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The Effect of Circadian Intervention on Sleep and EEG in Model of Huntington's Disease

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The Effect of Circadian Intervention on Sleep and EEG in Model of Huntington's Disease

A thesis submitted in partial satisfaction

of the requirements for the degree Master of Science

in Physiological Science

by

Emily Chiem

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ABSTRACT OF THE THESIS

The Effect of Circadian Intervention

on Sleep and EEG in Model of Huntington's Disease

by

Emily Chiem

Master of Science in Physiological Science

University of California, Los Angeles, 2021

Professor Ketema N. Paul, Chair

Sleep and circadian rhythm disturbances are common features of Huntington's disease (HD). Since the daily feed/fast cycle is a powerful entrainer of the circadian clock, we implemented a time-restricted feeding (TRF) protocol, which limits food availability to 6h during the mid-active phase, as a tool to improve outputs of a dysfunctional clock. Electroencephalography (EEG) was used to measure sleep/wake states and EEG patterns in the BACHD mouse model of HD. Our findings show that male, but not female, BACHD mice display disrupted sleep/wake architecture and sleep fragmentation early in the disease. The delta and gamma frequency bands in the EEG were altered in male, but not female, BACHD mice when compared to their WT counterparts. TRF was sufficient to improve the sleep/wake cycle and induce changes in the EEG in male and female BACHD mice. These findings suggest that TRF can improve early HD symptoms and delay disease progression.

The thesis of Emily Chiem is approved.

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2021

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INTRODUCTION

Sleep is an evolutionarily conserved behavior that is essential for maintaining many physiological functions, such as immunity (Besedovsky *et al.*, 2012), learning and memory (Rasch and Born, 2013), and glymphatic regulation (Xie *et al.*, 2013). There are 3 states of consciousness: wake, non-rapid eye movement (NREM) sleep, and rapid eye movement (REM) sleep, and each state can be defined by distinct electrophysiologic parameters. The waking electroencephalogram (EEG) is characterized by a low amplitude and mixed frequency signal. The NREM EEG signal is dominated by slow delta (0.5-4 Hz) frequencies, and the amount of delta activity is proportional to the prior amount of wake. During REM, the EEG signal is low amplitude and dominated by theta (4-8 Hz) frequencies (Scammell *et al*., 2017). Although the precise function of sleep has yet to be elucidated, there are profound consequences of sleep loss. Sleep deprivation has been shown to negatively impact memory retrieval (Lo *et al*., 2016), cardiovascular health (Liu and Chen, 2019), and metabolism (Zimberg *et al*., 2012). Therefore, maintaining consistent sleep/wake practices is crucial in sustaining a healthy lifestyle.

Sleep disorders are a common feature of many neurodegenerative diseases, such as Huntington's disease (HD). Disruptions in the sleep/wake cycles of HD patients manifest early in disease progression, and are characterized by increased wakefulness during the night, daytime sleepiness, and delayed sleep onset (Morton *et al*., 2005; Cuturic *et al*., 2009; Goodman *et al*., 2011). By the end of life, the suprachiasmatic nucleus (SCN), the central circadian clock, shows signs of extensive neurodegeneration (van Wamelen *et al*., 2014). Mouse models of HD also exhibit disruptions in the circadian rest/activity cycles similar to those observed in affected individuals. For example, the R6/2 mouse model of HD displays disruptions in the diurnal sleep/wake cycle, and increased sleep fragmentation (Kantor *et al*., 2013). Similar changes in sleep behavior were seen in the Q175 mouse model, which shows increased wakefulness and decreased NREM sleep during the light phase (Loh *et al*., 2013; Fisher *et al.*, 2016). The BACHD mouse model of HD, which offers the advantage of carrying the human mutation (Gray

et al., 2008), also exhibits sleep/wake architecture disruptions (Kudo *et al*., 2011). Furthermore, there are significant sex differences observed in the circadian dysfunction of BACHD mice, with males exhibiting an advanced and more severe phenotype than females (Kuljis *et al*., 2016).

Sex differences in the development and manifestation of neurodegenerative diseases can offer important insights into the disease pathogenesis. However, the literature on sex differences in HD patients and mouse models remains complex and limited. While some studies report sex differences in the age of onset, progression, and severity of the disease (Zielonka *et al*., 2013; Foround *et al*., 1999), others show no effect of sex (Wexler *et al*., 2004; van Dujin et al., 2014; Dale *et al.*, 2016). Additionally, sex differences in behavioral and neurochemical measures have been shown in the 140 CAG (Dorner *et al*., 2007) and the Q175 (Padovan-Neto *et al*., 2019) knock-in mouse models, and the BACHD mouse model (Kuljis *et al*., 2016). The lack of definitive evidence of sex differences may be due to the fact that such effects on these various components are influenced by other genetic or hormonal factors (Kehoe *et al*., 1999; Bode *et al*., 2008). Importantly, there has not been extensive investigation of how sleep/wake cycles differ by sex during the early stages of HD progression.

Alterations in sleep behavior occur in parallel with changes in the sleep EEG during HD progression (Leuchter *et al*., 2017). EEG signal is generated by cortical brain activity, and rhythms in EEG oscillations are produced through interactions between thalamic and cortical neurons (Feyissa and Tatum, 2019). Neural oscillations occur in different frequency bands, (delta (0.5-4 Hz), theta (4-8 Hz), alpha (8-14 Hz), beta (14-20 Hz), gamma (>20 Hz)), each with distinct functional characteristics (Saby and Marshall, 2012). Aberrant EEG patterns have been observed in many different disorders (Sebastián-Romagosa *et al*., 2020; Horvath *et al*., 2018; Fitzgerald and Watson, 2018; Han *et al*., 2013). In HD patients, the quantitative EEG (qEEG) shows abnormal delta and alpha EEG power (Hunter *et al*., 2010). Similarly, mouse models of HD display characteristic changes in the qEEG spectra. In the Q175 mouse HD model, there is an overall decrease in EEG power, with significant increases in beta and gamma power during

wake and REM, and significant decreases in delta power during NREM (Fisher *et al*., 2016). Additionally, the R6/2 model exhibits a shift in the theta peak during REM, a decrease in slow wave activity in NREM, and an overall increase in gamma power (Kantor *et al*., 2013). These alterations to the EEG spectra act as a sensitive biomarker for HD, and thus represent a target that can be used to examine the effects of an intervention to improve disease progression.

Interventions to improve sleep must be viewed in the context of the two-process model, which proposes that the interaction between homeostatic and circadian processes regulate sleep (Borbély, 1982). The daily feed/fast cycle is a powerful entrainer of the circadian clock (Stephan, 1983). Irregular feeding patterns can severely disrupt physiological outputs, and contribute to disease (Zarrinpar *et al*., 2016). Thus, a time-restricted feeding (TRF) protocol, which limits food availability to a few hours each day, can be a robust tool to synchronize the clock. Previous studies have shown that mice under TRF consume equivalent calories from a high fat diet as *ad libitum* controls, and yet, are protected against obesity and metabolic diseases (Hatori *et al*., 2012). In addition, TRF has also been shown to improve circadian output in mouse models of HD. Improvements in locomotor activity, sleep behavioral patterns, and heart rate variability have been shown in Q175 mice (Wang *et al.*, 2018) and BACHD mice (Whittaker *et al*., 2018) following TRF-treatment. Thus, regulation of the feed/fast cycle may be used to improve the output of a dysfunctional circadian clock. Additionally, feeding has been shown to affect sleep homeostatic mechanisms. For example, a population of neurons in the arcuate nucleus of the hypothalamus has been shown to promote sleep in food-deprived mice (Goldstein *et al*., 2018). Additionally, the sleep/wake architecture is reorganized in food restricted mice (Northeast *et al*., 2019).

In this study, I explored sex differences in sleep/wake architecture, EEG spectral power, and the homeostatic response to sleep deprivation in the BACHD mouse model. Then, I examined the impact of TRF on these parameters, and whether this intervention can attenuate pathological changes in the sleep/wake cycle.

MATERIALS AND METHODS

Animals

The BACHD mouse model HD used in this study contains a human mutant Htt gene encoding 97 glutamine repeats. BACHD females backcrossed on a C57BL/6J background were bred in house with C57BL/6J (wildtype, WT) males from the Jackson Laboratory in order to obtain male and female offspring, either WT or heterozygous for the BACHD transgene. The WT littermates were used as WT controls in this study. Both 3 month old male (WT, n=10; BACHD, n=13) and female (WT, n=16; BACHD, n=17) mice were used in this study in order to further our knowledge on the sex difference previously observed in their circadian phenotypes and other behaviors (Kuljis *et al*., 2016). There were 6 males and 8 females that had severe artifacts in their recordings, and thus were partially or fully excluded from analysis. Animals were group housed (4 per cage), entrained to a 12:12 LD cycle, in sound-proof, humidity controlled chambers until experimentation began.

Surgery

All animals were surgically implanted at 3 months with electroencephalograph (EEG) and electromyograph (EMG) electrodes for polysomnography recordings. A prefabricated headmount (Pinnacle Technologies, Lawrence, KS) was used to position three stainless-steel epidural screw electrodes. The first electrode (frontal- located over the front cortex) was placed 1.5 mm anterior to bregma and 1.5 mm lateral to the central suture. The second two electrodes (interparietal- located over the visual cortex and common reference) were placed 2.5 mm posterior to bregma and 1.5 mm on either side of the central suture. The resulting two leads (frontal-interparietal and interparietal-interparietal) were referenced contralaterally. A fourth screw served as ground. Silver epoxy was used to aid electrical continuity between the screw electrode and headmount. Stainless-steel teflon-coated wires were inserted bilaterally into the nuchal muscle to record EMG activity. The headmount was secured to the skull with dental

acrylic. Mice were allowed to recover for at least 7 days prior to being hooked up to the preamplifier tethers, and given 7 days to acclimate to the tether system. Mice were singly housed during this period.

