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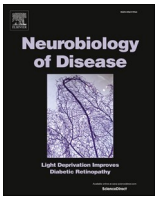

Abnormal patterns of sleep and EEG power distribution during non-rapid eye movement sleep in the sheep model of Huntington's disease

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ABSTRACT

Sleep disruption is a common invisible symptom of neurological dysfunction in Huntington's disease (HD) that takes an insidious toll on well-being of patients. Here we used electroencephalography (EEG) to examine sleep in 6 year old OVT73 transgenic sheep (*Ovis aries*) that we used as a presymptomatic model of HD. We hypothesized that despite the lack of overt symptoms of HD at this age, early alterations of the sleep-wake pattern and EEG powers may already be present. We recorded EEG from female transgenic and normal sheep (5/group) during two undisturbed 'baseline' nights with different lighting conditions. We then recorded continuously through a night of sleep disruption and the following 24 h (recovery day and night). On baseline nights, regardless of whether the lights were on or off, transgenic sheep spent more time awake than normal sheep particularly at the beginning of the night. Furthermore, there were significant differences between transgenic and normal sheep in both EEG power and its pattern of distribution during non-rapid eye movement (NREM) sleep. In particular, there was a significant decrease in delta (0.5–4 Hz) power across the night in transgenic compared to normal sheep, and the distributions of delta, theta and alpha oscillations that typically dominate the EEG in the first half of the night of normal sheep were skewed so they were predominant in the second, rather than the first half of the night in transgenic sheep. Interestingly, the effect of sleep disruption on normal sheep was also to skew the pattern of distribution of EEG powers so they looked more like that of transgenic sheep under baseline conditions. Thus it is possible that transgenic sheep exist in a state that resemble a chronic state of physiological sleep deprivation. During the sleep recovery period, normal sheep showed a significant 'rebound' increase in delta power with frontal dominance. A similar rebound was not seen in transgenic sheep, suggesting that their homeostatic response to sleep deprivation is abnormal. Although sleep abnormalities in early stage HD patients are subtle, with patients often unaware of their existence, they may contribute to impairment of neurological function that herald the onset of disease. A better understanding of the mechanisms underlying EEG abnormalities in early stage HD would give insight into how, and when, they progress into the sleep disorder. The transgenic sheep model is ideally positioned for studies of the earliest phase of disease when sleep abnormalities first emerge.

1. Introduction

Sleep disturbance is common in Huntington's disease (HD) patients (for references see Morton, 2013 and below), with symptoms often emerging at the premorbid stage of HD (Arnulf et al., 2008; Diago et al., 2018; Goodman et al., 2011; Lazar et al., 2015). Sleep is an essential circadian behaviour that plays an important role in restorative functions in the brain (Hauglund et al., 2020). Insufficient sleep impairs cognitive function (Killgore, 2010), and probably contributes to neurodegenerative changes in the brain (Anderson and Bradley, 2013). Treatments of sleep disorder in HD should therefore ameliorate some sleep deficit-related symptoms. Consistent with this idea, we showed that pharmacological restoration of a sleep-wake cycle in a transgenic mouse model of HD ameliorated cognitive decline and delayed onset of symptoms

Abbreviations: NREM, Non-rapid eye movement; REM, rapid eye movement; qEEG, quantitative EEG; EMG, electromyogram; EOG, electrooculogram; FFT, Fast Fourier Transform.

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(Pallier and Morton, 2009). Unfortunately, despite increasing recognition that sleep abnormalities might contribute to mood and cognitive decline in HD, few advances have been made to determine if sleep-based therapeutic interventions would be helpful. A better understanding of the sleep deficits in HD is essential if potentially deleterious consequences of sleep disruption are to be avoided.

HD is an autosomal dominant neurodegenerative condition caused by an unstable trinucleotide repeat expansion in the *HD* gene (MacDonald et al., 1993, for references see Bates et al., 2014). Progressive changes in sleep structure and quantitative EEG (qEEG) have been widely reported in HD patients (Aziz et al., 2010; Cuturic et al., 2009; Goodman et al., 2011; Goodman and Barker, 2010; Lazar et al., 2015; Nguyen et al., 2010; Painold et al., 2011, 2010; Piano et al., 2017a, 2017b) and mouse models of HD, including the R6/1 (Pignatelli et al., 2012), R6/2 (Fisher et al., 2013; Kantor et al., 2013) and Q175 (Rothe et al., 2015). In prodromal HD individuals, decreased sleep continuity, prolonged sleep onset latency, lower sleep efficacy as well as delayed and shortened REM sleep episodes have been reported as the earliest sleep-wake pattern changes (Arnulf et al., 2008; Diago et al., 2018; Goodman et al., 2011; Lazar et al., 2015). In the qEEG the first alterations emerge in the theta and alpha frequency ranges (Lazar et al., 2015; Ponomareva et al., 2014). To date, however, there is little understanding of what causes the earliest changes in the sleep-wake pattern and qEEG.

Here we use the OVT73 transgenic (hereafter called transgenic) sheep model of HD (Jacobsen et al., 2010) to study sleep-wake qEEG patterns. The sheep is an excellent species for studying cortical EEG because they have large brains with convoluted cortices that give high quality EEG signal uncontaminated by the subcortical activity that confounds EEG studies using mice (Campbell and Tobler, 1984; Morton and Howland, 2013). We have previously used sheep to study sleep in normal (Perentos et al., 2015; Schneider et al., 2020) and Batten's disease (Perentos et al., 2016, 2015) sheep. Sheep are diurnal, and the sleep-wake pattern and EEG signatures are similar to those seen in humans and non-human primates (Hsieh et al., 2008; Silber et al., 2007). Transgenic sheep show no signs of disease until at least 10 years of age, although they have measurable abnormalities in circadian behaviour (Morton et al., 2014), circadian hormonal regulation (Morton et al., 2020), and metabolism (Handley et al., 2016; Skene et al., 2017) as well as some brain aggregate pathology (Handley et al., 2017; Reid et al., 2013). Thus, at the age we tested them here, they are at a stage equivalent to a pre-symptomatic/early symptomatic phase in humans.

The regulation of sleep-wake cycle is known to require the complex interplay between multiple cortical and subcortical brain structures (Scammell et al., 2017), thus identifying the alterations in this regulation in transgenic sheep can reveal crucial aspects of the pathophysiology of HD. The most widely accepted index of homeostatic sleep need is the power of cortical EEG delta oscillation (0.5–4 Hz) during NREM sleep, that peaks at the onset of sleep and decreases gradually with the decline in sleep need (Achermann and Borbély, 2003; Borbely, 1982). To test the integrity of the homeostatic sleep regulation in response to sleep deprivation in transgenic and normal sheep, we established a sleep disturbance method that was aimed at keep the sleep of the sheep disrupted overnight. We used gentle human interventions by known individuals to make the procedure as stress-free as possible for the sheep. We investigated whether alterations of the sleep-wake pattern and the qEEG spectra during NREM sleep emerge in the pre-symptomatic stage of transgenic sheep despite the lack of any overt symptoms at this age. We also tested the potential differences between transgenic and normal sheep in their homeostatic response to (1) experimental manipulation of melatonin secretion by using different light conditions, (2) to increasing sleep need as a result of overnight sleep disturbance, and (3) in the magnitude of 'sleep rebound' that reflects the ability of the brain to recover following sleep deprivation.

We hypothesized that genotype-specific differences appear in the pre-manifest stage of the transgenic sheep affecting both the pattern of sleep-wake cycle and the distribution of EEG oscillations as a consequence of disrupted homeostatic and circadian sleep regulation.

2. Materials and methods

2.1. Animals

Subject animals were 10 female merino sheep of the OVT73 line (Jacobsen et al., 2010) that had been reared in a larger flock on open pasture at a livestock research facility in South Australia. The sheep were aged ~5 years old at time of surgery and weighed 84 ± 2 kg. Five were transgenic for the human HD transgene carrying 73 CAG repeats; five were genetically normal flock-mates. Sheep were transported from the breeding facility where they had lived up to 4 years of age to a research facility (South Australian Health and Medical Research Institute, Gilles Plains, South Australia) where they were housed under physical containment (PC2) conditions (Australian Government, Dept. of Health and Aging, Office of the Gene Technology Regulator, 2013) for at least 4 weeks before surgery. Three transgenic and three normal sheep were prepared for chronic EEG recording in August – September 2016;

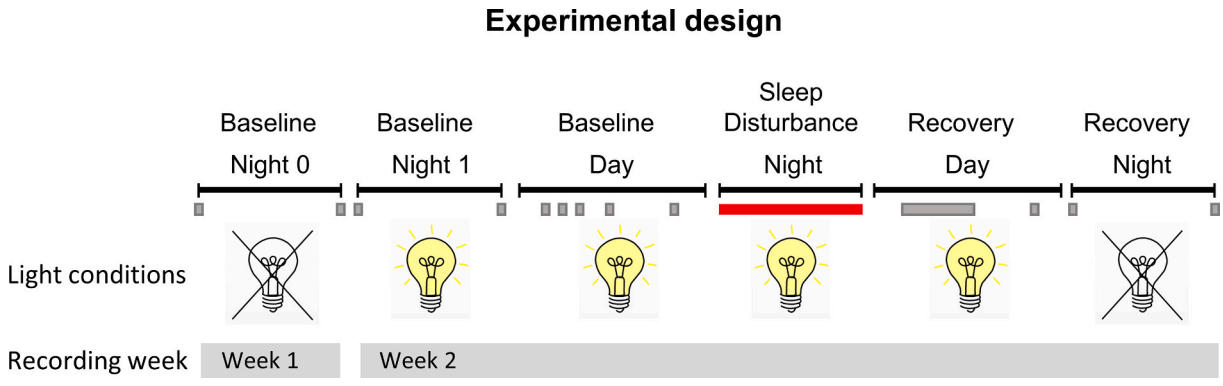

Fig. 1. Experimental design of the study. EEGs were recorded in an indoor area on normal and transgenic sheep during light-off (white lightbulb) and light-on (yellow lightbulb) baseline nights, baseline day, sleep disturbance night as well as during sleep recovery day and night. During sleep disturbance night the sheep were disturbed regularly by two experimenters who entered the recording rooms alternately and kept the sheep awake by cleaning, moving objects in the room or gentle handling the sheep when they showed the sign of drowsiness (red bar). During baseline day and sleep recovery day, sheep were undisturbed apart from routine husbandry activities (grey bars).(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the remaining sheep were prepared in January 2017. All procedures were conducted at the Preclinical Imaging and Research Laboratories (PIRL) of the South Australian Health and Medical Research Institute (SAHMRI) and followed the requirements of the SAHMRI Animal Ethics Committee including the Australian Code for the Care and Use of Animals for Scientific Purposes (8th Edition 2013). All handling of these sheep conformed to physical containment conditions as approved by the Institutional Biosafety Committee and the Office of the Gene Technology Regulator (OGTR, Australia).

