Late Breaking Abstracts

LBA 1

Circadian Misalignment in Everyday Life: Associations with Body Fat and Dietary Intake

Baron KG, Reid KJ, Attarian H, Wolfe L, Bardsley L, Siddique J, Zee PZ
Feinberg School of Medicine, Northwestern University, Chicago, IL

Introduction: Circadian misalignment is hypothesized to lead to obesity through metabolic and behavioral pathways. The goal of this study was to evaluate the relationship between the phase angle of melatonin and sleep with body mass index (BMI), body fat and dietary intake among healthy, freely living adults. We hypothesized that shorter phase angle (sleeping closer to dim light melatonin onset; DLMO) would be associated with higher BMI, body fat and poorer dietary habits.

Methods: This observational study included healthy adults with sleep duration >6.5 hours. Participants completed self-report questionnaires and 7 days of wrist actigraphy. DLMO was evaluated by blood samples. Body mass index (BMI) was calculated from height and weight at the hospital admission and body fat was evaluated using dual axis absorptiometry (DXA). Data were analyzed using correlations and regression analyses controlling for relevant covariates (age, gender and sleep duration).

Results: Participants included 97 adults (61 f, age 26.7 $\pm$ 7.3 years) Average sleep duration was 443.7 (SD= 50.4) minutes, average BMI and body fat % were non-obese (BMI M= 24.0, SD= 4.5 kg/m^2, body fat M=30.4%. SD=8.4%). Average DLMO was 22:48 (SD= 1:27), average sleep midpoint was 04:42 (SD=1:25). Phase angle between DLMO and sleep midpoint was 6:06 (SD= 1.07) hours. Phase angle was inversely associated with body fat (r= -.35, p=.04) among male participants only. Phase angle was not associated with BMI or body fat percentage among women. Shorter phase angle was associated with higher caloric intake, as well as intake of carbohydrates and fats for men and women (p values <.05). Associations with dietary intake remained significant after controlling for age, gender and sleep duration.

Conclusion: This is the first study demonstrating associations between phase angle with dietary behavior and body fat. Results indicate that circadian misalignment in everyday life may impact risk for obesity.

LBA 2

FMRFamide Signaling Promotes Stress-induced Sleep in *Drosophila*

Lenz O[1], Xiong J[1,2], Nelson MD[2,3], Raizen DM[1,2] , Williams JA[1]
[1]Center for Sleep and Circadian Neurobiology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, [2]Department of Neurology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, [3]Department of Biology, Saint Joseph's University, Philadelphia, PA.

Introduction: Although stress induces sleep among multiple species, the mechanism of this adaptive behavior has yet to be fully elucidated. Septic and aseptic injury increase sleep in *Drosophila melanogaster*, and *Caenorhabditis elegans* responds to high temperatures with a sleep-like quiescence. FMRFamide neuropeptides are involved in the behavioral response to environmental stress in *Drosophila*, and a *C. elegans* homolog was recently identified as necessary for stress-induced quiescence. Here, we test whether heat stress in *Drosophila* similarly augments sleep, as well as the role of FMRFamide and its receptor (FR) in the stress-induced sleep response.

Methods: We exposed flies lacking either the FMRFamide or FR genes and wild-type controls to one of two types of stimuli: heat shock (HS) or bacterial infection, and quantified the effects on sleep. Flies were subjected to a 1h HS at ZT0, 6, 12, and 18; or infection with *Serratia marcescens* at ZT18, as previous work showed that treatment at this time produced the most robust effects on sleep.

Results: We show that *Drosophila* respond to heat stress with an increase in sleep, and that mutants of either FMRFamide or FR exhibit a reduced sleep response to HS and infection. FR mutants also succumbed to infection more rapidly than wild-type controls. Unlike sleep following infection, HS-induced sleep is not dependent on time-of-day of the heat pulse, nor on the NFκB transcription factor Relish.

Conclusion: These findings indicate that different types of stress induce sleep via multiple mechanisms, but converge on FMRFamide and its receptor FR to promote an adaptive stress-induced sleep. Due to an FMRFamide-like neuropeptide that acts in an analogous manner in *C. elegans*, we propose that FMRFamide signaling is an ancient regulator of recovery sleep that occurs in response to cellular stress.

LBA 3

Melanin-concentrating Hormone Neurons Release Glutamate

Chee MJ[1], Arrigoni E[2], Maratos-Flier E[1]
[1] Division of Endocrinology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA,
[2] Department of Neurology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA.

