EFFECTS OF ANTISENSE-INDUCED DOWNREGULATION OF CIRCADIAN GENE PERIOD1 IN THE BASOLATERAL AMYGDALA ON SPONTANEOUS SLEEP-WAKEFULNESS AND CONSOLIDATION OF TRAUMATIC MEMORY

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INTRODUCTION

❖ The basolateral amygdala (BLA) significantly influences both the sleep-wake cycle and various memory functions, highlighting its intricate role in governing sleep regulation.

❖ The basolateral amygdala plays a crucial role in regulating the timing and coordination of the sleep-wake cycle through its involvement in circadian processes.

❖ Although many studies have not distinguished functional roles of the BLA, this study suggests that modulating the expression of the Period1(Per1) gene in the BLA has a role in regulation of the sleep-wake cycle.

Hypothesis

The basolateral amygdala regulates the circadian process of sleep in BL6J/c57 Wild-type (WT) mice.

Research Question

Does antisense-induced expression of Per1 in the BLA reverses sleep-wake cycle?

Conclusion

Antisense-induced downregulation of the Per1 gene in the BLA increases wakefulness during sleep period and decreases wakefulness during active period.

Salient Findings

❖ Local infusion of Per1 AS ODNs in the BLA, at light onset (ZT=0), downregulates Per1 expression in the BLA, without having any effect in the SCN.

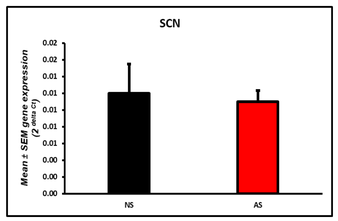
❖ Antisense-induced down regulation of Per1 expression in the BLA reverses sleep-wake cycle as evident by:

1. **<u>During normal sleep (light) period</u>:-** Increased latency to sleep, reduced amount of time spent in NREM and REM sleep along with increased wakefulness were observed.
2. **<u>During normal wake (dark) period</u>:-** Reduced latency to sleep, increased amount of time spent in NREM and REM sleep along with reduced wakefulness were observed.

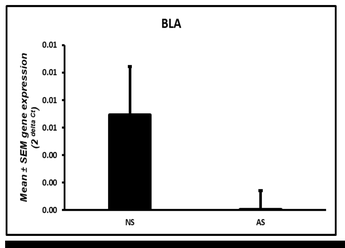
Result

Period 1 Gene expression

No significant changes observed in SCN



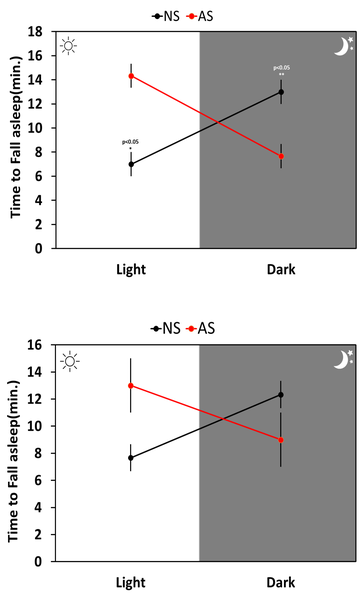
Significant downregulation of per1 gene in the BLA



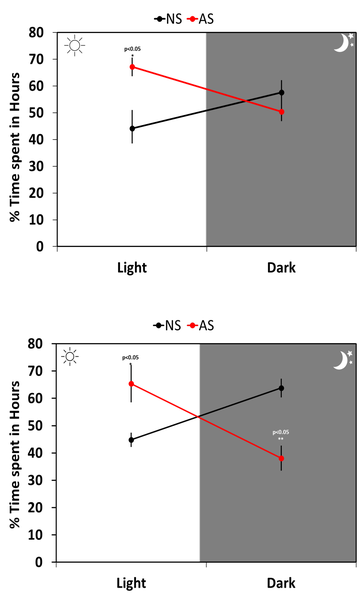
Post Infusion Result

Significant decreases in NREM latency and the percentage of time spent in sleep (NREM and REM) were observed during the active (dark) period in the AS group as compared to the NS group on Post-infusion Day 1 and Post-infusion Day 2.

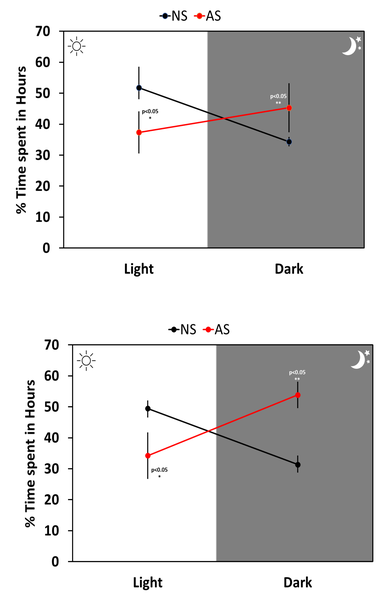
Sleep Latency



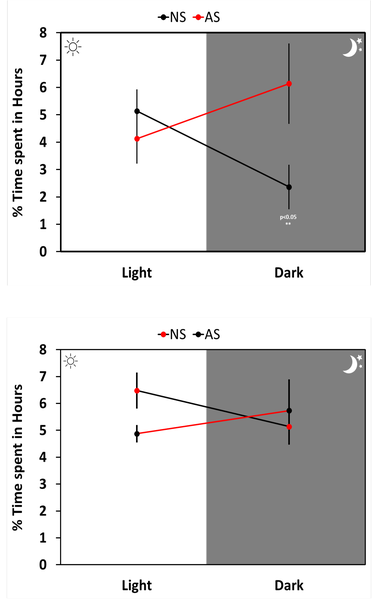
Wakefulness



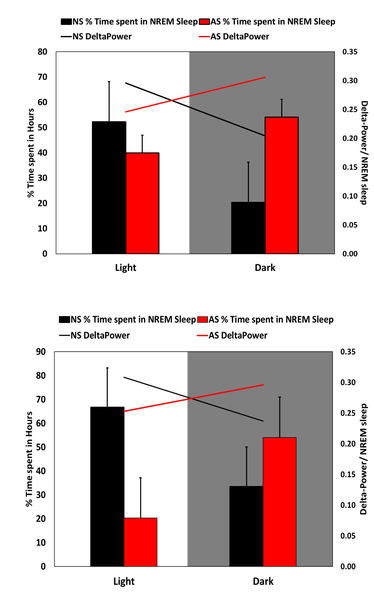
NREM



REM



NREM-Delta(▲)



Experimental Design

Surgery

Animals: C57BL/6J Wildtype mice.

EEGs: Screw electrodes were implanted on the skull to record sleep.

EMGs: EMGs were implanted to record the muscle activity.

Bilateral cannulas were implanted to guide microinjection (2mm above the BLA).

Habituation

5-7 Days

Baseline recorded; Animals were allocated in two groups

Experiment Timeline

Antisense oligonucleotides (AS-ODNs; Antisense group)

Nonsense oligonucleotides (NS-ODNs; Nonsense group)

Micro-injection

ZT0=7AM

Day-1

Sleep-wake analyzed

Day-2

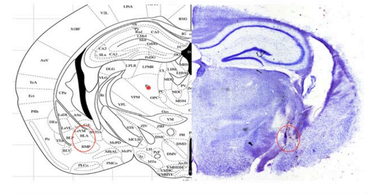
Sleep-wake analyzed

Gene expression

Animals were euthanized; BLA and SCN were punched out for the RNA isolation at Light-onset on Day-3.

Histology

Injection site verification



Tissue Punch