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The Effects of Circadian Misalignment on Astrocyte Morphology in the Nucleus Tractus Solitarius

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The Effects of Circadian Misalignment on Astrocyte Morphology in the Nucleus Tractus Solitarius

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INTRODUCTION

- Approximately 2 in 5 workers in the US are engaged in non-standard shifts, including evenings, nights, rotating, and weekends.
- Clinical studies have shown that shift-work schedule may increase risk of cancer, cardiovascular disease, gastrointestinal disorders, and metabolic disorders.
- Healthy individuals typically experience blood pressure dips overnight due to less need for circulation, but night shift workers have exhibited non-dipping blood pressures that may correlate with a worse prognosis in cardiovascular disease, due to a prolonged hypertensive state.
- Limited research exists on the relationship between shift work, circadian misalignment, and associated adverse health effects.
- The caudal nucleus tractus solitarius (nTS) plays a crucial role in autonomic control for the cardiorespiratory system and mediates baroreflex control of arterial pressure in part through the excitatory neurotransmitter glutamate.
- Decreased levels of glutamate in the nTS have been linked with increased blood pressure during wake. However, how glutamate changes in shift work is unknown.
- Astrocytes not only provide structural filament support through glial fibrillary acidic protein (GFAP), but also contain excitatory amino acid transporters responsible for glutamate uptake.
- Astrocytes are capable of gliosis which is a reactive response to neurological stress that results in hypertrophy or proliferation of glia.
- Immunohistochemistry (IHC) was used to visualize GFAP and S100β, enabling the observation of astrocyte branches and cell bodies, respectively.
- Analyzing changes in the number or length of astrocyte branches, along with astrocyte counts themselves, during hypertensive periods can provide insights.

HYPOTHESIS

We hypothesize that Sprague Dawley rats experiencing circadian misalignment (CM) will exhibit reduced glutamatergic activity in the nTS due to increases in astrocytes, astrocyte branches, and/or longer branch lengths, resulting in an increase in blood pressure.

METHODS

- Sprague Dawley male (7-11 weeks) rats were subjected to a six-hour light-dark (LD) phase delay every 2 days to simulate a shift work schedule, while control (CTL) rats were maintained on a standard twelve-hour LD phase for twenty-two days.
- ***Four time points were used for data collection, however we only show data taken at 9am and 9pm.***

Immunohistochemistry (IHC)

- Rats were perfused with 0.01M phosphate buffered saline (PBS) and underwent fixation with 4% paraformaldehyde.
- The brainstem containing the nTS was removed and sliced at 30µm. Tissue was stored in cryoprotectant until use.
- Primary antibodies against GFAP (Synaptic Systems #713004, 1:500) and S100β (AbCam #AB41548, 1:100), followed by secondary antibodies Cy2 and Cy5, respectively were used.
- Brain slices were incubated with the primary antibodies overnight and washed before being incubated with the secondary antibodies for a two-hour period. The specimens were washed with PBS between each antibody staining and were transferred to microscope slides to be stored at refrigerated until use.

Confocal Imaging

- The nTS was located and imaged using confocal microscopy at magnification of 10x, 40x, and 60x.
- 10x magnification was used to determine the location of the nTS (caudal, rostral, anterior-posterior)
- Cell body counts were conducted using 40x Z stack (0.5 µm increments and oil immersion).

Analysis

- ImageJ/Fiji software was used to optimally visualize the nTS astrocytes with high intensity and max entropy immunofluorescence.
- Z project function was used to combine the stack and identify the total number of cell bodies and perform threshold analysis using the analyze particles function. Size of particles ranged between 5-300 microns^2 and circularity remained between 0.00-1.00 units. Final cell counts were averaged and graphed with respective standard deviations.

Statistical Analysis

- Analysis was performed using FIJI and GraphPad Prism 9.02.
- Student t-test was used to examine astrocyte count in CTL/CM at the two time points. Statistical significance is considered p<0.05.

SPECIFIC AIMS

To analyze the shape and quantity of astrocytes, located in the nTS, between CTL and CM rats in hypertensive environments.

