

SLEEP 2020

Late Breaking Abstracts

LBA 1

Sleeping While Diving: First Non-Invasive Recording of Sleep in Freely Swimming Marine Mammals

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Introduction: Studies examining sleep in marine mammals have traditionally relied on either behavioral observation exclusively or invasive surgical procedures. Our study represents the first non-invasive electroencephalogram (EEG) investigating the neurophysiology of sleep in freely swimming elephant seals, using surface-mounted Genuine Grass gold-cup electrodes.

Methods: After developing our methods with anesthetized animals (N=11), we ruggedized and waterproofed a Neurologger3 (©Evolocus) device to record several days of polysomnography, motion, and environmental data for a juvenile elephant seal, on land and diving in a 5-foot pool under temporary human care. Heart rate artifacts during submersion were removed from polysomnography signals using Independent Components Analysis from the MATLAB EEGLAB toolbox. We identified sleep stage transitions visually and quantified spectral power (δ SP) in the delta frequency range (0.5-4 Hz) with fast Fourier transform (Hanning window; resolution 1024) in LabChart (©ADInstruments).

Results: For the 32.5 hours during which the animal had access to water, the animal spent 72.5% under the water holding its breath, in apneas lasting up to 18.5 minutes and 3.8 minutes on average (SD= 4.3 minutes). It spent 11% of that time in slow wave sleep across 60 episodes lasting 3.6 ± 2.8 minutes. In water, the seal slept in short cycles tied to apneas with a stereotypic pattern from quiet waking (QW: δ SP baseline $\sim 38.7 \mu$ V2 – animal prone at bottom of pool) to slow wave sleep (SWS: δ SP $3.8 \times$ QW – animal often supine at bottom of pool). It sometimes entered a brief period of very low voltage electrophysiological activity resembling REM sleep (REM: δ SP $0.70 \times$ QW – animal often supine at bottom of pool) before returning to quiet or active waking (AW: δ SP $> 200 \times$ QW baseline due to motion artifacts while animal swims).

Conclusion: This stereotyped pattern closely matches certain drift diving patterns observed in wild, migrating elephant seals. It suggests these animals survive on short (~ 10 minute) apneic naps over 600 feet below the ocean's surface for over 7 months at a time.

Support: This project received support from the Office of Naval Research Defense University Research Instrumentation Program, National Geographic, and UCSC's Committee on Research. We declare no competing interests.

LBA 2

The Unfolded Protein Response Sensor IRE1 is Required for Sleep

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Introduction: The maintenance of protein homeostasis – or proteostasis – is vital to the proper functioning of the organism. One of the effects of sleep deprivation is an activation of pathways in the cell that regulate proteostatic balance, such as the inositol requiring element (IRE1) pathway. IRE1 activation increases chaperone transcription and the degradation of unwanted mRNA transcripts in response to misfolded protein accumulation and endoplasmic reticulum (ER) stress. Given our recent findings that proteostasis mechanisms do influence sleep we examined the role of IRE1 in sleep.

Methods: Using pharmacological and genetic approaches, we investigated the effect of manipulating the IRE1pathway on sleep in *Drosophila melanogaster*. We used the small molecule inhibitors STF to inhibit IRE1 pathway activity in the fly. We also employed the *Drosophila* GAL4/UAS system to transgenically knockdown and increase IRE1 pathway activity in *Drosophila* neurons.

Results: Oral administration of STF, significantly reduced nighttime sleep in wildtype flies compared to vehicle controls. Constitutive transgenic expression of IRE1 RNAi in neurons also reduced sleep.

Furthermore, acute genetic expression of IRE1 RNAi in adult neurons also reduced sleep in *Drosophila*. Finally, we demonstrate that knockdown of IRE1 in wake-promoting clock neurons enhances the transcription of the wake-promoting pigment dispersing factor (PDF) neuropeptide thus revealing a mechanism through which proteostatic pathways may affect sleep/wake behavior.

Conclusions: Together, these results demonstrate that IRE1 is a regulator of sleep and wake that that changes in IRE1 signaling may be an upstream regulator of sleep- and clock-relevant neuropeptide signaling. Our results have important implications for understanding the cross talk between sleep regulation and protein homeostasis in the brain. Furthermore, since proteostasis is regulated by both circadian and homeostatic processes, our findings provide a critical juncture from which to further examine the relationship between sleep and the clock.

LBA 3

Sleep Duration and Plasma Metabolites: A Metabolome-Wide Association Study in US Women

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Introduction: Short and long sleep duration are associated with adverse health outcomes, such as type 2 diabetes, and cardiovascular disease. To delineate potential underlying biological mechanisms, we examined the association of self-reported short and long sleep duration with human plasma metabolites.