2.2. Anaesthesia and surgery

Surgery was performed under aseptic conditions with appropriate anaesthesia and analgesia as described previously (Nicol and Morton, 2020; Perentos et al., 2017). Subdural Ag/AgCl disc electrodes (NDimension [Science and Engineering] Ltd., Cambridge, UK) were implanted ~25 mm, ~15 mm and ~5 mm anterior, and ~10 mm posterior to the bregma, and 10 mm lateral to the midline over both hemispheres. These positions correspond approximately to the post cruciate gyrus, the ansatus sulcus, the front third of the ectolateral sulcus, and the lateral sulcus near the anterior part of the entolateral sulcus. Electrodes were also implanted in the dorsal neck muscles for recording electromyogram (EMG) and near the anterior and posterior canthi of each eye for recording eye movements (electrooculograms, EOG). Wires from each electrode terminated at a miniature multi-pin connector (Omnetics Connector Corporation, MN, USA). A 3D-printed chamber with a screw cap that allowed easy access to the connector for fitting the amplifier for wireless transmission of signals during recordings was fixed to the acrylic cap to house the connector (Perentos et al., 2017). After surgery, the sheep remained under observation indoors for 1 week before being transferred to individual pens in an outdoor facility with overhead cover where they were exposed to natural daylight and climate. The pens were lined with Perspex to minimize damage to the implant from pen bars. During the whole experiment fresh water and food was provided ad libitum. Baseline data from all sheep were collected regularly before the sleep disruption experiment, so they were well accustomed to being handled.

2.3. Sleep disruption experimental paradigm

Sheep were placed in individual pens in two adjacent indoor recording rooms with controlled environmental conditions (artificial light, with lights turned on and off at the hour of sunrise and sunset, respectively) and equal number of transgenic and normal sheep in each room (Supplementary Fig. 1). Relative sheep location in the pens was the same for all of the recording periods. The protocol (Fig. 1) started with an undisturbed baseline night under light-off conditions (BL N0). A week later, sheep were returned to the recording room and recordings were made during a second undisturbed baseline night and day (BL N1 and BL D, respectively) with the lights kept on. On the sleep disturbance night (SD N), the sheep were disturbed regularly by two experimenters who entered the recording room and performed husbandry activities such as sweeping and cleaning. During SD N each pen also contained three coloured empty feed bins as novel objects, in addition to their usual containers for food and water. These, and other objects around the room were moved regularly to provide additional stimulation for the sheep. When a sheep showed signs of drowsiness, the experimenter entered the pen. Often this would rouse the sheep, but if not the experimenter would rouse the sheep by gentle handling. Over the following 24 h, designated sleep recovery day (SR D) and night (SR N) sheep were undisturbed apart from routine husbandry activities. The facility is in an area prone to electrical blackouts, and we had some technical issues with the lighting system during the experiment. On SR D, the light went off for 84 min affecting hours 5–6. On SR N, the light went on for 52 min affecting hours 9–10.

2.4. Electrophysiological recording

EEG data were recorded from eight cortical EEG channels: left and right anterior 1 and 2 (A1L, A1R and A2L, A2R, respectively), left and right central (CL, CR), and left and right posterior (PL, PR). EOGs were recorded from the left and right at anterior and posterior positions. EMG was recorded via electrodes implanted in the left and right neck muscles. Signals recorded from all of the electrodes were differential to the common reference electrode. At the start of each recording session, each sheep was gently restrained, and the screw cap was removed from the chamber to allow connection of a 16 channel wireless transmitter-amplifier (W2100-HS16, Multichannel Systems Gmbh, Germany). During recordings, the amplifier was held in the chamber and connected also to a battery in an integrated housing that fitted in place of the screw cap. Recordings were conducted using a wireless data acquisition system (Advanced W2100-System, Multichannel Systems Gmbh, Germany) and Multichannel Experimenter software (Multichannel Systems Gmbh, Germany). All signals were low-pass filtered at 200 Hz. Prior to this study, recordings were made approximately once per month for each sheep. The sheep quickly became accustomed to being handled,

Table 1
Sleep-wake pattern parameters of normal and transgenic sheep during baseline, sleep disturbance and sleep recovery nights.

Experimental period	Light-off baseline night		Light-on baseline night		Sleep disturbance night		Sleep recovery night	
Genotype	Normal	Transgenic	Normal	Transgenic	Normal	Transgenic	Normal	Transgenic
Total time (min/10 h)								
WAKE	263.9 ± 32.9	323.9 ± 19.8	254.3 ± 44.2	354.3 ± 20.3	525.1 ± 21.7*#	521.7 ± 13.1*#	325.4 ± 36.4	347.1 ± 25.2
NREMS	256.8 ± 29.3	229.2 ± 18.0	293.3 ± 41.7	216.5 ± 15.1	70.7 ± 20.1*#	74.0 ± 13.6*#	217.9 ± 32.8	208.2 ± 27.7
REMS	79.3 ± 8.6	46.9 ± 8.9&	52.4 ± 8.7*	29.2 ± 8.1	4.2 ± 1.9*#	4.3 ± 2.8*	48.7 ± 4.0*	36.3 ± 5.7
Number of bouts								
WAKE	20.6 ± 1.4	22.6 ± 2.1	17.0 ± 1.4	21.0 ± 2.1	13.2 ± 3.4	14.0 ± 2.2*	18.6 ± 2.1	22.4 ± 1.0
NREMS	25.0 ± 3.4	21.6 ± 1.5	26.2 ± 2.3	21.0 ± 2.1	8.0 ± 2.6*#	10.0 ± 1.9*#	22.8 ± 3.7	21.0 ± 1.4
REMS	25. 2 ± 3.5	17.0 ± 3.7	18.6 ± 2.9	11.0 ± 3.5	1.4 ± 0.7*#	0.8 ± 0.4*	15.0 ± 2.2	12.8 ± 2.5
Mean bout duration (min)								
WAKE	12.4 ± 1.9	14.4 ± 2.1	13.7 ± 2.1	9.7 ± 0.4	65.5 ± 24.6*#	43.5 ± 8.2	18.6 ± 3.8	15.2 ± 1.0
NREMS	9.4 ± 0.6	8.3 ± 0.9	11.4 ± 1.3	9.7 ± 0.4	5.9 ± 0.3*#	5.7 ± 0.5#	9.0 ± 0.4	9.1 ± 1.3
REMS	3.1 ± 0.1	2.5 ± 0.3	2.6 ± 0.2	2.3 ± 0.4	2.0 ± 0.6	1.8 ± 0.2	3.4 ± 0.4	2.7 ± 0.2
Latency times (min)								
NREM sleep	34.4 ± 20.0	49.7 ± 20.9	30.6 ± 11.1	40.9 ± 11.1	322.0 ± 98.1*#	269.8 ± 74.1*#	75.7 ± 34.1	31.7 ± 8.2
REM sleep	56.9 ± 21.5	68.9 ± 18.4	56.8 ± 19.2	147.1 ± 94.1	351.8 ± 101.2*#	462.8 ± 68.4*#	90.2 ± 36.3	69.3 ± 16.3
Inter-REM interval (min)	55.6 ± 8.4	89.3 ± 20.1	109.8 ± 20.9	318.1 ± 146.2	454.9 ± 146.1*#	172.5 ± 84.5	187.3 ± 46.0	165.5 ± 23.9
Sleep fragmentation	159.6 ± 6.0	167.4 ± 10.5	153.0 ± 10.6	124.2 ± 11.5	65.8 ± 15.2*#	72.8 ± 14.4*	135.8 ± 17.2	141.6 ± 15.1

Data are shown as mean ± SEM. *$P < 0.05$ vs. light-off baseline night, #$P < 0.05$ vs. light-on baseline night, &$P < 0.05$ normal vs. transgenic (Bonferroni post hoc comparison after significant result of the two-way ANOVA).

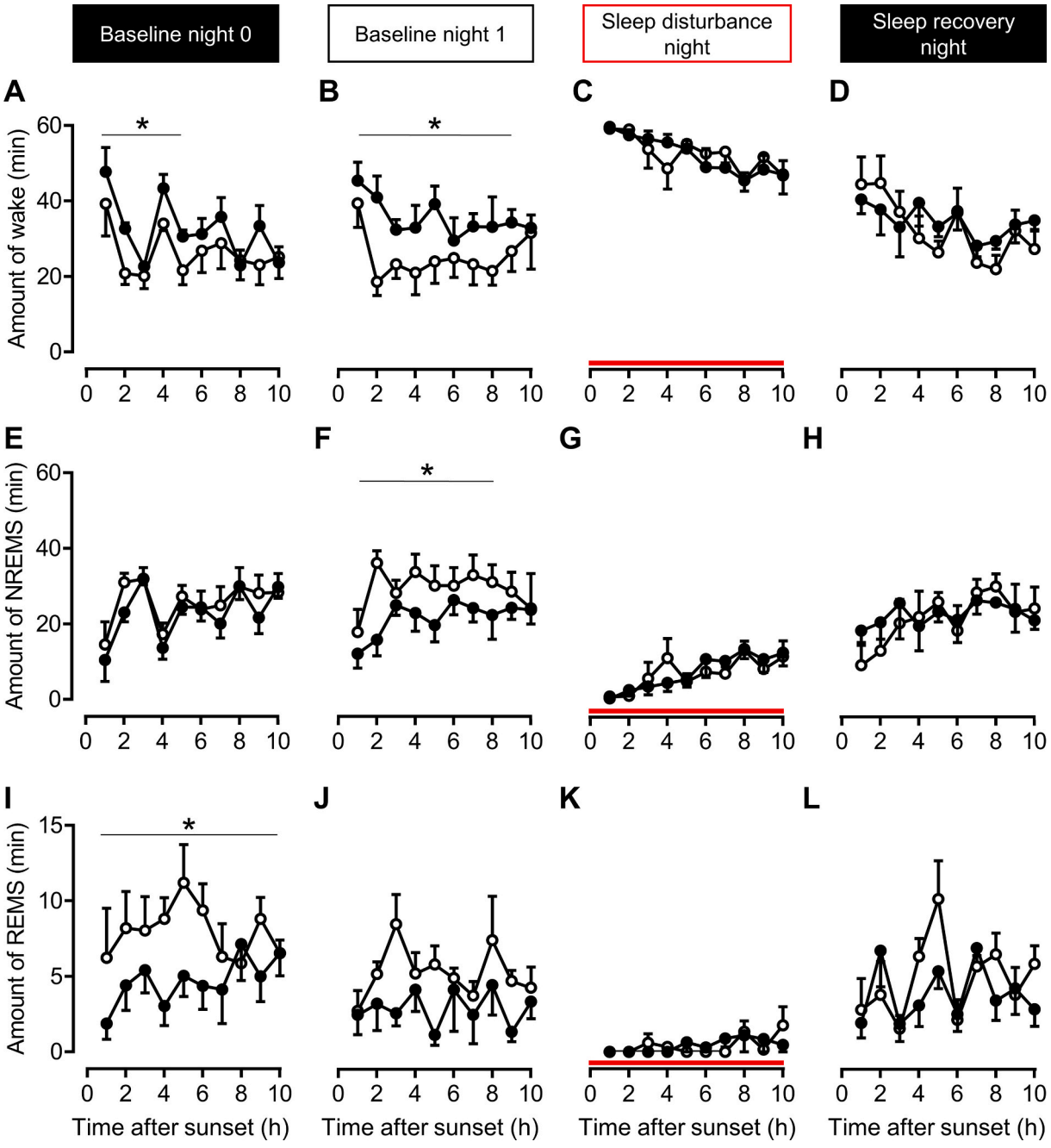

Fig. 2. Quantification of sleep-wake states during the night time in normal and transgenic sheep. The amount of wakefulness (A–D), non-rapid eye movement sleep (NREMS; E–H), and rapid eye movement sleep (REMS; I–L) is shown during light-off and light-on nights without disturbance (Baseline nights 0 and 1, respectively), sleep disturbance night and sleep recovery night in normal (○) and transgenic (●) sheep. Values are mean ± SEM. * = $P < 0.05$, two-way ANOVA (repeated factor: hours). The red bars show the period of overnight sleep disturbance.(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