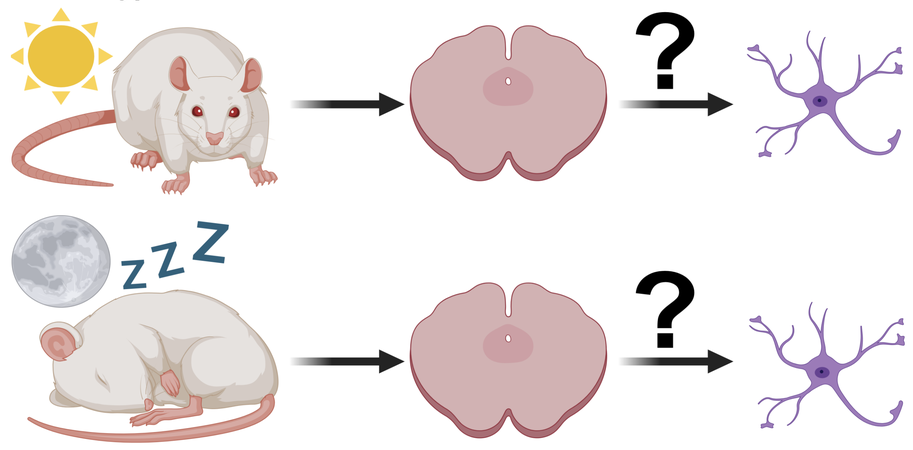


Figure 1: A rodent model of circadian misalignment with a six-hour light:dark (LD) phase delay established, while the control rats were exposed to twenty-two days of a standard twelve-hour L:D phase.

TIMELINE

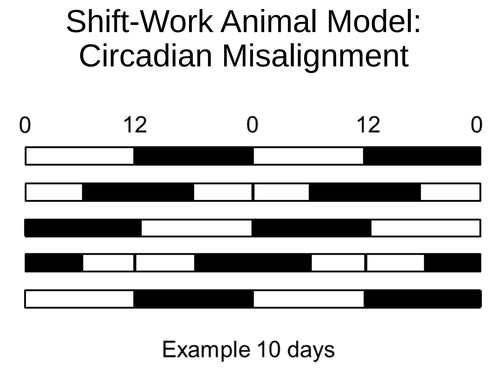
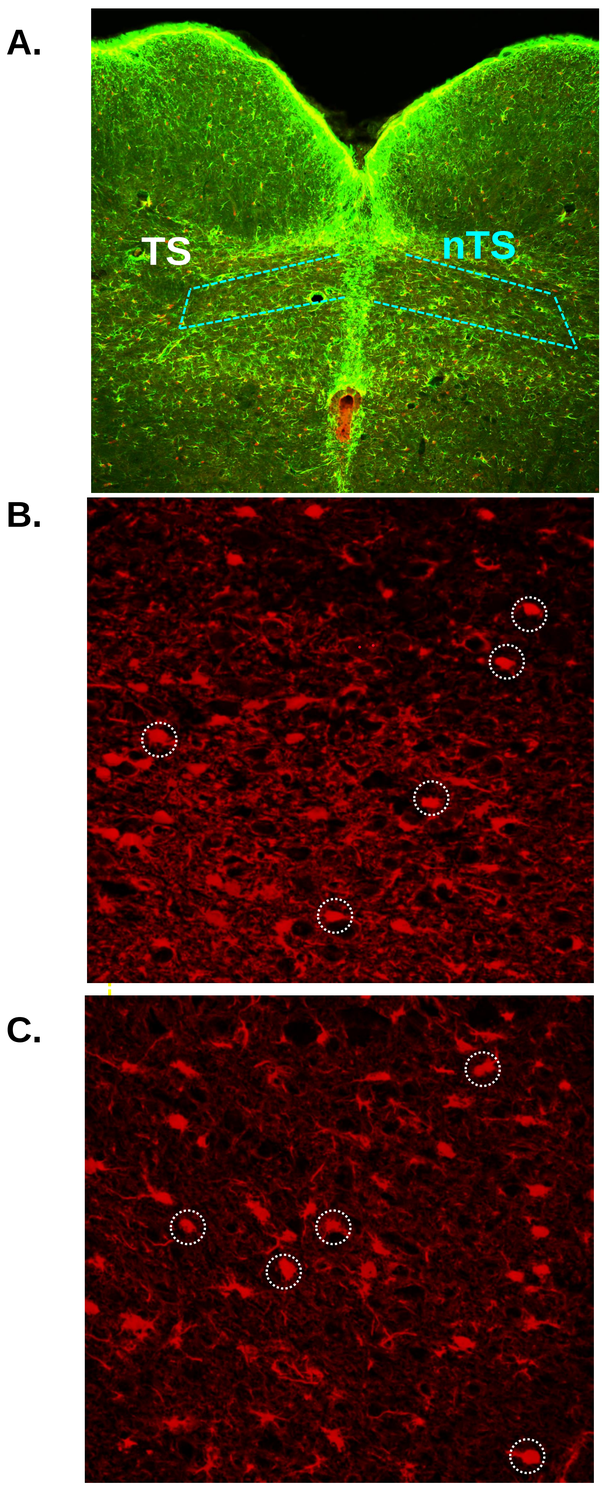
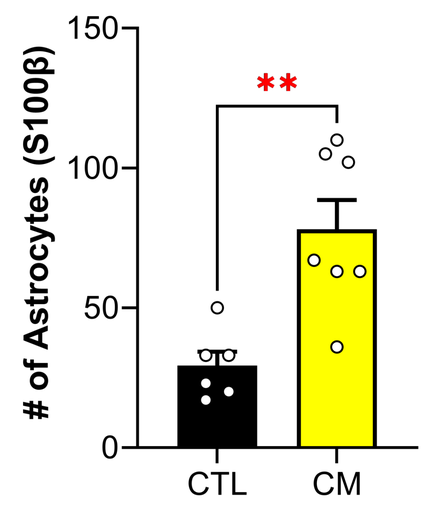