Methods: We analyzed 313 annotated metabolites profiled via liquid chromatography-mass spectrometry. The discovery data consisted of 6,210 women from the Nurses' Health Study (NHS; blood collection 1989-1991, mean age 57.0 ± 6.9) and 3,185 women from the Nurses' Health Study II (NHSII; 1996-2000, mean age 44.6 ± 4.5). We tested the replication of findings in an independent study of 2,294 women from the Women's Health Initiative (WHI; 1994-1998, mean age 67.0 ± 6.9). We used linear regression models to cross-sectionally evaluate differences in metabolites levels between women with short (<7h), adequate (7-8h), and long (≥ 9 h) self-reported sleep duration, adjusted for age, body mass index, physical activity, diet quality, alcohol consumption, signs of depression, smoking, fasting, and case-control status. Statistical significance was based on $P < 0.05$, false-discovery rate adjusted for both discovery and replication.

Results: The prevalence of long sleep was low and similar across cohorts (NHS: 4.4%, NHSII: 5.6%, WHI: 4.6%). Short sleep occurred frequently (NHS: 27.8%, NHSII: 25.8%, WHI: 40.6%). In the NHS/NHSII, 38 metabolites were significantly associated with short sleep duration. Of these, 8 were replicated in the WHI: 5 triglycerides, 2 diglycerides, and 1 glycolithocholate. In long sleep duration analysis, 5 metabolite associations were identified, but none were replicated.

Conclusion: Metabolites associated with short and with long sleep duration did not overlap, suggesting that mechanisms underlying disease-specific associations may be different between short and long sleepers. The low replication rate may be explained by marked differences in cohort profiles between discovery and replication cohorts and, for long sleep, by insufficient power.

Support: R21HL145421; Max-Kade-Foundation; NHS/NHSII: UM1CA186107, P01CA87969, R01CA49449, R01HL034594, R01HL088521, UM1CA176726, R01CA67262, R01HL35464; WHI: HHSN268201300008C, HHSN268201600018C, HHSN268201600001C, HHSN268201600002C, HHSN268201600003C, HHSN268201600004C.

LBA 4

A Phase 3, Multi-Center, Double-Blind, Randomized, Placebo-Controlled, Polysomnography Study to Assess Efficacy and Safety of Daridorexant in Adult and Elderly Insomnia Patients

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Introduction: Insomnia affects sleep and daytime functioning. Treatment of insomnia should address both; enabling sleep and improving daytime functioning, an outcome lacking in many trials. The first phase-3 trial of daridorexant, a dual orexin receptor antagonist was completed in April 2020 (NCT03545191).

Methods: In this phase-3, double-blind, placebo-controlled, parallel-group study, 930 patients with insomnia were evenly randomized to daridorexant 25mg, 50mg or placebo, administered nightly for 3-month (M), after a week placebo baseline and followed by a week placebo run-out. Primary endpoints were change from baseline in polysomnography sleep parameters wake time after sleep onset (WASO) and latency to persistent sleep (LPS) at 1M and 3M. Secondary endpoints were change from baseline in subjective total sleep time (sTST) and daytime functioning using the validated Insomnia Daytime Symptoms and Impacts Questionnaire (IDSIQ) sleepiness score (assessing energy, sleepiness, mental and physical tiredness).

Results: WASO improvement from baseline (minutes) at 1M for placebo, 25mg and 50mg was -6.20, -18.40, and -28.98 and at 3M -11.11, -22.97 and -29.41, respectively. For LPS, improvement from baseline at 1M for placebo, 25mg and 50mg was -19.85, -28.17 and -31.20 and at 3M, -23.13, -30.73 and -34.80, respectively (all p values vs. placebo <0.002).

sTST increase from baseline (minutes) at 1M for placebo, 25mg and 50mg was 21.56, 34.18(p=0.0013), and 43.62(p<0.0001), and at 3M 37.90, 47.85(p=0.0334), 57.67(p<0.0001), respectively. Daytime functioning improved at 1M for placebo, 25mg and 50mg: -2.02, -2.77(p=0.0547) and -3.77(p<0.0001), and at 3M: -3.79, -4.78(p=0.0534) and -5.70(p=0.0002) for placebo, 25mg, and 50mg, respectively p-values vs placebo).

The most frequent AEs, nasopharyngitis and headache, were balanced between arms. Six, 11, and 5 subjects experienced somnolence on placebo, 25mg and 50mg daridorexant, respectively.

Conclusions: This study showed that daridorexant improved objective and subjective sleep parameters, and daytime performance using a validated instrument, with an acceptable safety profile.