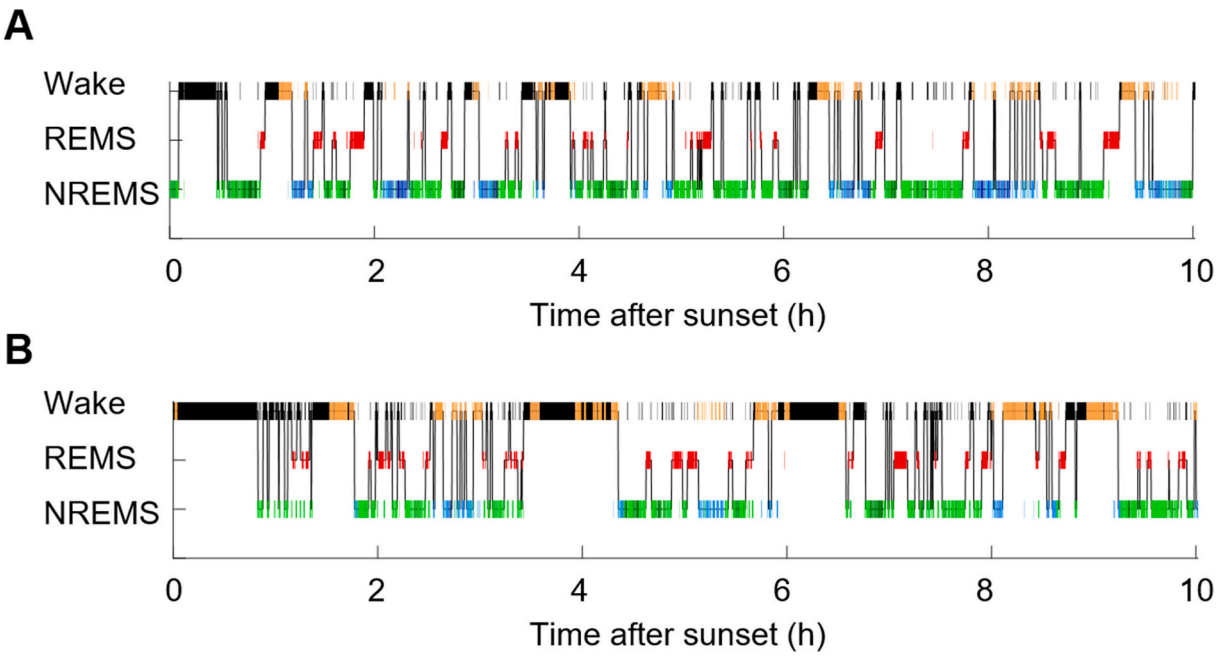

Fig. 3. Representative hypnograms showing the pattern of different states of the sleep-wake cycle during the light off baseline night in normal and transgenic sheep. The pattern of wakefulness, non-rapid eye movement sleep (NREMS) and rapid eye movement sleep (REMS) are shown for typical normal (A) and transgenic sheep (B). Transitions between vigilance states are marked by vertical lines when the ongoing vigilance state was interrupted for at least 1 min by a different vigilance state. Colour codes on the hypnograms: orange and black: wakefulness with and without rumination, respectively; red: REMS; blue and green: NREMS with and without rumination, respectively.(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

allowing a single handler to fit and remove the devices. Nevertheless, with the recording device and battery fitted, the extended chamber, had a higher profile than the screw-on cap, so the sheep were fitted with protective collars (Buster transparent collar, 25 cm, Kruuse, Denmark) to protect against damage to the device or to the sheep. During recordings the behaviour of the sheep was captured by stills taken every 10 s using GoPro cameras.

2.5. Off-line pre-processing of data

Data were collected in serial 1 h files in the native format for the MCS system. After recording, these files were translated to the format used in Spike2 software (Cambridge Electronic Design, Cambridge, UK) before further off-line processing. Pre-processing of the data included down-sampling to 250 Hz, and compiling the individual 1 h files to form a single continuous data file through the entire period of recording. The EEG data collected from the subdural electrodes were globally referenced by computing an average for all EEG channels, then subtracting this average reference from each EEG channel. EMG and EOG signals remained as sampled, and differential to the global reference. The data were exported as text files for further processing with SleepSign software (Kissei Comtec, Matsumoto, Japan) and Matlab.

2.6. Analysis of sleep-wake pattern and EEG spectra

Before further analysis, EEG, EOG and EMG signals were filtered digitally (EEG: band pass filtered at 0.5–30 Hz, EOG: band pass filtered at 0.5–10 Hz and EMG: high pass filtered at 35 Hz). Automatic detection of vigilance states was performed using Sleep Sign software in 10 s epochs on the A2L channel because this was a stable channel in all of the sheep. Automatic staging was followed by visual supervision by an expert who was blind to genotype of the sheep. For separating vigilance states, we used the criteria published for sheep (Perentos et al., 2015) and humans (Silber et al., 2007). Wakefulness (Wake) was defined by low voltage mixed fast frequency EEG (desynchronized EEG) accompanied by increased level of EMG activity and intensive but irregular ocular movements. Non-rapid eye movement (NREM) sleep was characterized by large voltage slow waves (synchronized EEG), decreased muscle tone compared to Wake, and sometimes slow rolling eye movements were present. During rapid eye movement (REM) sleep, desynchronized EEG activity was accompanied by characteristic ocular movements appearing in bursts. Muscle tone in REM sleep was reduced compared to NREM sleep but interrupted by transient muscle activity. For the characterization of sleep-wake pattern, the following parameters were calculated: time spent in each sleep-wake state; latency to persistent NREM sleep, comprising the time spent until the occurrence of the first 2 min of continuous NREM sleep; REM sleep onset latency was defined as the time spent until the emergence of the first 1 min of continuous REM sleep; sleep fragmentation was defined as the number of awakenings; inter-REM sleep period intervals; number and average length of sleep-wake bouts. Bouts of wake and NREM sleep were defined as at least a 2 min episode without interruptions of more than 1 min of any other vigilance state. Bouts of REM sleep were defined as at least a 1 min episode without interruptions of more than 0.5 min of any other vigilance state.

To compute EEG power (mV^2) content of each vigilance state, qEEG analysis was performed for all EEG channels using a code generated in Mathworks Matlab R2020a. Fast Fourier Transform (FFT) was performed applying 2-s Hanning windows with 1-s overlaps between windows which were then combined to obtain a mean spectra for each 10-s window. Epoch bins were of size 0.5 Hz between 0 and 120 Hz. Epochs containing extra-physiological (for example, amplifier saturation) or intra-physiological (for example, movement-related) artefacts, and epochs on the border of vigilance states were excluded from the qEEG analysis. The ratio of data excluded from the qEEG analysis was the following: BL N0: 21.04%; BL N1: 25.27%; BL D1: 28.68%; SD N: 15.07%; SR D: 17.85%; SR N: 14.15% (as an average ~20% of the data across all sheep and experimental time periods). In qEEG analysis the frequency bands we used were delta (0.5–4 Hz), theta (4–9 Hz), alpha (9–14 Hz), beta (14–35 Hz), low gamma (35–55 Hz) and high gamma (35–120 Hz; Nicol and Morton, 2020). Due to the large inter-individual differences in absolute EEG spectral power, EEG power values in each

