Prenatal Infection and Circadian Disruption: fat distribution, microglial morphology and clock gene expression

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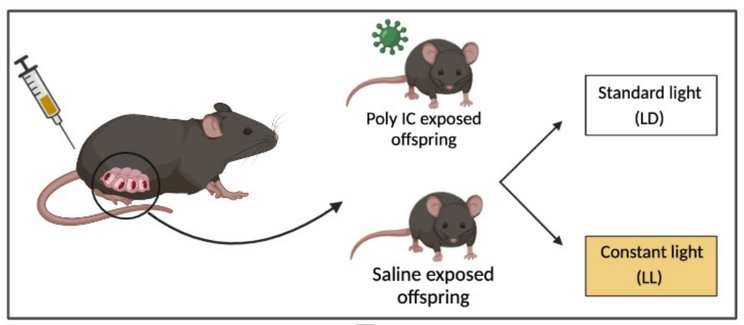
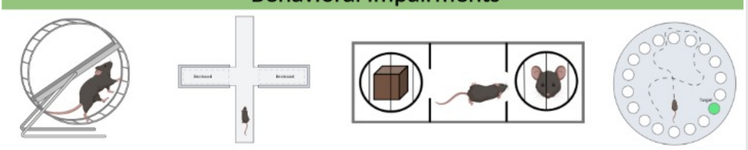
Introduction

Up to 80% of individuals with neurodevelopmental disorders (NDDs, such as schizophrenia) exhibit disrupted circadian rhythms[1]. A known risk factor for schizophrenia is prenatal infection during the mother's pregnancy[2]. Aside from being a symptom of schizophrenia, circadian disruption may also act as a risk factor to the disease[3]. Additionally, recent findings in our laboratory have shown that the interaction between circadian disruption and prenatal infection leads to a number of behavioral impairments characteristic of NDDs[4], including decreased social preference. Therefore, we hypothesized that prenatal infection will interact with circadian disruption and lead to changes in fat distribution[5], microglia morphology[6], and clock gene expression[7], three parameters known to be disrupted in NDDs. To evaluate this, we used the Maternal Immune Activation protocol that models prenatal infection in mice. Pregnant dams were injected with the viral mimic poly IC (or saline) at embryonic day 9.5, and resulting offspring were exposed at adolescence to constant lighting (LL), a treatment known to disrupt circadian rhythms, or to standard lighting (LD).

Methods

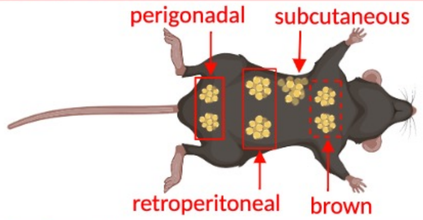


Behavioral Impairments



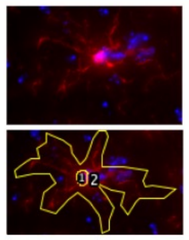
Experiment 1: fat distribution

4 fat regions were dissected and weighed: *subcutaneous, retroperitoneal, perigonadal, brown*



Experiment 2: microglia morphology

Images of microglia from the PFC were obtained through IHC, then analyzed on ImageJ.
Each microglia was characterized using morphology after the tracing of their processes and soma.



Experiment 3: clock gene expression

RNA was extracted from hippocampal and PFC brain slices, then reverse transcribed to cDNA. qPCR was performed to evaluate the expression of clock genes Bmal1 and Per2.

Figure 1. The neurodevelopmental mouse model used and its known and hypothesized effects

Results

Experiment 1: fat distribution

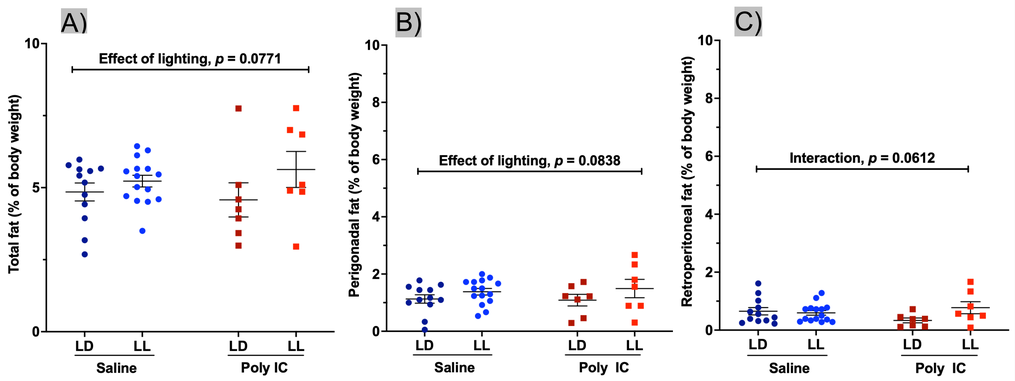


Figure 2. Effect of poly IC and constant light (LL) exposure on percent fat distribution in males. LL exposure led to a trending increase in (A) total (p=0.0771) and (B) perigonadal (p=0.0838) fat. A trending interaction between lighting condition (LL or LD) and group (poly IC or saline) was observed in (C) retroperitoneal fat (p=0.0612).

