

Investigating the Impact of Chronic Sleep Deprivation on Hippocampal Subregion Gene Expression in Young Male Mice

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Abstract

Sleep deprivation is a widespread public health concern affecting multiple demographics around the world. Previous studies have shown deficits in cognitive function and memory consolidation following sleep deprivation, and these changes occur alongside gene expression alterations. More recent studies have shown these alterations occur differentially between hippocampal subregions.

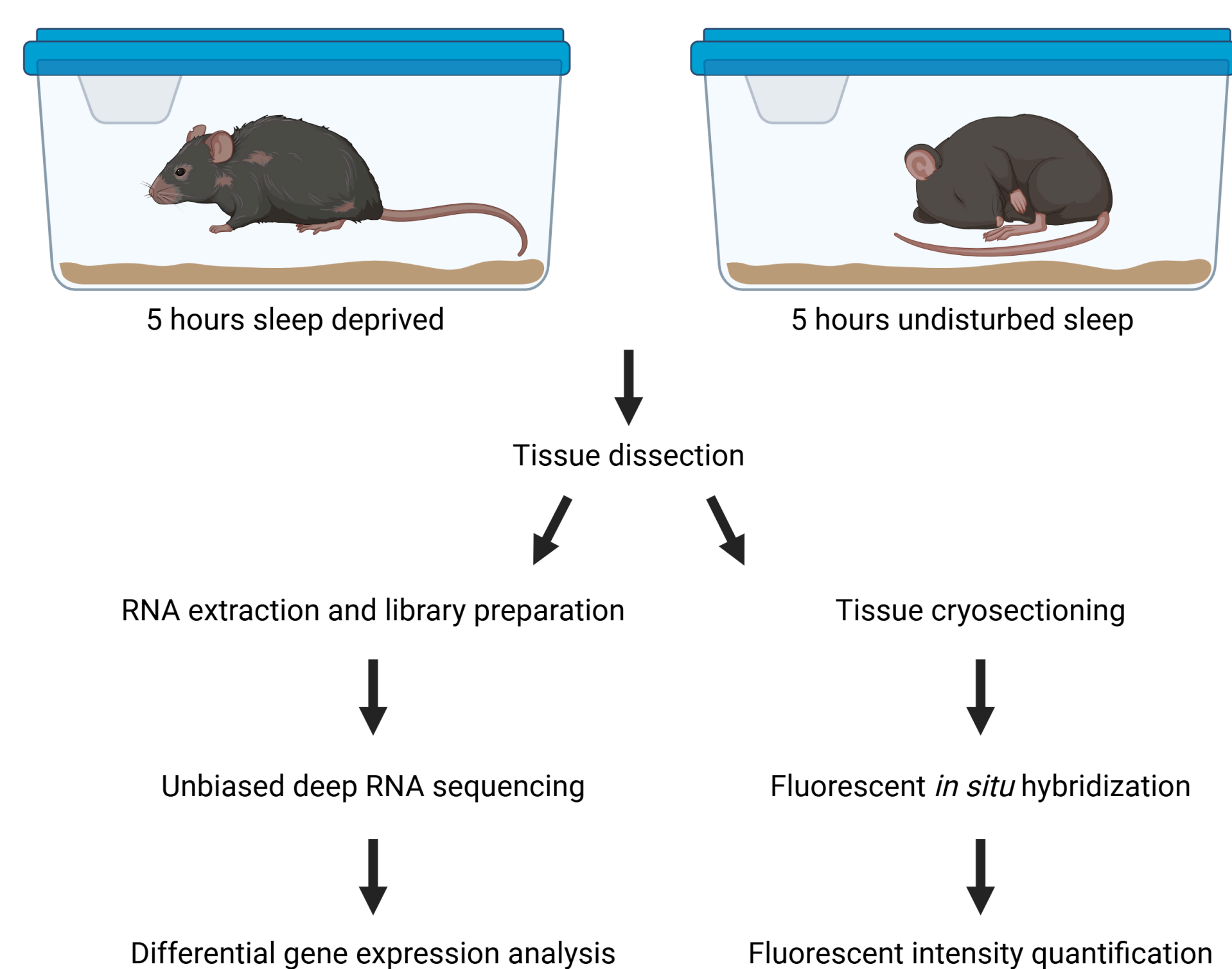
In this study, we use fluorescent in situ hybridization (FISH) to investigate region-specific changes in RNA within the hippocampus in response to chronic sleep deprivation in young male mice. These subregions were then examined with imaging analysis tools (FIJI) to quantitatively measure spatial RNA expression. This work aims to explore that chronic sleep deprivation has major implications on molecular mechanisms that relate to memory storage and synaptic functions.

