Longitudinal analysis of the electroencephalogram and sleep phenotype in the R6/2 mouse model of Huntington’s disease


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Deficits in sleep and circadian organization have been identified as common early features in patients with Huntington’s disease that correlate with symptom severity and may be instrumental in disease progression. Studies in Huntington’s disease gene carriers suggest that alterations in the electroencephalogram may reflect underlying neuronal dysfunction that is present in the premanifest stage. We conducted a longitudinal characterization of sleep/wake and electroencephalographic activity in the R6/2 mouse model of Huntington’s disease to determine whether analogous electroencephalographic ‘signatures’ could be identified early in disease progression. R6/2 and wild-type mice were implanted for electroencephalographic recordings along with telemetry for the continuous recording of activity and body temperature. Diurnal patterns of activity and core body temperature were progressively disrupted in R6/2 mice, with a large reduction in the amplitude of these rhythms apparent by 13 weeks of age. The diurnal variation in sleep/wake states was gradually attenuated as sleep became more fragmented and total sleep time was reduced relative to wild-type mice. These genotypic differences were augmented at 17 weeks and evident across the entire 24-h period. Quantitative electroencephalogram analysis revealed anomalous increases in high beta and gamma activity (25–60 Hz) in all sleep/wake states in R6/2 mice, along with increases in theta activity during both non-rapid eye movement and rapid eye movement sleep and a reduction of delta power in non-rapid eye movement sleep. These dramatic alterations in quantitative electroencephalographic measures were apparent from our earliest recording (9 weeks), before any major differences in diurnal physiology or sleep/wake behaviour occurred. In addition, the homeostatic response to sleep deprivation was greatly attenuated with disease progression. These findings demonstrate the sensitivity of quantitative electroencephalographic analysis to identify early pathophysiological alterations in the R6/2 model of Huntington’s disease and suggest longitudinal studies in other preclinical Huntington’s disease models are needed to determine the generality of these observations as a potential adjunct in therapeutic development.

Keywords: EEG; biomarkers; sleep; Huntington’s disease; R6/2 transgenic mice

Abbreviation: T_b = core body temperature
Introduction

Huntington’s disease is a debilitating neurodegenerative disorder characterized by progressive motor, psychiatric and cognitive impairments. Huntington’s disease is caused by an unstable CAG repeat expansion in exon 1 of the huntingtin gene, resulting in the expression of an extended polyglutamine tract that is thought to confer a ‘toxic gain of function’ (The Huntington’s Disease Collaborative Research Group, 1993). A primary feature of the disease is the preferential loss of striatal medium spiny neurons, but neurodegeneration also occurs in the cerebral cortex, hippocampus and hypothalamus (Vonsattel et al., 1985; Vonsattel and DiFiglia, 1998; Rosas et al., 2008). Huntington’s disease typically manifests during mid-adult life, but increasing evidence suggests that neurodegeneration is preceded by a period of neuronal dysfunction that occurs decades earlier than previously appreciated (Zimbelman et al., 2007; Paulsen et al., 2008; Georgiou-Karistianis et al., 2013).

A high prevalence of circadian and sleep abnormalities has recently been identified in clinical studies of patients with Huntington’s disease. These symptoms include increased night time activity, prolonged sleep latency, delayed sleep phase, insomnia, daytime sleepiness and, in a subset of patients, rapid eye movement (REM) sleep behaviour disorder (Morton et al., 2005; Arnulf et al., 2008; Cuturic et al., 2009; Aziz et al., 2010). These changes in sleep characteristics are early features of the disease that correlate with cognitive dysfunction and disease severity (Arnulf et al., 2008; Aziz et al., 2010; Goodman et al., 2011). EEG abnormalities have also been found in patients with symptomatic Huntington’s disease, with a reduction in alpha power being the most reliable alteration detected (Scott et al., 1972; Pokrovskia and Insarova, 1988; Bysma et al., 1994; Painold et al., 2011). A recent study employing quantitative EEG analysis demonstrated that such abnormalities were detectable in the waking EEG of premanifest Huntington’s disease gene carriers (Hunter et al., 2010). This finding supports the utility of quantitative EEG measures as biomarkers to predict the onset and objectively track longitudinal changes in the Huntington’s disease brain, years in advance of conventional clinical diagnosis. The identification of EEG biomarkers in premanifest Huntington’s disease could facilitate the development of novel therapies aimed at delaying disease progression and further our understanding of the early pathophysiological alterations in the disease.

The circadian abnormalities reported in patients with Huntington’s disease have been replicated in animal models of the disease (Morton et al., 2005; Bode et al., 2009; Kudo et al., 2011; Oakeshott et al., 2011). Among these models, the R6/2 mouse, which expresses a fragment of exon 1 of the human huntingtin gene, is the most widely studied and is extensively employed in preclinical Huntington’s disease trials (Li et al., 2005; Gil and Rego, 2009). These mice display a progressive Huntington’s disease phenotype characterized by motor and cognitive deficits, brain atrophy, and widespread neuronal intranuclear inclusions of mutant huntingtin (Mangiarini et al., 1996; Davies et al., 1997). R6/2 mice also exhibit disrupted activity rhythms that worsen with disease development, culminating in the disintegration of the circadian cycle (Morton et al., 2005). Intriguingly, treatment of these mice with a sedative to exogenously impose a rest/activity cycle ameliorated the circadian disruption and increased cognitive function and survival (Pallier et al., 2007). Although these observations suggest that sleep and circadian disruption may contribute to and/or exacerbate disease progression in Huntington’s disease, to this point, the existence of sleep abnormalities in preclinical Huntington’s disease models are inferred and remain to be directly determined. In the present study, we performed a longitudinal characterization of the changes in EEG-defined sleep/wake behaviour that accompany the documented circadian disruption in the R6/2 mouse. We specifically evaluated changes in quantitative EEG measures to determine whether we could identify EEG ‘signatures’ associated with disease onset and progression.

