Exploring the Impact of the Microbiome on Sleep Homeostasis in *Drosophila melanogaster*

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Abstract

The study of the Brain-Gut-Microbiome axis is an exciting avenue of research. The microbiome has been shown to modulate behaviors and physiology associated with activity and movement including Parkinson's Disease, locomotion, and sleep. There are competing studies which argue whether the microbiome modulates the sleep homeostasis response. Sleep homeostasis in response to sleep deprivation in *Drosophila melanogaster* is regulated by *nur*, a gene which produces the secreted peptide nemuri. In order to test the effect of the microbiome on the sleep homeostasis response, wild-type, dechorionated, streptomycin-treated, and *L. brevis*-treated flies were sleep deprived. Relative expression of *nur* was compared across treatment groups to determine if one of the key regulatory mechanisms of sleep homeostasis is affected by microbial composition. Semiquantitative gel electrophoresis PCR revealed that differences in microbial composition modulated baseline sleep levels and rebound sleep, specifically in dechorionated flies. Differences in relative expression level of *nur* could not be detected between experimental groups, indicating that there is a relatively small difference in expression of *nur* induced by sleep deprivation. Microbial composition had no observable effect on expression of *nur.* For more precise differentiation between experimental groups, future studies should compare exact levels of transcription. Much more work needs to be done to probe the mechanisms through which the microbiome can regulate the sleep homeostasis response.

Introduction

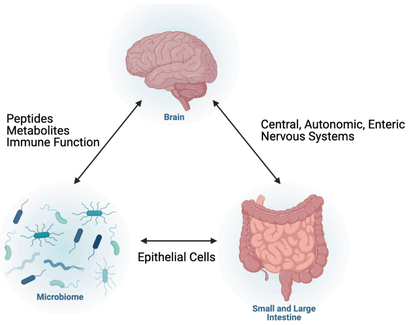


Figure 1: Mechanisms of communication through the Brain-Gut-Microbiome Axis. Diagram inspired by review from Martin et al., 2018.

- The Brain-Gut-Microbiome Axis is a bidirectional channel of communication. Through this axis, changes in the microbiome can have profound effect on physiology and behavior.
- *Lactobacillus brevis* can modulate locomotion in *Drosophila melanogaster* through secretion of xylose isomerase, which alters downstream metabolism of sugar in octopaminergic neurons (Schretter et al., 2018).

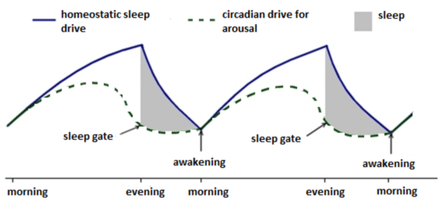
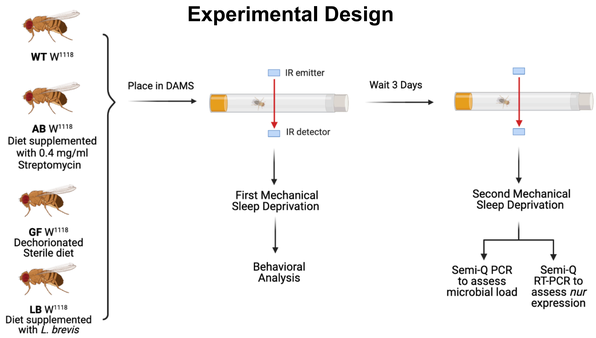


Figure 2: The interaction between the circadian and homeostatic mechanisms which control sleep. Diagram from Angeloff, 2017.

- Sleep is regulated through two interacting mechanisms. A circadian signaling network controls the timing of sleep, while the homeostasis response controls the drive to initiate and maintain sleep (Allada et al., 2017).
- *Nur* expressed at higher levels after a period of sleep deprivation in *D. melanogaster*. It produces the secreted peptide nemuri, which is required for a strong sleep homeostasis response (Toda et al., 2019).
- This study assessed the effects of the microbiome, with a focus towards *L. brevis*, on the sleep homeostasis response. I measured this effect through analysis of sleep behavior and transcription levels of *nur* to probe the regulatory mechanisms through which this effect occurs.

