Abstract #: 199.7/AAA7

Waking EEG as a measure of homeostatic increase in wild-type and *Clock* mutant mice

Erik Naylor[1], Fred W. Turek[2] and Phyllis C. Zee[1]

1- Department of Neurology, Northwestern University Medical School, Chicago, IL, USA

2- Department of Neurobiology and Physiology, Northwestern University, Evanston, IL, USA

Introduction

- Mice homozygous for the *Clock* mutation have been shown to have less NREM sleep and greater wakefulness than wild-types[1,2].
- One hypothesis for this finding is that the *Clock* mutation slows the build-up of homeostatic sleep load.
- Slow-wave activity (0.5 - 7 Hz) EEG data measured during waking had been shown to increase with sleep deprivation in manner corresponding with homeostatic load[3]. As such, it has been demonstrated to be an accurate marker of sleepiness in humans[4] and rats[5].
- In this experiment we explored whether slow-wave activity during enforced waking also increases in the mouse and whether this phenomenon may be altered by the presence of the *Clock* mutation.

Methods

•Nine wild-type C57Bl/6 mice (age:16.0 ± 0.2 wks) and six C57Bl/6 mice homozygous for the *Clock* mutation (age:17.3 ± 0.2 wks) were obtained from the Northwestern University breeding colony.

•Mice were housed under a 12 hour light / 12 hour dark cycle (LD 12:12) for ten weeks with food and water available *ad lib*.

•All mice were surgically implanted with electroencephalograph (EEG) and electromyograph (EMG) recording electrodes.

•After recovery from surgery (10 days), EEG and EMG waveforms were recorded for a 48 hour baseline period.

•Mice were forced to maintain wakefulness using the gentle method handling for six hours beginning at lights on (ZT 1-6).

•Recovery sleep was recorded for the next 18 hours.

Data Analysis:

•EEG and EMG waveforms were recorded and analyzed using the 4100 recording system and Sirenia software package (Pinnacle Technology Inc, Lawrence, KS).

•All recorded waveforms were evaluated by an expert scorer into 10 sec. epochs of WAKE, NREM or REM sleep.

•Fast Fourier Transform analysis was applied to each epoch for the following frequency ranges (delta 0.5-4 Hz, theta 4-8 Hz, alpha 8-15 Hz, beta 15-25 Hz and gamma 25-40 Hz). Epochs containing artifact were flagged and excluded from spectral analysis.

•All power measurements during the six hour enforced wake period were averaged by hour and normalized to the power in that band during the first hour of the forced waking period.

Results

• FFT analysis was completed on 87% of the epochs during the forced waking period (Table 1).

•Sleep amounts for both wild-type and *Clock* mutant mice did not differ during the six hour forced waking period (P=0.32; t-test) (Table 1).

	wake	NREM	artifact	total
Wild-type	**1800** ± 268	**68** ± 49	**292** ± 255	2160
Clock/Clock	**1778** ± 259	**103** ± 84	**279** ± 243	2160

Table 1: Average (mean ± SD) 10 sec. epochs of wake, NREM sleep and artifact (wake but unsuitable for FFT) epochs for all wild-type and *Clock* mutant mice during the enforced wake period.

Results (continued)

• Similar to previous studies[1,2], *Clock* mice demonstrated more wakefulness and less NREM sleep than wild-types (Figure 1).

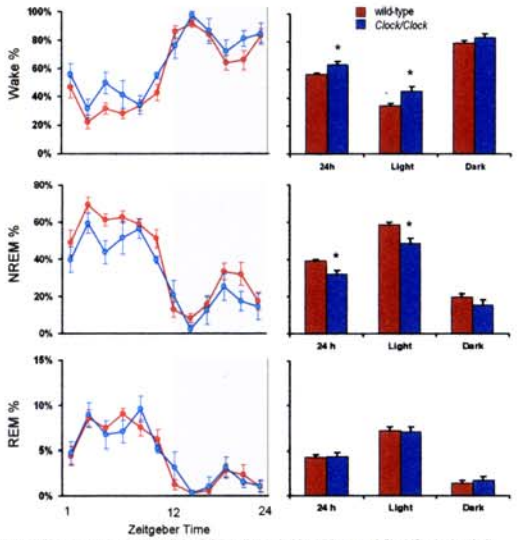
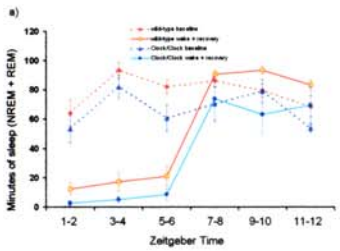