Materials and methods

Animals

Male R6/2 mice (mean CAG repeat length of 241 ± 5) were obtained from Jackson Laboratories together with wild-type controls on a C57BL/6 × CBA/CaJ background (n = 11–15 per genotype for temperature/activity data; n = 8 per genotype for EEG data). Genotyping was conducted on tissue samples by Laragen before the start and after completion of the study. Mice were housed in ventilated, light-tight, sound-isolated chambers under a standard 12:12 h light/dark cycle (lights on at 0900, ZT0, white LED-based light source, 40 lx) with food and water available ad libitum. Room temperature and relative humidity were maintained at 22 ± 2°C and 50 ± 20%, respectively. Animals were inspected daily in accordance with The Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) and SRI guidelines. All experimental procedures involving animals were approved by SRI’s Institutional Animal Care and Use Committee and were in accordance with U.S. National Institute of Health guidelines. To enhance the health status of R6/2 mice, soft chow was introduced to all cages daily at ZT0 beginning at 15 weeks of age.

Surgical procedures

All surgeries were performed under general anaesthesia in sterile conditions. Mice (~6–7 weeks of age) were implanted with prefabricated headmounts (Pinnacle Technologies) to record the EEG and EMG. Core body temperature (Tb) and gross motor activity were recorded using Mini-Mitter telemeters (G2 E-Mitter, Philips Respironics). Mice were anaesthetized with isoflurane (5% induction, 2–3% maintenance) and the fur was shaved from the top of the head and from the mid-abdominal region. After the skin was disinfected with betadine and alcohol, a midventral incision was made through the peritoneum parallel to the linea alba. The peritoneum was irrigated with 0.9% physiological saline and a sterile telemetry device was sutured to the inner layer of the abdominal muscle within the peritoneal cavity using PolysiliconeTM (5–0) suture. The abdominal musculature was then closed with absorbable suture (plain gut suture 5–0), and the outer skin was closed with polyvinyl and wound clips. Next, a dorsal midline incision was made on top of the head, the temporalsis muscle was retracted, and the skull was cleaned with a 3% hydrogen peroxide solution. The headmount (three channels: two EEG and one EMG) was affixed to the skull using cyanoacrylate and stainless steel screws (0.10 in and 0.12 in) that contacted the dura and also functioned as EEG electrodes. The front edge of the
headmount was placed 3.0 mm anterior to bregma aligned centrally along the sagittal suture. In this configuration, EEG electrodes were positioned 1.5 mm lateral to the sagittal suture, 2.0 mm anterior of bregma and –4.0 mm posterior to bregma. The threads of the EEG electrodes were coated with silver epoxy (Pinnacle Technologies) to ensure a solid connection with the headmount. EMG electrodes attached to the headmount were inserted into the trapezius muscle and sutured in place. The implant was secured to the cranium using dental acrylic (Lang Dental Manufacturing Co) and the incision was closed with Polyviolene®. For 1–2 days post-surgery, mice were administered analgesics (buprenorphine, 0.05–0.1 mg/kg and ketoprofen, 2−5 mg/kg), hydration (0.9% physiological saline) subcutaneously and provided thermal support and soft chow. During post-surgery recovery (12–14 days) and for all subsequent experiments, mice were individually housed in cages (280 × 175 × 130 mm) with extended vertical Plexiglas sides that permitted the animal to be tethered for EEG/EMG recordings and minimized the risk of escape.

Electrophysiological data collection

EEG/EMG data were collected using iox2 (Version 2.8.0.11, EMKA Technologies) installed on a personal computer (Windows 7). Electrophysiological signals were collected from the mice using cables consisting of ultra-flexible six-channel cable (NMUF6/30-4046U, Cooner Wire) and double-sided six-pin connectors (Pinnacle Technologies). These were connected to swivel commutators (Pinnacle Technologies) mounted above the cage, allowing the animal to move freely at all times. Animals were habituated to the recording cable for 4–5 days before data collection. Additional custom-made cables connected the commutator to an amplifier system (M15 bipolar amplifier, 8x15A54 modules, Grass Instruments). Signals were routed from the amplifier to iox2 interface boxes (EMKA Technologies). EEG and EMG signals were sampled at 200 Hz. The EEG was low-pass filtered at 100 Hz and high-pass filtered at 0.1 Hz; EMG was low-pass filtered at 6 KHz and high-pass filtered at 3 Hz.

Activity and core body temperature recordings

Activity and Tb were collected continuously from ~8 weeks of age using inductive telemetry (Mini-Mitter, Philips Respironics), which consisted of a network of receivers (ER4000, Philips Respironics) located beneath each cage connected to the serial port of a personal computer. Data were sampled every minute using Vitalview software (Version 5.0, Philips Respironics).