Background

Sleep deprivation is an increasingly prevalent issue that affects all demographics including children, adolescents, and adults. Sleep loss interferes with spatial memory consolidation and storage, cognitive ability, and neuronal function. Long-term sleep loss has been linked to the development and progression of comorbid disorders, including diabetes, hypertension, and Alzheimer's disease. Despite this, the effects of chronic sleep deprivation on the brain are not well characterized. However, understanding the molecular and long-term effects is crucial, as chronic sleep deprivation poses a significant public health risk. In this study, we use spatial analysis to quantify these molecular changes in the brain associated with chronic sleep deprivation.

Preliminary data has shown transcriptomic differences following chronic sleep deprivation, and these changes are brain region-specific, especially in the prefrontal cortex and the hippocampus. Additionally, previous literature has shown differences in gene expression between hippocampal subregions following chronic sleep deprivation. Based on these findings, we hypothesize that chronic sleep deprivation induces RNA level changes in these hippocampal subregions. To investigate, we assessed these RNA changes spatially using a RNAscope fluorescent in situ hybridization (FISH) against SNAP25 and Rbfox1, two genes important for synaptic plasticity.

Experimental Design



Materials and Methods

Male mice models were housed under standard laboratory conditions and were subjected to chronic sleep deprivation consisting of 5 hours per day for 5 consecutive days. All procedures were conducted in accordance with institutional and NIH guidelines. Mice were group housed in cages using soft bedding with food and water using a light-dark schedule.

Following the final sleep deprivation period, the sleep deprived and the non-sleep deprived mice's hippocampus were dissected into subregions. Half of each brain was subduced in OCT, frozen in a dry ice ethanol bath. They were then cut dry and sectioned through the dorsal hippocampus using a cryostat to 12 microns and placed onto Fisherbrand Superfrost slides. RNAscope in situ hybridization was performed where the slides were processed using commercially available RNAscope fluorescent reagent kits. Tissue sections were pretreated, hybridized with target-specific RNA probes, and protected by DAPI for visualization of nuclei. Images were obtained with a Nikon Confocal CSU W1 at 40x magnification.

Fluorescent images were quantified and analyzed by FIJI. Hippocampal subregions were identified, and rolling-ball background subtraction was applied to each channel. Mean fluorescence intensity (MFI) was measured within defined regions of interest for each subregion. MFI values were then averaged per subregion for each sample per animal, then normalized to the corresponding control condition, where the values were statistically compared by using student's t-test.

Hippocampal subregional gene expression following chronic sleep deprivation in young males

