Sleep disturbances in EcoHIV-infected mice

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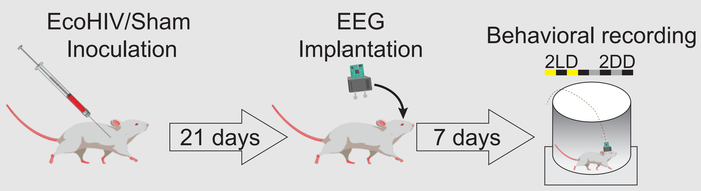
Abstract

Background: In patients living with HIV infection, the prevalence of insomnia and other sleep disturbance is nearly 2.5 times higher than healthy controls and affects nearly 70% of this population. The importance of sleep in healthy cognition has been well-established, and its disruption may contribute to neurocognitive deficits observed in infected individuals. Additionally, both HIV infection and sleep have established bidirectional relationships with neurodegenerative diseases of aging, which represent a rising affliction in these patients. This connection presents a novel opportunity for pharmacological intervention-- we may ameliorate HIV-associated sleep disturbances by treating the disease itself, or improve neurocognitive function in these patients by treating the sleep disruption. In order to assay the efficacy of novel therapeutics and treatment modalities, we assessed the sleep phenotype exhibited in the EcoHIV mouse model of infection.

Results: By multi-day polysomnography recordings of electroencephalography (EEG) and electromyography (EMG), we examined the uninterrupted sleep-wake patterns of EcoHIV infected mice, and uninfected control littermates. Across the entire 24-hour period, and particularly during their daytime period of deep sleep, mice infected with EcoHIV exhibited more wakefulness and less consolidated sleep than their healthy counterparts. This effect manifested in more frequent arousals, shorter sleep bouts, and decreased slow-wave power. Additionally, the amount of rapid eye movement (REM) sleep was significantly decreased.

Conclusions: Similarly to people with HIV infection, the EcoHIV mouse model exhibited sleep disturbances suggestive of insomnia. These data suggest that this model effectively replicates the disease-relevant sleep phenotype, and should be usable to trial possible therapeutics. In addition to assessing novel drug-efficacy, we will use established sleep-restorative compounds to determine if improved sleep can slow the progression of HIV-associated neurocognitive consequences.

Experimental Design



Materials & Methods

EEG/EMG: 3 channel headmount with 2 EEG/1EMG; Pinnacle Technology, Lawrence, KS.

In brief; mice were deeply anesthetized with Ketamine-Xylazine, and an incision made down the skin covering the skull. 4 guide holes were hand-drilled, and stainless steel screws inserted through the headcap and skull, to rest on top of the dura. Twisted stainless steel EMG leads were tucked into the nuchal muscles and affixed with sutures and cyanoacrylate glue. After 3 days of recovery, 50x preamplifiers were attached to the implanted headcaps, and the mice were placed into the recording arena. They acclimatized for another 4 days before recording. Following all experiments, animals were perfused and brains collected for future analysis.

EcoHIV (EcoNDK): EcoNDK was chosen as the strain for evaluating the effect of EcoHIV infection on mouse sleep because of its high virulence and early entrance into the CNS. The EcoNDK plasmid was generously shared by Dr. David Volsky's lab, and we produced the virus in house using HEK293T culture and purified via ultracentrifugation. All injections contained 4e10^6 viral particles as quantified by the p24 sandwich ELISA, and infectivity was confirmed for each cohort by running the p24 ELISA on the spleen of 3 sentinel mice per injection group.

Figure 1: Electroencephalographic recording (EEG) of sleep/wake behavior.