Electrophysiological signal analysis

The electrophysiological data presented here are derived from 1152 h of recordings of eight wild-type and eight R6/2 mice during baseline conditions and another 1680 h of sleep deprivation and recovery, all of which were analysed using ecgAUTO (Version 3.2, EEG Module, EMKA Technologies). EEG/EMG data were scored offline by experienced scorers, blinded to genotype, as either wakefulness (Wake; mixed-frequency, low amplitude EEG and high amplitude, variable EMG), REM sleep (theta dominated EEG and EMG atonia) or non-REM sleep (low frequency, high amplitude EEG and low amplitude, steady EMG) in 12 s epochs. Hourly sleep/wake amounts and sleep consolidation measures (bout duration and number of bouts) were assessed for all conditions. A bout was defined as either two consecutive epochs of waking or non-REM sleep or a single epoch of REM sleep. For spectral analysis of the EEG data, a Fast Fourier transform (range: 0.3–60 Hz, Hanning window function) was performed on artifact-free epochs (12 s) for each behavioural state. EEG spectra were analysed in 1 Hz bins and in standard frequency bands (delta: 0.5–4 Hz; theta: 4–9 Hz; alpha: 9–12; beta: 12–30; and gamma: 30–60 Hz). Baseline spectral data for all mice were normalized to the wild-type Week 9 condition (average group value).

Activity and core body temperature data analyses

Activity and Tb data were exported from VitalView into Excel (Microsoft Office, 2010). Hourly average values were then calculated for all experimental groups and plotted as 48 h line graphs for each baseline period at 9, 13 and 17 weeks of age. Activity data were analysed as actograms using Actiview software (version 1.3, Philips Respironics) in which raw activity counts were plotted as vertical bars in a double-plot format. Temperature data were similarly analysed in ClockLab (Actimetrics, Evanston) on a percentile distribution basis. To determine the amplitude of diurnal periodicity of activity and Tb rhythms, chi-square periodograms (period lengths: 10–36 h) were calculated using ClockLab. Periodogram analyses were performed on data collected over a 7-day interval preceding the EEG/EMG recordings at 9, 13 and 17 weeks of age. For all periodogram analyses, the significance level was set at P < 0.01. The peak periodicity in the circadian range (23–25 h) was calculated for each individual animal and mean data values were plotted as bar graphs for wild-type and R6/2 groups.

Sleep deprivation

To probe the integrity of the sleep homeostat, after 24 h undisturbed baseline EEG/EMG recordings at 9, 13 and 17 weeks of age, mice were sleep deprived on alternate days for either 1, 3 or 6 h by progressive stimulation (e.g. cage tapping, movement of bedding, light and brief stroking with a brush). The initiation of sleep deprivation was adjusted to ensure that recovery sleep occurred at the same circadian time (ZT6) regardless of the duration of the sleep deprivation. Due to deteriorating health, four R6/2 mice were removed from the study before the 6 h sleep deprivation recording at 17 weeks of age.

Statistical analyses

Statistical analyses were performed using SigmaPlot 12.0 (Systat Software Inc). Differences among group means were assessed using appropriately designed two-way repeated measures ANOVA. For activity and Tb data, comparisons were made between genotypes at each age (factors: ‘genotype’ and ‘time of day’). For all sleep/wake and normalized EEG analyses, both between (wild-type versus R6/2) and within genotype (9 versus 13 and 9 versus 17 weeks) comparisons were performed. If an ANOVA test indicated significance, multiple comparisons were made using post hoc Bonferroni t-tests, where differences were considered statistically significant if P < 0.05.

Results

Diurnal rhythms of activity and core body temperature are disrupted in R6/2 mice

Gross motor activity was progressively disrupted in R6/2 mice over the duration of the study (Supplementary Fig. 1A) in agreement
with previous findings (Morton et al., 2005; Kudo et al., 2011). At 9 weeks of age, both genotypes exhibited robust diurnal rhythms; however, the peak amplitude at dark onset was gradually attenuated in R6/2 mice (Supplementary Fig. 1B). In addition, the amplitude of the 24 h periodicity was significantly reduced compared with wild-type mice (Supplementary Fig. 1C, inset bar graph). At 17 weeks, a small increase in activity at light onset became evident that coincided with the addition of mashed chow to all cages. Despite this artifact, a significant 24 h rhythm in activity was not evident in R6/2 mice (Supplementary Fig. 1C). Average activity counts during the 12 h dark period were significantly lower in R6/2 mice relative to wild-type at all ages and in R6/2 mice at 9 weeks of age (Supplementary Table 1).

R6/2 mice exhibited robust Tb rhythms during the initial weeks of recording but the amplitude of these rhythms gradually diminished, although a small peak in Tb at dark onset was still evident in the latter stages of the study (Fig. 1A). At 9 weeks, both wild-type and R6/2 mice exhibited robust diurnal rhythms in Tb that were synchronized to the light/dark cycle, although the peak amplitude of this rhythm at dark onset was reduced in R6/2 mice (Fig. 1B). The diurnal rhythm of Tb was significantly attenuated by 13 weeks of age, primarily due to a reduction in Tb during the dark period. Although this decline in Tb rhythm amplitude continued with age, a detectable 24 h rhythm remained at 17 weeks (Fig. 1C).

The waveform of the Tb rhythm was dramatically altered at this age, with hypothermia evident in R6/2 mice in which the average Tb in the light period was actually higher (34.8 ± 0.2°C) than during the dark period (33.9 ± 0.3°C). Mean Tb at 13 and 17 weeks of age was also significantly lower than both age-matched wild-type mice and 9 week old R6/2 mice (Supplementary Table 1).