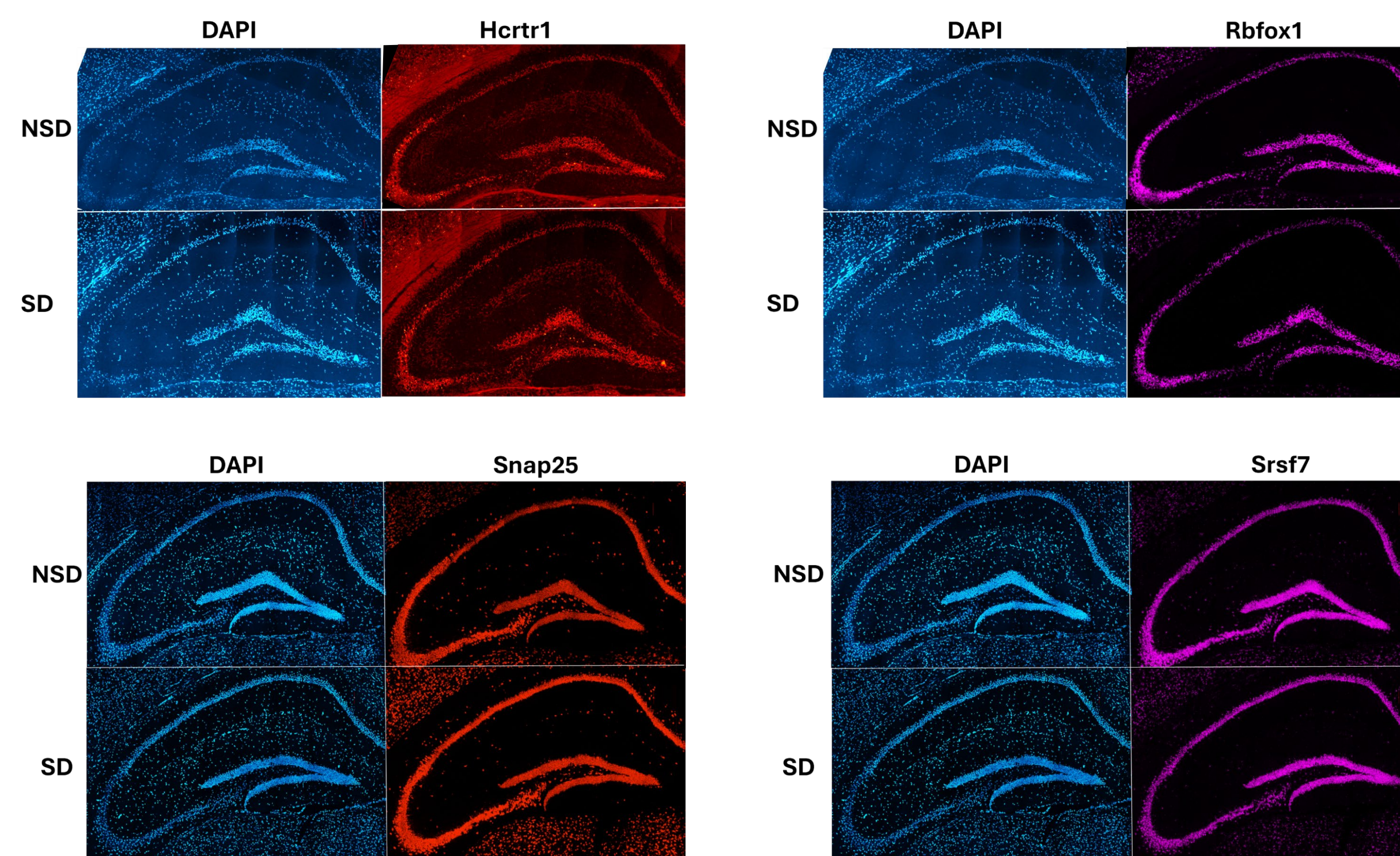
