, 37.21)=542.1$; $p<0.001$). After 70 mA stimulation, a PPR of CBKO hippocampus showed pair-pulse depression at 10ms, followed by decreased facilitation at higher pulse intervals, indicating a presynaptic effect on short-term plasticity ($F(1,11)=14.00$, $p<0.01$). Using a tetanic stimulation protocol (100Hz, 0.5s, 2x: t=20 min) we observed a 55.22% decreased LTP of CBKO ($t(14)=28.19$, $p<0.0001$). The gene expression of circadian genes, such as *Per1* and *Nr1d1* showed loss of day/night differences in CBKO mice, confirming loss of rhythmicity on these neurons. Additionally, we observed a loss of rhythmicity in the expression of *Tmem106b*, a risk factor gene for AD associated with cognitive decline and brain aging whose function is linked to regulation of lysosomal activity and exocytosis.

Conclusions (100 word limit)

Our findings underscore the critical role of circadian rhythmicity in hippocampal excitatory neurons in regulating synaptic plasticity and memory formation. The observed loss of LTP and short-term plasticity in CBKO mice highlights the functional consequences of disrupting *Bmal1* in these neurons. Additionally, the disruption of

rhythmic expression of genes like *Tmem106b*, associated with lysosomal function and AD, suggests a mechanistic link between circadian regulation and neurodegenerative processes. These results provide new insights into the relationship between circadian dysfunction and memory deficits, offering a potential pathway to better understand and target Alzheimer's disease-related cognitive decline.

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Circadian rhythms in cortical network excitability in mice with molecular clock impairment in Parvalbumin-expressing interneurons

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Introduction (100 word limit)

Gamma oscillations - high frequency signals vital for memory, cognitive functioning, and sensory processing- are generated through a network of pyramidal cells and interneurons, including the fast-spiking PV+ (Parvalbumin-expressing) interneurons. Patients with Alzheimer's Disease (AD) have abnormal gamma oscillations and PV+ interneuron activity, resulting in dysfunctions in memory, cognition, emotion, and coordination. Moreover, AD patients have dysregulated sleep-wake activity and impaired circadian rhythms.

Methods (200 word limit)

Therefore, we sought to determine whether the molecular clock in PV+ interneurons is important for rhythmic gamma oscillations. We hypothesized that knockdown of the core molecular clock gene *Bmal1* in PV+ interneurons disrupts the diurnal circadian rhythmicity of gamma oscillations. To test this hypothesis, we crossed *Bmal1*$^{Flox/Flox}$ mice with PV-Cre/TdTomato mice to selectively knockout *Bmal1* from PV+ interneurons to generate PV-BKO (PV-Cre+:TdTomato+: *Bmal1*$^{Flox/Flox}$) offspring. We then performed subdural EEG recordings of PV-BKO mice and control (PV-Cre-:TdTomato+:*Bmal1*$^{+/+}$) mice and examined cortical network activity, including gamma oscillations (gamma 1: 30-56Hz and gamma 2: 64-80Hz), theta oscillations (4-8 Hz) and sub-epileptiform spikes.

Results (200 word limit)

EEG recordings showed significant day-night differences in theta (higher during day/inactive phase) and gamma oscillations (higher during night/active phase) in both genotypes. However, no significant genotype differences were observed in rhythmic gamma oscillations between PV-BKO and control mice. To investigate potential differences in sleep-wake cycles, we analyzed sleep-scored EEG recordings and compared sleep parameters (% time in NREM/REM/wake, bout count and length, etc.) of both genotype groups. There were no significant differences in sleep-wake cycles between PV-BKO and control mice.

Conclusions (100 word limit)

Sample size (n=4, n=3 for controls and PV-BKO respectively) was a limitation to this study, therefore we will repeat these studies in a larger cohort for future analysis. Based on our current results, other factors are able to compensate for the loss of the molecular clock in PV+ interneurons to drive circadian rhythmicity of cortical network activity.

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Metaplastic sleep regulation in Drosophila determined by microscale circadian neural dynamics

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Introduction (100 word limit)

Drosophila sleep allows us to identify molecules and circuits that regulate sleep, but the biophysical and computational basis of sleep regulation remains elusive. Using the Drosophila circadian network, we investigate how microscale biophysical dynamics induced by environmental noise influence macroscale membrane potential dynamics of clock neurons in sleep regulation. We investigate the stability and instability of the circadian network membrane potential and how metaplastic properties of these biophysics contribute to the circuit dynamics process that affects sleep/arousal states in *Drosophila*.

Methods (200 word limit)

We performed dual quadruple patch-clamp recordings on the Drosophila circadian network regulating sleep to capture the dynamics of membrane potential stability and instability. To characterize this stability and instability, we conduct a hysteresis analysis using the Preisach model of hysteresis. In the absence of hysteresis, past and future states are independent, resulting in no change in neural activity over time; this allows the activity to remain constant and stable. Conversely, when hysteresis is present, past and future states are interlinked, leading to continuous fluctuations in neural activity, making it dynamic and unstable. In addition, by conducting a large-scale screen to identify molecules that influence these dynamics, we identified Rabphilin (Rph) as a candidate for stabilizing membrane potential of the circadian network. Through extensive electrophysiological analysis assisted by genetic perturbation, we examined Rph's role in synaptic plasticity, focusing on its promotion of non-canonical synaptic depression and cross-synaptic synchrony. We also assess the effects of environmental light exposure on neuronal stability and synaptic potentiation, aiming to understand the broader implications of light pollution on sleep quality.

Results (200 word limit)

Our results show that microscale fluctuations in membrane potential, driven by noise, play a critical role in shaping the macroscale dynamics of circadian clock neurons. Stable membrane potential dynamics enhance nocturnal sleep quality by maintaining precise spike timing and promoting non-canonical synaptic depression along with cross-synaptic synchrony. In contrast, dynamic instability in clock neurons increase the unpredictability of spike timing, leading to synaptic potentiation, reduced sleep quality and increased arousal. We identified the essential role of Rph in the stabilization of these dynamics, particularly under nighttime light exposure, by counteracting the effects of synaptic potentiation induced by environmental light pollution.

Conclusions (100 word limit)

Our result underscore the importance of membrane potential stability in Drosophila clock neurons for reliable sleep regulation, suggesting that environmental noise-induced dynamic instability influences sleep and arousal. The findings identify Rph as a molecular stabilizer of sleep quality through its role in modulating synaptic plasticity. The study also highlights environmental light pollution as a disruptor of sleep quality, with Rph-based synaptic depression and cross-synaptic synchrony offering protective mechanisms by hysteresis. This work provides insight into the biophysical and computational basis of sleep regulation and presents potential avenues for mitigating environmental effects on sleep through molecular modulation.

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