Developmental changes in wakefulness, non-rapid eye movement sleep and rapid eye movement sleep in R6/2 mice

In parallel with the activity rhythm, the diurnal rhythm of sleep and wakefulness in R6/2 mice was abolished over the study duration (Fig. 2). Normal distributions and hourly amounts of waking and non-REM sleep across the light/dark cycle were observed in R6/2 mice at 9 weeks of age (Fig. 2A and B). Changes in the distribution of wakefulness and non-REM sleep were evident at 13 weeks with increased wakefulness and decreased non-REM sleep in the light period relative to wild-type mice. By 17 weeks, the hourly percentages of wakefulness and non-REM sleep showed little variation across the 24 h cycle, resulting in an attenuated diurnal distribution of sleep and waking. Interestingly, REM sleep at 9 weeks of age was significantly enhanced in R6/2 mice during the light period (Fig. 2C). By 13 weeks, this increase in REM sleep also became evident in the dark period, a change that persisted through 17 weeks.

Whereas the total amounts of sleep and wakefulness across the 24 h period were unchanged in wild-type mice across this study, the total amounts of wakefulness significantly increased and non-REM sleep decreased in R6/2 mice with age (Table 1). At 9 weeks, total non-REM sleep time was 95 min less in R6/2 than in wild-type mice, which increased to a difference of 163 min by 17 weeks. As suggested in Fig. 2C, total REM sleep during the 12 h dark period was greater in R6/2 mice at 13 and 17 weeks compared to both age-matched wild-type mice and to 9 week old R6/2 mice (Table 1). There was also a significant increase in REM sleep for the overall 24 h baseline period in R6/2 mice compared with wild-type mice at each of the recording weeks.

Developmental changes in sleep/wake architecture in R6/2 mice

The changes in the diurnal distribution of sleep and wakefulness in R6/2 mice described above were accompanied by significant changes in sleep architecture with age. Although sleep/wake architecture in R6/2 mice was indistinguishable from that of wild-type mice at 9 weeks of age, the number of sleep/wake bouts progressively increased across the three recording periods in R6/2 mice (Fig. 3). Increases in the number of wake bouts were evident in R6/2 mice across the entire 24 h baseline period at 13 and 17 weeks of age while similar increases in the number of non-REM and REM sleep bouts were largely confined to the dark period. The prominent increase in wake bout duration during the dark period seen in wild-type mice was absent in R6/2 mice at 13 and 17 weeks (Fig. 4). Therefore, as wake bout duration decreased from 9 to 17 weeks in R6/2 mice, the number of wake bouts increased, resulting in a highly fragmented pattern of wakefulness. Non-REM bout duration was shorter in the light and dark phases at 17 weeks in R6/2 mice (Fig. 4). As no significant differences were found in REM bout duration between R6/2 and wild-type mice, the increased time in REM in these mice at week 13 and 17 was due to an increase in the number of REM episodes without a change in REM bout duration. In contrast with these age-related changes in R6/2 mice, sleep/wake architecture was unchanged in wild-type mice across the study.

Quantitative electroencephalographic analysis in R6/2 and wild-type mice

We identified complex genotype-specific alterations in the raw power distribution of the EEG spectra in all sleep/wake states (Supplementary Fig. 2). In order to better visualize changes in the EEG, particularly in frequency bins >20 Hz, we normalized the power in all recordings to the 24 h average power derived from the 9 week wild-type condition per 1 Hz bin (Fig. 5). Full statistical analysis of the normalized EEG power spectra can be found in Supplementary Table 2. The most prominent abnormal feature in the EEG of R6/2 mice was a large increase in high beta and gamma power from ~25–60 Hz in each of the sleep/wake states. Surprisingly, increased gamma activity was evident in R6/2 mice from our earliest recording at 9 weeks of age. As the disease progressed, the peak frequency of these oscillations slowed and the distribution of frequencies became narrower (Fig. 5). For non-REM and REM sleep, the peak amplitude increased with age, particularly in the light/inactive phase. During wakefulness, the increase in normalized high frequency power (25–45 Hz)
EEG and sleep in Huntington’s disease

Figure 1 Diurnal rhythms in core body temperature are severely attenuated in R6/2 mice. (A) Representative double-plotted actogram-style plots for body temperature of wild-type (WT, left) and R6/2 mice (right) recorded continuously from ~8–17 weeks of age maintained under a regular 12:12 light/dark cycle (indicated by black and white bars at the top of the actogram). Each horizontal line represents two consecutive 24 h periods in which the second half of each line is repeated on the first half of the following line. One, 3 and 6 h sleep deprivations performed at 9, 13 and 17 weeks of age are highlighted in red. (B) Forty-eight hour body temperature profiles during baseline recordings at 9, 13 and 17 weeks of age for wild-type (blue circles) and R6/2 mice (gold circles), n = 11–15, mean hourly values ± SEM. Black bars above the x-axis indicate times with significant differences (P < 0.05) between wild-type and R6/2 mice. For full statistical information refer to the Supplementary material. (C) Chi-square periodogram analysis of the amplitude of diurnal rhythmicity (10–35 h) of body temperature. Dotted diagonal line represents P < 0.05 significance level. Inset bar graph represents the magnitude of periodicity in the circadian (23–25 h) range for body temperature of each genotype (*P < 0.05, two-tailed t-test).

in R6/2 mice was fairly consistent from 9–17 weeks (~5-fold greater versus wild-type mice) but also slowed over the progression of the disease. In the waking EEG spectra of wild-type mice, there was a much smaller increase in the 43–59 Hz range with age.