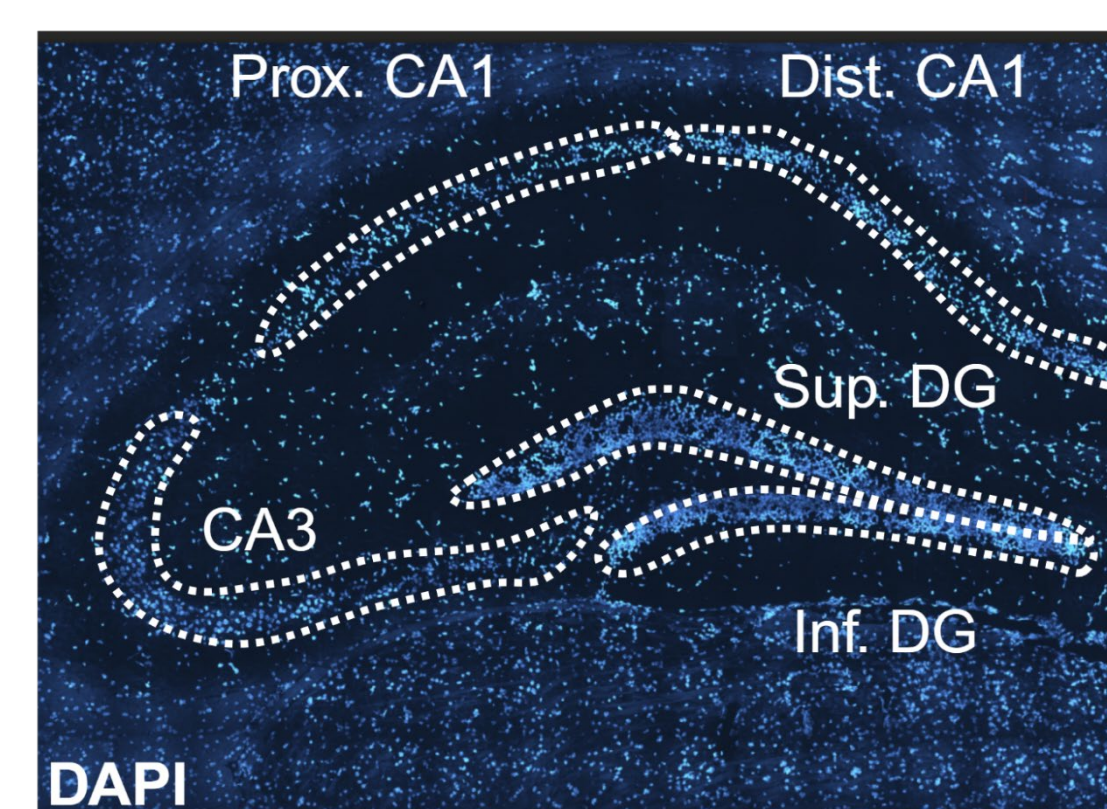