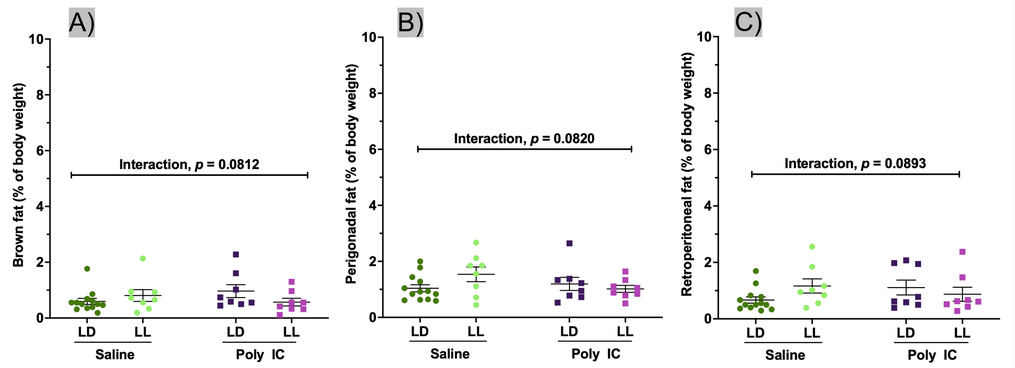


Figure 3. Effect of poly IC and LL exposure on percent fat distribution in females. There was a trending lighting by group interaction in (A) brown (p=0.0812), (B) perigonadal (p=0.0820) and (C) retroperitoneal fat (p=0.0893).

Experiment 2: microglia morphology

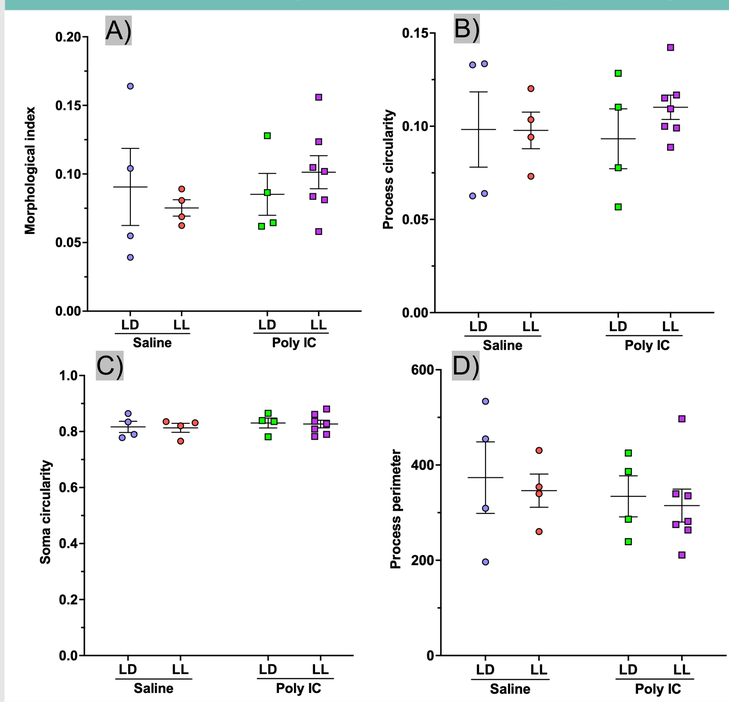


Figure 4. Effect of poly IC and LL exposure on microglia morphology in the prefrontal cortex (PFC) of male mice. No significant nor trending changes were observed in the (A) morphological index, (B) process circularity, (C) soma circularity, and (D) process perimeter of the microglia.

Experiment 3: clock gene expression

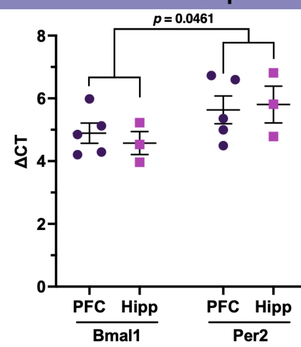


Figure 5. The ΔCT values of Bmal1 and Per2 (two clock genes) expressed in the PFC and hippocampus (Hipp) of wild type mice. Per2 was significantly more expressed than Bmal1 across both brain regions (p=0.0461).

Discussion

Experiment 1:

As demonstrated in this study, constant light led to a trending increase in total and perigonadal fat percentage in males. A trending lighting by group interaction was observed in retroperitoneal fat in both males and females. Females additionally experienced the same interaction in brown and perigonadal fat.

Future direction: Other metabolic factors than weight gain and fat percentage could be observed to examine the presence or not of a response to circadian disruption in the gut-brain axis.

Experiment 2:

In the PFC, we report no changes in microglia morphology in response to circadian disruption and prenatal infection. And surprisingly, no morphological differences were observed between poly IC and saline-exposed mice[6].

Future direction: To further validate our findings, we could increase the length of constant light exposure from four weeks and the number of mice per group from six.

Experiment 3:

The results obtained constitute preliminary data from wildtype mice. The purpose of this study was to help establish, troubleshoot and validate a protocol that will be used to perform qPCR on experimental samples in future studies.

References

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Acknowledgments

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