The non-REM EEG power spectra in R6/2 mice was redistributed towards faster frequencies relative to wild-type mice, reflected by a decrease in delta power (1–4 Hz) at Weeks 13 and 17 and an increase in theta power (6–9 Hz) at 9 and 13 weeks of age (Fig. 5 and Supplementary Fig. 2). This increase in theta power during non-REM sleep was no longer apparent by 17 weeks of age. In addition, we also identified an increase in the amplitude of theta activity during REM sleep that increased with age in R6/2 mice. During wakefulness, normalized power in the 1 Hz range decreased at 13 and 17 weeks in R6/2 mice compared with dark period baseline levels at 9 weeks of age. In contrast with a previous study in R6/1 mice (Pignatelli et al., 2012), these abnormalities in the EEG spectra were not associated with any spontaneous behavioural or electrophysiological evidence of seizure activity.

Statistical analysis was also performed on normalized EEG power binned into standard frequency bands (delta, theta, alpha, beta and gamma). The most prominent change was the increase in EEG gamma band activity in all three states that was evident across the entire 24 h day from 9 weeks but, by 17 weeks, was only significantly different from wild-type in the light period (Supplementary Fig. 3E, 4E and 5E). In the waking EEG, there were increases in theta and beta power at 9 weeks and alpha activity decreased relative to wild-type at 17 weeks (Supplementary Fig. 3). Delta power in non-REM sleep progressively decreased in R6/2 mice at 13 and 17 weeks of age whereas beta power increased primarily in the light period during these ages. Beta power also increased in
Figure 2  Distribution of sleep/wake states during baseline recordings. Percentage time spent in wakefulness (A), non-REM (NREM, B) and REM sleep (C) in wild-type (WT, blue circles) and R6/2 mice (gold circles) across the 24 h day at 9, 13 and 17 weeks of age (n = 8, mean hourly values ± SEM). In all panels, black horizontal bars above the x-axis indicate times when P < 0.05 between the two genotypes. For full statistical information refer to the Supplementary material.

Table 1  Time spent in wake, non-REM and REM during baseline recording at 9, 13 and 17 weeks of age in R6/2 and wild-type mice

<table>
<thead>
<tr>
<th>Week</th>
<th>Wake 24 h</th>
<th>12 h light</th>
<th>12 h dark</th>
<th>Non-REM 24 h</th>
<th>12 h light</th>
<th>12 h dark</th>
<th>REM 24 h</th>
<th>12 h light</th>
<th>12 h dark</th>
</tr>
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<tbody>
<tr>
<td>Week 9</td>
<td>WT</td>
<td>10.66 ± 0.40</td>
<td>4.64 ± 0.19</td>
<td>6.02 ± 0.29</td>
<td>12.13 ± 0.40</td>
<td>6.68 ± 0.19</td>
<td>5.45 ± 0.27</td>
<td>1.11 ± 0.06</td>
<td>0.60 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>R6/2</td>
<td>11.87 ± 0.37</td>
<td>5.15 ± 0.15</td>
<td>6.72 ± 0.29</td>
<td>10.54 ± 0.38*</td>
<td>5.89 ± 0.17</td>
<td>4.65 ± 0.26*</td>
<td>1.57 ± 0.11*</td>
<td>0.96 ± 0.08</td>
</tr>
<tr>
<td>Week 13</td>
<td>WT</td>
<td>10.41 ± 0.32</td>
<td>4.36 ± 0.15</td>
<td>6.04 ± 0.20</td>
<td>12.39 ± 0.33</td>
<td>6.93 ± 0.17</td>
<td>5.46 ± 0.19</td>
<td>1.17 ± 0.12</td>
<td>0.70 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>R6/2</td>
<td>12.24 ± 0.29*</td>
<td>6.09 ± 0.13*</td>
<td>6.15 ± 0.22</td>
<td>9.88 ± 0.30*</td>
<td>4.96 ± 0.14*</td>
<td>4.92 ± 0.20</td>
<td>1.87 ± 0.20*</td>
<td>0.96 ± 0.13</td>
</tr>
<tr>
<td>Week 17</td>
<td>WT</td>
<td>10.69 ± 0.26</td>
<td>4.87 ± 0.22</td>
<td>5.82 ± 0.24</td>
<td>12.24 ± 0.24</td>
<td>6.50 ± 0.18</td>
<td>5.74 ± 0.21</td>
<td>1.05 ± 0.10</td>
<td>0.63 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>R6/2</td>
<td>12.89 ± 1.10*</td>
<td>6.69 ± 0.73*</td>
<td>6.21 ± 0.48</td>
<td>9.53 ± 0.98*</td>
<td>4.59 ± 0.69*</td>
<td>4.94 ± 0.41*</td>
<td>1.55 ± 0.24*</td>
<td>0.73 ± 0.16</td>
</tr>
</tbody>
</table>

Two-way ANOVA

- **Genotype:**
  - WT 0.010, R6/2 0.002
  - Mean ± SEM: (± 0.010) ± 0.002
- **Week:**
  - 0.421
  - Mean ± SEM: 0.421 ± 0.016
- **Interaction:**
  - 0.525
  - Mean ± SEM: 0.525 ± 0.057

Mean (± SEM) time spent (h) in wake, non-REM and REM during baseline in male R6/2 and wild-type mice across the 24 h period, the 12 h light period and 12 h dark period at 9, 13 and 17 weeks of age. Bonferroni t-test (P < 0.05): * = significant difference between wild-type and R6/2 mice for a given week; # = significant difference from 9 weeks of age within genotype. P-values resulting from two-way ANOVA with ‘genotype’ and ‘week’ factors indicated.

**WT** = wild-type.
REM sleep from 17 weeks of age. Theta band activity was elevated in R6/2 mice from 9 weeks during non-REM and REM sleep but was not apparent in non-REM sleep by 17 weeks (Supplementary Figs 4 and 5).