EEG/EMG recording

One week after surgery, mice were moved to sound-proof sleep-recording chambers and connected to a lightweight tether attached to a low-resistance commutator mounted over the cage (Pinnacle Technologies). Mice were allowed free range of movement throughout the cage while being tethered. Mice were given one week to acclimate to the tether and recording chambers. EEG and EMG recordings began at zeitgeber time (ZT) 0 (light onset) and continued for 24h. Data acquisition was performed on a personal computer running Sirenia Acquisition software (Pinnacle Technologies), a software system specific to rodent polysomnographic recordings. EEG signals were low-pass filtered with a 40-Hz cutoff and collected continuously at a sampling rate of 400 Hz. After data collection, waveforms were scored by the same trained operator as wake (low-voltage, high-frequency EEG; high-amplitude EMG), NREM sleep (high voltage, mixed frequency EEG; low-amplitude EMG), or REM sleep (low-voltage EEG with a predominance of theta activity (4-8Hz); low amplitude EMG). EEG epochs containing artifact due to scratching, moving, eating, or drinking were excluded from analysis. Recordings were scored in 10-sec epochs.

Signal Analysis

Spectral analysis was performed on the frontal-interparietal lead. Power spectral analysis was performed by applying a fast Fourier transform (FFT) to raw EEG waveforms. Only epochs classified as NREM sleep were included in this analysis. Delta power was measured as spectral power in the 0.5-4 Hz frequency range and expressed as a percentage of total spectral power in

the EEG signal (0.5-100 Hz). Power in the 0.5-4 Hz range (delta) was then averaged for all NREM epochs in a 24h period. Gamma power was measured as spectral power in the 20-40 Hz frequency range and expressed as a percentage of total spectral power in the EEG signal (0.5-100 Hz). Any changes in spectral power seen in the mid-active phase (ZT16-ZT22), were defined as changes in the siesta period. Sleep fragmentation was measured by the number of NREM bouts, duration of NREM bouts, and number of stage shifts.

Total sleep deprivation

Immediately following a 24h baseline recording, mice underwent 6h of total sleep deprivation using a gentle-handling protocol, which includes cage tapping, introduction of novel objects, and delicate touching when mice displayed signs of sleep onset. Sleep deprivation began at the onset of the light phase in a 12h:12h light/dark cycle. Recordings continued for 18h of recovery sleep following the period of forced wakefulness.

Time restricted feeding (TRF)

Immediately following recovery from sleep deprivation, male and female WT and BACHD mice (3-4 months old) were exposed to one of two feeding conditions for 1 month: *ad libitum* (*ad lib*) feeding or feeding restricted to 6h during the middle of the active phase (ZT15-21) **(Fig. S1)**. All mice had *ad lib* access to water. Mice were singly housed, and the bedding was changed twice a week as mice are coprophagic. For the first three weeks of TRF, mice were housed in cages with a custom-made programmable food hopper that controls food access. At the start of the fourth week of TRF, mice were re-connected to tethers and allowed one week to acclimate to the tethers and chambers before the next set of 24h recordings began. TRF was performed manually during this time, as the programmable food hoppers did not fit in the recording cages. Control mice were singly housed and given *ad lib* access to food and water.

Statistical Analysis

Data were analyzed using GraphPad Prism (version 9.0.1). All values are reported as group mean ± standard error of the mean (SEM). We used a two-way analysis of variance (two-way ANOVA) with genotype and time as factors for all sleep and spectral data, and sex and genotype as factors for the fragmentation data. Two-way ANOVA with treatment and time as factors was used to determine the impact of TRF on sleep and spectral measures, as well as on fragmentation with genotype and treatment as factors. We followed up with Bonferroni *post hoc* analyses when appropriate. Between-group differences were determined significant if $p < 0.05$.

RESULTS

Disrupted sleep/wake cycle in male BACHD mice

It has been reported that the temporal patterning of the sleep/wake cycle is disrupted in several mouse models of HD (Kantor *et al*., 2013; Loh *et al*., 2013; Fisher *et al*., 2016). While there have not been previous studies examining the sleep/wake architecture or specific sleep/wake states in female BACHD mice, it has been shown that male BACHD mice exhibit more severe motor and circadian system dysfunction than females (Kuljis *et al*., 2016). Therefore, we tested the hypothesis that there are early sleep/wake cycle disruptions in male, but not female, BACHD mice. Under *ad lib* conditions, there were significant differences between WT and BACHD male mice in the 24h pattern of wake **(Fig. 1A)**, and NREM **(Fig. 1B)** sleep. A two-way ANOVA revealed no significant effect of genotype, but a significant effect of time and of their interaction **(Table 1)**. Also, a significant effect of both genotype and time was observed in the 24h pattern of REM **(Fig. 1C & Table 1)**. Conversely, no genotypic differences were observed in female mice **(Fig. 1D-F & Table 1)**.

Furthermore, male BACHD mice displayed greater sleep fragmentation than females, with a significantly greater number of NREM bouts than WT male mice in both the light and dark phases, while female BACHD mice did not differ from their WT counterparts **(Fig. 2A, B).** A two-

way ANOVA revealed a significant effect of genotype, but not of sex, as well as a significant interaction between the two factors during the light phase, while both genotype and sex had significantly influenced the number of NREM bouts in the dark phase **(Table 2)**, but not their interaction. In addition, male BACHD mice exhibited a significantly shorter NREM bout duration than WT mice in the light phase, but not the dark phase. The average NREM bout duration in the mutant females was not significantly different from that in WT females **(Fig. 2C, D)**. A two-way ANOVA showed significant effects of genotype and sex, and a significant interaction between the two factors in the light phase **(Table 2)**. The number of stage shifts (shifts between wake, NREM, or REM) was not significantly different between WT and mutants **(Fig. 2E, F)**, however, a significant effect of the interaction between genotype and sex was observed in the light, but not the dark phase **(Table 2)**. This suggests that while the sleep/wake cycle of male BACHD mice is heavily disrupted early in the disease, female BACHD mice remain protected.

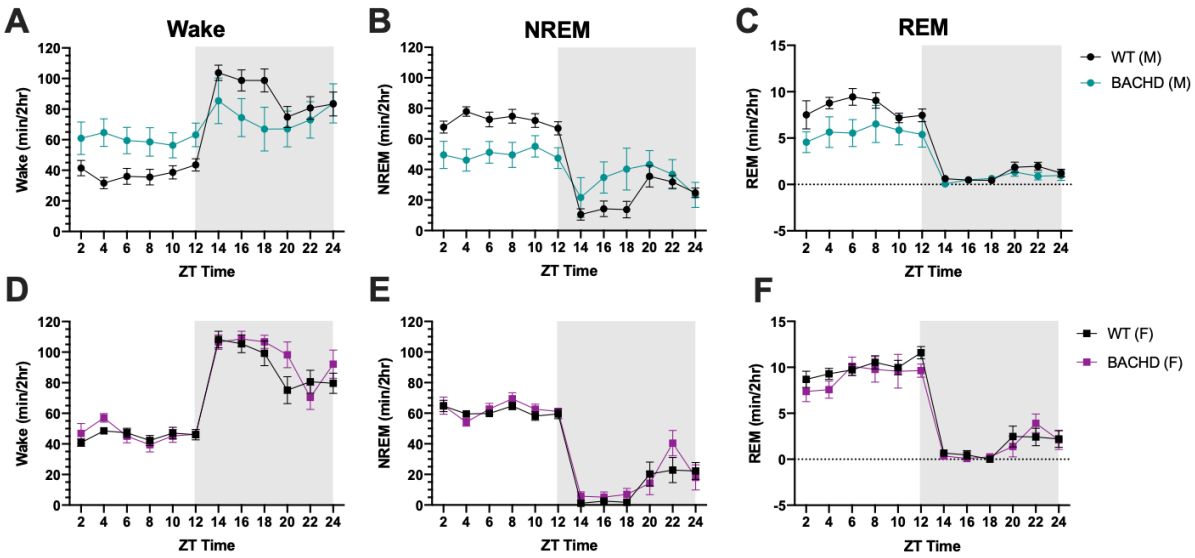

Figure 1. Disrupted sleep/wake architecture in male BACHD mice.
24h EEG recordings were conducted in undisturbed mice in a 12:12 light/dark cycle. The 24h pattern of (A) wake, (B) NREM, (C) REM in male, but not (D-F) female, BACHD mice was significantly different when compared to WT male and female mice. The gray-shaded area represents the dark (active) phase. Data are presented as mean ± SEM (n=9-10/group). Data was analyzed using a two-way ANOVA with time and genotype as factors, followed by Bonferroni's multiple comparisons test (see table 1).