Introduction. Melanin-concentrating hormone (MCH) neurons fire maximally in rapid eye movement (REM) sleep and optogenetic activation of MCH neurons increase the number and duration of REM sleep episodes. In addition to the peptide MCH, these neurons coexpress other neurotransmitters that can mediate MCH actions, including GABA, as recently shown, or glutamate. Since some MCH terminals show immunoreactivity for vesicular glutamate transporter 2 (vGLUT2), we tested whether MCH neurons release glutamate.

Methods. We determined if MCH neurons express vGLUT2 by colocalizing MCH with green fluorescent protein (GFP) in *vGLUT2-cre;L10-gfp* mice. To test the functional connectivity between MCH and downstream neurons, we stereotaxically injected *MCH-cre* mice with a cre-dependent channelrhodopsin (ChR2)-mCherry adeno-associated virus and obtained whole-cell recordings from lateral septum (LS) neurons, which received the densest ChR2-expressing MCH terminals. We photostimulated and recorded optogenetically-evoked inhibitory (eIPSC) and excitatory postsynaptic currents (eEPSC) in the LS.

Results. Almost all MCH neurons expressed vGLUT2, suggesting MCH neurons may release glutamate. Photostimulating MCH axons/terminals evoked two types of postsynaptic responses in LS neurons. GABAergic eIPSCs were blocked by bicuculline, a $GABA_A$ receptor antagonist, and glutamatergic eEPSCs were blocked by kynurenic acid, a non-selective glutamate receptor antagonist. Interestingly, kynurenic acid also abolished eIPSCs. This indicates that GABA release depends on glutamatergic inputs from MCH cells. Furthermore, blocking polysynaptic events with tetrodotoxin extinguished GABAergic eIPSCs but did not affect glutamatergic eEPSCs. This indicated a monosynaptic glutamate release from MCH terminals that feeds forward to trigger GABA release onto LS cells.

Conclusion. MCH neurons release glutamate in the LS. MCH-immunoreactive fibers have been shown in the pontine REM-executive region. While previous models propose that GABA-releasing MCH neurons could disinhibit REM-on neurons in the pons, our findings suggest MCH neurons can promote REM sleep by directly activating the pontine REM generator.

LBA 4

PR Domain Containing Protein 13, a Dorsomedial Hypothalamus-Enriched Gene, Connects Sleep, Metabolism, and Aging in Mammals

Satoh A, Brace CS, Rensing N, Imai S
Washington University School of Medicine, St. Louis, MO.

Introduction: It has been reported that the hypothalamus functions as a high-order "control center of aging," counteracting age-associated functional changes and thereby promoting longevity in mammals. We demonstrated that brain-specific *Sirt1*-overexpressing (BRASTO) transgenic mice display lifespan extension and also that maintaining sleep quality is one of the striking phenotypes in old BRASTO mice. This result reveals an idea that sleep quality could be one of the determinants that control the process of aging and longevity in mammals. In the

present study, we investigated the role of Prdm13, a newly identified DMH-enriched gene, in the connection of sleep, metabolism, and aging.

Methods: *Prdm13* expression was quantified by real-time qRT-PCR using hypothalamic RNA samples from *Ad libitum*/diet restriction (DR) and young/old in C57BL/6 mice, or DMH samples from old BRASTO/wild-type mice. DMH-specific *Prdm13*-knockdown mice and control mice were generated by stereotactic injection of lentiviruses carrying *Prdm13* or *firefly Luciferase* shRNA into the DMH. The mice were instrumented with EEG/EMG electrodes. Sleep parameters were assessed one month after the injection, and body weigh were monitored over the experimental period.

Results: The expression level of *Prdm13* increased under DR, whereas its expression decreased with advanced age. Moreover, *Prdm13* expression in the DMH of long-lived BRASTO mice was significantly higher than the level in wild-type mice. *Prdm13* in the DMH has a minimal effect on the normal diurnal oscillation of sleep. On the other hand, the level of EEG delta power during NREM sleep was significantly reduced in DMH-specific *Prdm13*-knockdown mice, compared to that of control mice during both light and dark periods. DMH-specific *Prdm13*-knockdown mice also exhibited progressive increases in body weight and adiposity.

Conclusion: Prdm13 plays an important role as one of key regulators in the DMH that mediate age-associated pathophysiologies, particularly sleep quality and adiposity (Satoh *et al.*, *Aging Cell* 2015).