**Homeostatic regulation of sleep is impaired in R6/2 mice**

A strong compensatory increase in sleep occurs after sleep deprivation, which reflects the homeostatic regulation of sleep (Borbély, 1982; Franken et al., 1991). One of the best characterized markers of sleep homeostasis is non-REM EEG delta power (0.5–4 Hz), which increases as a function of the duration of wakefulness and declines exponentially during sleep (Borbély, 1982). To compare the function of the sleep homeostat between R6/2 and wild-type mice, on alternate days, we increased the duration of sleep deprivation from 1 to 3 to 6 h. At 9 weeks of age, both genotypes exhibited the expected increase in non-REM delta power (relative to each mouse’s age-matched baseline delta power) that was proportional to the sleep deprivation duration and which decayed exponentially across the 6 h recovery period (Fig. 6). By 13 weeks, the ability of R6/2 mice to increase delta power in response to sleep loss was impaired, particularly during the first 2 h of the recovery period. This impairment was further augmented at 17 weeks, particularly for the 3 and 6 h sleep deprivation.

We also determined the amount of non-REM sleep during the 6 h recovery period following sleep deprivation for both genotypes. R6/2 mice increased non-REM sleep less than wild-type mice across all sleep deprivations with the exception of the shortest sleep deprivation at 9 weeks (Fig. 7). This difference between genotypes was amplified with age and sleep deprivation duration. By 17 weeks, the typical compensatory increase in non-REM sleep following sleep deprivation was significantly blunted in R6/2 mice. Overall, R6/2 mice displayed an age-dependent decrease in both measures of homeostatic sleep regulation, non-REM sleep time and intensity (EEG delta power) when compared with wild-type mice.

**Discussion**

We performed a comprehensive longitudinal analysis of the sleep phenotype of R6/2 Huntington’s disease mice during the period
when circadian regulation has been shown to deteriorate (Morton et al., 2005). We identified sleep abnormalities that progressively worsen with disease development and accompany the deterioration in diurnal rhythms. In addition, we found distinct EEG ‘signatures’ in R6/2 mice present from 9 weeks of age when basic sleep/wake parameters, activity and Tb rhythms were largely comparable with wild-type. The finding that quantitative EEG measures exhibit robust alterations that precede the occurrence of other sleep and behavioural abnormalities demonstrates the sensitivity of this approach to detect early pathophysiological changes in Huntington’s disease. These data suggest further comprehensive EEG studies in other preclinical Huntington’s disease models are needed to validate these conclusions and to determine the potential translational utility of such biomarkers.

Sleep and circadian abnormalities are evident in the R6/2 mouse model of Huntington’s disease

Sleep and circadian disturbances are a prominent early feature of patients with Huntington’s disease and have been proposed to have a negative impact on the progression of other Huntington’s disease symptoms (Arnulf et al., 2008; Aziz et al., 2010). We confirmed the previously reported loss of diurnal activity rhythms in R6/2 mice (Morton et al., 2005). In addition, we identified a disruption of the 24 h rhythm in Tb, accompanied by hypothermia in these mice. This dysregulation of diurnal physiology is a common feature of multiple Huntington’s disease animal models (Bode et al., 2009; Kudo et al., 2011; Oakeshott et al., 2011) and is consistent with the disrupted day/night activity patterns observed in patients with Huntington’s disease (Wiegand et al., 1991; Morton et al., 2005).

Although the existence of sleep disruption in preclinical Huntington’s disease models has been inferred from the disintegration of circadian activity, EEG studies have not previously been performed. Here, we identified progressive sleep deficits in the R6/2 mouse. The distribution of sleep/wake states in R6/2 mice at 9 weeks was indistinguishable from wild-type mice, with the exception of increased REM sleep during the light period. At 13 and 17 weeks of age, the 24 h variation in all sleep/wake states attenuated and sleep/wake behaviour became highly fragmented. Total non-REM sleep amount progressively decreased with age in R6/2 mice relative to wild-type, an effect that reached a daily difference of nearly 3 h by 17 weeks of age and was associated with a marked decrease in non-REM EEG delta power. This loss of non-REM sleep and disruption of sleep/wake patterns may contribute to the progression of the disease, as normalization of
rest/activity cycles with appropriately timed administration of the hypnotic alprazolam and the stimulant modafinil ameliorated cognitive decline and increased survival in R6/2 mice (Pallier et al., 2007; Pallier and Morton, 2009). The extent to which these treatments consolidated bouts of sleep was not evaluated, but nevertheless suggest that enhancement of sleep may be a therapeutic avenue in Huntington’s disease (Pallier et al., 2007; Pallier and Morton, 2009).

As the diurnal variation in all sleep/wake states attenuated, the increase in REM sleep during the light period seen at 9 weeks of age in R6/2 mice was lost and REM became more evenly distributed across the 24 h period. Interestingly, total daily REM sleep amount was increased at all ages in R6/2 mice. In contrast, reduced REM sleep duration, increased REM sleep latency and the occurrence of REM sleep behaviour disorder have been reported in patients with premanifest and mild Huntington’s disease (Arnulf et al., 2008). The reason for the discrepancy between the R6/2 mouse model and human disease is unclear and warrants further investigation.

Sleep is regulated by homeostatic and circadian processes (Borbély, 1982) and dysfunction of either one or both of these systems may underlie the sleep disruption in Huntington’s disease. Circadian cycles of gene expression in the suprachiasmatic nucleus and efferent projection sites are attenuated and phase advanced in R6/2 mice relative to wild-type (Morton et al., 2005). When isolated in vitro, the firing rate of the suprachiasmatic nucleus in...
R6/2 mice was relatively normal during the night time (Pallier et al., 2007), however, a more recent study in the BACHD mice identified a reduction in spontaneous firing during the light period (Kudo et al., 2011). This resulted in the abolition of the typical day/night difference in suprachiasmatic nucleus activity, suggesting pathophysiology directly at the level of the circadian clock.