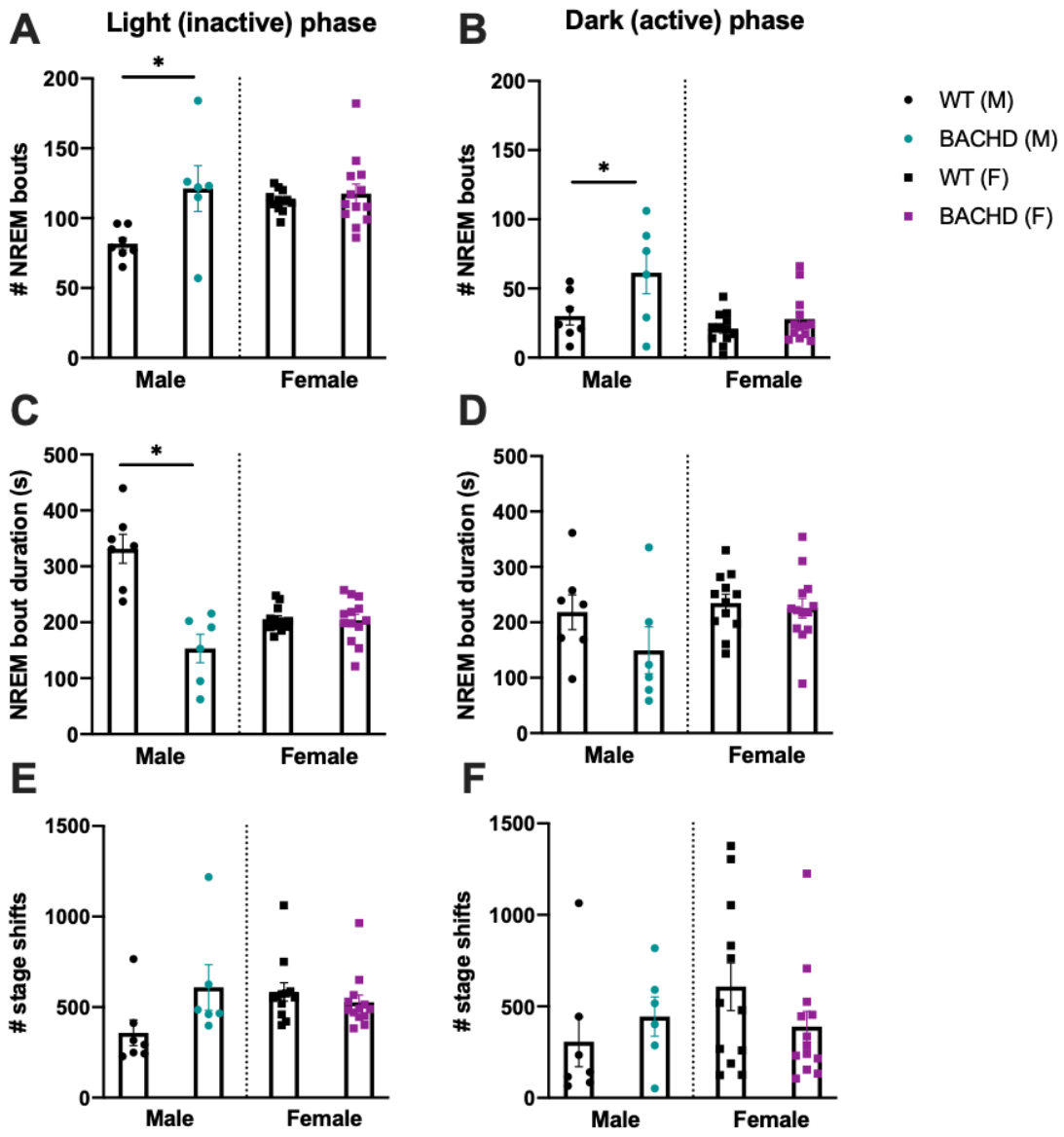

Figure 2. Highly fragmented sleep in male BACHD mice.
Measures of sleep fragmentation were calculated from 24h EEG recordings in undisturbed mice. The (A) number of NREM bouts, (C) NREM bout duration, (E) number of stage shifts are shown in the light phase and (B, D, F) dark phase. Male, but not female, BACHD mice exhibit a significantly greater number of NREM bouts in both phases, and shorter NREM bout duration in the light phase when compared to WT mice. Data are presented as mean ± SEM (n=6-13/group). Data was analyzed using a two-way ANOVA with sex and genotype as factors, followed by Bonferroni's multiple comparisons test (see table 2).

Sex differences in EEG spectral power

It has been reported that HD mouse models display characteristic changes to the EEG spectra (Fisher *et al*., 2016; Kantor *et al*., 2013), which recapitulate those displayed by HD patients (Leuchter *et al*., 2017). To investigate differences in the EEG spectra, we quantified the relative power values in the frontoparietal cortical region. Gamma power (20-40 Hz) has been shown to be disrupted in both HD mouse models and patients, hence, it has been suggested to serve as a reliable biomarker. The gamma power rhythm was altered in BACHD males but not in BACHD females, as compared to their WT counterparts **(Fig. 3A, C)**, with a significant effect of time, but not of genotype or the interaction between the two factors **(Table 1)**.

We were also interested in examining delta power (0.5-4 Hz) during NREM sleep, as this serves as an indicator of sleep pressure (Borbely and Tobler, 1989). We found that male BACHD mice had altered rates of dissipation and accumulation of NREM delta power during the active phase when compared to WT mice. A two-way ANOVA confirmed a significant effect of time, no significant effect of genotype, and a significant interaction between the two factors **(Fig. 3B & Table 1)**. Conversely, the pattern of NREM delta power was not significantly different in female BACHD mice when compared to WT females **(Fig. 3D & Table 1)**. This suggests that there is an impairment in the sleep homeostatic system under undisturbed conditions in male, but not female, BACHD mice.

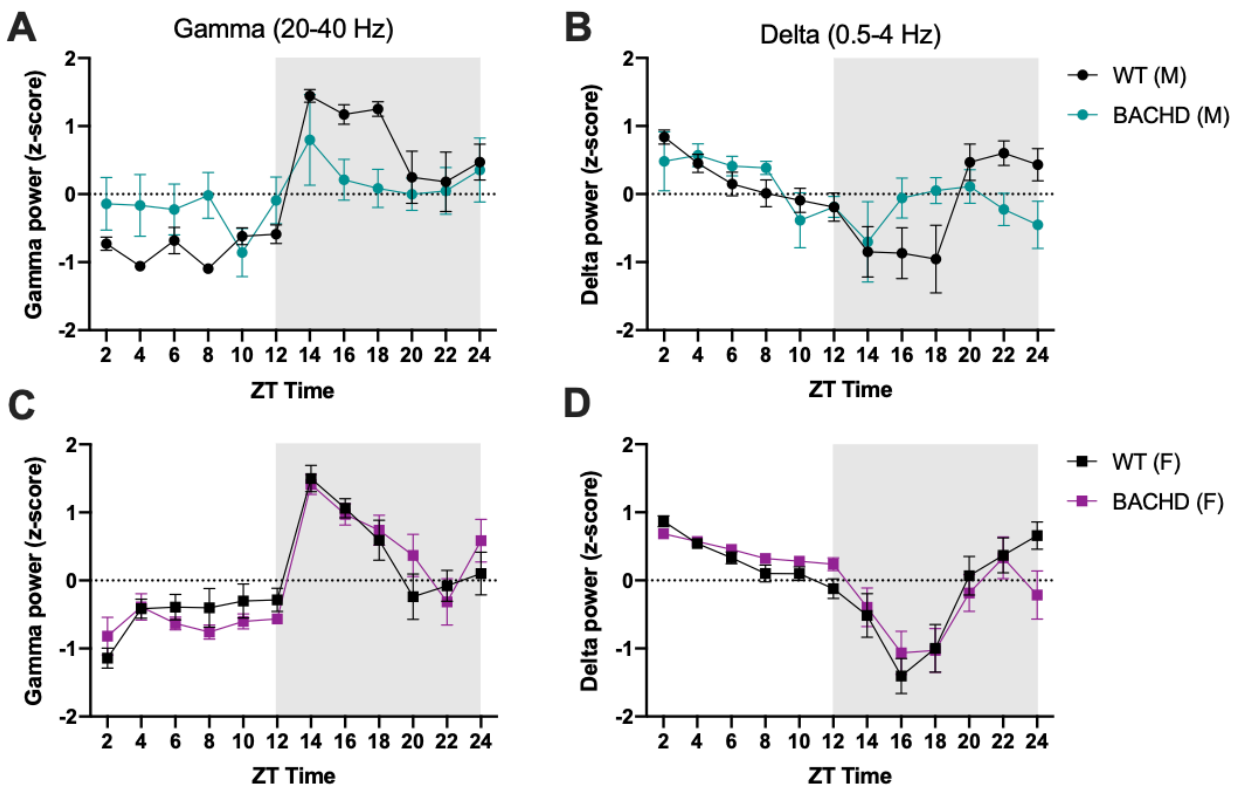

Figure 3. Altered EEG patterns in male BACHD mice.
Power spectral analysis was performed by applying a fast Fourier transform to raw 24h EEG waveforms. The 24h pattern of gamma power across sleep/wake states is altered in (A) male, but not (C) female, BACHD mice when compared to WT mice. Similarly, the 24h pattern of NREM delta power is altered in (B) male, but not (D) female, BACHD mice when compared to WT mice. The gray-shaded area represents the dark (active) phase. Data are presented as mean ± SEM (n=4-13/group). Data was analyzed using a two-way ANOVA with time and genotype as factors, followed by Bonferroni's multiple comparisons test (see table 1).

The ability to recover from sleep deprivation is disrupted in male BACHD mice

In order to investigate whether the ability to recover from sleep loss remained intact in the BACHD mutants, we examined 18h of recovery sleep following 6h of sleep deprivation (SD). Recovery sleep in response to forced wakefulness is commonly used to assess changes in sleep homeostasis, and this recovery is often characterized by an increase in sleep amount, as well as in NREM delta power. No significant changes in the amount of NREM recovery-sleep was observed in male and female BACHD mice when compared to their WT counterparts **(Fig.**

4A & Table 2). Additionally, there were no statistically significant differences in NREM delta power between male and female mutants and their WT counterparts **(Fig. 4B-C & Table 1)**. While more animals will need to be added, there is a trend towards increased NREM delta power in BACHD males as compared to WT males during the mid-active phase. This suggests that the regulation of sleep following a homeostatic challenge may be altered in male BACHD mutants.

