Identification of Brain Regions Activated with Arousal-Induced Clock Resetting in Mice

Jonathan E. Schenk, Andrew T. Schultz, and Robert G. Olson with Daniel S. Janik (Faculty Sponsor)

Department of Biology, University of Wisconsin-Eau Claire

Introduction

The circadian clock in the suprachiasmatic nucleus (SCN) of the brain is involved with many important biological functions. This 24-hour clock is experienced by nearly all organisms.

Previously, it has been shown that transitioning mice into complete darkness in the middle of the day period arouses them and causes a 2.5 hour circadian phase advance. However, the brain regions involved with transmitting arousal information to the circadian clock in the SCN remain unidentified. The purpose of this experiment was to investigate these possible regions by determining activation patterns in mouse brains given a transition to complete darkness.

Methods

Subjects were male C57BL/6 mice of seven to eight months of age housed in internal wheel cages with food and water provided *ad libitum*. All subjects were entrained to a 12-hour bright light (200 lux)/dim light (< 0.1 lux) cycle for at least two weeks prior to experimentation. Circadian phase onsets were measured by locomotor activity using ClockLab.

A total of 20 subjects were used. Twelve of these were sacrificed for brain analysis and the remaining eight served as behavioral controls. Half of the sacrificed subjects were transitioned into complete darkness (lights out group) at the middle of the day period (CT6) and the other half remained in light as control. Brains of these subjects were extracted at CT7. The behavioral controls were also transitioned into darkness at CT6 but kept alive to measure clock resetting.

After extraction, the subject brains were sectioned and stained for FOS protein. The staining procedure was the ABC method of immunohistochemistry with diaminobenzedine as the chromagen. Sections were then plated for microscopy. Images of the regions of interest, including the periaqueductal gray, thalamic paraventricular nucleus, bed nucleus of the stria terminalis, and the hypothalamic periventricular nucleus, were taken using Leica Acquire.

The images of these regions were processed for background and threshold. Immunopositive nuclei were counted in defined boundaries within the regions of interest using ImageJ.

Average nuclei counts of each group were compared using t-tests and graphed using Prism.

Acknowledgements

University of Wisconsin-Eau Claire Office of Research and Sponsored Programs

University of Wisconsin-Eau Claire Faculty/Student Research Collaboration

Results

Actigram data indicated an average phase shift of 2.50 hours (Figure 1).

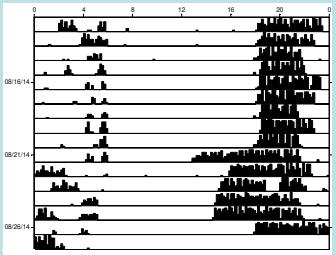

Figure 1. Actigram of mouse transitioned into complete darkness from a 12-hour light/dark cycle at CT6. Vertical bars indicate the number of exercise wheel revolutions in a given time interval. The day of transition is indicated as 8/21/14.

Images of each brain structure can be seen in Figures 2-5. Comparison images between control and experimental groups are shown for brain structures displaying a significant difference in FOS-positive nuclei count ($p < 0.05$). Graphs comparing the average nuclei counts can also be seen in Figures 2-5. Average counts of each group are displayed in Table 1.

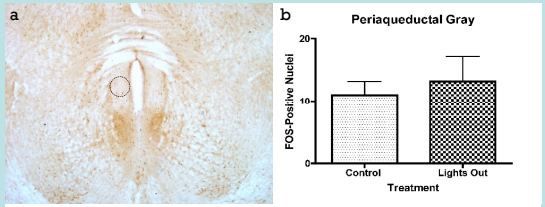

Figure 2. Periaqueductal gray brain region. *a*) FOS staining of the periaqueductal gray. The area used for measurement is outlined. *b*) Average counts of FOS-positive nuclei in the defined area of interest. Error bars indicate standard error of the mean.