Post-mortem studies of patients with Huntington’s disease indicate that there is a preferential loss of striatal medium spiny neurons, although neurodegeneration also occurs in brain regions involved in sleep and circadian regulation including the hypothalamus, cerebral cortex, brainstem and thalamus (Vonsattel et al., 1985; Vonsattel and DiFigiglia, 1998; Rosas et al., 2008). Recently, altered morphology was identified in the suprachiasmatic nucleus of patients with Huntington’s disease (Van Wamelen et al., 2013). Furthermore, loss of the wake-promoting peptide hypocretin from the lateral hypothalamus is observed in both patients with Huntington’s disease and R6/2 mice and may contribute to the sleep disruption (Petersén et al., 2005; Williams et al., 2011). A comprehensive histopathological analysis of the effect of mutant huntingtin expression in major sleep and wake-promoting areas has not yet been performed but may provide valuable insights into the mechanistic basis of sleep disruption in Huntington’s disease.

Alterations in quantitative electroencephalographic measures in R6/2 mice

Altered electrophysiology and corticostriatal processing is a salient feature of preclinical Huntington’s disease models (Rebec et al., 2006; Miller et al., 2008, 2011; Walker et al., 2008) and is evident at the level of individual neurons (Cepeda et al., 2010). We hypothesized that these modifications would result in distinct EEG ‘signatures’ that could be identified across the course of the disease in R6/2 mice. We found dramatic alterations in quantitative EEG measures that included large increases in high frequency gamma activity and enhanced theta activity evident in non-REM sleep. In rats, gamma activity changes with vigilance state and is associated with wakefulness and REM sleep (Franken et al., 1994); however, in R6/2 mice we identified an increase in gamma activity independent of state. The peak gamma frequency slowed with age, which may be associated with the reduction in $T_b$ in these mice as EEG frequencies vary in a temperature-dependent manner (Deboer and Tobler, 1995).

Theta and gamma activity are known to co-occur in both awake rodents and humans, with a positive correlation between the amplitude and frequency of these oscillations (Bragin et al., 1995;...
Canolty et al., 2006). The coupling of these frequencies is thought to reflect communication between distinct neuronal populations and be important for memory and learning (Tort et al., 2009). Analogous gamma and theta spectral changes have recently been identified in local field potential recordings in the motor cortex and dorsal striatum of R6/2 mice (Hong et al., 2012). Similarly, R6/1 mice that underwent a procedural learning task exhibited enhanced gamma activity in medium spiny neurons, local interneurons and cortical neurons across both hemispheres, suggesting that this neuronal disruption may have multiple origins within corticostriatal networks (Cayzac et al., 2011).

The functional implications of these EEG abnormalities are unclear; however, the increase in theta and gamma activity indicates an augmentation in neuronal excitability in the R6/2 brain. The cortex provides the major excitatory glutamatergic input to the striatum via the corticostriatal pathway and dysfunction of these neuronal circuits is central to the development of motor and cognitive deficits characteristic of Huntington’s disease (Cepeda et al., 2007). In vitro studies in R6/2 mouse brain slices have also identified hyperexcitability in both striatal medium spiny neurons (Klapstein et al., 2001) and within cortical pyramidal neurons (Cummings et al., 2009). Increased neuronal excitability may result from the loss of inhibition regulating striatal and cortical neurons that may include fast spiking parvalbumin interneurons which are known to generate gamma oscillations (Bracci et al., 2003). Alternatively, hyperexcitability and gamma oscillations in R6/2 mice may be a result of the glutamate excitotoxicity (Estrada-Sánchez et al., 2009), resulting in activation of metabotropic glutamate receptors (Whittington et al., 1995).

Prominent baseline reductions in non-REM delta power were evident in R6/2 mice from 13 weeks, with increased theta activity shifting the non-REM EEG spectra towards faster frequencies. Non-REM delta power is a correlate of sleep intensity and homeostatic sleep drive (Borbély, 1982). The apparent decrease in sleep intensity in R6/2 mice could be due to the inability to maintain long bouts of waking, thus preventing the build-up of sleep pressure. Conversely, these observations could be due to impairments in the ability to generate and propagate slow-wave oscillations throughout the cortex during sleep, possibly due to the hyperexcitability of cortical pyramidal neurons leading to the intrusion of aberrant theta and gamma oscillations. We also identified differences in the homeostatic sleep response of R6/2 mice to sleep deprivation. The typical increase in non-REM delta power and sleep time after sleep deprivation (Franken et al., 1991) was greatly diminished in R6/2 mice from 13 weeks. Therefore, not only did R6/2 mice have a blunted sleep rebound following sleep deprivation, the ‘depth’ or ‘intensity’ of the non-REM sleep was less than in wild-type mice, suggesting that the sleep homeostat is compromised in this Huntington’s disease model.
Neuronal loss is not a core feature of the R6/2 model and, although significant brain atrophy does occur, the majority of symptoms are thought to be due to functional reorganization of neuronal networks (Davies et al., 1997; Cepeda et al., 2010). As significant electrophysiological changes occurred during our initial recordings and before disruptions to sleep and diurnal rhythms, compensatory mechanisms may exist that prevent early neuronal alterations from resulting in an overt behavioural phenotype. Alternatively, the changes identified in sleep, EEG and diurnal rhythms in R6/2 mice may be due to the effect of mutant huntingtin expression in peripheral tissues. In support of this, Tb was profoundly disrupted in R6/2 mice (Fig. 1) and metabolic alterations are present in these mice including reduced food intake, increased energy expenditure and abnormalities in adipocyte and mitochondrial function (Fain et al., 2001; Weydt et al., 2006; Goodman et al., 2008). Abnormal peripheral cytokine release is also observed in R6/2 mice and patients with Huntington’s disease (Björkqvist et al., 2008), suggesting that innate immune activation is a feature of the disease that could additionally modulate brain pathology and the EEG.