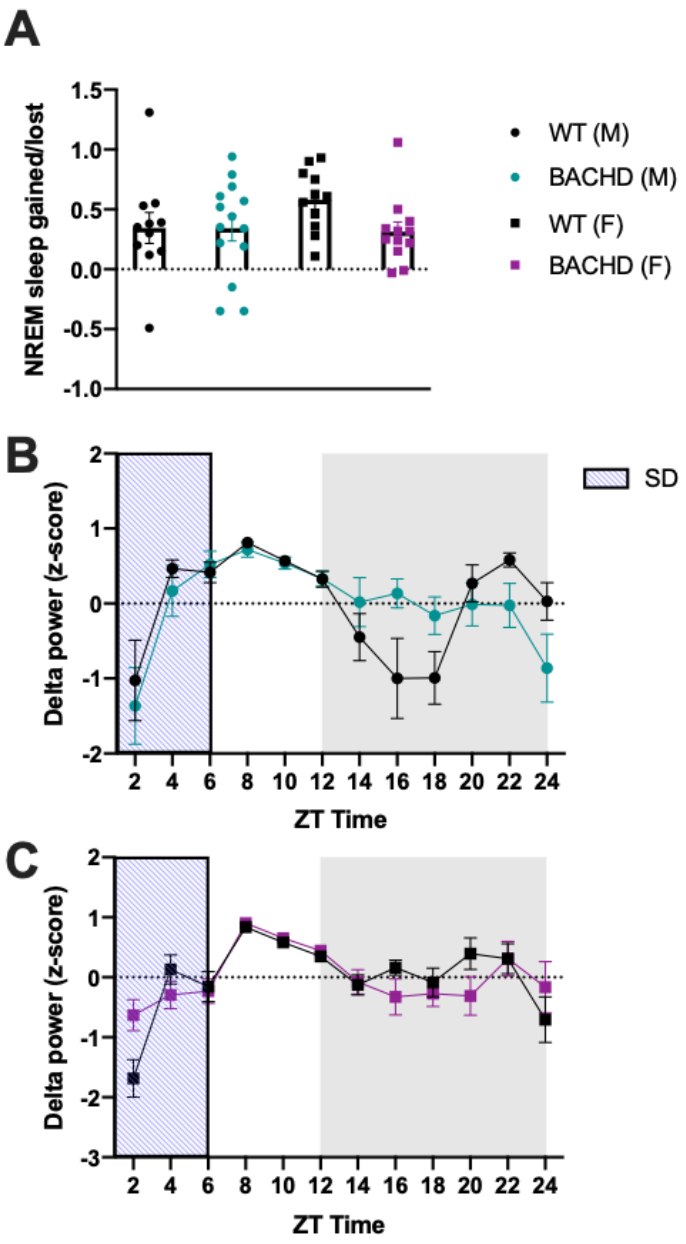

Figure 4. Ability to recover from sleep loss is disrupted in male BACHD mice.
Mice were exposed to sleep deprivation at the beginning of their inactive phase (ZT0-6) using a gentle-handling protocol. (A) NREM recovery sleep amount following 6h of sleep deprivation was calculated as the ratio of sleep gained during the 18h recovery period (ZT6-24) over sleep lost during the sleep deprivation period (ZT0-6). The pattern of NREM delta power during recovery is altered in (B) male, but not (C) female, BACHD mice when compared to WT mice. The blue-shaded area represents 6h of sleep deprivation, while the gray-shaded area represents the dark (active) phase. Data are presented as mean ± SEM (n=7-13/group). Data was analyzed using a two-way ANOVA with sex and genotype, or time and genotype, as factors, followed by Bonferroni's multiple comparisons test (see tables 1 and 2).

Time restricted feeding (TRF) improves sleep in male and female mice

TRF has been shown to improve sleep behavior in male BACHD mice (Whittaker *et al.*, 2018). Therefore, we wanted to further examine its effects on the 24h pattern of EEG-defined wake, NREM, and REM. In both WT and BACHD male mice, TRF significantly improved the temporal pattern of wake and NREM **(Fig. 5A, C)**, but had no effects on REM **(Fig. 5E)** as compared to their *ad lib* counterparts. In particular, significant effects of treatment were observed on the wake waveform in WT males, while the NREM waveforms were significantly influenced in both genotypes by the interaction between time and treatment. Such interaction also significantly affected wake in the male mutants **(Table 3)**.

In WT female mice, there was a significant improvement in the temporal pattern of NREM in the TRF-fed mice as compared to *ad lib*-fed mice **(Fig. 5D)**. A two-way ANOVA of the NREM waveform confirmed a significant effect of time and treatment, and a significant interaction between the two factors **(Table 3)**. However, TRF did not influence wake or REM in WT females **(Fig. 5B, F)**. TRF-fed female mutants showed improvements in the temporal patterning of wake, NREM, and REM **(Fig. 5B, D, F)**. A significant effect of time and treatment was observed on REM, while the interaction between the two factors positively impacted all sleep/wake states **(Table 3)**.

Due to our limited sample size, the effect of TRF on sleep fragmentation in male and female mutants was not statistically significant. There were no significant differences in number of NREM bouts, NREM bout duration, or the number of stage shifts between TRF-fed mice and *ad lib*-fed controls in either the light or dark phase **(Fig. 6 & Table 4)**. However, there is a trend towards decreased number of NREM bouts, increased NREM bout duration, and decreased number of stage shifts in TRF-fed BACHD mice as compared to the *ad lib*-fed controls. Although preliminary, these results would suggest that TRF can decrease sleep fragmentation in male mutants.

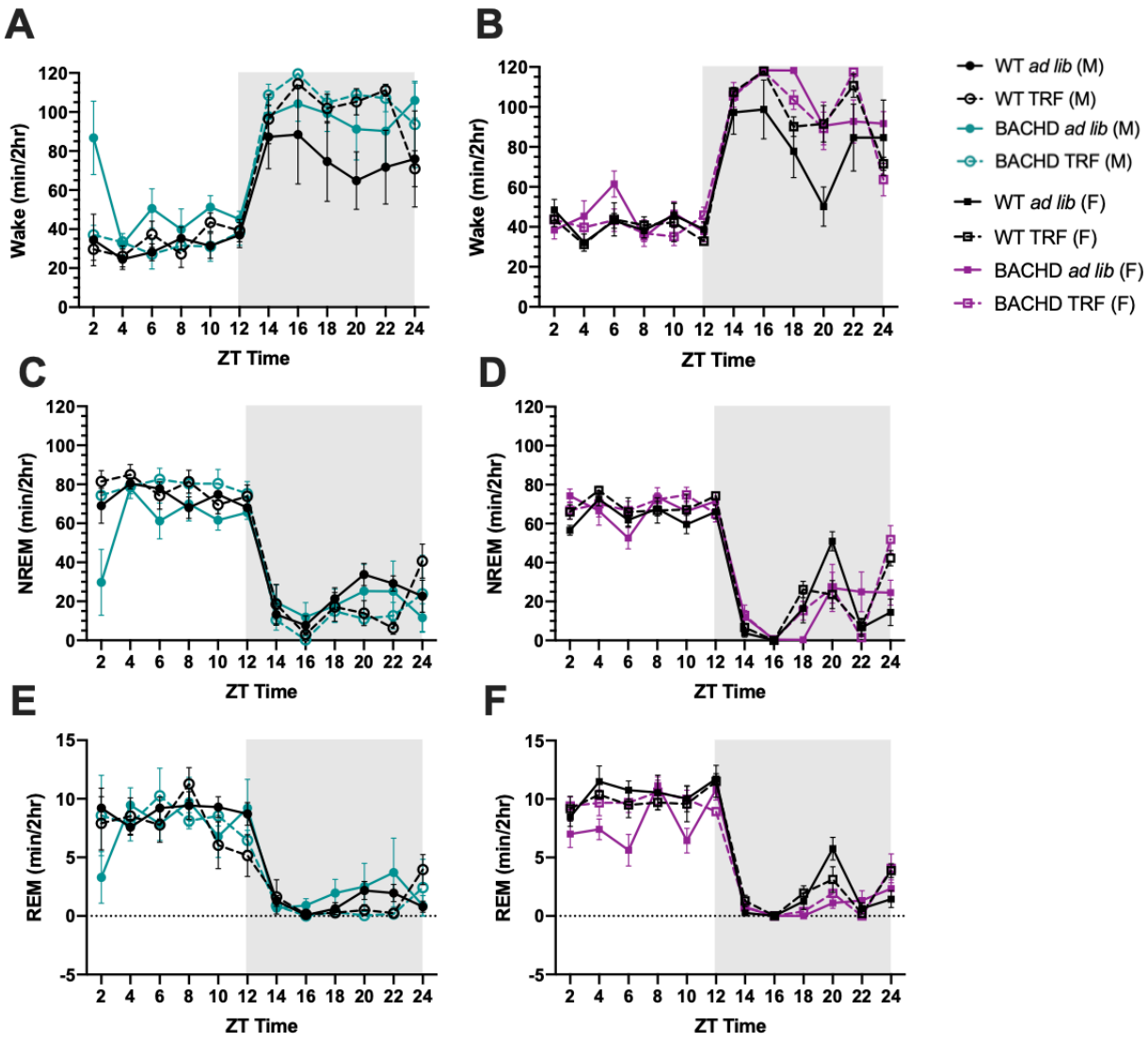

Figure 5. Time-restricted feeding (TRF) improves sleep/wake architecture.
Mice were put on a time-restricted feeding (TRF) schedule, with food access from ZT15-21, or kept as controls on *ad libitum* (*ad lib*) feeding. The 24h pattern of wake, NREM, and REM is shown in TRF-fed or *ad lib*-fed (A, C, E) male and (B, D, F) female WT and BACHD mice. TRF was sufficient to elicit improvements in male and female WT and BACHD mice. The gray-shaded area represents the dark (active) phase. Data are presented as mean ± SEM (n=4-6/group). Data was analyzed using a two-way ANOVA with time and treatment as factors, followed by Bonferroni's multiple comparisons test (see table 3).

