

LATE-BREAKING ABSTRACTS

LBA 1

Genetic Modeling of Restless Legs Syndrome in *Drosophila*

Freeman A¹, Rye DB², Sanyal S¹

¹Department of Cell Biology

²Department of Neurology, Emory University, Atlanta, GA, United States

Introduction: Common variants in multiple genes have recently been associated with Restless Legs Syndrome (RLS), a widely prevalent disorder that negatively impacts the sleep quality and quantity in humans. An intronic variant of BTBD9 accounts for nearly 50% of the population attributable risk for RLS. While it is dose-dependently related to periodic limb movements in sleep, a recognized endophenotype of RLS, there is no data implicating this gene or its variant as causative to RLS. Here, we report the modeling of BTBD9-dependent sleep phenotypes using *Drosophila* as a model organism.

Methods: Deletion mutations were generated in the single fly homolog of BTBD9 (gene CG1826). Sleep architecture was assessed using the *Drosophila* Activity Monitor and established protocols. Flies transgenic for the full length CG1826 protein tagged with a FLAG epitope were generated to rescue sleep phenotypes in mutant animals and for cellular localization and biochemical analysis of this novel protein.

Results: Deletion of CG1826/BTBD9 yielded viable animals with normal locomotor behavior and intact circadian rhythms. However, reminiscent of sleep disruption in RLS patients, the continuity of sleep in multiple CG1826 mutant alleles is markedly disturbed with prominent sleep fragmentation. Additionally, these phenotypes were mimicked by way of dopaminergic neuron-specific RNAi mediated knock-down of CG1826. CG1826 is expressed in the brain (including dopaminergic neurons) and localizes in distinct elongated specializations in the plasma membrane, suggesting functions in signaling and/or membrane physiology.

Conclusions: This is the first reverse genetic demonstration of sleep associated roles for a gene implicated in a common human sleep-related disorder. Our results also provide evidence for a long appreciated, though poorly understood, role for dopaminergic transmission in RLS associated sleep disturbance. Novel cellular localization of CG1826 and ongoing efforts to map protein binding partners and compartmental identities for CG1826 should illuminate fundamental molecular functions for CG1826/BTBD9 in sleep physiology.

LBA 2

Per2 Genotype Impacts Reward-related Brain Function Associated with Circadian Characteristics in Adolescents

Forbes EE, Phillips ML, Ferrell RE, Nimgaonkar VL, Mansour H, Sciarillo SR, Dahl RE

University of Pittsburgh, Pittsburgh, PA, United States

Introduction: The circadian shift at puberty is a key part of adolescents' sleep deprivation, and it is accompanied by increased reward-seeking behavior, changes in reward-related brain function, and onset of reward-related psychopathology (Crowley, Acebo, & Carskadon, 2007; Forbes et al., 2010). Circadian genes influence behavioral indices of reward function, including cocaine sensitization, in animals (McClung et al., 2005). The *Period2* (*Per2*) gene is expressed in reward-related brain regions (Amir & Stewart, 2009), and is associated with cocaine sensitization in animals (Abarca et al., 2002) and psychosis in humans (Mansour et al., 2009). Circadian disturbances in psychiatric disorders suggest that circadian genes also play a role in disruptions of human reward function (Shulz & Steimer 2009; Benca et al., 2009). Gene-brain-behavior studies are essential to elucidate mechanisms by which circadian genes influence complex behavioral phenotypes.

Methods: We tested associations between two *Per2* single nucleotide polymorphisms (rs2304672, rs2304674) and neural response to monetary reward using functional magnetic resonance imaging in 96 healthy adolescents. Sleep midpoint, a behavioral circadian characteristic, was measured using actigraphy. Puberty was measured by physical exam.

Results: A whole-brain regression revealed that neural response to reward was associated with sleep midpoint in several reward-related regions. Constraining our genotype analyses to those brain regions correlated with sleep midpoint, we found that CC homozygotes for rs2304672 had greater medial prefrontal cortex response to reward outcome than G carriers. The other polymorphism was unrelated to circadian-associated reward function.

Conclusion: In this innovative genes-brain-behavior study using circadian genes, *Per2* was associated with reward-related brain function that is relevant to psychiatric disorders and circadian-influenced behavior. Findings were evident in the mPFC, a region of the brain critical to reward function, affect, and psychopathology. These results have implications for understanding interrelations of circadian systems, affective systems, and psychiatric disorders.

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LATE-BREAKING ABSTRACTS

LBA 3

The Effectiveness of a 34h Restart Break to Sustain Performance across Two Consecutive 5-Day Work Periods Depends on the Circadian Timing of the Work Shifts

Van Dongen H, Vila BJ, Belenky G

Sleep and Performance Research Center, Washington State University, Spokane, WA, United States

Introduction: U.S. commercial truck drivers are allowed to be on duty up to 14h per day; to accumulate 60h/70h on duty in a work period of 7/8 consecutive days; and to begin another work period after a 34h restart break. We investigated the effectiveness of this break for sustaining performance in daytime and nighttime work schedules.

Methods: N=27 healthy subjects (ages 22-39y; 14f) participated in a 14-day in-residence laboratory study, which included a 5-day work period, a 34h restart break, and another 5-day work period. 14 subjects were randomized to a daytime condition, involving nocturnal sleep (TIB 22:00-08:00) every day. 13 subjects were randomized to a nighttime condition, involving nocturnal wakefulness and diurnal sleep (TIB 10:00-20:00) daily during the two 5-day work periods, while reverting to diurnal wakefulness and nocturnal sleep during the restart. Performance on cognitive tasks and on a high-fidelity driving simulator was tested four times per day, except during the restart.