### Regulation of core body temperature is severely disrupted in R6/2 mice

The diurnal variation in Tb was attenuated in R6/2 mice, in agreement with findings in BACHD mice (Kudo et al., 2011). By 17 weeks, the 24h distribution of Tb became severely disrupted with R6/2 mice displaying hypothermia, particularly evident in the dark period (Fig. 1B). These thermoregulatory and metabolic abnormalities in R6/2 mice are thought to contribute to both weight loss and early death (Weydt et al., 2006; Van der Burg et al., 2008). Surprisingly, despite these metabolic disturbances, there was an increase in amplitude of the 24h periodicity of Tb in R6/2 mice from 13 to 17 weeks of age (Fig. 1C). This was likely a result of a ‘masking effect’ imposed by the daily supplementation of all cages with mashed chow at lights onset. The suprachiasmatic nucleus-independent food-entrainable oscillator remains functional in R6/2 mice (Maywood et al., 2010) and scheduled feeding may have partially restored 24h rhythms of Tb. Nonetheless, the distribution of Tb was virtually inverted relative to wild-type mice (Fig. 1B).

### The electroencephalogram as a prognostic biomarker in Huntington’s disease

In Huntington’s disease, brain atrophy and significant cell loss is already evident upon clinical diagnosis (Zimbelman et al., 2007; Paulsen et al., 2008). Therefore, a critical goal in the development of Huntington’s disease therapeutics is to identify interventions that will delay disease progression before neuronal loss occurs. Evaluation of the efficacy and safety of novel therapeutics will remain a challenge until reliable, sensitive biomarkers can be identified during the prodromal phase of Huntington’s disease. Clinical assessment of quantitative EEG measures has been recognized as a promising indicator of disease onset and progression in Huntington’s disease (Hunter et al., 2010; Nguyen et al., 2010) and, consequently, there is a compelling need for translational markers in preclinical disease models.

To this point, clinical EEG studies in patients with Huntington’s disease have been driving the research direction of preclinical studies. The characterization of the EEG in animal models of Huntington’s disease is still largely in its infancy and, therefore, it is difficult to translate the conclusions of a single model directly to findings in humans. There will also be differences that result from mimicking the human disease in a nocturnal rodent that exhibits polyphasic sleep. Direct comparisons can also be problematic because mouse EEG studies typically record across a single pair of electrodes whereas clinical studies typically employ electrode arrays capable of performing topographical EEG analysis. Despite these limitations, evaluation of EEG ‘signatures’ across multiple animal models of Huntington’s disease may identify common biomarkers that correlate with EEG changes that accompany disease progression in patients with Huntington’s disease.

The R6/2 Huntington’s disease mouse is one of the most widely-used preclinical models, although it has a number of limitations. The major caveat is the absence of a significant presymptomatic phase due to the aggressive phenotype, with expression of mutant huntingtin occurring in the developing brain (Heng et al., 2007). We used R6/2 mice with ~240 CAG repeats, which exhibit a delay in phenotype onset compared with R6/2 CAG 110 mice (Li et al., 2005; Morton et al., 2009). However, quantitative EEG analysis revealed that large alterations were already present from 9 weeks of age. Implanting and recording longitudinally from mice earlier than this would be problematic, due to the large increases in the juvenile skull size that would compromise the viability of the implants. The R6/2 model also lacks genetic elements present in patients with Huntington’s disease, as mutant huntingtin expression occurs in the absence of the natural genomic and protein context (Ehrnhoefer et al., 2009). Investigation of sleep abnormalities and EEG ‘signatures’ in other preclinical Huntington’s disease models is warranted, particularly as no single model recapitulates all features of the human disease and convergence of evidence across models is essential to validate findings.

In summary, we have identified sleep abnormalities and EEG ‘signatures’ present in the R6/2 mouse model of Huntington’s disease that indicate a progressive dysfunction of both the diurnal and homeostatic regulation of sleep. The rapid phenotype of the R6/2 mouse was largely unsuitable for characterizing the prodromal EEG phenotype of Huntington’s disease, but nevertheless demonstrates the sensitivity of quantitative EEG analysis to provide insights into early pathophysiological alterations that precede the disruption of behaviour. Evaluating sleep and EEG abnormalities across different Huntington’s disease models may result in the identification of convergent translational EEG ‘signatures’ that could be valuable in preclinical studies designed to evaluate novel therapeutics for the treatment of Huntington’s disease. Similar results in R6/2 mice have been obtained by Kantor et al., ‘Progressive sleep and electroencephalogram changes in mice carrying the Huntington’s disease mutation’, Brain, in press.
Acknowledgements

We thank David Howland for advice on the experimental design, Kristy Silveria and Deepa Ramamurthy for technical assistance, and the members of the Center for Neuroscience and Mammalian Toxicology for assistance with sleep deprivation.

Funding

This work was supported by the CHDI Foundation, Inc. and internal funds from SRI International.

Supplementary material

Supplementary material is available at Brain online.

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