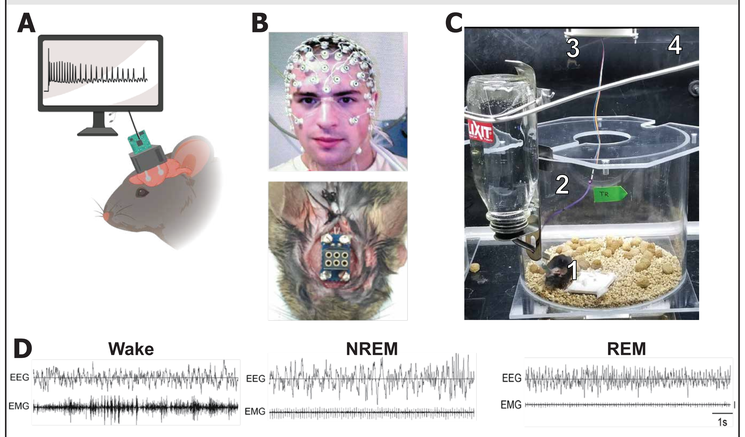


Figure 1: (A) Mouse EEG schematic, based on Pinnacle Technology 3-channel polysomnography recording headmount. **(B)** Cortical EEG performs similarly in both humans and mice, as well as most mammalian species. Coherent activity in thousands of cortical neurons may be read non-invasively through the skull (top), or via implanted transcranial recording electrodes (bottom). **(C)** One mouse with surgically affixed headcap (1) plugged into the preamplifier (2) on a commutator swivel (3), which passes recorded data through an analog-digital converter and to the recording computer. All experiments are conducted within a light-tight box (4). **(d)** Representative EEG/EMG waveforms which indicte waking brain activity, slow-wave activity characteristic of NREM sleep, and the 'paradoxical' waking EEG pattern during REM sleep.

Figure 2: EcoHIV-infected mice exhibit reduced daytime NREM and REM sleep.

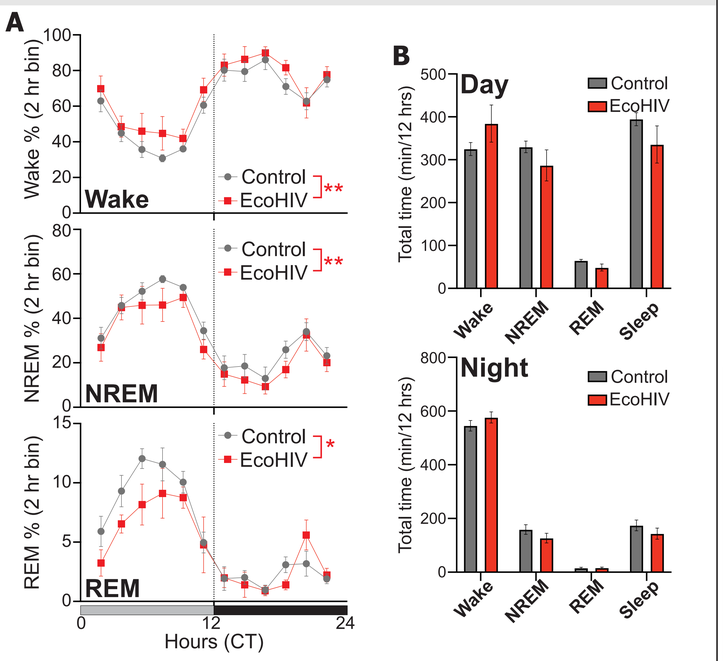


Figure 2: (A) Mouse EEG recordings were scored by hand, and the amount of each vigilance state is plotted as a percent of total time in 2 hour bins. Whole-day differences in each trace were evaluated via two-way ANOVA, and a post hoc Sidak test was used for multiple comparisons of each time point. For all experiments, 'Control' (Gray bars and traces) mice were IP injected with a Sham while 'EcoHIV' (Red bars and traces) mice were injected with 4e10^6 of EcoHIV virus. **B)** Plotted here is the total time (in minutes) each mouse spent in each vigilance state, divided into 'Day' (when the lights are putatively on, CT 0-12) and 'Night' (lights putatively off, CT 12-24). 'Sleep' includes both NREM and REM. In both (A) and (B), All data represents mean +/- SEM, Control n=8 and EcoHIV n=6, Two-way ANOVA with Sidak Post-Hoc tests was performed on all data, *=p<0.05,**=p<0.01

Figure 3: EcoHIV-infection in mice disrupts normal sleep architecture.