Figure 1: Representative images of whole hippocampi stained with DAPI (left column) and RNAscope probes (right column) from non-sleep deprived (top row) and sleep deprived mice (bottom row). * indicates P-Value < 0.1

Fluorescent quantification of gene expression in hippocampal subregions

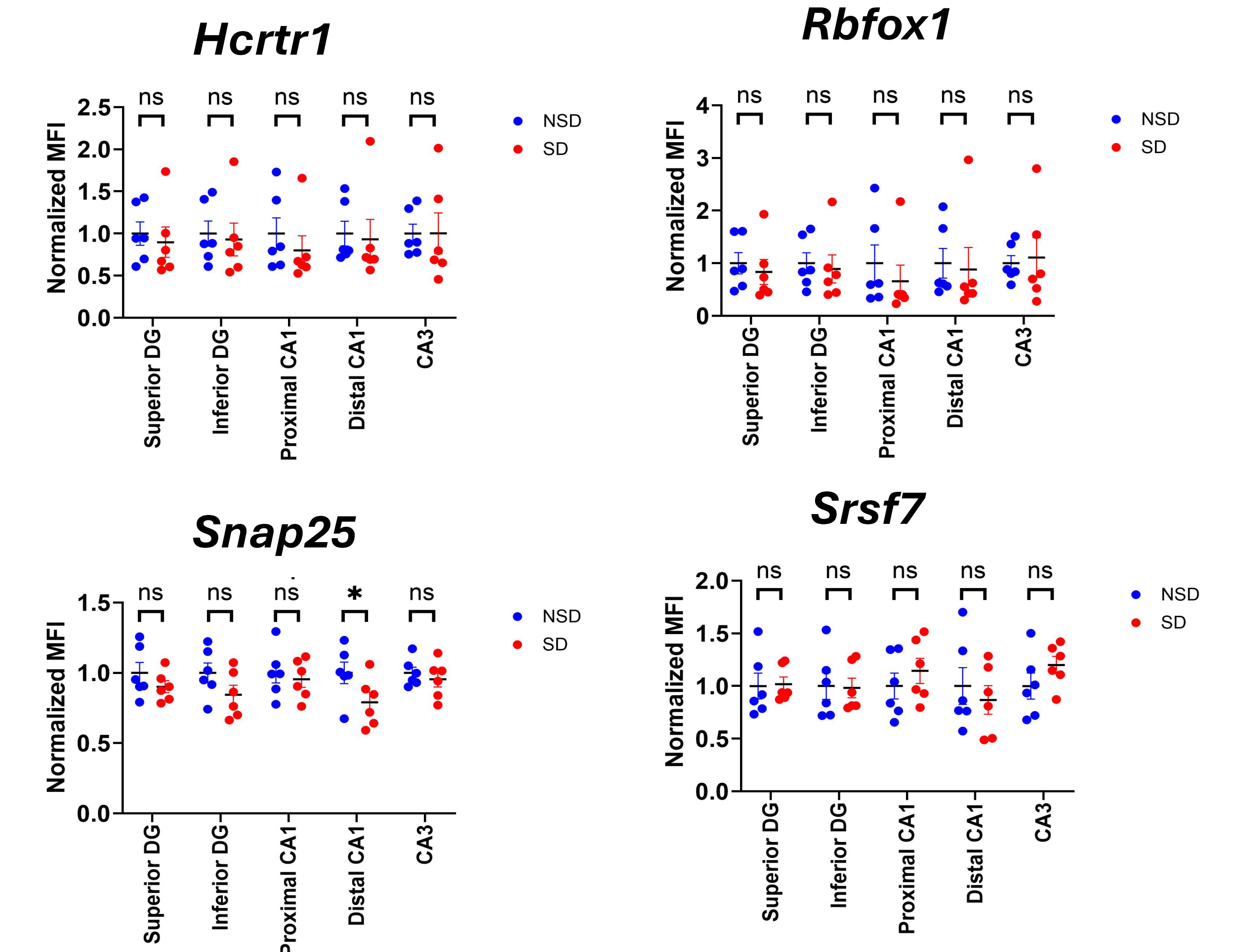


Figure 2: Scatter dot plot of batch normalized MFI values for each hippocampal subregion and 40x representative images of RNAscope probes from non-sleep deprived (top row) and sleep deprived controls (bottom row).

Conclusions

Snap25, a presynaptic plasma membrane protein involved in the regulation of neurotransmitter release shows decreased gene expression following chronic sleep deprivation. In this study *Snap25* was found to be significantly downregulated in the distal CA1 subregion, a major output area for the hippocampus tied to spatial learning and memory.

Rbfox1, a transcription factor, was found to be upregulated at the RNA level following chronic sleep deprivation. Conversely, *Srsf7*, an RNA binding protein, and *Hcrtr1*, a G protein coupled receptor, were found to be downregulated following chronic sleep deprivation. However, no significance was found through our fluorescent quantification.

One limitation of this study is use of lower resolution imaging in this dataset (40x magnification vs 60/100x magnification). Shifting to puncta counting in the future may provide more informative results from this dataset.

Our results demonstrate a subregional downregulation of *Snap25* specific to the distal CA1 region which may help explain the deficits to spatial learning and memory observed following sleep deprivation, both chronically and acute.

Acknowledgments

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References

- Gainetdinov, Marie E., et al. "Altered Hippocampal Transcriptome Dynamics following Sleep Deprivation." *Molecular Brain*, vol. 14, 2021, p. 125, <https://doi.org/10.1186/s13041-021-00835-1>. Accessed 17 Nov. 2025.
- Lyons, Lisa C., et al. "Sleep and Memory: The Impact of Sleep Deprivation on Transcription, Translational Control, and Protein Synthesis in the Brain." *Journal of Neurochemistry*, vol. 166, no. 1, 2023, p. 24, <https://doi.org/10.1111/jnc.15787>. Accessed 17 Nov. 2025.
- Vanrobaeys, Yann, et al. "Spatial Transcriptomics Reveals Unique Gene Expression Changes in Different Brain Regions After Sleep Deprivation." *Nature Communications*, vol. 14, no. 1, 2023, p. 7095, <https://doi.org/10.1038/s41467-023-42751-z>. Accessed 17 Nov. 2025.