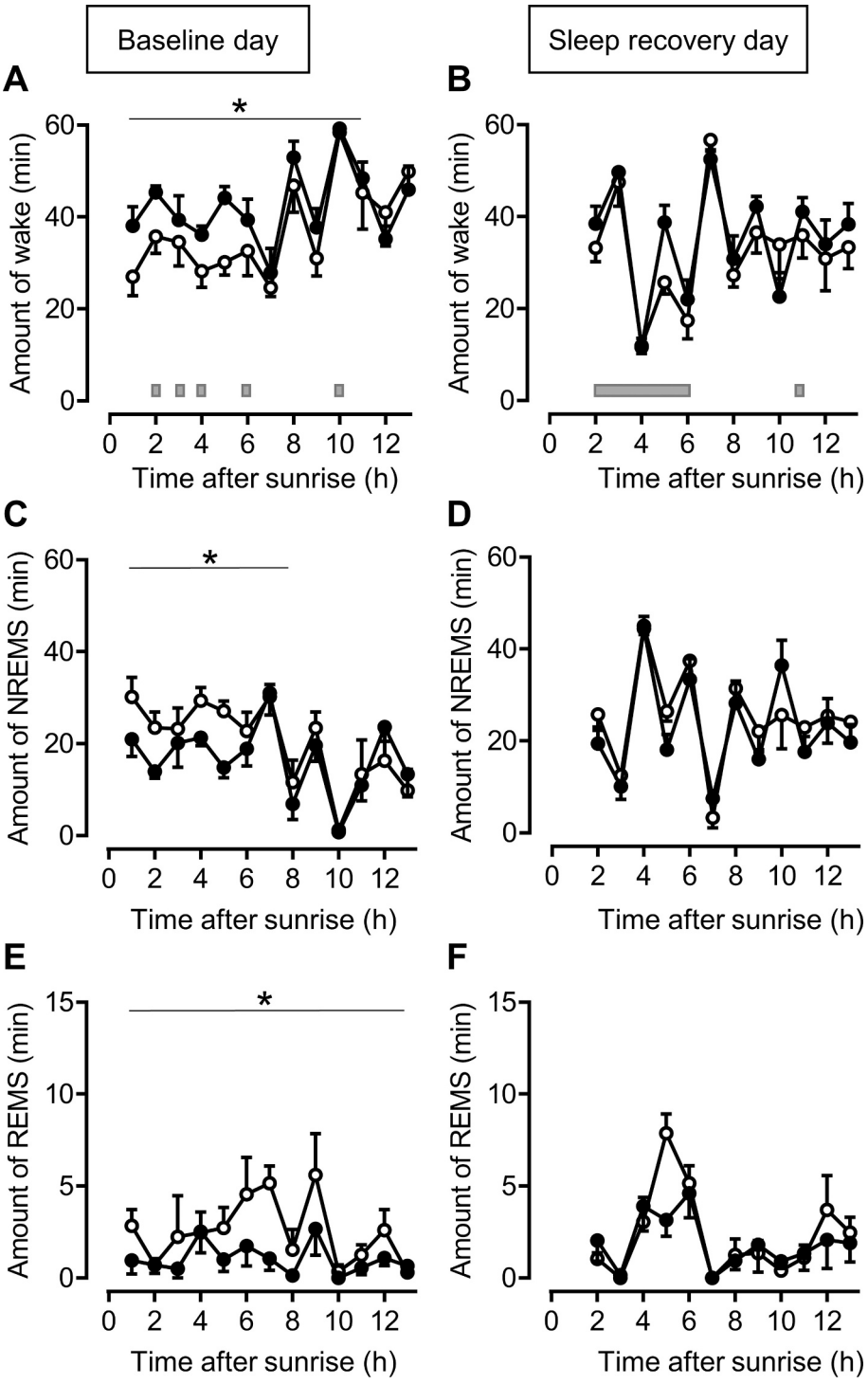

Fig. 4. Quantification of sleep-wake states during the day time in normal and transgenic sheep. The amount of wakefulness (A–B), non-rapid eye movement sleep (NREMS; C—D) and rapid eye movement sleep (REMS; E-F) is shown during baseline day and sleep recovery day, respectively following the sleep disturbance night in normal (○) and transgenic (●) sheep. Values are mean ± SEM. Due to extensive signal loss, the first hour of the sleep recovery day was omitted. * = $P < 0.05$, two-way ANOVA (repeated factor: hours). Grey rectangles (■) show the hours when normal behaviour of the sheep was disturbed by routine husbandry activities, such as feeding, watering, cleaning and checking.

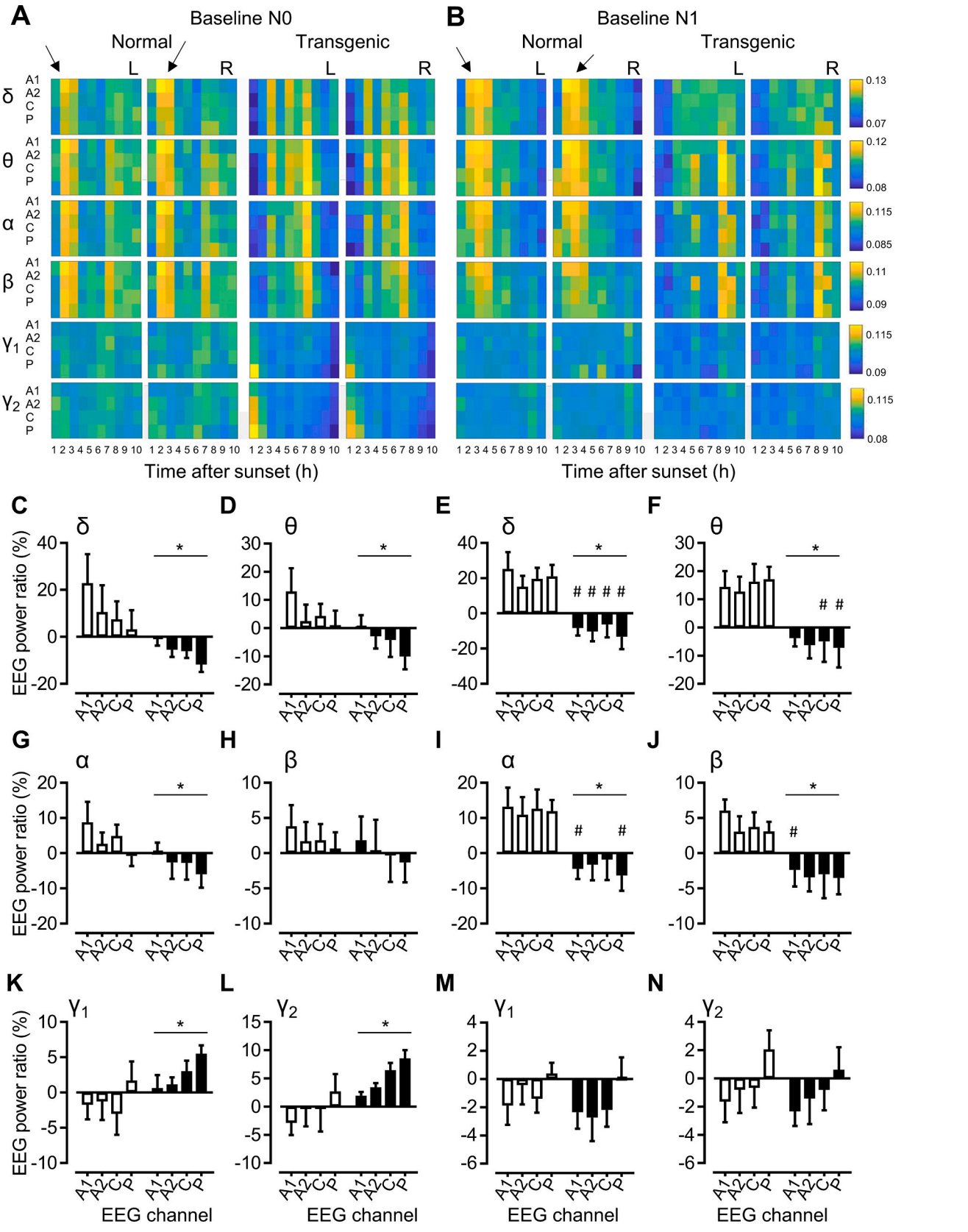

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Fig. 5. EEG powers during non-rapid eye movement sleep are different in transgenic and normal sheep under baseline conditions. Heatmaps in A and B show the relative distribution of EEG powers in different frequency ranges denoted by Greek letters (δ, delta [0.5–4 Hz]; θ, theta [4–9 Hz]; α, alpha [9–14 Hz]; β, beta [14–35 Hz]; γ1, low-gamma [35–55 Hz]; γ2, high-gamma [55–120 Hz]) during undisturbed (A) light-off and (B) light-on baseline nights (Baseline N0 and N1, respectively) in transgenic and normal sheep. Normalised EEG powers are presented as group means of normal and transgenic sheep for each hour and EEG channel (A1 = anterior 1; A2 = anterior 2; C = central; P = parietal) on the left (L) and right (R) hemispheres. Panels C-—N show ratios of changes of EEG power content of the first versus the second half of the night in normal (open columns) an\d transgenic (closed columns) sheep in the δ, θ, α, β, γ1 and γ2 frequency ranges during Baseline N0 (C, D, G, H, K, L) and Baseline N1 (E, F, I, J, M, N) measured on A1, A2, C and P channels. For clarity, data are shown only for right hemisphere. * = $P < 0.05$: transgenic versus normal sheep (two-way ANOVA), # = $P < 0.05$: transgenic versus normal sheep (Bonferroni post hoc test). The arrows on A and B point to the period when the distribution of EEG powers show striking difference between normal and transgenic sheep. Colour scales in each frequency range show the mean normalised EEG power values (per channel, per hour).

frequency bin were normalised on a per-subject and per-channel basis to the average power (0.5–120 Hz) of the BL N1 that was calculated by averaging (arithmetic mean) the EEG power values during wakefulness, NREM and REM sleep states in each hour. Then the hourly mean powers in wake, NREM and REM sleep states were averaged for the whole time period (1−10h) of BL N1 ($mean_W$, $mean_{NR}$ and $mean_R$, respectively) to avoid the potential biasing effect of the different spectral qEEG profile that is typically changing from hour-to-hour. Then, to represent all vigilance states with the same weight, we calculated the arithmetic mean of $mean_W$, $mean_{NR}$ and $mean_R$ ($MEAN_{W\text{-}NR\text{-}R}$) that number served the basis of normalisation for all further calculation. The $MEAN_{W\text{-}NR\text{-}R}$ (calculated for each channel of each sheep) represents the variability in the EEG power that exists between sheep (and channels). This variability is due to both biological differences and technical practicalities that are beyond our control (including depth of the EEG screws, recording conditions etc.). This normalisation method was used for data presented in Fig. 6A, B, 7C, F, G, J, K, N, O, and 9E, F, I, J, M, N, and Supplementary Fig. 2A–R. For the heatmaps (Figs. 5A,B, 7A, 8A and 9A) the EEG power values in each frequency bin, and in each hour were 'self-normalised' on a per-subject and per-channel basis to the total power of the same frequency range during the whole period.

Sheep are ruminants, and the rumination can be present during both NREM sleep and wakefulness (Perentos et al., 2015). We analysed NREM sleep without rumination in this study, since NREM sleep with rumination can be classified as a different vigilance state (Perentos et al., 2016, 2015). Nevertheless, because the ratio of these items vs. the total amount of NREM sleep was similar between the genotype groups (17.44% and 16.86%, respectively) and did not differ significantly within any of the investigated periods (two-way repeated measure ANOVA: $F(1,8) = 0.011$, $P = 0.9197$), it seems unlikely that excluding NREM sleep accompanied by rumination from the analysis biased the qEEG analysis of NREM sleep.

To exclude mains electrical interference, values between 49.5 and 51 Hz bins were discarded from the qEEG analysis.

2.7. Statistics

Graph Pad Prism 7 (GraphPad Software, Inc.) and Matlab 2020a (Mathworks, Inc. U·S) were used to create figures and to perform statistical analyses. All data are presented as mean ± standard error of mean (SEM). To calculate the hour-to-hour differences in the times spent in different vigilance states a two-way ANOVA (repeated: hours) was used. The difference between the genotypes in sleep-wake pattern parameters (number and mean duration of bouts, sleep latency times and inter-REM sleep interval) was analysed by two-way ANOVA. Due to extensive signal loss (>20 min) of the 1st h during SR D in case of seven sheep, the 1st h was omitted from the sleep-wake pattern analysis. Hours 4, 7 and 8 from normal sheep number 4, and hour 10 from transgenic sheep number 5 during SR N were excluded for similar reasons. In all other time bins at least 70% of the data were included (see details on the ratio of data excluded due to artefacts in the previous section of the Methods). To analyse the difference in the ratio of EEG powers for the first and second half of the night between the genotypes, hemispheres and EEG channels, a two-way ANOVA was used. The effect of genotype and EEG channels on normalised EEG powers in different frequency ranges was analysed using a two-way ANOVA with Bonferroni test for post hoc analysis. Results were considered statistically significant at $P < 0.05$.