Experimental Design



- Fruit flies were placed in a Drosophila Activity Monitoring System (DAMS) to assess the sleep homeostasis response after the first mechanical deprivation.
- Treatment groups were deprived of sleep a second time to induce the expression of *nur*.
- Control flies for each treatment group were not deprived of sleep.

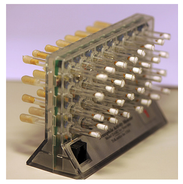


Figure 3: Flowchart detailing experimental design. Photo of DAMS from "CECAD: Prof. Dr. Linda Partridge."

Dechorionation Protocol

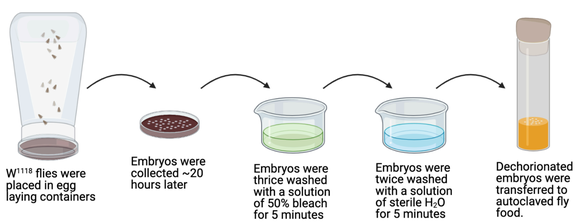


Figure 4: Embryos were washed with bleach to remove the microbiome of w1118 *D. melanogaster.* GF flies were reared in a sterile environment. Protocol adopted from Koyle et al., 2016.

W1118 flies were successfully deprived of sleep

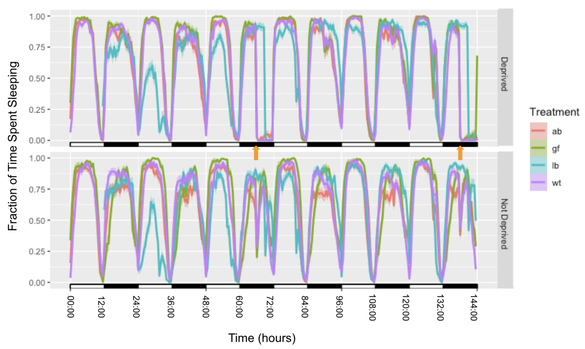


Figure 5: Fruit flies were successfully deprived of sleep. Fraction of time spent sleeping (Calculated as the average proportion of time spent sleeping in a period of 30 minutes per fly) throughout the duration of the experiment. Orange arrows indicate initiation of sleep deprivation. Flies were kept on a 12h:12h light:dark cycle.

Dechorionated flies had longer baseline sleep

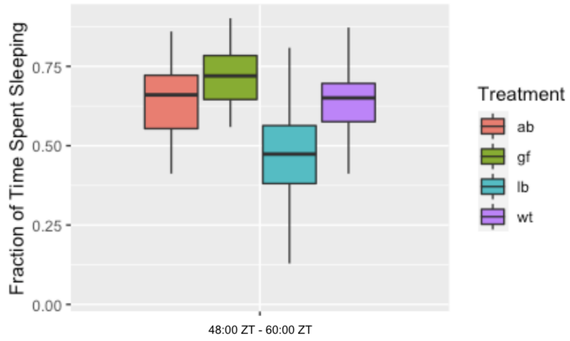


Figure 6: Microbial Treatment affected baseline sleep behavior. Fraction of time spent sleeping during the light phase prior to deprivation (median ± interquartile range) is shown for AB (n = 86), GF (n = 72), LB (n = 72), and WT (n = 89) flies from 48:00 - 60:00. The fraction of time spent sleeping during the 12 hours prior to deprivation was significantly different between treatment groups (Kruskal Wallis, χ^2 = 97.956, df = 3, p = 2.2e-16). Post hoc testing revealed significant differences between all groups (Pairwise Wilcoxon Test, p ≤ 2.3e-05) except WT and AB flies (p = 0.83).

Dechorionated flies had decreased rebound sleep compared to antibiotic and wild type w1118 flies