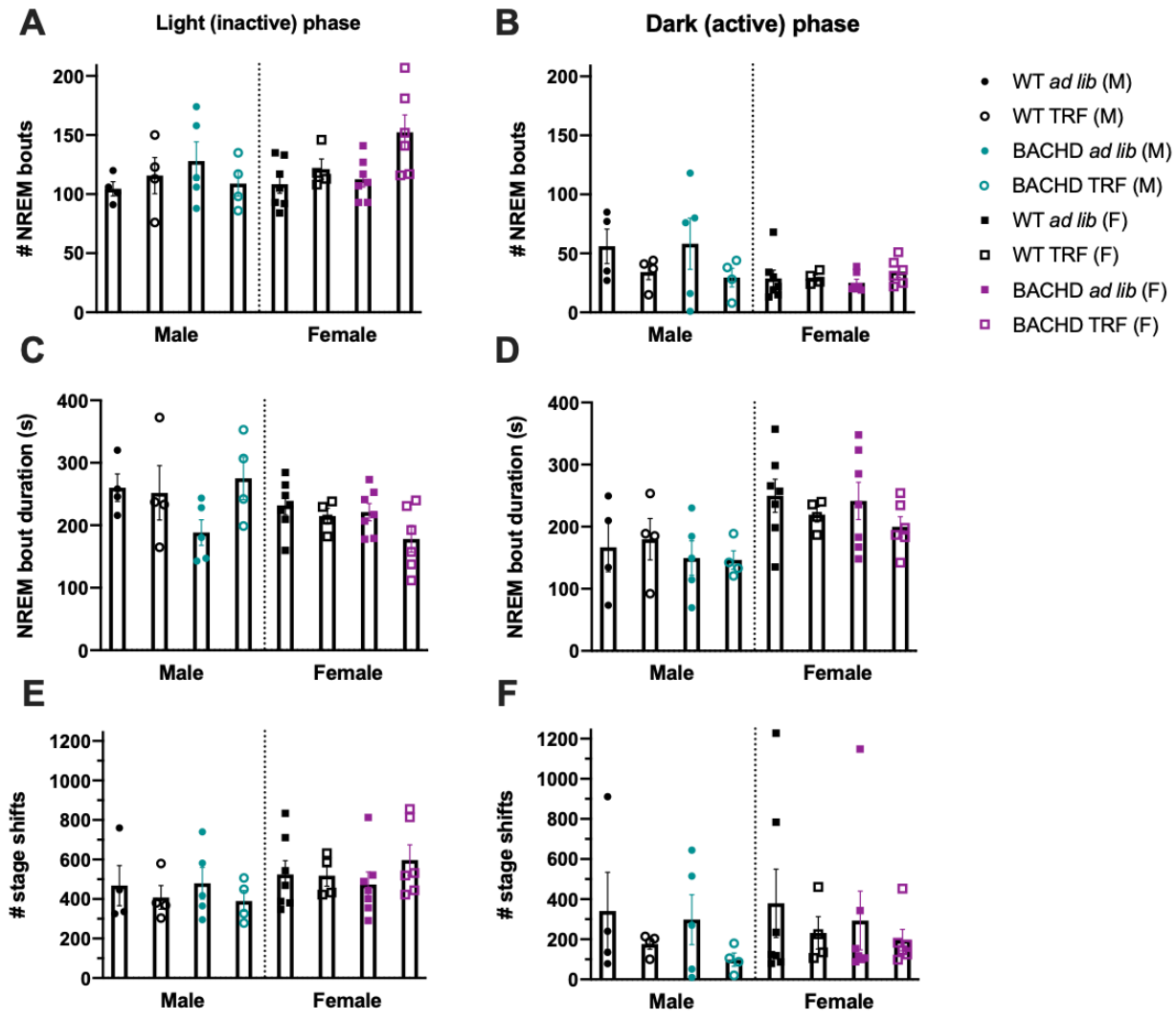

Figure 6. TRF decreases sleep fragmentation in male BACHD mice.
Measures of sleep fragmentation were calculated from 24h EEG recordings in undisturbed TRF-fed or *ad lib*-fed male and female mice. The number of NREM bouts, NREM bout duration, and number of stage shifts in the (A, C, E) light phase and (B, D, F) dark phase, is shown in male and female WT and BACHD mice. TRF seems to elicit a decrease in number of NREM bouts, an increase in NREM bout duration, and a decrease in number of stage shifts in TRF-fed male BACHD mice as compared to *ad lib*-fed male BACHD mice. Data are presented as mean ± SEM (n=4-7/group). Data was analyzed using a two-way ANOVA with genotype and treatment as factors, followed by Bonferroni's multiple comparisons test (see table 4).

Time restricted feeding (TRF) alters EEG spectral power

Next, we investigated the effects of TRF on certain frequency bands of the EEG power spectra. No statistically significant differences in gamma power were seen between TRF-fed and *ad lib*-fed WT male mice **(Fig. 7A)**. However, a non-significant increase in gamma power can be observed in TRF-fed BACHD males as compared to *ad lib*-fed controls during the early-active phase **(Fig. 7B & Table 3)**. In female mice, there was no effect of TRF on gamma power in either WT or BACHD mice **(Fig. 7E-F & Table 3)**.

Additionally, there were no statistically significant effects of TRF on NREM delta power in WT and BACHD male mice under undisturbed conditions **(Fig. 7C-D)**. Interestingly, there was an effect of TRF on NREM delta power in WT and BACHD female mice as compared to their *ad lib* counterparts **(Fig. 7G-H & Table 3)**. This suggests that TRF is able to alter sleep pressure in female, but not male, mice.

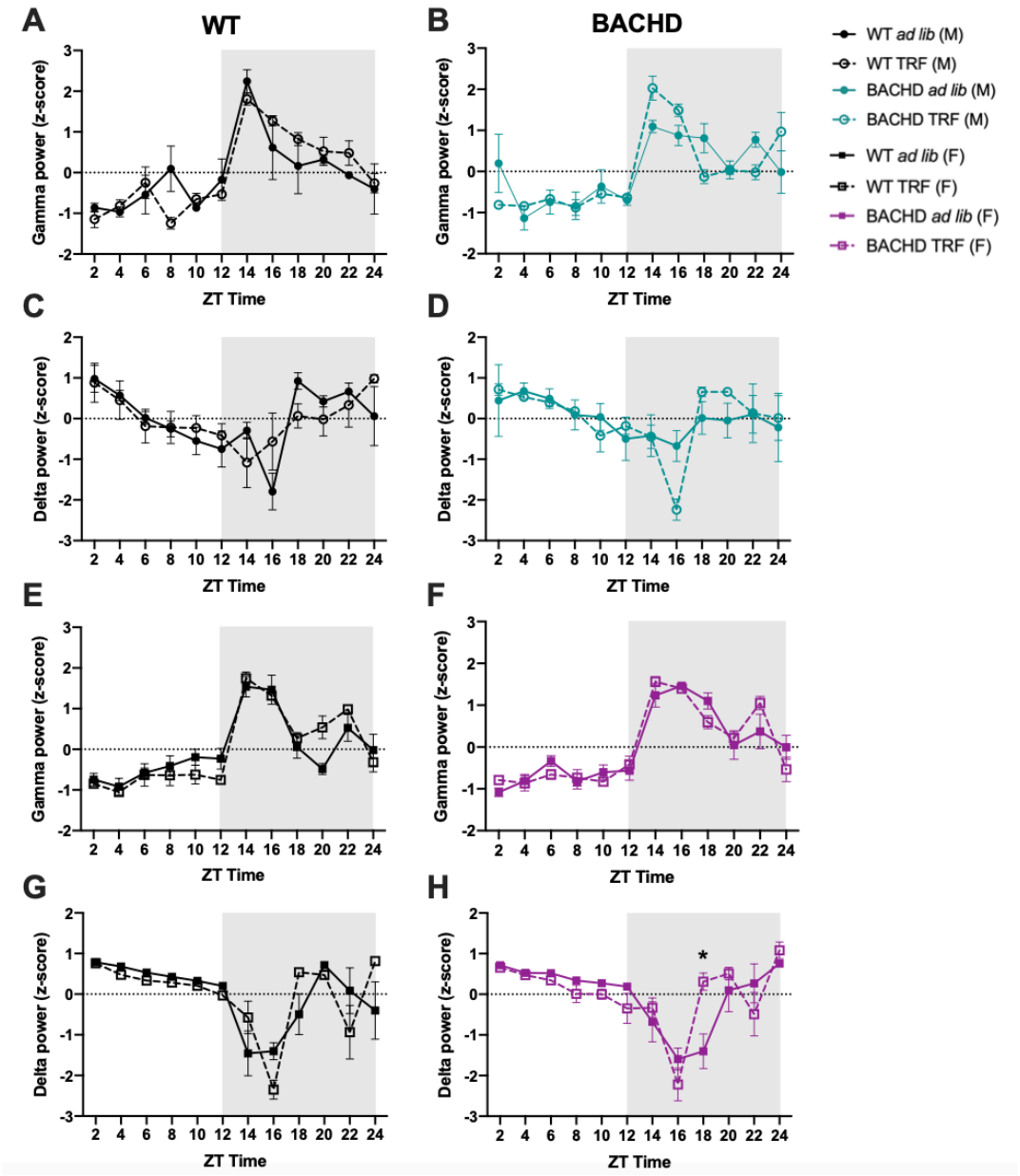

Figure 7. TRF alters EEG patterns in male and female WT and BACHD mice.
Power spectral analysis was performed by applying a fast Fourier transform to raw 24h EEG waveforms of TRF-fed or *ad lib*-fed male and female mice. The 24h pattern of gamma power across sleep/wake states is shown in (A) WT and (B) BACHD males, and in (E) WT and (F) BACHD females. There is a nonsignificant increase in gamma power in TRF-fed male BACHD mice as compared to their *ad lib*-fed counterparts. The 24h pattern of NREM delta power is shown in (C) WT males, (D) BACHD males, (G) WT females, and (H) BACHD females. The gray-shaded area represents the dark (active) phase. TRF seems to alter NREM delta power in TRF-fed male BACHD mice, and female WT and BACHD mice as compared to their *ad lib*-fed counterparts. Data are presented as mean ± SEM (n=2-6/group). Data was analyzed using a two-way ANOVA with genotype and treatment as factors, followed by Bonferroni's multiple comparisons test (see table 3).

