Chronobiology/Circadian Disorders

CIRCADIAN CLOCK PROTEIN PERIOD3 CONTRIBUTES TO SLEEP HOMEOSTASIS THROUGH HISTAMINE AND GABA SIGNALING IN ZEBRAFISH

H. Wang, Center for Circadian Clocks, Soochow University, Suzhou, China

Introduction: The zebrafish (Danio rerio) has figured prominently as a vertebrate model for studying circadian clocks and sleep. It is known that both the homeostatic process (S) and the circadian process (C) contribute to regulation of sleep homeostasis. While we have a good understanding of circadian regulation, relatively little is known about molecular mechanisms underlying circadian regulation of sleep homeostasis and/or interaction between the circadian clock system and sleep.

Materials and methods: TALEN, a genome-editing tool, was used to generate a number of zebrafish per3 mutant lines. Behavioral assays, qRT-PCR, luciferase reporter assays, and RNA-seq were used to characterize zebrafish period3 (per3) null mutants.

Results: Locomotor assays showed that per3 mutant fish display 0.5-hour shortened period and approximately 3-hour phase advance compared with wild types under constant dark, and are completely arrhythmic under constant light; and also per1a, per1b and per2 are down-regulated in per3 mutant fish; indicating that per3 is essential for zebrafish circadian regulation. Intriguingly, per3 mutant fish display less sleep time, reduced arousal threshold and difficulty to restore sleep after sleep deprivation. As shown by ELISA, the GABA level is reduced while the histamine level is increased in per3 mutant fish at night-time, indicating that the disturbed sleep pattern of per3 mutant fish may be resulted from altered levels of endogenous GABA and histamine. Deep sequencing-based transcriptome analysis leads us to focus on two candidate genes, GABA A receptor gene rha2a and histamine decarboxylase gene hdc, both up-regulated in the per3 mutant fish. Luciferase reporter assays showed that both rha2a and hdc are circadian clock-controlled genes and Per3 negatively regulates their expression.

Conclusions: Taken together, these results ascertain Per3’s essential roles in the circadian rhythm system, demonstrate that Per3 acts through both histamine signaling and GABA signaling to contribute to sleep regulation, and provide an ideal sleep disorder vertebrate model for drug screen and pathogenesis analysis.

Acknowledgements: This work was supported by the grants from the National Basic Research Program of China (973 Program) (2012CB947600) and the National Natural Science Foundation of China (NSFC) (31030062, 81070455).

Narcolepsy

EVIDENCE FOR A NARCOLEPSY SPECTRUM DISORDER IN FAMILY MEMBERS OF PATIENTS WITH TYPE 1 NARCOLEPSY

P. Wang1,2, H. Yan1, F. Han1, L. Lin2, E. Mignot2. 1 Peking University People’s Hospital, Beijing, China; 2 Stanford University Center for Sleep Science and Medicine, Palo Alto, United States

Introduction: The existence of a narcolepsy spectrum has been suggested in family members of patients with narcolepsy, but this has never been confirmed through systematic evaluation using sleep study and hypocretin evaluation.

Materials and methods: Narcolepsy cases (n=496) were identified among 5,462 patients visiting the Peking University People’s Hospital Sleep Center from 09/01/2012 to 12/03/2014, including 307 children (<18y) meeting inclusion criteria. Two hundreds and one families (66%) with at least one parent available accepted further evaluation. The resulting 378 parents underwent HLA typing, polysomnography, multiple sleep latency test (MSLT), and questionnaire evaluations. CSF hypocretin-1 was tested in 4 subjects. Three subjects with a positive MSLT underwent a second MSLT for confirmation.

Results: We found 3 parents (0.8%) with narcolepsy-cataplexy (100% DQB1*06:02) and 9 with a positive MSLT but no cataplexy (78% DQB1*06:02). In the 6 parents tested for CSF hypocretin-1 level, two cases (one with and one without cataplexy) had low CSF hypocretin-1 (<110 pg/ml), and one case without cataplexy had intermediary level (153 pg/ml). Repeat PSG-MSLT was positive in 2 of 3 relatives retested. Further analysis suggests that between 2 (0.5%) and 6 (1.6%) of the 9 subjects with narcolepsy but no cataplexy have hypocretin deficiency.

