Sleep, Circadian Rhythms and Interval Timing

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Timing is a fundamental property of many biological systems, however, a systems biology approach to timing is still missing. Indeed, timing can be investigated at genetic/epigenetic, cellular, neuronal and behavioral level [1].

The temporal interplay between genetic and epigenetic mechanisms determines behavioral, physiological and molecular phenotypes across the organism. The understanding of how genetic sequences translate in complex phenotypes is a major challenge in current functional genomics. This difficulty is due to the fact that modules of genes co-express [2] and a particular phenotype results from the precise temporal dynamics (i.e., daily oscillations) of such modules.

Single-cell organisms adapted to the environment by entraining to external stimuli (caused by the earth's rotation) in order to set the time of their metabolic processes and sleep-wake rhythms evolved from this rest-activity cycle. The success in discovering molecular loops that set the circadian clock has favored the diffusion of clock-like models in other fields of investigation. For instance, the presence of a pacemaker-like mechanism has been hypothesized in timed biological processes across many time-scales (microseconds, seconds, hours). Thus, lots of effort in research has been dedicated to develop comprehensive clock-like computational models across different timescales.

Timing is also fundamental in cognitive processes although the mechanism by which the brain codes for timing is still unknown [3-5]. In mice, as in many other species, conditioning behaviors are subjected to temporal determinants and a brief (seconds to minutes) time interval (namely interval timing) itself may embody the proper information to be learned.

However, as the mechanisms to time information across different timescales vary, we investigate timing at different timescales and at systems level [6]. An integrated investigation of timing mechanisms represents a powerful instrument to understand strategies that evolution has developed to timestamp information in daily life [1]. Here we will present evidence that support the use of mice in understanding the biology of timing properties in cognitive, circadian and sleep homeostatic processes. In particular, by studying mouse models in which specific genetic and epigenetic variables were manipulated, we identified a series of abnormal timing and sleep phenotypes.

We investigated cognitive timing in circadian and genomic imprinting mutant mouse lines. We have paralleled the behavioral analysis with long-term electrophysiological measures in home-cage. An analysis of the sleep-wake patterns of mice reveals a close relation between timing behaviors, circadian and sleep physiology.

We will show evidence that interval timing and temporal uncertainty [7] are compromised in a circadian mouse model. Circadian mutants showed asynchrony daily activity compared to littermate controls. Moreover, the performance during the dark phase of the light/dark cycle was significantly worse in the mutants compared to controls. Interestingly, we found that during the dark period (12 hours, from 7 p.m. to 7 a.m.) the control mice made significantly less mistakes respect to the mutants in every experimental phase. The control mice had a better performance compared to the mutants and they also showed a circadian trend in the error rate that is disrupted in the mice carrying the mutation in both heterozygosity and homozygosity.

A reduction of the spread of the timing peak of the behavioral responses in control mice during probes (not rewarded trials) occurred when the probability of probes was high, which testify a reduction of temporal uncertainty. On the contrary, the spread of responses was high and not significantly different in both heterozygous and homozygous mutant mice at each change of the probability of probes. This suggests that under different risk assessments the temporal uncertainty of mutants does not decrease. The reduction of the

uncertainty, due to different risk assessments, produced also a change in the strategy. When the control mice become confident in a low probability condition they decide to anticipate the switch to the long location, while when the probability to receive the reward in the long location is lower, they persist more in the short location, postponing the switch.

Moreover, we will present evidence for a role of an imprinted gene, *Gnas* [8], in sleep and sleep-related timing functions. Sleep homeostasis refers to a process in which the propensity to sleep increases and decreases as wakefulness and sleep, respectively, progress [1]. Importantly, sleep modulates cognitive performance. Recent advances in functional studies of sleep states strongly suggest that non-REM sleep facilitates memory consolidation via modulation of synaptic plasticity through highly synchronized slow cortical activity, while REM sleep sustains consolidation by means of hippocampus-generated rhythms [9]. However, a direct measurement of synaptic plasticity and sleep and EEG activity is lacking. We have used a unique *in vivo* setup that allows the simultaneous and synchronized monitoring of EEG and behavior (by a home-cage 24-h monitoring system) to study sleep-cognition interactions. REM and NREM sleep are two distinct stages of the sleeping brain which are involved in the modulation of metabolic, physiological and cognitive processes. Despite these advances in functional understanding of REM and NREM sleep states, genetic and epigenetic mechanisms of sleep-dependent plasticity and memory processing are not currently well understood.

Several clinical and experimental evidence pointed out so far that imprinting genes are important in the regulation of sleep. However, the fundamental question whether genomic imprinting exerts a direct role in sleep, has remained elusive up to now. To address this crucial question we have started, a few years ago, to study sleep physiology and cognition in mouse models with an altered parent-of-origin expression profile. In this work we show that REM and NREM brain states are differentially modulated by the maternally expressed imprinted gene, *Gnas*. In particular, NREM-dependent physiologic and cognitive functions are enhanced while REM and REM-linked functions are inhibited. This is the first demonstration that we are aware of a specific effect of genomic imprinting on sleep states and their associated effects on cognition.

Interestingly, we observe a trade-off across different behavioral measures. For example, an increased precision in timing estimation is negatively correlated with the performance in behavioral fear responses.

References

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