Results: Mixed-effects ANOVA revealed significant interactions of work period (before versus after the restart) by condition (daytime versus nighttime) for lapses (RTs>500ms) on a psychomotor vigilance test (PVT; $F=20.1$, $p<0.001$), for ability to orient a map in a cardinal direction decision task (CDDT; $F=17.8$, $p<0.001$), and for lane deviation on the driving simulator ($F=9.2$, $p=0.003$). In the daytime condition, performance after the restart was the same or better than before the restart. In the nighttime condition, performance after the restart was degraded (PVT lapses) or showed less improvement from practice than in the daytime condition (CDDT, driving) relative to before the restart.

Conclusion: A 34h restart break between two 5-day work periods was adequate to sustain performance in subjects scheduled to daytime shifts, but not in subjects scheduled to nighttime shifts. This stresses the importance of circadian effects on sleep and performance for effective hours-of-service regulations.

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LBA 4

Dose Effects of MK-4305, A Dual Orexin Receptor Antagonist for Insomnia, in Healthy Male Subjects

Kennedy WP¹, Lewis N1, Calder N2, Li X¹, Yee K¹, Perlstein I¹, Wilbraham D³, Murphy GM¹

¹Merck Research Laboratories, Clinical Pharmacology, Westpoint, PA, United States

²Hoddesdon, United Kingdom

³Quintiles Guy's Drug Research Unit, London, United Kingdom

Introduction: MK-4305 is a novel sedative-hypnotic agent that antagonizes the orexin system that regulates alertness and sleep being developed for insomnia therapy.

Methods: This is a report on a double-blind, placebo-controlled 5-period cross-over study of the polysomnography (PSG) effects of MK-4305 in single dose administration to healthy male subjects. Healthy male subjects without a history of sleep disorders or recent use of sedating medications were eligible for the study. Following a habituation night in the sleep lab, subjects received placebo, 10-mg, 50-mg or 100-mg of MK-4305 in a balanced cross-over design one hour before PSG recording. In the last period, plasma concentrations of MK-4305 were collected from subjects following PM administration of MK-4305 without PSG recording.

Results: The 50 mg and 100 mg doses of MK-4305 significantly decreased latency to persistent sleep. The 10, 50 and 100 mg doses of MK-4305 significantly decreased the wake after sleep onset time and the 50 and 100 mg doses of MK-4305 significantly increased the sleep efficiency. There were no significant changes in slow wave activity (sleep stage S3+S4) or increases in total REM stage duration. Subjective evaluations of sleep by the Leeds Sleep Questionnaire were remarkable for a decrease in alertness for the 50 to 100 mg at 10 hours post-dose. There was an increase in reaction time measures for the 100 mg dose alone.

Conclusion: Single doses of MK-4305 from 50 to 100 mg demonstrated clinically significant improvement in sleep onset and maintenance without disproportionate changes in sleep stages. MK-4305 was generally safe and well tolerated but there was evidence of residual effects by subjective and objective criteria at the highest dose of 100 mg.

LATE-BREAKING ABSTRACTS

LBA 5

Treatment of Obstructive Sleep Apnoea Reduces Post-Prandial Lipidemia: Evidence from a Randomised, Placebo Controlled Trial of Continuous Positive Airway Pressure

Yee BJ^{1,2,3}, Phillips CL^{2,3}, Marshall NS^{2,3}, Liu PY^{2,3}, Sullivan DR^{1,3}, Grunstein RR^{1,2,3}

¹Royal Prince Alfred Hospital, Sydney, NSW, Australia

²Woolcock Institute of Medical Research, Sydney, NSW, Australia

³University of Sydney, Sydney, NSW, Australia

Introduction: Studies have proven that OSA causes some of the observed hypertension, there are no robust data to support a causal relationship with dyslipidemia. We conducted a randomised, placebo-controlled crossover trial to assess whether post-prandial lipidemia would improve because of treatment with continuous positive airway pressure (CPAP).

Methods: Adult patients with OSA who met all inclusion and exclusion criteria were recruited. Lipid profiles including triglycerides (TAG) were measured at 7 time points over a 24-hour period (inclusive of sleep) on each of three occasions – at baseline prior to treatment and then after 2 months of either sham (placebo) CPAP or therapeutic CPAP (with 1 month washout). Patients consumed western style meals and snacks (55% carbohydrate, 30% fat, 15% protein) at set times during the daytime (wake) period.

Results: 29 Patients (3 females, 26 males), middle-aged (48+/-13 yrs), obese (BMI 31.7+/-4.1 kg/m²) and with moderate-severe OSA (TRDI=50+/- 23/hr) completed the study. Mean compliance was lower on sham CPAP than therapeutic CPAP (3.5 +/- 2.3 vs 4.4+-2.2 hrs/night; p<0.05). TAG concentrations peaked at 2 time points (2pm and 3am). Intention-to-treat analysis revealed that peak TAG levels (mean +/- SEM) were significantly worse on sham CPAP (2 pm : 3.45 +/- 0.09 mmol/L; 3am : 3.48 +/- 0.09 mmol/L) than therapeutic CPAP (2 pm : 2.97 +/- 0.09 mmol/L; 3am : 3.08 +/- 0.09 mmol/L; p for difference < 0.01 at both time points. Moreover, TAG, HDL and Total Cholesterol levels across the whole 24 hour period were improved by CPAP (p<0.01). Free fatty acids were not different between treatments (p=ns).

Conclusion: Treatment of OSA with CPAP improves post-prandial lipidemia (Triglycerides) and HDL cholesterol – both strong markers of cardiovascular risk. This implies that the association between OSA and cardiovascular disease may, in part, be caused by direct effects on dyslipidemia.