Effect of time-restricted feeding on recovery following sleep deprivation

Finally, we examined if a TRF regimen would influence the animals' ability to recover from sleep deprivation. In both males and females, there were no significant differences in NREM recovery-sleep amount between TRF-fed and *ad lib*-fed WT or BACHD mice **(Fig. 8A & Table 4)**. However, the trend towards a decrease in NREM recovery-sleep amount could be observed in TRF-fed mice. Additionally, TRF did not affect delta power during NREM recovery sleep in WT or BACHD male **(Fig. 8B-C)** and female mice **(Fig. 8D-E & Table 3)**. Although these results are preliminary, the findings suggest that TRF may influence NREM recovery-sleep amount, but not NREM delta power.

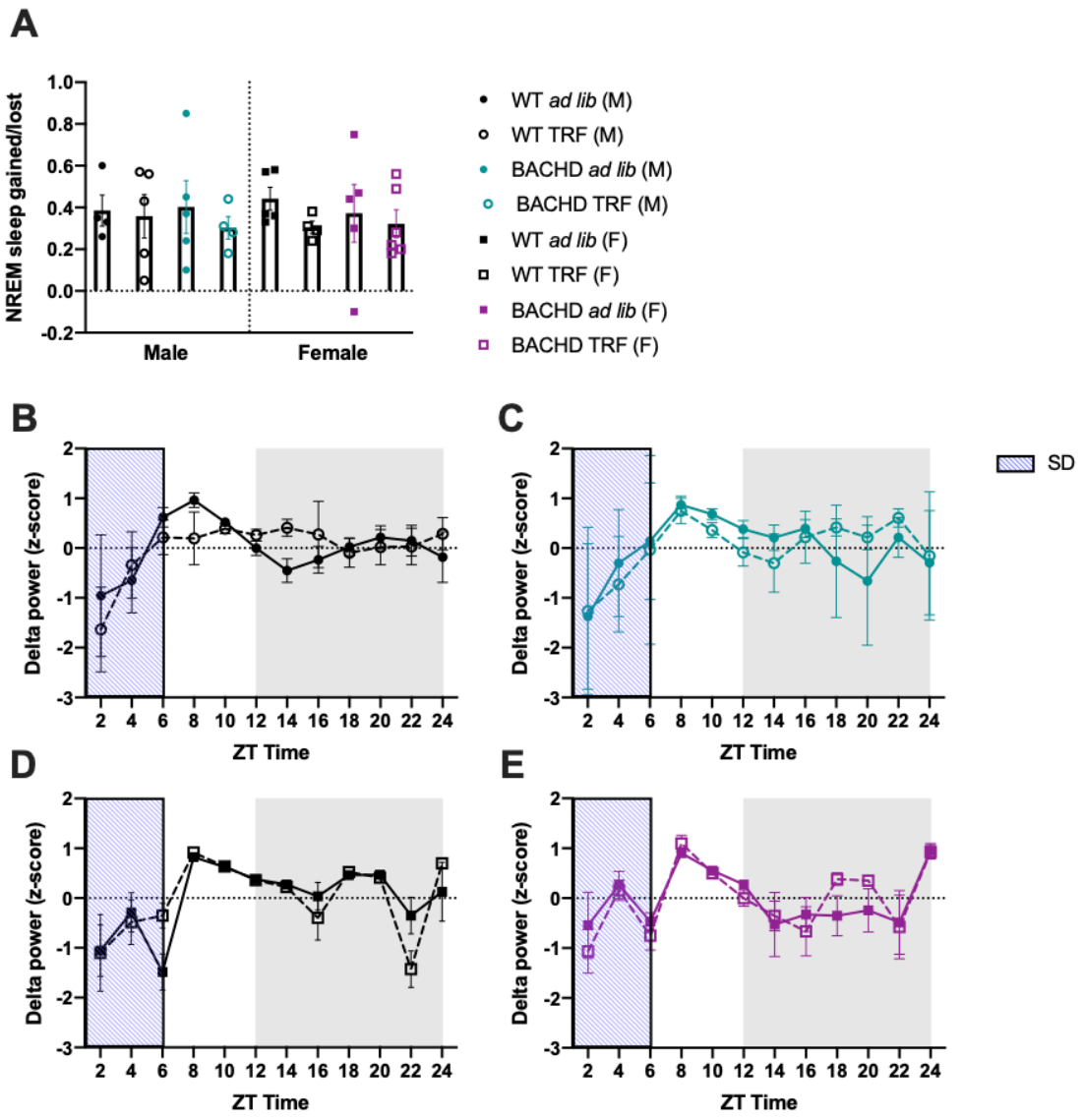

Figure 8. Effect of TRF on the ability to recover from sleep loss.
TRF-fed and *ad lib*-fed mice were exposed to sleep deprivation at the beginning of their inactive phase (ZT0-6) using a gentle-handling protocol. (A) NREM recovery sleep amount was calculated as the ratio of sleep gained during the 18h recovery period (ZT6-24) over sleep lost during the sleep deprivation period (ZT0-6). The pattern of NREM delta power during recovery was not significantly different between TRF-fed and *ad lib*-fed (B) WT and (C) BACHD male, and (D) WT and (E) BACHD female mice. The blue-shaded area represents 6h of sleep deprivation, while the gray-shaded area represents the dark (active) phase. Data are presented as mean ± SEM (n=4-6/group). Data was analyzed using a two-way ANOVA with genotype and treatment, or time and treatment, as factors, followed by Bonferroni's multiple comparisons test (see tables 3 and 4).

DISCUSSION

HD patients often exhibit sleep and circadian disruptions before the onset of motor and cognitive symptoms (Morton, 2013; Goodman *et al*., 2011). Mouse models of HD have been shown to mirror the patient condition, typified by increased wake during the inactive phase, increased sleep during the active phase, and loss of consolidated sleep (Kantor *et al*., 2013; Fisher *et al*., 2016; Loh *et al*., 2013). Prior work has shown that implementing a circadian-based treatment can improve sleep patterns in BACHD mice (Whittaker *et al*., 2018); however, this study only examined sleep through video analysis, which does not allow for identification of specific sleep stages. Therefore, our present study investigated the effect of TRF on sleep architecture, EEG patterns, and sleep homeostasis in the BACHD mouse model of HD.

Our findings demonstrated clear dysfunction in the sleep/wake architecture, sleep fragmentation, and alterations in the EEG spectra of male BACHD mice. Compared to WT controls, BACHD mice showed increased wake and decreased NREM and REM during the light phase. During the dark phase, the BACHD mutants exhibited decreased wake and increased NREM and REM **(Fig. 1A-C)**. Male BACHD mice also exhibited highly fragmented sleep during the light phase, as shown by increased number of NREM bouts and shorter NREM bout duration when compared to WT mice **(Fig. 2A, C)**. Additionally, male BACHD mice exhibited abnormal changes in the EEG spectra. Although this data set is underpowered, there was a trend towards a difference in gamma power between BACHD and WT males **(Fig. 3A)**. BACHD males exhibited a distinct phase difference and a reduction in the amplitude of gamma power. Alterations in neural oscillations in the gamma frequency band (20-40 Hz) have been detected in patients with Parkinson's disease (Pal *et al*., 2020), and Alzheimer's disease (van Deursen *et al*., 2008), and thus has been suggested as a potential biomarker for disease progression. BACHD male mice also displayed disrupted NREM delta power **(Fig. 3B)**. NREM delta power serves as a reliable indicator of sleep pressure (Borbely et al., 1981). Our findings show that BACHD male mice have altered rates of dissipation of delta power during the dark phase, which

might suggest a disruption in their homeostatic regulation of sleep. These results are consistent with previous findings that have shown similar disruptions in both the sleep/wake patterns and EEG spectra in the R6/2 (Kantor *et al*., 2013), and Q175 (Fisher *et al.*, 2016; Loh *et al.*, 2013) mouse models of HD. Thus, there are sleep/wake pattern and spectral changes that accompany the disrupted rhythms in heart rate, body temperature, and locomotor activity that have been previously identified in the BACHD mouse model (Kudo *et al*., 2011).

There are sex differences in the progression of HD symptoms in patients (Zielonka *et al*., 2018; Ullah *et al*., 2019) that are recapitulated in the BACHD mouse model. Male BACHD mice exhibit more severe deficits in activity rhythms and motor coordination than females (Kuljis *et al*., 2016). Our findings reveal a similar sex difference in the sleep/wake cycle and EEG power spectrum of BACHD mice. Unlike males, BACHD female mice do not display differences in the temporal patterning of wake, NREM, or REM **(Fig. 1D-F)**, in measures of sleep fragmentation **(Fig. 2)**, or in gamma or NREM delta power **(FIg. 3C-D)** when compared to WT female mice. Previous studies have suggested a protective role of estrogens on neurodegeneration. It has been shown that 17**β**-estradiol protects against **β**-amyloid toxicity (Guerra *et al.*, 2004), and plasma 17**β**-estradiol was found to be correlated with striatal neuron loss in male HD rats (Bode *et al*., 2008). In conjunction with the literature, our findings suggest that females are protected from sleep/wake deficits early in the HD progression.

Importantly, we show that a time-restricted feeding (TRF) intervention can not only attenuate these pathological changes in the sleep/wake cycle and EEG spectrum of male BACHD mice, but also can benefit WT mice and female BACHD mice, who do not show any initial deficits. In this study, mice designated to the TRF group were provided food from ZT 15-21. TRF improved the temporal patterning of wake and NREM in WT and BACHD males **(Fig. 5A, C)**. Also, improvements were seen in the temporal patterning of NREM in WT females **(Fig. 5D)**, and of wake, NREM, and REM in BACHD females **(Fig. 5B, D, F)**. Although additional animals will be added in order to get an accurate representation of how TRF affects sleep

fragmentation, there is a trend towards decreased number of NREM bouts, longer NREM bout duration, and decreased number of stage shifts in TRF-fed male mice **(Fig. 6)**. As an entrainer of the circadian clock, the feed/fast cycle is an attractive target for manipulation in order to improve outputs of the central clock. Previous studies have shown that TRF prevents obesity and metabolic diseases (Hatori *et al.*, 2012; Chaix *et al*., 2014). TRF has also been shown to improve sleep behavior patterns and fragmentation in the Q175 (Wang *et al*., 2018) and BACHD (Whittaker *et al*., 2018) mouse models of HD. The orexin system has a functional role in both appetite and sleep regulation (Scammell *et al*., 2017). In particular, orexin-1 receptors have been implicated in mediating the transition between feeding and sleeping behaviors (Rodgers *et al*., 2002). Therefore, we speculate that the ability of TRF to alter orexin signaling may act to gate the downstream effects of TRF on sleep/wake patterns in this present study.