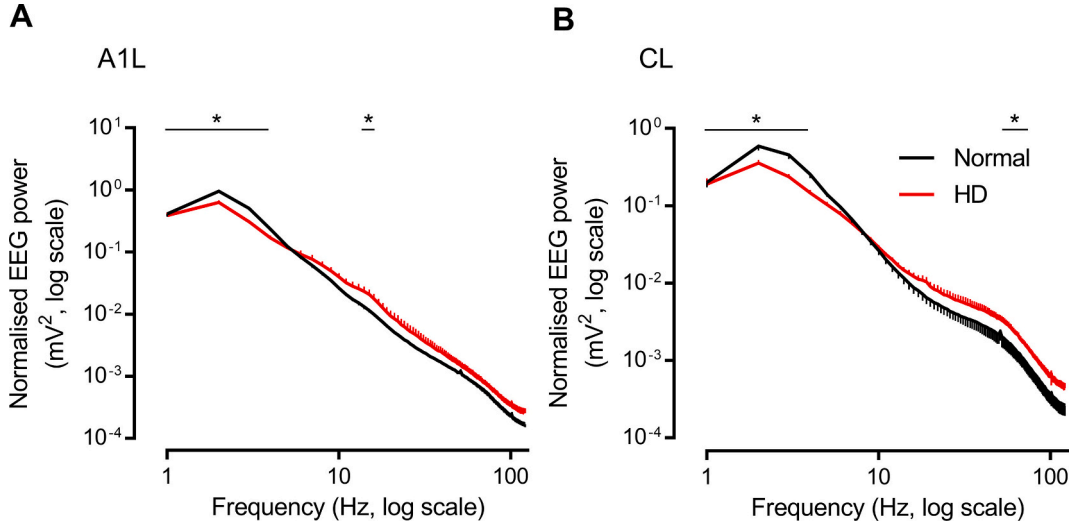

Fig. 6. Quantitative EEG power spectra during non-rapid eye movement sleep is different in normal and transgenic sheep during light-on baseline night. Normalised EEG powers in the 0–120 Hz frequency range was averaged in the first 3 h of the night on the anterior 1 left (A) and central left channels (B). Data are mean ± SEM EEG power per 1 Hz frequency bins. * = $P < 0.05$: transgenic versus normal sheep (two-way ANOVA and Bonferroni post hoc comparison).

Table 2
Statistical analysis of the normalised EEG powers during NREM sleep in normal and transgenic sheep during baseline, sleep disturbance and sleep recovery periods.

EEG Frequency range	Experimental period	Results of two-way ANOVA (Genotype)	
		F value	*P* value
Delta (0.5–4 Hz)	BL N0	**$F_{(1,64)} = 28.33$**	**< 0.0001**
	BL N1	**$F_{(1,64)} = 8.08$**	**0.0060**
	SD N	$F_{(1,64)} = 3.79$	0.0561
	SR D	**$F_{(1,64)} = 28.81$**	**<0.0001**
	SR N	**$F_{(1,64)} = 7.56$**	**0.0078**
Theta (4–9 Hz)	BL N0	**$F_{(1,64)} = 11.47$**	**0.0012**
	BL N1	$F_{(1,64)} = 0.07$	0.7992
	SD N	$F_{(1,63)} = 0.29$	0.5934
	SR D	$F_{(1,64)} = 3.60$	0.0623
	SR N	$F_{(1,64)} = 0.82$	0.3697
Alpha (9–14 Hz)	BL N0	$F_{(1,64)} = 1.78$	0.1875
	BL N1	**$F_{(1,64)} = 15.03$**	**0.0003**
	SD N	**$F_{(1,64)} = 8.61$**	**0.0046**
	SR D	$F_{(1,64)} = 1.53$	0.2201
	SR N	**$F_{(1,64)} = 6.39$**	**0.0140**
Beta (14–35 Hz)	BL N0	$F_{(1,64)} = 2.87$	0.0954
	BL N1	**$F_{(1,64)} = 12.13$**	**0.0009**
	SD N	**$F_{(1,64)} = 19.03$**	**<0.0001**
	SR D	$F_{(1,64)} = 3.59$	0.0627
	SR N	**$F_{(1,64)} = 5.10$**	**0.0273**
Low-gamma (35–55 Hz)	BL N0	$F_{(1,64)} = 1.61$	0.2094
	BL N1	**$F_{(1,64)} = 4.33$**	**0.0416**
	SD N	**$F_{(1,63)} = 4.42$**	**0.0395**
	SR D	$F_{(1,63)} = 1.07$	0.3040
	SR N	$F_{(1,64)} = 1.77$	0.1881
High-gamma Hz (55–120)	BL N0	**$F_{(1,63)} = 4.15$**	**0.0459**
	BL N1	**$F_{(1,63)} = 6.33$**	**0.0145**
	SD N	**$F_{(1,63)} = 7.21$**	**0.0092**
	SR D	$F_{(1,64)} = 2.94$	0.0912
	SR N	$F_{(1,64)} = 3.86$	0.0539

BL N0, light-off baseline night; BL N1, light-on baseline night 1; SD N, sleep disturbance night, SR D, sleep recovery day, SR N, sleep recovery night. Data are shown as mean ± SEM. Statistically significant results of two-way ANOVA between normal and transgenic sheep are shown in bold.

The polygonal plots were performed by adapting the code of Víctor Martínez-Cagigal (2020) [Polygonal Plot (https://www.mathworks.com/matlabcentral/fileexchange/62200-polygonal-plot), MATLAB Central File Exchange. Retrieved April 16, 2020].

2.8. Data availability

The original data included in this study are available on request to the corresponding author.

3. Results

3.1. Sleep-wake patterns in transgenic sheep differ from those of normal sheep

There was no difference in the total amount of NREM sleep and wakefulness between genotypes when the whole baseline nights were considered (Table 1). The distribution pattern of sleep and wake, however, differed markedly between normal and transgenic sheep on both nights (Fig. 2). During the first half of the lights-off baseline night (BL N0) the amount of wake in the transgenic sheep was greater than that of normal sheep (two-way ANOVA for hours 1–5, genotype effect: $F(1,8) = 5.87$, $P = 0.0417$). During the light-on baseline night (BL N1), the period of increased wakefulness in the transgenic sheep was more pronounced than it was during BL N0 (two-way ANOVA for hours 1–9, genotype effect: $F(1,8) = 6.10$, $P = 0.0387$) and there was now also a significantly less NREM sleep in transgenic compared to normal sheep (two-way ANOVA for hours for hours 1–8; genotype effect: $F(1,8) = 5.72$, $P = 0.0437$). There was significantly less REM sleep in the transgenic compared to normal sheep across the whole BL N0 (two-way ANOVA for hours 1–10, genotype effect: $F(1,8) = 6.84$, $P = 0.0309$), but not on BL N1. When the number of bouts of NREM and REM sleep were counted, there was a genotypic difference only in the number of REM sleep bouts (fewer in transgenic sheep during BL N0; Table 1 and Supplementary Table 1). Representative hypnograms of normal and transgenic sheep during BL N0 are shown in Fig. 3.

Sheep of both genotypes spent a considerable amount of time asleep during the baseline day (BL D), particularly in the first half (Fig. 4A, C and E). Although there was no difference in the total time of NREM sleep and wakefulness during BL D, transgenic sheep spent significantly more time awake than normal sheep (two-way ANOVA for hours 1–11; genotype effect: $F(1,8) = 5.75$, $P = 0.0433$), and less time in NREM sleep during BL D (two-way ANOVA for hours 1–8 h; genotype effect: $F(1,8) = 5.68$, $P = 0.0443$). REM sleep was also reduced in transgenic sheep across the entire daytime period (two-way ANOVA for hours 1–13, genotype effect: $F(1,8) = 9.62$, $P = 0.0146$).

3.2. Distributions of EEG powers during NREM sleep are altered in transgenic sheep under baseline conditions

Using qEEG, we examined the distribution of EEG powers, using classical frequency bandings (for details see Methods and Fig. 5 legend). Heat-maps of self-normalised EEG powers illustrate the differences between normal and transgenic sheep in the distribution of different powers across both baseline nights (Fig. 5A and B). In normal sheep, delta, theta, alpha and beta EEG powers were highest in the first few hours of the night (arrows, Fig. 5A and B). By contrast, in transgenic sheep these powers were spread more diffusely across the night on BL N0, and were predominant in the second half of the night during BL N1. The percent change in the EEG powers in the first (hours 1–5) versus the second (hours 6–10) half of the night (EEG power ratios) on both nights were used to quantify these changes (Fig. 5C–N). EEG power ratios showed significant differences between the genotype groups (two-way ANOVA genotype effect) in the distribution of delta (Fig. 5C; $F(1,32) = 10.30$, $P = 0.0030$), theta (Fig. 5D; $F(1,32) = 5.84$, $P = 0.0216$) and alpha (Fig. 5G; $F(1,32) = 5.57$, $P = 0.0245$) powers during BL N0, and in delta (Fig. 5E; $F(1,32) = 39.77$, $P < 0.0001$), theta (Fig. 5F; $F(1,32) = 27.42$, $P < 0.0001$), alpha (Fig. 5I; $F(1,32) = 24.18$, $P < 0.0001$) and beta (Fig. 5J; $F(1,32) = 20.17$, $P < 0.0001$) powers during BL N1. In normal sheep the pattern of gamma oscillations was the opposite to that of delta oscillations during BL N0, with higher power seen in the second half of the night (Fig. 5K and L). In the first half of BL N0, there was significantly more gamma power in transgenic than in normal sheep (Fig. 5K; γ_1: $F(1,32) = 6.05$, $P = 0.0195$; Fig. 5L; γ_2: $F(1,32) = 10.24$, $P = 0.0031$). During BL N1 the pattern of gamma power was similar in both genotypes (Fig. 5M and N).

Analysis of EEG powers during NREM sleep showed genotype-specific differences during both BL N0 and BL N1. Power-frequency plots are shown on Fig. 6.

Analysis of mean normalised EEG powers during the 1–10 h of both baseline nights (shown on polygon plots in Supplementary Fig. 2), showed less delta and more high-gamma power in transgenic compared to normal sheep on both baseline nights. Thus, these differences were not influenced by lighting conditions. The differences in other frequencies were light condition-dependent. A decrease in theta was seen

