UNDERSTANDING THE MOLECULAR IMPACTS OF SLEEP DEPRIVATION IN *MUS MUSCULUS*

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INTRODUCTION

Sleep deprivation is a global health epidemic impacting those that work irregular hours such as shift workers and students. Studies have shown that in the U.S. alone, more than one-third of the adult population restricts their sleep to less than 7 hours per weeknight (Basner *et al*., 2013). Sleep deprived individuals have an increased risk of neurodegenerative diseases, impaired neurobehavioral functioning, and an increased susceptibility to metabolic and cardiac diseases as well as other health problems throughout the body (Basner *et al*., 2013). The consequences of sleep deprivation make it important to understand on a neurological level, with previous research indicating that a lack of sleep causes epigenetic alterations in the mRNA pool. Our studies focus mainly on post-transcriptional gene regulation in three regions of the brain that are particularly susceptible to the impacts of sleep deprivation: the hippocampus, prefrontal cortex, and cerebellum (Lyons *et al*., 2020). The Tau protein is known to be modified by sleep deprivation and is a neuropathological hallmark for Alzheimer's Disease and Related Dementias (ADRD). Our future research hopes to uncover whether the effects of sleep deprivation on the Tau protein correlates with the development of ADRD. We also hope to examine the effects of chronic sleep fragmentation when compared with acute sleep deprivation We utilized *Mus musculus* strain C57BL/6J as our model organisms for sleep deprivation due to their physiological and genetic similarities to that of humans. Mice are also cost effective and easy to look after under experimental conditions.

METHODS

ANIMAL CARE

Mus Musculus strain C57BL/6J, housed in groups of four, were obtained from our Jackson Labs distributor and ultimately split into individual housing (food, water, corn cob flooring, and cotton fluff bedding) while being kept on a 12-12 light-dark cycle for at least one week before any procedures began. Daily wellness checks and weekly cage changes were performed.

Figure 1- Sleep Deprivation Experiment

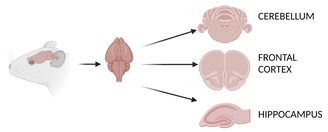


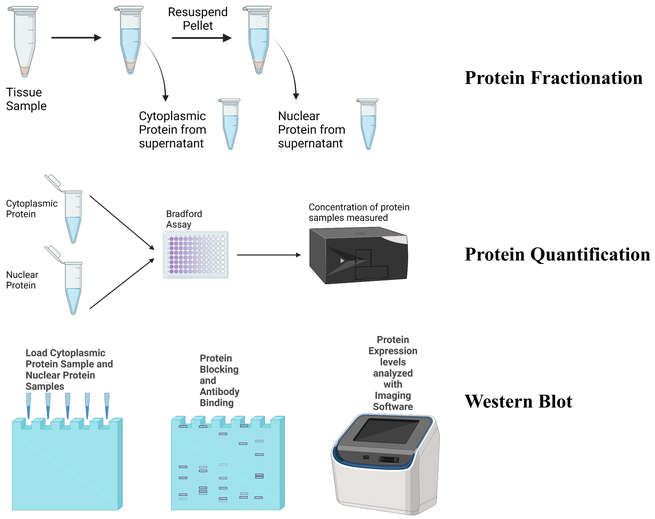
Figure 2- Brain Regions of Interest under Sleep Deprivation

SLEEP DEPRIVATION

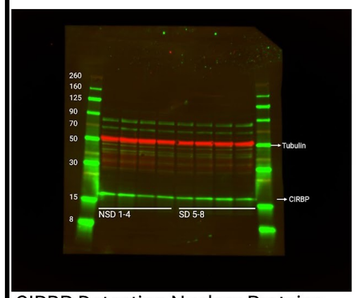
Three days before sleep deprivation experiments, groups of three mice (both experimental and control) had their cages tapped for three minutes. On the fourth day, mice were sleep deprived for five consecutive hours by tapping their cages to ensure that none of the mice would fall asleep or enter a period of micro-sleep. All sleep deprivation experiments started at the beginning of the light-dark cycle to avoid circadian confounds. After sleep deprivation, experimental and control mice were taken to the surgical room one at a time. After mice were euthanized, their brain tissue was collected. Samples were taken from the prefrontal cortex, the hippocampus, and the cerebellum. The samples then were stored in solutions of PBS that has been kept on ice.