Our findings demonstrate that TRF might ameliorate pathological alterations in the EEG spectra observed in BACHD mice. There is a trend towards increased gamma power in TRF-fed male BACHD mice **(Fig. 7B)** and altered NREM delta power in TRF-fed WT and BACHD female mice **(Fig. 7G-H)**. The effects of mutant *huntingtin* protein on brain function can be visualized through EEG. HD patients exhibit changes in the EEG spectra that are correlated with severity of cognitive and motor dysfunction, and number of CAG repeats (Hunter *et al.*, 2010). Therefore, EEG serves as a sensitive biomarker of disease, and alterations in the EEG spectra act as a reliable read-out for the effectiveness of treatments (Leuchter *et al*., 2017). Given this information, our findings that TRF attenuates pathological changes in the EEG spectra suggests that TRF works to alter disease progression.

Sleep homeostasis refers to the ability to recover from sleep loss, which is essential in maintaining cognitive function (Olaithe *et al*., 2018; Cousins *et al*., 2019; Pinheiro-da-Silva *et al*., 2017). Following a period of extended wakefulness, a prolonged period of recovery sleep, characterized by increased duration and increased slow-wave activity, is generated (Borbely and Tobler, 1989). In this study, we show that BACHD mice may have an altered ability to

recover from sleep loss. Following 6h of sleep deprivation by gentle-handling, there was a trend towards less NREM recovery sleep in female BACHD mice as compared to WT littermates **(Fig. 4A)**. Additionally, the rate of NREM delta power dissipation following sleep deprivation in male BACHD mice differs from that of WT males **(Fig. 4B)**. This suggests that BACHD mice may exhibit deficits in their homeostatic sleep regulation, and thus are unable to properly induce a rebound response. Although additional animals will need to be added to this data set, our findings suggest that TRF alters the amount of NREM recovery sleep following sleep deprivation in male and female BACHD mice **(Fig. 8A),** while having no effect on NREM delta power **(Fig. 8B-E)**. The relationship between energy and sleep homeostasis has been extensively investigated. Food deprivation has been shown to increase wake episodes and disrupt sleep/wake architecture (Borbely, 1977; Deswasmes *et al*., 1989; Goldstein *et al*., 2018). However, when mice were placed on a restricted feeding protocol, the increase in wake associated with food restriction was diminished after the first few days of scheduled feeding and sleep pressure was attenuated (Northeast *et al*., 2019). This body of evidence suggests that while food deprivation acts to disrupt sleep homeostasis, stable food entrainment may work to increase the robustness of the sleep homeostatic system. Thus, the results of our study suggest that TRF acts to improve the ability of BACHD mice to recover from sleep loss by strengthening the sleep homeostatic system.

There is an important distinction to be made between TRF and caloric restriction. While caloric restriction involves reducing daily energy intake, TRF restricts the feeding window to certain hours of the day without reducing the total number of calories (Hatori *et al*., 2012; Longo and Panda, 2016). Although caloric restriction has been effective in many animal models (Ito *et al*., 2015; Yamada *et al*., 2018; Li *et al*., 2017), the level of motivation required in humans makes it difficult to implement in patients (Moreira *et al*., 2011). Thus, TRF presents as a more feasible therapeutic alternative. While HD is a genetically determined disease with no known cure, our findings suggest that scheduled feeding can improve disruptions to the sleep/wake cycle, and

thus dramatically improve quality of life. This lifestyle change has great therapeutic potential for HD patients, and may alter the trajectory of disease progression.

SUPPLEMENTARY FIGURES

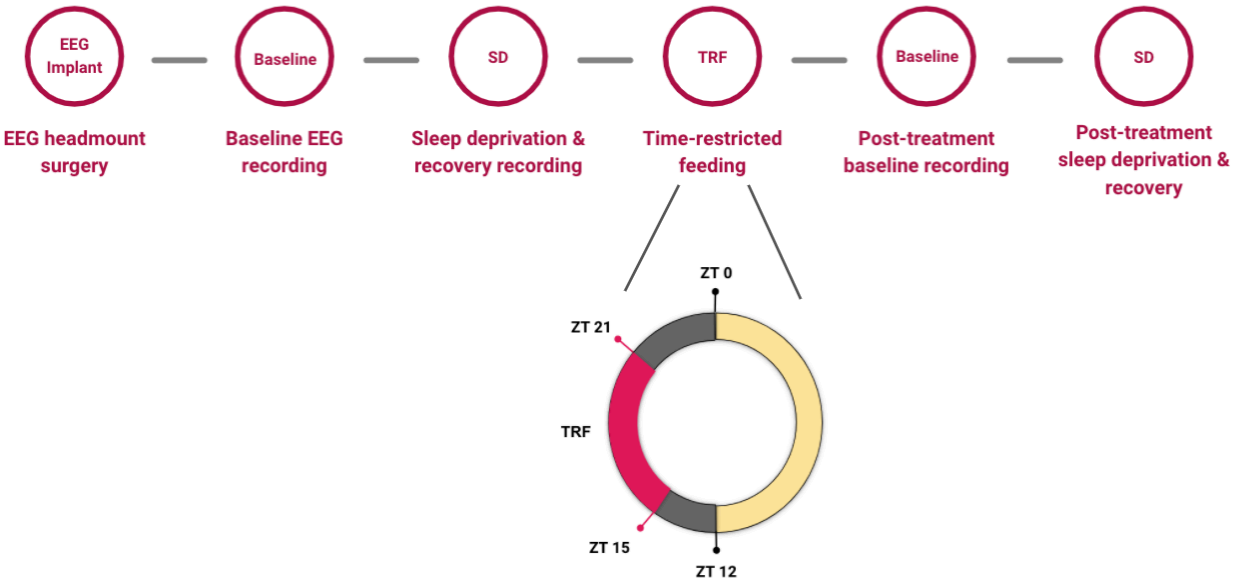

Figure S1. Experimental design.
Animals underwent EEG headmount surgery at 3 months old. After 1 week of recovery from surgery and 1 week of acclimation to the recording chambers, there was a 24h baseline recording, followed by 6h of sleep deprivation by gentle-handling, and a 18h recovery recording. Animals on TRF had access to food from ZT15-21. The light phase is from ZT0-12, and the dark phase is from ZT12-24. Control animals had *ad libitum (ad lib)* access to food and water. After 1 month of TRF or *ad lib*, the baseline and recovery recordings were repeated.

TABLES

Table 1. Disrupted sleep/wake cycle and EEG power in male BACHD mice

		WT	BACHD	Time	Genotype	Interaction
Male	Wake	63.92	67.81	**F(1.761, 29.93)=12.70; p=0.0002**	F (1,17)=0.2779; p=0.6049	**F(11,187)= 4.375; p<0.0001**
	NREM	46.91	41.60	**F(1.831, 31.14)=15.50; p<0.0001**	F (1,17)=1.255; p=0.2782	**F(11, 187)= 4.273; p<0.0001**
	REM	4.671	3.159	**F(3.035, 51.59)=31.25; p<0.0001**	**F (1,17)=4.577; p=0.0472**	F(11,187)=1.450; p=0.1537
	Gamma	6.276e-005	-0.0001096	**F(2.388, 19.10)=5.069; p=0.0134**	F (1, 8)=0.9980; p=0.3470	F(11,88)=1.723; p=0.0811
	Delta	1.646e-011	1.788e-011	**F(4.890, 78.24)=4.086; p=0.0026**	F (1,16)=0.002137; p=0.9637	**F(11,176)= 1.854; p=0.0484**
	Delta Recovery	-7.734e-005	6.874e-008	**F(4.113, 53.47)=7.064; p=0.0001**	F (1,13)=1.539; p=0.2367	F(11,143)= 1.803; p=0.0585
Female	Wake	68.38	71.90	**F(3.835,61.36)=53.07; p <0.0001**	F (1,16) = 0.8978; p=0.3575	F(11,176)=1.404; p=0.1748
	NREM	36.45	38.81	**F(3.598,57.56)=64.68; p<0.0001**	F (1, 16) = 1.635; p=0.2192	F(11,176)=0.9074; p=0.5345
	REM	5.673	5.181	**F(4.714,75.43)= 52.32; p<0.0001**	F (1, 16) = 1.065; p=0.3175	F(11,176)=0.6311; p=0.8006
	Gamma	-1.197e-005	-6.221e-005	**F(3.499,55.99)=19.34; p<0.0001**	F (1, 16) = 0.5210; p=0.4808	F(11,176)=0.9507; p=0.4934
	Delta	1.338e-005	-1.785e-005	**F(5.192,119.4)=14.11; p<0.0001**	F (1, 23) = 0.4717; p=0.4991	F(11,253)=1.056; p=0.3980
	Delta Recovery	-3.283e-006	-1.608e-005	F(5.076,111.7)=8.805; **p<0.0001**	F (1.22) = 0.1312; p = 0.7206	F(11,242)=1.737; p=0.0661

Comparisons of wake, NREM, and REM waveforms and EEG spectral power in age-matched WT and BACHD male and female. Data was analyzed with a two-way ANOVA with time and genotype as factors. The Bonferroni test for multiple comparisons was used when appropriate. p values < 0.05 were considered significant and are shown in bold.