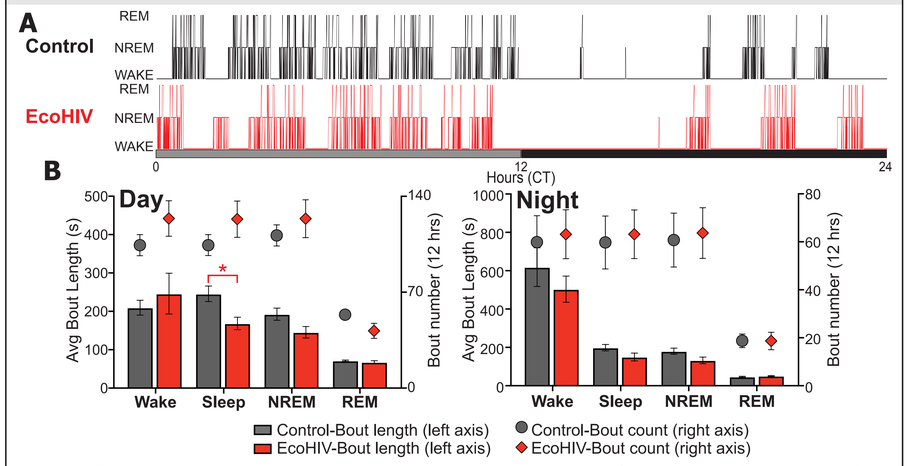


Figure 3: (A) Representative hypnograms plotting the transitions between vigilance states within two mice (Control in black, EcoHIV in red) over one 24 hour DD day. Each point on the line reflects a single scored 10 s epoch, and vertical lines indicate transitions between waking and sleep states. **(B)** Sleep architecture is assessed by evaluating the length of each bout of sleep and wakefulness, with poorer quality sleep containing more, shorter sleep bouts. These plots show both the mean length of bouts of wake, sleep, NREM, and REM (bar plots, left axes), as well as the occurence count for bouts of each subtype (symbols, right axes). The analysis is divided between putative day and nighttime hours. All data represents mean +/- SEM. Control n=8 and EcoHIV n=6, Student's ttest with Sidak Post-Hoc correction for multiple comparisons was used for all of (B), *=p<0.05

Figure 4: EcoHIV-infection induces suppression of cortical delta-power.

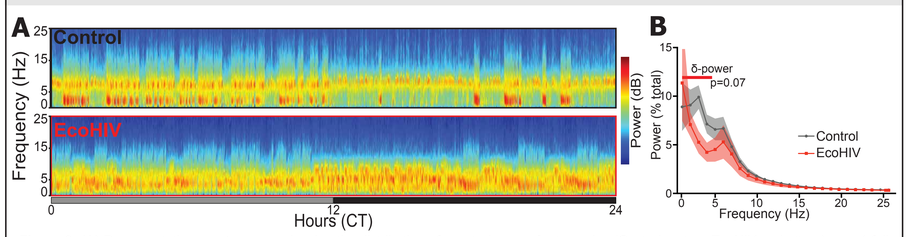


Figure 3: (A) Representative spectrograms showing the distribution of spectral power for two mice (Control on top, EcoHIV on bottom) over 1 full DD day. The spectrogram is computed using a short-time fourier transform, creating 30 second windows of time in which the mixed frequency is deconvoluted and the power is plotted as a heat map with frequency increasing up the y-axis. **(B)** This Welch's periodogram contains the unbiased fourier transform of 30 minutes (CT 6-6.5) of EEG trace. The data is smoothed by rolling window, and frequency power binned into 1 Hz bins. All data represents mean +/- SEM. Delta-power (0.5-4 Hz) was summed and compared via Students t-test, p>0.05, Control n=5 and EcoHIV n=5.

Summary, Future Directions, References

> EcoHIV-infected mice exhibit reduced daytime sleep and broad disruptions to sleep depth, quality, and architecture.

> These phenotypes recapitulate the presentation of insomnia in patients living with HIV, and indicate the murine models may be beneficial to better understand the physiology underlying this neurocognitive effect.

> Future studies will utilize a higher-throughput piezoelectric sleep-screening system, enabling us to test novel therapeutics against their effect on sleep in both infected and uninfected mouse models.

> Many neuroaffective diseases have a bidirectional relationship with sleep, and we plan to unravel the roles each play in EcoHIV mice, to determine possible therapeutic targets.

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