MOLECULAR ANALYSIS

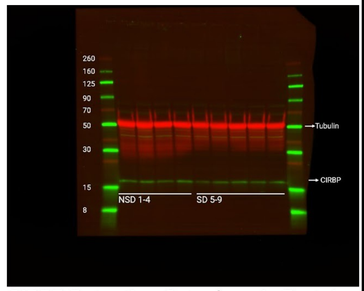
Sample tissues from the three brain regions of interest were homogenized and separated into fractionated nuclear and cytoplasmic proteins. Protein abundance was quantified using a colorimetric Bradford Assay at a wavelength 595 nm. Samples and standards were both measured in triplicates. A precast gradient (4-20%) SDS-PAGE acrylamide gels (Bio-Rad) were used to separate proteins by size. Gels were loaded equally by sample type based on total protein amount. Proteins were blotted onto a PVDF membrane using a Turbo-blot system. Membranes were blocked in the blocking buffer and the primary antibody Cold Inducible RNA Binding Proteins (CIRBP) at 1:500 concentration was added. Membranes were then blocked in secondary in order to fluoresce under imaging. Membranes were imaged with the Bio-Rad ChemiDoc MP Imaging System.



RESULTS



CIRBP Detecting Nuclear Proteins



CIRBP Detecting Cytoplasmic Proteins

WESTERN BLOT

Membrane was blocked using the Odyssey TBS blocking buffer (Li-Cor) prior to antibody exposure for 60 minutes to prevent nonspecific binding. Blocking buffer was poured out. Then a solution of blocking buffer and primary antibody (hnRNP, A2/B1) at 1:10,000 concentration was added. The membranes were tumbled at 4°C overnight. The primary antibody solution was then removed, and the membrane was washed with TBST three times for 5 minutes. Exposure to fluorescent secondary antibody (IRDye 680RD Goat anti-Rabbit) and a fluorescent antibody for tubulin (anti-tubulin, hFAB Rhodamine antibody) was done at room temperature for 1 hour in a solution of blocking buffer and TBST. Tubulin was used as a housekeeping protein for normalization. The samples were washed twice more in TBST on the tumbler for 5 minutes. Samples were washed with TBS on the tumbler for 5 minutes. Membranes were images with the Bio-Rad ChemiDoc MP Imaging System.

DATA ANALYSIS

CIRBP in the nuclear and cytoplasmic fractions of specific brain regions for sleep deprivation (SD) and non-sleep deprivation (NSD) mice were quantified with fluorescence relative to the housekeeping gene, Tubulin. Tubulin was selected because it is equally expressed in mice regardless of sleep deprivation. We will normalize our results using a ratio of CIRBP to Tubulin.

CONCLUSION

- 5 hours of sleep deprivation in a mouse model can be sufficient to cause molecular changes in protein expression.
- Changes in protein expression can be seen within western blotting with CIRBP, as we see concentration changes between nuclear and cytoplasmic samples.
- Multiple brain tissue samples are in storage to continue work with Tau antibodies and RNA extraction.

FUTURE DIRECTIONS

Experiments are still ongoing as we pursue more work with protein analysis and changes in RNA. Preliminary results with the Tau protein have so far been inconclusive due to difficulties with getting the antibody to fluoresce, but the next steps include further studies involving Tau and the molecular connections that bridge sleep deprivation and the development of Alzheimer's Disease.

Further work also includes the extraction and sequencing of multiple RNA targets that could explain a difference in gene expression between sleep deprived males and females. Preliminary results with female mice have shown different upregulations of genes during certain stages of the estrus cycle. Experiments are also planned to investigate sleep fragmentation, which is a harsh 28-day protocol and may give us more insight into how shift work in humans can negatively impact health.

ACKNOWLEDGEMENTS

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