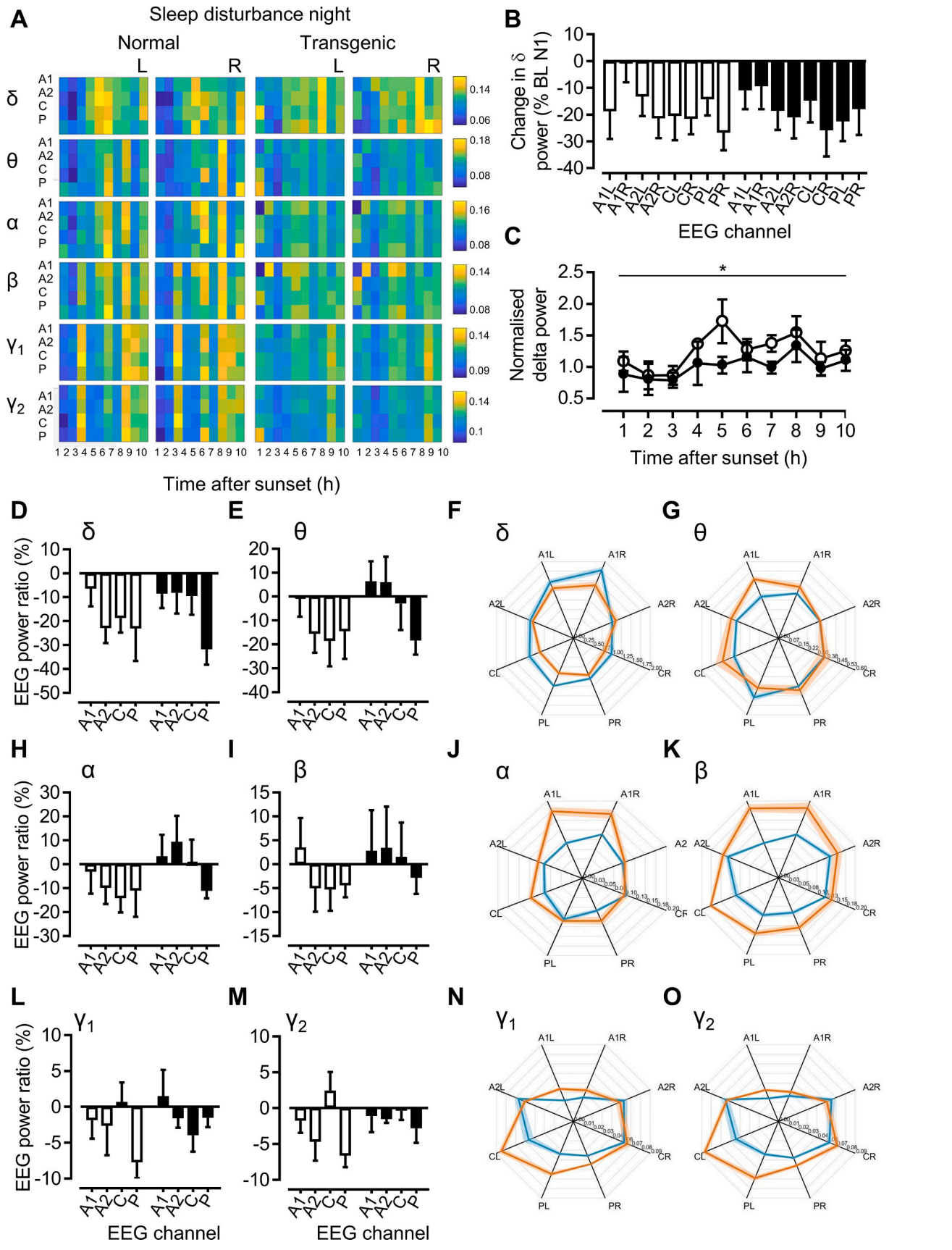

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Fig. 7. EEG powers during non-rapid eye movement sleep are different in transgenic and normal sheep during the sleep disturbance night. Heatmaps in A show the relative distribution of EEG powers in different frequency ranges denoted by Greek letters (for power frequency ranges see Fig. 4 legend) in normal and transgenic sheep. Normalised EEG powers are presented as group means of normal and transgenic sheep for each hour and EEG channel. Bar chart in B shows changes in the delta power relative to the light-on baseline night for each EEG channel in normal (open columns) and transgenic (closed columns) sheep. Graph in C shows relative delta power across the night (mean of all EEG channels). Bar charts (D, E, H, I, L, M) show ratios of changes of EEG power content of the first versus the second half of the night in normal (open columns) and transgenic (closed columns) sheep in the δ (D), θ (E), α (H), β (I), γ1 (L) and γ2 (M) frequency ranges. Polygon plots (F, G, J, K, N, O) show normalised EEG powers in the δ (F), θ (G), α (J), β (K), γ1 (N) and γ2 (O) frequency ranges in normal (blue) and transgenic (orange) sheep recorded on all channels on both hemispheres. In C and all bar charts and polygon plots, (A1 = anterior 1; A2 = anterior 2; C = central; P = parietal) on the left (L) and right (R) hemispheres. Data in C and all bar charts are shown as mean ± SEM. Data in polygon plots are mean (solid line) ± SEM (shading). * = $P < 0.05$: transgenic versus normal sheep (two-way ANOVA).(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

on BL N0 but not BL N1. An increase in alpha, beta and low-gamma was seen in transgenic sheep compared to normal on BL N1 but not BL N0. (For statistical analysis see Table 2 and Supplementary Table 2.)

3.3. Despite differences in qEEG, global response to sleep disturbance is similar in both genotypes

During the overnight sleep disturbance, sheep were able to fall asleep for short periods of times (normal: 7.07 ± 1.08; transgenic: 7.40 ± 0.98 min hourly). The distributions of self-normalised EEG powers during these NREM sleep episodes showed genotype-specific differences (Fig. 7A), although the amount of NREM sleep (two-way ANOVA for hours 1–10, genotype effect: $F(9,72) = 5.34$, $P < 0.0001$, Fig. 2G) and the number of NREM sleep episodes ($F(9,72) = 5.92$, $P < 0.0001$) increased consistently from hour-to-hour during SD N, in both genotypes. Compared to BL N1, the delta power decreased in both genotypes (Fig. 7B). While the normalised delta power (Fig. 7C) increased in both genotypes over the night, it was still significantly reduced in transgenic sheep compared to the normal sheep (two-way ANOVA for hours 1–10, genotype effect: $F(1,65) = 6.11$, $P = 0.0161$). Together these data suggest that the extent of the accumulating sleep pressure was different in the genotype groups. Interestingly, the differences in the EEG power ratios seen between the genotypes in the first versus the second half of the night during both baseline nights were not evident during SD N (Fig. 7D, E, H, I, L and M). This is attributable to a change in the distribution of EEG powers in normal sheep, where the predominance of delta, theta, alpha and beta (two-way ANOVA genotype effect: δ: $F(1,16) = 59.16$, $P < 0.0001$, θ: $F(1,16) = 26.38$, $P < 0.0001$, α: $F(1,16) = 25.0$, $P = 0.0001$ and β: $F(1,16) = 6.64$, $P = 0.0203$) powers during NREM sleep shifted from the first to the second half of the night. Notably, the distribution of EEG powers in sleep-disturbed normal sheep shifted to a pattern of distribution similar to that of transgenic sheep on the baseline nights.

Some genotype differences in the normalised EEG powers (Fig. 7F, G, J, K, N and O) remained significant during SD N, with an increase in alpha, beta, low-gamma and high-gamma powers seen in transgenic compared to normal sheep. We also detected local differences in the EEG powers: alpha (Fig. 7J) and beta (Fig. 7K) were increased in the A1 left channel, while high-gamma power (Fig. 7O) increased on the CL channel in transgenic sheep compared to the normal sheep ($P < 0.05$, Bonferroni post hoc test after significant genotype effect of two-way ANOVA, Table 2).

3.4. Sleep recovery in sheep occurs during the day and differs between transgenic and normal sheep

On the recovery day (SR D), the total amount of NREM, REM sleep and wake in normal and transgenic sheep was similar. Despite ongoing daytime husbandry activities, all sheep spent considerable amount of time asleep during the first half of SR D (Fig. 4D and F). Differences between normal and transgenic sheep persisted in the percent changes of delta, theta, alpha and beta powers during SR D relative to BL N1 (Fig. 8B–E). In normal but not in transgenic sheep a homeostatic increase in delta power relative to BL N1 was detected during NREM sleep (two-way ANOVA, genotype effect: $F(1,64) = 7.63$, $P = 0.0075$), presumably in response to the accumulated sleep pressure. This emerged predominantly during SR D (Fig. 8A and B) rather than during SR N (Fig. 9A and B). The change in delta was significantly higher in normal than in transgenic sheep (two-way ANOVA, genotype effect: $F(1,64) = 31.39$, $P < 0.0001$). Interestingly, there was a peak in the relative distribution of delta power in transgenic sheep during the early hours of SR D (Fig. 8A), resembling the distribution of delta power seen in normal sheep during the baseline nights.

During SR D the changes in the powers of theta, alpha and beta powers relative to BL N1 were significantly greater in normal than in the transgenic sheep (two-way ANOVA, genotype effect: θ: $F(1,63) = 14.58$, $P = 0.0003$, α: $F(1,62) = 31.89$, $P < 0.0001$, β: $F(1,64) = 15.39$, $P = 0.0002$; Fig. 8C–E). The increase in delta showed local differences by appearing predominantly on A1 channels in the normal but not in transgenic sheep (Fig. 8B). Of the normalised EEG powers (Supplementary Fig. 2), only delta power was decreased significantly in transgenic compared to normal sheep (two-way ANOVA, genotype effect: $F(1,64) = 28.81$, $P < 0.0001$).

From the qEEG heatmaps it appears that the pattern of distribution of powers did not revert completely on SR N to that seen on the baseline nights in sheep of either genotype. This was more apparent for the normal than transgenic sheep (Fig. 9A). Also, the EEG power ratios (Fig. 9C, D, G, H and K) did not revert back to the baseline pattern, apart from the high-gamma power (Fig. 9L) that was significantly increased in the second half of the night in transgenic compared to normal sheep (two-way ANOVA, genotype effect: $F(1,32) = 6.88$, $P = 0.0133$).

The normalised EEG powers showed similar genotype differences (Fig. 9E, F, I, J, M and N) to those detected during BL N0, with a decrease in delta, and an increase in alpha and beta powers, although the increased gamma powers in transgenic sheep did not emerge during SR N. Detailed statistical analyses of the genotype- and EEG channel- specific differences in the EEG powers are shown in Table 2 and Supplementary Table 2.

4. Discussion

This is the first study providing a detailed comparison of sleep-wake patterns and distribution of EEG spectra in normal and transgenic sheep carrying the mutation that causes HD. We found a number of important differences between genotypes. First, transgenic sheep spent more time awake during the first part of the light-off night than normal sheep, suggesting their response to sleep pressure is abnormal. This effect was exaggerated when the lights were left on. Second, a detailed qEEG analysis revealed marked genotype-specific differences in the pattern of distribution of EEG powers across the night. The distribution of delta, theta, alpha, beta and gamma powers was skewed in transgenic sheep compared to normal sheep. Furthermore, while the response to