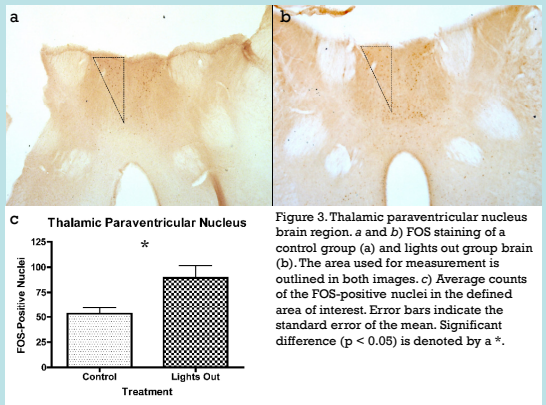

Figure 3. Thalamic paraventricular nucleus brain region. *a* and *b*) FOS staining of a control group (a) and lights out group brain (b). The area used for measurement is outlined in both images. *c*) Average counts of the FOS-positive nuclei in the defined area of interest. Error bars indicate the standard error of the mean. Significant difference ($p < 0.05$) is denoted by a *.

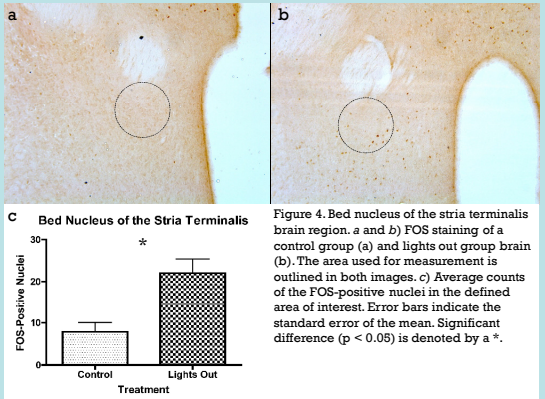

Figure 4. Bed nucleus of the stria terminalis brain region. *a* and *b*) FOS staining of a control group (a) and lights out group brain (b). The area used for measurement is outlined in both images. *c*) Average counts of the FOS-positive nuclei in the defined area of interest. Error bars indicate the standard error of the mean. Significant difference ($p < 0.05$) is denoted by a *.

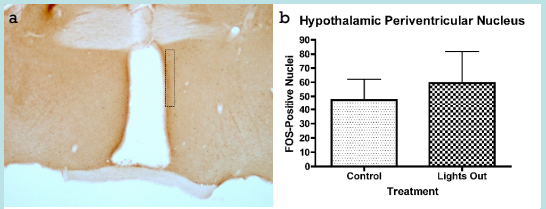

Figure 5. Hypothalamic periventricular nucleus brain region. *a*) FOS staining of the region. The area used for counting is outlined. *b*) Average counts of FOS-positive nuclei in the defined area of interest. Error bars indicate standard error of the mean.

Table 1. Average FOS-positive nuclei of each structure for each group ± standard error of the mean. Significant differences are indicated by a * ($p < 0.05$).

	Control	Lights Out
Periaqueductal Gray	11.0 ± 2.2	13.2 ± 4.0
Thalamic Paraventricular Nucleus *	53.2 ± 6.2	89.4 ± 12.2
Bed Nucleus of the Stria Terminalis *	7.8 ± 2.2	22.0 ± 3.4
Hypothalamic Periventricular Nucleus	47 ± 14.8	59.4 ± 22.4

Conclusions

It has been shown in previous work that FOS protein expression is an indicator of neuronal activation. Therefore, regions with more FOS-positive nuclei can be established to have increased levels of activation. Our results indicate that the PVN and the ST display significantly increased levels of FOS-positive nuclei, suggesting increased activation between the control and experimental groups. The PVN and ST are brain regions involved with stress and arousal. We then conclude that neuronal activation in these areas could be involved the phase shift seen when mice are transitioned to complete darkness.

Future work in this area can include investigating possible activation differences in other stress and arousal involved brain regions such as, but not limited to, the amygdala, locus coeruleus, hippocampus, and anterior cingulate cortex.