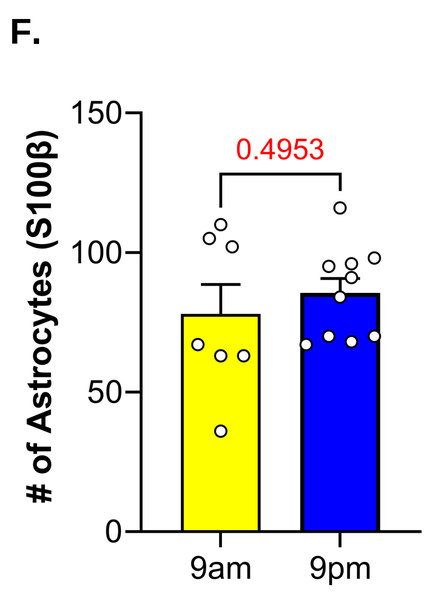
Figure 2: Every two days there was a six-hour delay to the L:D phase to mimic a shift work schedule. After the 10 days, circadian misalignment was established, and blood pressures were recorded.

RESULTS

Circadian misalignment causes an increase in astrocytes in the nTS.







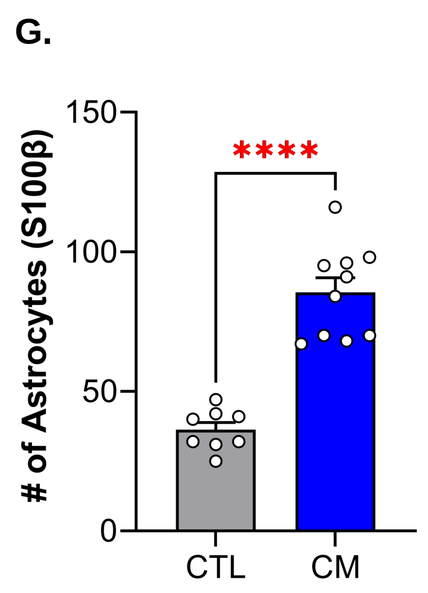


Figure 3:**nTS astrocytes increase in circadian misalignment. A.** 10x magnification of a CTL brainstem slice containing the nTS, showing astrocyte soma (S100β, red) and structure (GFAP, cytoskeleton, green) **B.** 40x magnification of only CTL astrocytes (S100β, red, circled) within the nTS. **C.** CM nTS at 40x magnification showing labeled soma (S100β, red). **D.** 9am and 9pm CTL groups are statistically similar. **E.** Number of astrocytes is greater in CM than CTL groups for 9am. **F.** 9am and 9pm CM groups are statistically similar. **G.** Number of astrocytes is greater in CM than CTL groups for 9pm. nTS= nucleus of solitary tract. TS = solitary tract. * p< 0.05, ** P< 0.01, *** p< 0.001, **** p < 0.0001

CONCLUSIONS

- Circadian Misalignment causes hypertension, data shown previously by lab.
- CM increases astrocytes (as measured through S100β) both 9am and 9pm timepoints.
- This supports that with more astrocytes present, there is an increased uptake of glutamate due to more excitatory amino acid transporters (EAATs), which results in a decrease of extracellular glutamate and an increase in blood pressure.

FUTURE DIRECTIONS

- We aim to collect more data for both CM and CTL rats at all time points.
- We will continue to collect 60x images, which will allow us to use simple neurite tracer and Sholl analysis to detect changes in branching of astrocytes.

Our study suggests that in shift work (circadian misalignment), astrocyte number increases which may lead to the high blood pressure seen in shift workers.