Conclusions: In parents of patients with cataplexy, 0.8% has narcolepsy-cataplexy, and an equivalent or larger number (0.5-1.6%) have mild type 1 narcolepsy without cataplexy due to hypocretin deficiency. These results substantiate the hypothesis that some subjects with hypocretin deficiency do not have cataplexy, and that subjects with cataplexy are the extreme of a disease spectrum. Mild symptomatology may explain why these subjects are rarely diagnosed in sleep centers.

Acknowledgements: This work was supported by research grants from the Ministry of Science and Technology (2015CB856405), NSFC(8142010B002, 81670087, 81300061, 81570083), Beijing Natural Science Foundation (7152153) and NIH grant (P50 NS-23624). We thank the parents and most importantly the children for their participation.

Chronobiology/Circadian Disorders

THE ROLE OF NUCLEAR RECEPTORS IN REGULATING CIRCADIAN PERIOD

T. Wang, Y. Xu. CAM-SU Genomic Resource Center, Soochow University, Suzhou, China

In mammals, circadian oscillation depends on the complex transcriptional network comprised of the interaction among core clock genes via E box, RRE, D box and other DNA elements. As important clock genes, the nuclear receptor genes Rev-Erbs and RORs participate in clock feedback loop by regulating RRE activity, which is essential for maintaining clock robustness. In addition to Rev-Erbs and RORs, most clock controlled nuclear receptors may also be involved in the regulation of circadian rhythms. We comprehensively investigated the influences of nuclear receptor knockout on circadian rhythms using CRISPR-CAS9 system. We found ablation of several nuclear receptors significantly changes clock period in human cells. While these nuclear receptors are transcriptionally clock controlled. We further predicted and found many potential nuclear receptor binding motifs near TSS of human core clock genes. Our data implicate a multi-circuit model in which nuclear receptors stabilize the circadian transcription-translation feedback loop (TTFL) and coupling circadian clock with diurnal physiological changes.

Behavior, Cognition and Dreaming

EXPERIENTIAL EMOTION REGULATION VERSUS COGNITIVE REAPPRAISAL: EFFECTS ON AFFECT AFTER STRESS AND FOLLOW-UP SLEEP PHYSIOLOGY

Y. Wang1, L. Depoortere2, L. Carlo Bulnes3, D. Dong4, M. Dhar4, D. Mannuzzo4, M. Vandekerckhove1. 1 Faculty of Psychology and Educational Sciences, VUB, Brussels, Belgium; 2 Department of Experimental and Applied Psychology, Vrije Universiteit Brussel, Brussels, Belgium; 3 Key Laboratory for NeuralInformation of Ministry of Education, School of Life Science and Technology, University of Electronic Science and Technology of China, Chengdu, China; 4 Department of Experimental and Applied Psychology, Vrije Universiteit Brussel, Brussels, Belgium; 5 University of Gent, Gent, Belgium; 6 Vrije Universiteit Brussel, Brussels, Belgium

Introduction: The interesting idea that emotion regulation (ER) plays a key role in modulating effects of stress on sleep, however, has received few research attention. Research findings suggest that emotion regulation plays a key role in the precipitating and perpetuating effects of stress on sleep (Vandekerckhove & Cluydts, 2010). In this study, we compared the impact of an induced experiential approach, defined as stressing the awareness of our feelings by paying attention to our bodily felt parts in an acceptable and welcoming way (Vandekerckhove & Kestemong, 2012), versus a cognitive approach by cognitive reappraisal, defined as reinterpreting a situation in order to eliminate or change one’s emotions about it (Gross, 1998), on sleep physiology.

Materials and methods: 43 participants were recruited and randomly assigned to 3 groups: 15, 13, 15 for experiential, reappraisal and neutral non-specific regulation respectively. 20-Item Toronto Alexithymia Scale (TAS) and Emotional Approach Coping Scale are used to address the individual difference. All participants spend 3 nights (adaptation, baseline and experimental night) in the sleep lab for 8 hours. An emotional failure induction was used to trigger stress, after which emotion regulation was induced twice (writing task). Subject negative affect was obtained by the
Long Sleep Duration and Health Outcomes: A Systematic Review, Meta-Analysis and Meta-Regression