Table 2. Sleep fragmentation and NREM sleep recovery

		WT		BACHD				
		Male	Female	Male	Female	Sex	Genotype	Interaction
# NREM bouts	Light	81.71	112.5	121.2	117.5	F(1,34)=3.143; p=0.0852	**F(1,34)=8.436; p=0.0064**	**F(1,34)=5.047; p=0.0313**
	Dark	30.00	21.00	61.33	27.85	**F(1,34)=9.614; p=0.0039**	**F(1,34)=7.763; p=0.0087**	F(1,34)=3.194; p=0.0828
Bout duration	Light	331.4	205.5	153.1	203.5	F(1,34)=5.792; **p=0.0217**	F(1,34)=33.04; **p<0.0001**	F(1,34) = 31.64; **p<0.0001**
	Dark	218.4	234.9	149.5	225.5	F(1,34)=3.504; p=0.0699	F(1,34)=2.512; p=0.1223	F(1,34)=1.452; p=0.2366
Stage shifts	Light	358.1	583.8	608.8	526.3	F(1,4)=1.147; p=0.2918	F(1,34)=2.087; p=0.1577	**F(1, 34)=5.314; p=0.0274**
	Dark	306.6	607.7	444.5	388.9	F(1,34)=0.9717; p=0.3312	F(1,34)=0.1053; p=0.7476	F(1, 34)=2.051; p=0.1613
NREM gained/lost		0.3445	0.5800	0.3436	0.3133	F(1,44)=1.010; p=0.3203	F(1,44)=1.719; p=0.1967	F(1,44)=1.694; p=0.1999

Comparisons of measures of sleep fragmentation and NREM recovery sleep amount in age-matched WT and BACHD male and female mice. Data was analyzed with a two-way ANOVA with sex and genotype as factors. The Bonferroni test for multiple comparisons was used. p values < 0.05 were considered significant and are shown in bold.

Table 3. Time-restricted feeding improves sleep/wake cycle and EEG power

		Ad Lib	TRF	Time	Treatment	Interaction
Males						
WT	Wake	54.46	66.92	**F(11,84)=14.70; P<0.0001**	**F(1,84)=7.646; P=0.0070**	F(11,84)=1.181; P=0.3125
	NREM	47.16	47.07	**F(11,84)=49.44; P<0.0001**	F(1,84)=0.001352; P=0.9708	**F(11,84)=2.145; P=0.0254**
	REM	5.028	4.450	F(11,84)=19.57; **P<0.0001**	F(1,84)=1.303; P=0.2569	F(11,84)=1.282; P=0.2492
	Gamma	-0.03572	6.250e-005	**F(11,23)=11.72; P<0.0001**	F(1,23)=0.05687; P=0.8136	F(11,23)=1.243; P=0.3159
	Delta	1.007e-005	4.375e-012	**F(11,72)=5.092; P<0.0001**	F(1,72)=3.922e-009; P>0.9999	F(11,72)=1.263; P=0.2635
	Delta Recovery	3.102e-005	-8.125e-012	**F(11,72)=2.295; P=0.0180**	F(1,72)=2.717e-008; P=0.9999	F(11,72)=0.5725; P=0.8449
BACHD	Wake	74.57	70.07	**F(11,72)=21.39; P<0.0001**	F(1,72)=1.228; P=0.2716	F(11,72)=2.024; **P=0.0381**
	NREM	39.77	45.42	**F(11,72)=22.25; P<0.0001**	F(1,72)=2.484; P=0.1194	F(11,72)=1.976; **P=0.0435**
	REM	4.750	4.469	**F(11,72)=9.829; P<0.0001**	F(1,72)=0.1741; P=0.6778	F(11,72)=1.218; P=0.2909
	Gamma	-7.083e-005	-0.0001417	**F(11,48)=8.785; P<0.0001**	F(1,48)=2.001e-007; P=0.9996	F(11,48)=1.469; P=0.1746
	Delta	6.722e-005	6.521e-005	**F(11,72)=3.673; P=0.0004**	F(1,72)=1.366e-010; P>0.9999	F(11,72)=0.9807; P=0.4718
	Delta Recovery	-6.982e-006	-1.389e-006	**F(11,72)=3.062; P=0.0021**	F(1,72)=9.503e-010; P>0.9999	F(11,72)=0.5182; P=0.8850
Females						
WT	Wake	61.67	68.69	**F(11,96)=16.21; P<0.0001**	F(1,96)=3.006; P=0.0862	F(11,96)=1.234; P=0.2754
	NREM	39.71	43.57	**F(11,96)=70.87; P<0.0001**	**F(1,96)=3.969; P=0.0492**	**F(11,96)=3.413; P=0.0005**
	REM	6.030	5.858	**F(11,96)=52.93; P<0.0001**	F(1,96)=0.2167; P=0.6426	F(11,96)=1.021; P=0.4345
	Gamma	-2.654e-005	-4.543e-005	**F(11,96)=23.28; P<0.0001**	F(1,96)=3.460e-008; P=0.9999	F(11,96)=1.468; P=0.1564
	Delta	9.921e-005	-7.292e-012	**F(11,72)=13.27; P<0.0001**	F(1,72) = 6.688e-007; P=0.9993	**F(11,72)=2.825; P=0.0040**
	Delta Recovery	-2.843e-005	6.478e-005	**F(11,108)=8.967; P<0.0001**	F(1,108)=5.213e-007; P=0.9994	F(11,108)=1.371; P=0.1972
BACHD	Wake	73.51	70.14	**F(11,108)=59.48; P<0.0001**	F(1,108)=1.925; P=0.1682	**F(11,108)=2.658; P=0.0047**
	NREM	41.19	43.68	**F(11,108)=52.07; P<0.0001**	F(1,108)=1.170; P=0.2818	**F(11,108)=2.610; P=0.0055**
	REM	4.494	5.454	**F(11,108)=57.28; P<0.0001**	**F(1,108)=8.767; P=0.0038**	**F(11,108)=2.788; P=0.0031**
	Gamma	1.475e-006	-0.0003179	**F(11,108)=39.68; P<0.0001**	F(1,108)=1.570e-005; P=0.9968	F(11,108)=1.661; P=0.0921
	Delta	3.637e-005	-5.855e-005	**F(11,96)=14.37; P<0.0001**	F(1,96)=7.007e-007; P=0.9993	**F(11,96)=2.829; P=0.0030**
	Delta Recovery	2.461e-006	-9.431e-012	F(11,108)=5.867; P<0.0001	F(1,108)=2.959e-010; P>0.9999	F(11,108)=0.5651; P=0.8532

Comparisons of wake, NREM, and REM waveforms and EEG spectral power in age-matched WT and BACHD male and female mice under *ad lib* or time-restricted feeding (TRF) treatment. Data was analyzed with a two-way ANOVA with time and treatment as factors. The Bonferroni test for multiple comparisons was used when appropriate. p values < 0.05 were considered significant and are shown in bold.

Table 4. Effects of time-restricted feeding on sleep fragmentation and recovery sleep

		WT		BACHD				
		Ad Lib	TRF	*Ad Lib*	TRF	Genotype	Treatment	Interaction
Males								
#NREM bouts	Light	104.5	115.5	128.0	109.0	F(1,13)=0.3980; P=0.5390	F(1,13)=0.08814; P=0.7712	F(1,13)=1.240; P=0.2857
	Dark	56.00	34.25	58.20	29.50	F(1,13)=0.0067; P=0.9359	F(1,13)=2.629; P=0.1290	F(1,13)=0.04988; P=0.8267
Bout duration	Light	260.0	252.0	188.4	275.1	F(1,13)=0.6320; P=0.4409	F(1,13)=1.665; P=0.2194	F(1,13)=2.411; P=0.1445
	Dark	166.8	179.8	149.6	146.4	F(1,13)=0.7055; P=0.4161	F(1,13)=0.0263; P=0.8737	F(1,13)=0.07239; P=0.7921
Stage shifts	Light	466.8	408.3	479.8	390.5	F(1,13)=0.0009; P=0.9763	F(1,13)=0.9080; P=0.3580	F(1,13) = 0.03943; P=0.8457
	Dark	341.3	176.5	298.2	98.50	F(1,13)=0.2577; P=0.6202	F(1,13)=2.336; P=0.1504	F(1,13) = 0.02148; P=0.8857
NREM gained/lost		0.3850	0.3580	0.4020	0.3025	F(1,14)=0.03605; P=0.8521	F(1,14)=0.3892; P=0.5427	F(1,14) = 0.1278; P=0.7260
Females								
# NREM bouts	Light	108.4	121.3	112.6	152.3	F(1,20)=3.016; P=0.0978	**F(1,20)=6.722; P=0.0174**	F(1,20)=1.764; P=0.1990
	Dark	28.71	29.25	25.14	34.50	F(1,20)=0.02504; P=0.8759	F(1,20)=0.8696; P=0.3622	F(1,20)=0.6915; P=0.4155
Bout duration	Light	231.9	214.7	221.1	178.3	F(1,20)=1.930; P=0.1800	F(1,20)=3.105; P=0.0933	F(1,20)=0.5681; P=0.4598
	Dark	249.9	219.6	241.5	199.8	F(1,20)=0.2912; P=0.5954	F(1,20)=1.903; P=0.1830	F(1,20)=0.04753; P=0.8296
Stage shifts	Light	524.7	518.0	473.6	597.8	F(1,20)=0.03986; P=0.8438	F(1,20)=0.6690; P=0.4230	F(1,20)=0.8306; P=0.3729
	Dark	379.3	232.3	293.7	196.2	F(1,20)=0.1854; P=0.6714	F(1,20)=0.7492; P=0.3970	F(1,20)=0.03067; P=0.8627
NREM gained/lost		0.4420	0.3050	0.3720	0.3217	F(1,16)=0.09491; P=0.7620	F(1,16)=1.171; P=0.2952	F(1,6)=0.2506; P=0.6234

Comparisons of measures of sleep fragmentation and NREM recovery sleep amount in age-matched WT and BACHD male and female mice under *ad lib* or time-restricted feeding (TRF) treatment. Data was analyzed with a two-way ANOVA with genotype and treatment as factors. The Bonferroni test for multiple comparisons was used. p values < 0.05 were considered significant and are shown in bold.

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