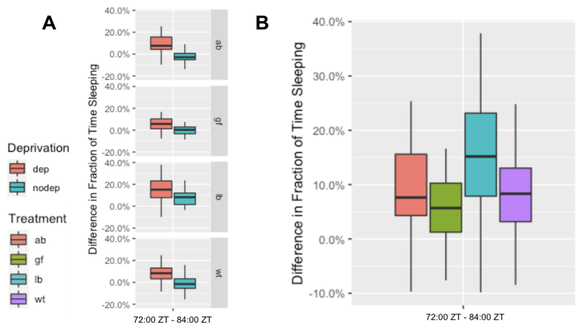


Figure 7: The microbiome caused a varied sleep homeostasis response in sleep deprived flies. A. Flies exhibited a sleep homeostasis response in the 12 hours following deprivation (72:00 ZT - 84:00 ZT). Median ± interquartile range is shown for AB dep (n = 43), AB nodep (n = 43), GF dep (n = 35), GF nodep (n = 37), LB dep (n = 38), LB nodep (n = 34), WT dep (n = 45), and WT nodep (n = 44). There was a significant difference in sleep rebound (difference in fraction of time sleeping before and after deprivation) between deprived and not deprived flies across all treatment groups (Wilcox Test, 988.5 < W< 1643.5, p < 0.001). **B**. Rebound sleep for deprived flies. There was a significant effect of microbial treatment on rebound sleep on deprived flies (Kruskal-Wallis χ^2 = 19.839, df = 3, p < 0.001). Post-hoc testing revealed a significant effect of treatment with *L. brevis* against all experimental groups (Pairwise Wilcoxon Test, p < 0.05). There was also a significant difference between AB and GF flies (p = 0.0325). There was no difference between WT and AB flies (p = 0.385) or between WT and GF flies (p = 0.125).

Dechorionation and antibiotic treatment did not eliminate total host microbiota, but did reduce load of *L. brevis*

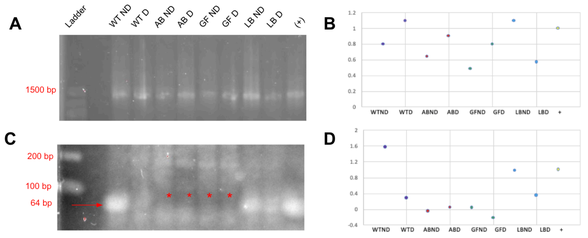


Figure 8: Semiquantitative PCR was used to measure microbial colonization across treatment groups. A. 16s Ribosomal DNA amplified by PCR were separated by electrophoresis on a 1% agarose gel. **B.** Microbial load in fruit flies was measured using relative fluorescence of 16s rRNA bands. Fluorescence for all experimental groups was normalized to the intensity of the 16s rRNA band from *L. brevis* genomic DNA. **C.** RecA gene amplified by PCR was separated by electrophoresis on a 3% agarose gel (* = no band). **D.** The relative amount of *L. brevis* in flies was quantified using the fluorescence of RecA bands of experimental groups normalized to the RecA band from *L. brevis.*

Nur expression did not significantly vary across experimental and treatment groups

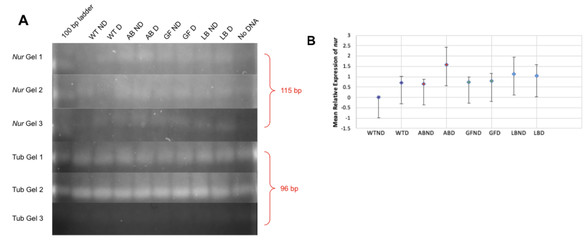


Figure 9: Semi-Quantitative PCR was used to assess levels of *nur* expression in *D. Melanogaster.* A. Gel electrophoresis of PCR reactions for *nur* and tubulin (Tub) are shown in triplicate. **B.** Relative expression of *nur* was quantified using band intensity normalized to the tubulin amplicon with the lowest intensity (mean ± standard error, n = 3). No statistically significant difference in relative expression of *nur* was found between experimental groups regardless of microbial treatment or deprivation status (Kruskal Wallis, χ^2 = 23, df = 23, p = 0.4608).

Conclusions

- Microbial composition affected the sleep homeostasis response.
- *L. brevis* is not required for regulating the sleep homeostasis response.
- Expression of nur was not significantly modulated by the microbiome.

Recommendations

- Sequencing of 16s amplicons to determine microbial composition of treatment groups.
- Determine microbial mechanisms of regulating the sleep homeostasis response in w1118 *D. melanogaster.*
- Use Droplet Digital™ PCR to determine the exact levels of *nur* transcripts in deprived and non deprived w1118 flies.
- Probe the mechanisms of *nur* regulation and nemuri functionality.

Works Cited

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