N. Watanabe1, J. Mak1, O. Itani3, D. Buyssse4, V. Kaneita2. 1Department of Health Promotion and Human Behavior, Kyoto University School of Public Health, Kyoto, Japan; 2Department of Social Medicine, Nihon University School of Medicine, Tokyo, Japan; 3Department of Public Health and Epidemiology, Oita University, Oita, Japan; 4Sleep Medicine Institute and Department of Psychiatry, University of Pittsburgh, Pittsburgh, United States

Introduction: The dose-response of long sleep duration in mortality and the incidence of important health outcomes such as diabetes mellitus, hypertension, cardiovascular diseases, stroke, coronary heart diseases, obesity, depression and dyslipidemia has been explored.

Materials and methods: We collected data from 5,134,036 participants from 137 prospective cohort studies. For the independent variable, we categorized participants at baseline as having long sleep duration or normal sleep duration. Risk ratios (RRs) for mortality and incident health conditions during follow-up were calculated through meta-analyses of adjusted data from individual studies. Meta-regression analyses were performed to investigate the association between each outcome and specific thresholds of long sleep.

Results: Long sleep was significantly associated with mortality (RR, 1.39; 95% CI, 1.31–1.47), incident diabetes mellitus (1.26, 1.11–1.43), cardiovascular disease (1.25, 1.14–1.37), stroke (1.46, 1.26–1.69), coronary heart disease (1.24, 1.13–1.37), and obesity (1.08, 1.02–1.15). Long sleep was not significantly related to incident hypertension (1.01, 0.95–1.07). Insufficient data were available for depression and dyslipidemia. Meta-regression analyses found statistically significant linear associations between longer sleep duration and increased mortality and incident cardiovascular disease.

Conclusions: Long sleep duration is significantly associated with multiple adverse health outcomes. A major strength of these analyses is the use of identical analytic methods for the different outcomes. Future studies should address whether the relationship between long sleep and health outcomes is causal and modifiable.

Acknowledgements: This study was funded by Industrial Disease Clinical Research Grants (160102-01), and by Health Labor Sciences Research Grant (H25-JUNKANNOTOU-JPAN-007) from the Japanese Ministry of Health Labor and Welfare.

Sleep Breathing Disorders

EVALUATION OF UPPER AIRWAY OBSTRUCTION OF OSA CHILDREN THROUGH SLEEPCENDOSCOPY (DISE)

S. Weber1, A.C. Marão1, J.L. Barros2, B. Zapponi1. 1Otolaryngology, Brazil; 2Sleep Lab, UNESP State University São Paulo, Botucatu, Brazil

Introduction: Obstructive sleep apnea syndrome (OSA) affects 1 to 4% of the children, the gold standard treatment being adenotonsillectomy (AT). However,10 to 35% of the patients submitted to AT remain with residual OSA. Drug-induced sleep endoscopy (DISE) may aid in the diagnosis of the levels of upper airway collapse (VAS) and thus, help in prediction of success or improve decision for best treatment.

Objectives: Evaluate the feasibility of drug-induced sleep endoscopy (DISE) in children with OSA and to correlate the research with OSA severity with apnea and hypopnea index (AHI).

Methods: Prospective study evaluating OSA children diagnosed by polysomnography, with indication of adenotonsillectomy (AT). They were submitted to DISE at the beginning of the surgery. The levels of obstruction were described by VOTE scale and associated to OSA severity.

Results: Out of 45 children, 7 had incomplete polysomnography data, 6 did not finish DISE due to hypersalivation and/or laryngospasm. 32 (20 male), mean age 7.5 years (3.25–9.92) and mean body mass index (Z-score) 1.24 (-2.06–10.42), completed all stages of evaluation. The mean AHI was 19.47 (2.9–54.8). DISE showed complete obstruction at the velopharynx in 15 (46.8%) patients and at oropharynx in 23 (71.8%). In 27 (84.3%) showed combined obstruction at velum and oropharynx. Complete obstruction of the tongue was observed in 9 (28.1%).

Conclusions: DISE can be considered an easy tool to evaluate other less common obstructive sites, but as there is manipulation of the airway, it must be realized in a safe environment. Untypical obstruction levels (tongue and epiglottis) were frequent, no significant association was found between obstruction of these sites and the severity of OSAS.