Figure 1: Sleep and wake amounts during 24h baseline period for wild-type and *Clock/Clock* mice. Left-hand graphs represent wake, NREM and REM sleep in 2-hour bins across the day (bars represent S.E.M.). Shaded regions represent time of lights off. Graphs on the right show the total amounts for 24h, as well as the 12h light and 12h dark period. * = P <0.05



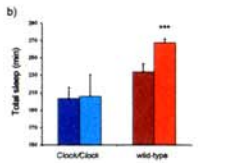


Figure 2: a) Sleep during baseline (dashed lines) compared to forced wake (ZT 1-6) and recovery period (solid line) for *Clock* mutant (blue) and wild-type (red) mice. b) Comparison of total sleep time during 6h baseline (dark color) and recovery period (light color). Bars represent S.E.M. *** = P <0.0005

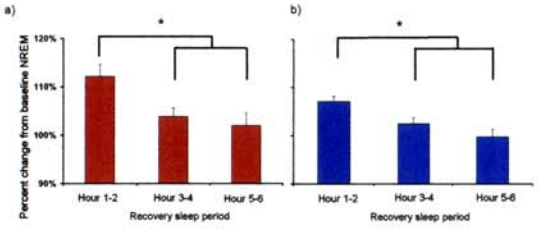


Figure 3: NREM delta power grouped in two hour periods during recovery (ZT 7-12) for a) wild-type (red) and b) *Clock/Clock* mice (blue). * = indicates P < 0.05; ANOVA & post-hoc comparisons.

Results (continued)

• *Clock* mice continue to sleep less during the recovery period (Fig 2a) and do not show a significant increase in overall sleep time compared to baseline (Fig 2b).

• Similar to wild-types, recovery NREM delta power in *Clock* mutants demonstrates a significant increase over baseline which declines over time (Fig 3).

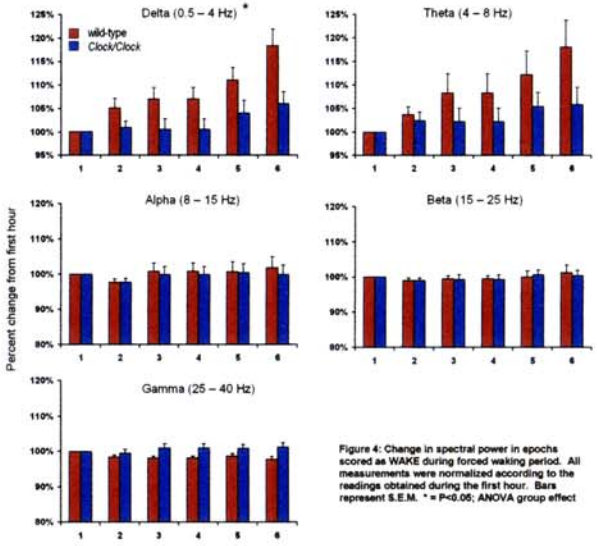


Figure 4: Change in spectral power in epochs scored as WAKE during forced waking period. All measurements were normalized according to the readings obtained during the first hour. Bars represent S.E.M. * = P<0.05; ANOVA group effect

• Waking delta power increases in both wild-type and *Clock* mutants over time.

•The increase in low frequency spectral power is significantly less than wild-types (Fig 4).

•Higher frequency spectral bands show no change from baseline over time.

Conclusions

•Increases in delta power can be reliably measured in a mouse species and may correlate with the increase in homeostatic load.

•*Clock* mice have less sleep and lower delta power increase than wild-types during recovery from forced waking.

•The increase in low frequency spectral power during waking is lower in *Clock* homozygotes.

•These results support the hypothesis that the *Clock* mutation influences the sleep homeostatic process.

References

1) **Naylor E, Bergmann BM, Krauski K, Zee PC, Takahashi JS, Vitaterna MH, and Turek FW.** The circadian clock mutation alters sleep homeostasis in the mouse. *J Neurosci* 20: 8138-8143, 2000.
2) **Easton A, Meerlo P, Bergmann B, and Turek FW.** The suprachiasmatic nucleus regulates sleep timing and amount in mice. *Sleep* 27: 1307-1318, 2004.
3) **Finelli LA, Baumann H, Borbely AA, and Achermann P.** Dual electroencephalogram markers of human sleep homeostasis: correlation between theta activity in waking and slow-wave activity in sleep. *Neuroscience* 101: 523-529, 2000.
4) **Tinguely G, Finelli LA, Landolt HP, Borbely AA, and Achermann P.** Functional EEG topography in sleep and waking: state-dependent and state-independent features. *Neuroimage* 32: 283-292, 2006.
5) **Vyazovskiy VV and Tobler I.** Theta activity in the waking EEG is a marker of sleep propensity in the rat. *Brain Res* 1050: 64-71, 2005.

Support

This research was supported by National Institutes of Health grant #: T32 AG020506-02