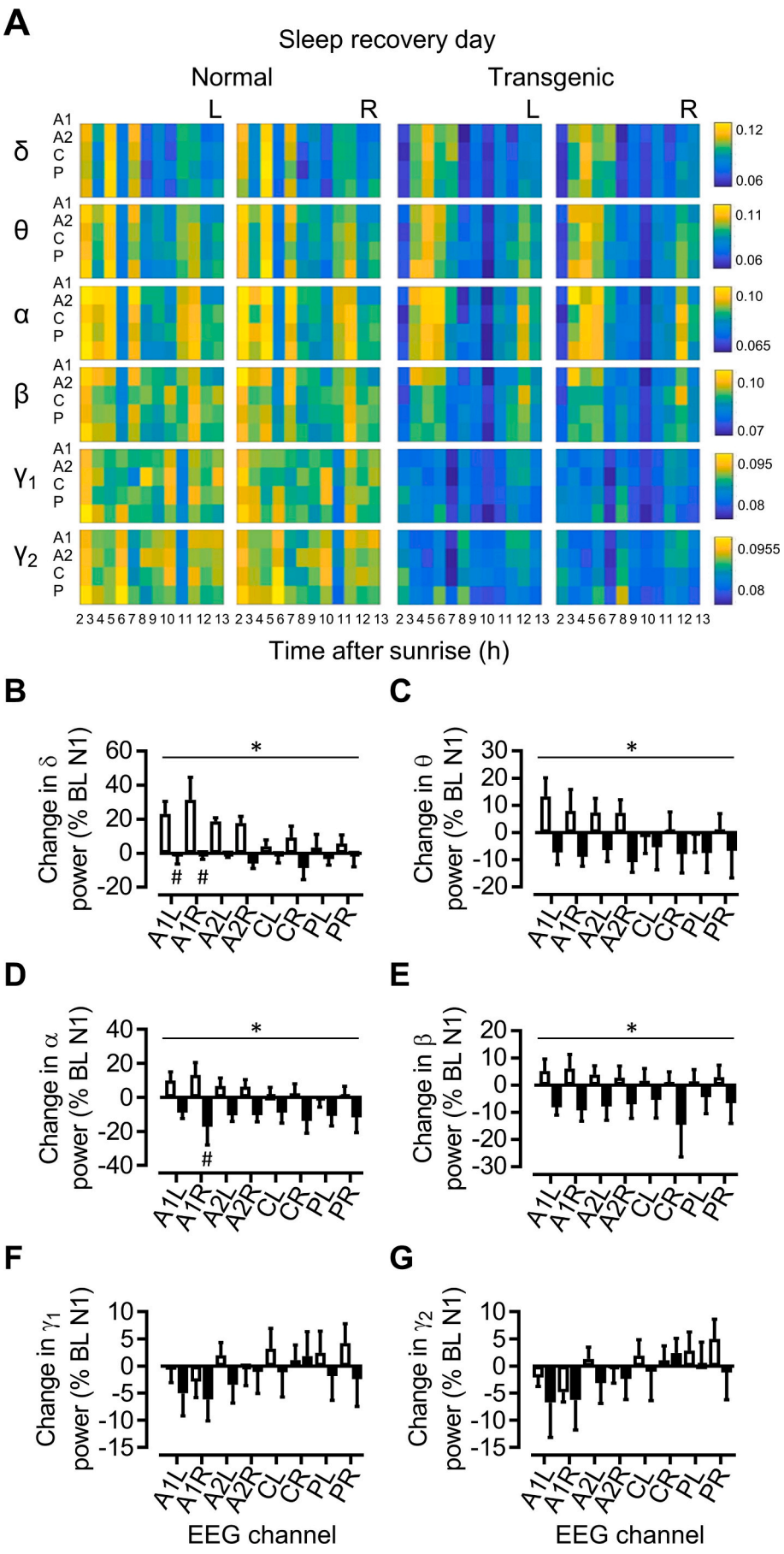

Fig. 8. EEG powers during non-rapid eye movement sleep are different in transgenic and normal sheep during the sleep recovery day. Heatmaps in A show the relative distribution of EEG powers in different frequency ranges denoted by Greek letters (for power frequency ranges see Fig. 4 legend) in normal and transgenic sheep. Normalised EEG powers are presented as group means of normal and transgenic sheep for each hour and EEG channel. Bar charts (B-G) show changes in EEG powers relative to the light-on baseline night in the δ (B), θ (C), α (D), β (E), γ1 (F) and γ2 (G) frequency ranges for each EEG channels in normal (open columns) and transgenic (closed columns) sheep. In all bar charts, (A1 = anterior 1; A2 = anterior 2; C = central; P = parietal) on the left (L) and right (R) hemispheres. Data in all bar charts are shown as mean ± SEM. * = $P < 0.05$: transgenic versus normal sheep (two-way ANOVA). # = $P < 0.05$: transgenic versus normal sheep (Bonferroni post hoc comparison).

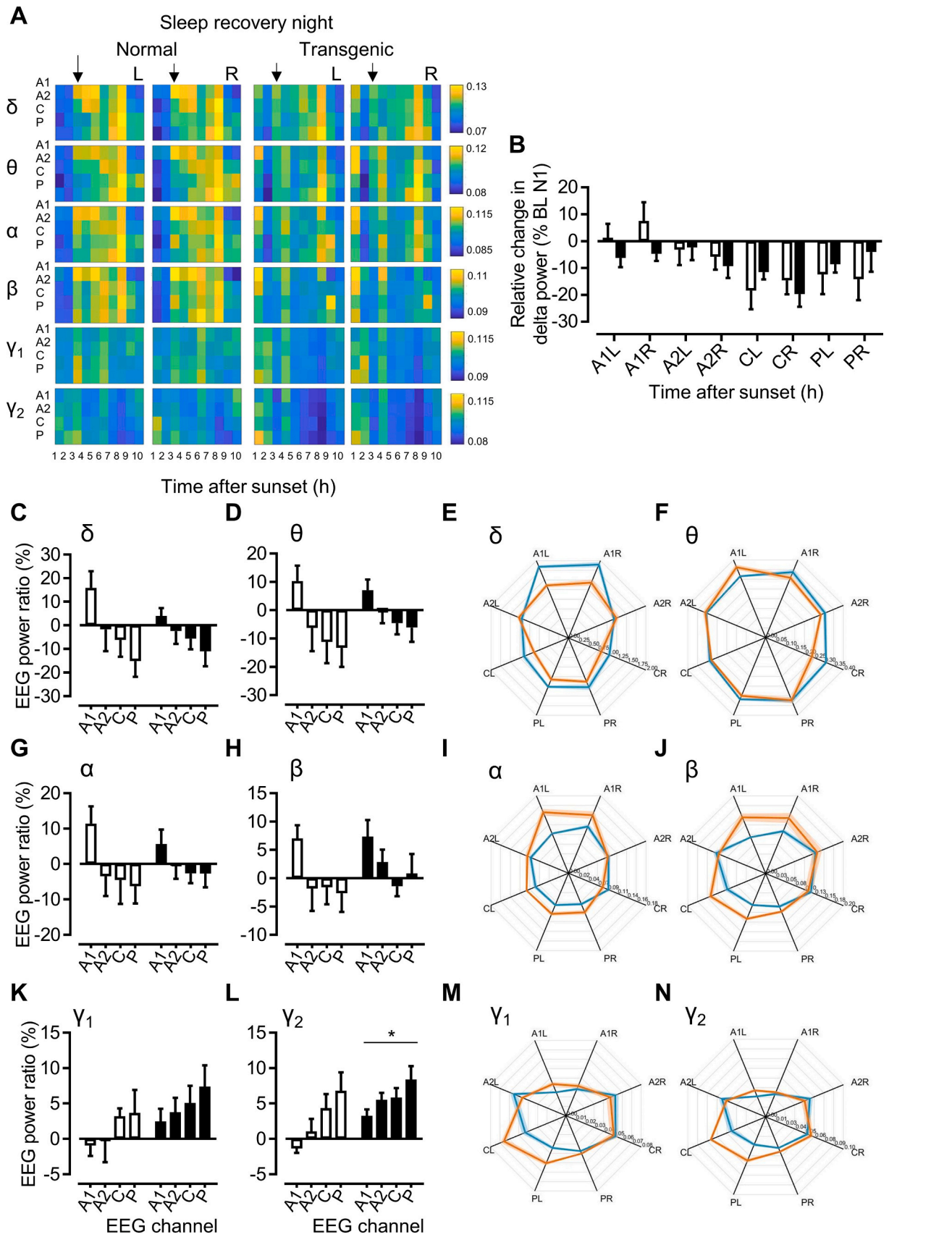

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Fig. 9. EEG powers during non-rapid eye movement sleep are different in transgenic and normal sheep during the sleep recovery night. Heatmaps in A show the relative distribution of EEG powers in different frequency ranges denoted by Greek letters (for power frequency ranges see Fig. 4 legend) in normal and transgenic sheep. Normalised EEG powers are presented as group means of normal and transgenic sheep for each hour and EEG channel (A1 = anterior 1; A2 = anterior 2; C = central; P = parietal) on the left (L) and right (R) hemispheres. Bar chart in B shows changes in the delta power relative to the light-on baseline night for each EEG channels in normal (open columns) and transgenic (closed columns) sheep. Bar charts (C, D, G, H, K, L) show the ratio of changes in the EEG power content of the first versus the second half of the night in normal (open columns) and transgenic (closed columns) in the δ (C), θ (D), α (G), β (H), γ1 (K) and γ2 (L) frequency ranges. Polygon plots (E, F, I, J, M, N) show normalised EEG powers in the δ (E), θ (F), α (I), β (J), γ1 (M) and γ2 (N) frequency ranges in normal (blue) and transgenic (orange) sheep recorded on all channels on both hemispheres. Data in all bar charts are shown as mean ± SEM. Data in polygon plots are mean (solid line) ± SEM (shading).(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

overnight sleep disturbance appeared overtly similar in both genotypes, the distribution of EEG rhythms seen in normal sheep shifted to an abnormal pattern of distribution similar to that seen on baseline nights in transgenic sheep. Additionally during the recovery period, while the accumulated sleep pressure induced a homeostatic 'rebound' increase in delta power in normal sheep, this was not seen in transgenic sheep. Thus, undisturbed, transgenic sheep exhibit an abnormal EEG profile of brain activity that is characteristic of disturbed sleep. We speculate that transgenic sheep under baseline conditions are in a state that resemble a chronic sleep-deprived state. Furthermore, transgenic sheep show an abnormal homeostatic response to sleep need. Together, these data are consistent with reports of abnormal sleep EEG in HD patients. It seems likely that some of the sleep deficits seen in presymptomatic HD patients arise because the mechanisms modulating response to sleep need are abnormal.

Our findings are consistent with a large body of evidence that shows disturbances of the circadian rhythmicity and sleep-wake patterns in HD patients. These abnormalities include a decrease in total sleep time, increased sleep fragmentation, insomnia, increased sleep onset latency, REM sleep disorders (Arnulf et al., 2008; Diago et al., 2018; Goodman et al., 2011; Hansotia et al., 1985; Lazar et al., 2015; Piano et al., 2017b; Wiegand et al., 1991) and often correlate with depression and deterioration of cognitive function (Aziz et al., 2010). We found that transgenic sheep spent more time awake in the first few hours of the night (when the sleep pressure is the typically highest) than normal sheep. This is consistent with insomnia reported in HD. Furthermore, the amount of REM sleep was decreased during the whole BL N0 and BL D, in line with studies reporting REM sleep abnormalities in HD patients (Arnulf et al., 2008; Wiegand et al., 1991). It is notable that sleep disruption and daytime sleepiness are not reported by many HD patients even when they have clear sleep-wake abnormalities. This suggests that either they lack awareness into their sleep abnormalities (Goodman et al., 2011), or they may not feel daytime sleepiness because the mechanisms regulating homeostatic sleep pressure are different in HD and the brain does not 'recognise' the sleep-deprived state.

Given that the qEEG and sleep patterns between normal and transgenic sheep were very different but their sleep behaviour (shown by the total amounts of sleep) were similar, it is possible that in the early stages of HD (whether in sheep or patients), compensatory changes 'mask' some features of the disturbed sleep-wake cycle so that they are not reflected in abnormal sleep-wake behaviour. One example of a possible compensation is via nocturnal melatonin secretion, that is increased in transgenic sheep aged 5–7 years (Morton et al., 2020). Melatonin is secreted at night by the pineal gland under the control of the suprachiasmatic nucleus of the hypothalamus, and known to possess sleep-promoting (Hughes and Badia, 1997) and neuroprotective functions (Brzezinski, 1997). Elevated melatonin levels in transgenic sheep may have preserved the amount of NREM sleep during BL N0, but not during BL N1 when the lights were on (since light inhibits the production of melatonin). No studies have been done examining melatonin levels under lights-on conditions, in either HD patients or transgenic sheep. It would be particularly interesting to investigate this, since social jetlag caused by the extended periods of artificial light at night is disruptive to sleep and has been linked to depression in normal people (Paksarian et al., 2020). Extended periods of artificial light (that is common in modern society) may be more deleterious to HD patients if their propensity to sleep is already disturbed as a consequence of their disease.

According to the theory of homeostatic regulation of sleep, the longer an animal stays awake, the longer and/or the deeper the following sleep will be (Borbely, 1982). The most widely accepted index of NREM sleep homeostasis is delta activity, that peaks shortly after sleep onset, and decreases gradually with the time spent asleep (Achermann and Borbély, 2003). As expected, in the normal sheep delta power during NREM sleep was dominant in the first half of both baseline nights. During sleep disturbance in normal sheep the dominance of delta shifted from the first to the second half of the night, reflecting the accumulating sleep pressure. However, in transgenic sheep the pattern of delta power distribution was abnormal, even during baseline conditions (with no delta predominance in the first half of the night) and there was no change to this pattern after sleep disruption. Also, in response to the accumulating sleep pressure, the homeostatic increase in delta power did not emerge in the transgenic sheep. Together these findings suggest that the homeostatic regulation of NREM sleep is disrupted in transgenic sheep. The generation of delta waves depends on the synchronous firing of large groups of cortical neurons coordinated by an underlying slow oscillation, the fundamental cellular phenomenon of NREM sleep (Steriade et al., 1993). Alterations of NREM sleep pattern, decrease in delta power and blunted homeostatic sleep rebound to sleep deprivation have also been reported in R6/2 mice (Fisher et al., 2013; Kantor et al., 2013; Vas et al., 2020). Disruption of delta waves are consistent with cortical brain pathology that is a key early event in HD mouse models (Burgold et al., 2019) and patients (Kassubek et al., 2004; Rosas et al., 2008).

One of the strengths of using EEG as a physiological readout of brain function is that regional changes in EEG power can be assessed. We observed a frontal dominance of delta power during the baseline nights in the normal sheep. This frontal predominance was further increased during recovery sleep and is consistent with human studies reporting frontal predominance of slow waves during baseline night (Roth et al., 1999; Werth et al., 1996) and after sleep deprivation (Finelli et al., 2000). It is thought that frontal predominance of delta power is the consequence of stronger cortico-cortical connections, leading to stronger neuronal synchronization in the frontal part of the brain (Finelli et al., 2000; Werth et al., 1996), which is crucial in neuronal plasticity (Tononi and Cirelli, 2006). Transgenic sheep, however, did not show the frontal predominance of delta power. It may be that changes in connectivity in the brain of HD patients are reflected in a lack of frontal dominance (Painold et al., 2012; Thiruvady et al., 2007). Following sleep recovery, genotype-related differences in the theta and gamma powers disappeared, although they were still present in low frequency ranges. We speculate that sleep deprivation may induce changes that abolished these differences, at least in the short term. Importantly, an imposed period of overnight sleep deprivation is known to evoke acute antidepressant effect of in humans, presumably by inducing beneficial changes in synaptic plasticity (Hines et al., 2013; Wolf et al., 2016).

A wide range of qEEG changes have been reported in HD patients (reviewed in Leuchter et al., 2017). During NREM sleep abnormal qEEG alterations have been found in the theta, alpha, beta and gamma frequency ranges (Lazar et al., 2015; Piano et al., 2017b). Similar changes have also been shown in mouse models of HD (Fisher et al., 2013; Kantor et al., 2013; Lebreton et al., 2015). The attenuation of differences in

gamma we saw in transgenic sheep is an intriguing finding given that increased gamma oscillation is considered a progressive pathological phenomena in patients (Lazar et al., 2015) and mouse models of HD (Fisher et al., 2013; Kantor et al., 2013). We showed recently that the increased gamma power seen in R6/2 mice was reduced by chronic treatment with modafinil, a wake-promoting compound (Vas et al., 2020). Given that qEEG changes are likely to reflect cortical and subcortical neuropathology of the brain, they could provide a basis for a sensitive readout of the disease progression in HD.

There are some obvious limitations of our study. First, for the qEEG analysis we focused on the NREM sleep. As alterations of REM sleep have been observed in HD we suggest that future studies that include investigation of qEEG during REM sleep would be useful. Second, our sleep deprivation protocol produced sleep disturbance rather than total sleep deprivation. Furthermore we did not disrupt sheep activity on the day following sleep disturbance, which meant they could sleep during the day. With hindsight a sleep-disruption protocol that included disrupting sleep that occurred in the recovery day would have been useful. Finally, it is likely that the way sheep sleep in laboratory settings will be different from that in the wild. Because under controlled, safe, familiar and uneventful conditions sheep typically spend significant amount of time sleeping during the day, this may explain the lower sleep pressure at the beginning of the night. On the other hand, sleeping pattern of the sheep may also be modulated by their innate behaviour, for example the threat from predators. It would be interesting to do a future study under more naturalistic conditions, for example in a field outdoors where increased vigilance would be expected.

Despite considerable effort in the field, there are few reliable biomarkers of HD progression. It is, however, becoming increasingly clear that a good understanding of early pathological changes as they emerge in HD will be critical for assessing efficacy of therapeutics, particularly given the drive to devise early interventions. EEG provides an affordable, non-invasive, and objective measure of the cortical brain activity that is ideally positioned to provide a better understanding of the sleep-wake abnormalities. There is a growing realisation that sleep is important not only for rest but also for maintenance of synapses and clearing of neurotoxic metabolites. Thus even if an individual is not aware of having a sleep abnormality, their brain health will suffer, because the normal function of sleep cannot be carried out. A better understanding of how an HD patient responds to accumulating sleep pressure and recovers from sleep deprivation may give insight into changes in functional brain circuitry that appear before neurodegeneration. A deeper awareness of sleep abnormalities is also a key factor in developing therapeutic interventions that could improve sleep quality in HD patients.

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Authors' contributions

Conceptualization AJM, AUN; Data curation AJM, AUN, SV, LK; Formal analysis AJM, AUN, SV, JM; Funding acquisition AJM; Investigation AJM, AUN; Methodology AJM, AUN, SV; Project administration AJM, AUN; Software LK, SV; Supervision AJM; Writing - original draft AJM, SV; Writing - review & editing All authors..

Declaration of competing interest

The authors report no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nbd.2021.105367.

References

Achermann, P., Borbély, A.A., 2003. Mathematical models of sleep regulation. Front. Biosci. 8, s683–s693. https://doi.org/10.2741/1064.

Anderson, K.N., Bradley, A.J., 2013. Sleep disturbance in mental health problemsand neurodegenerative disease. Nat. Sci. Sleep 5, 61–75. https://doi.org/10.2147/NSS.S34842.

Arnulf, I., Nielsen, J., Lohmann, E., Schieffer, J., Wild, E., Jennum, P., Konofal, E., Walker, M., Oudiette, D., Tabrizi, S., Durr, A., 2008. Rapid eye movement sleep disturbances in Huntington disease. Arch. Neurol. 65, 482–488. https://doi.org/10.1001/archneur.65.4.482.

Aziz, N.A., Anguelova, G.V., Marinus, J., Lammers, G.J., Roos, R.A.C., 2010. Sleep and circadian rhythm alterations correlate with depression and cognitive impairment in Huntington's disease. Parkinsonism Relat. Disord. 16, 345–350. https://doi.org/10.1016/j.parkreldis.2010.02.009.

Bates, G., Tabrizi, S., Lesley, J., 2014. Huntington's Disease, 4th ed. Oxford University Press - Oxford Monographs on Medical Genetics. https://doi.org/10.1093/med/9780199929146.001.0001.

Borbely, A.A., 1982. A two process model of sleep regulation. Hum. Neurobiol. 1, 195–204.

Brzezinski, A., 1997. Melatonin in humans. N. Engl. J. Med. 336, 186–195. https://doi.org/10.1056/NEJM199701163360306.

Burgold, J., Schulz-Trieglaff, E.K., Voelkl, K., Gutiérrez-Ángel, S., Bader, J.M., Hosp, F., Mann, M., Arzberger, T., Klein, R., Liebscher, S., Dudanova, I., 2019. Cortical circuit alterations precede motor impairments in Huntington's disease mice. Sci. Rep. 9, 6634. https://doi.org/10.1038/s41598-019-43024-w.

Campbell, S.S., Tobler, I., 1984. Animal sleep: a review of sleep duration across phylogeny. Neurosci. Biobehav. Rev. 8, 269–300. https://doi.org/10.1016/0149-7634(84)90054-X.

Cuturic, M., Abramson, R.K., Vallini, D., Frank, E.M., Shamsnia, M., 2009. Sleep patterns in patients with Huntington's disease and their unaffected first-degree relatives: A brief report. Behav. Sleep Med. https://doi.org/10.1080/15402000903190215.

Diago, E.B., Martinez-Horta, S., Lasaosa, S.S., Alebesque, A.V., Perez-Perez, J., Kulisevsky, J., Del Val, J.L., 2018. Circadian rhythm, cognition, and mood disorders in huntington's disease. J. Huntingtons. Dis. 7, 193–198. https://doi.org/10.3233/JHD-180291.

Finelli, L.A., Baumann, H., Borbély, A.A., Achermann, P., 2000. Dual electroencephalogram markers of human sleep homeostasis: correlation between theta activity in waking and slow-wave activity in sleep. Neuroscience 101, 523–529. https://doi.org/10.1016/S0306-4522(00)00409-7.

Fisher, S.P., Black, S.W., Schwartz, M.D., Wilk, A.J., Chen, T.M., Lincoln, W.U., Liu, H. W., Kilduff, T.S., Morairty, S.R., 2013. Longitudinal analysis of the electroencephalogram and sleep phenotype in the R6/2 mouse model of Huntington's disease. Brain 136, 2159–2172. https://doi.org/10.1093/brain/awt132.

Goodman, A.O.G., Barker, R.A., 2010. How vital is sleep in Huntington's disease? J. Neurol. 257, 882–897. https://doi.org/10.1007/s00415-010-5517-4.

Goodman, A.O.G., Rogers, L., Pilsworth, S., McAllister, C.J., Shneerson, J.M., Morton, A. J., Barker, R.A., 2011. Asymptomatic sleep abnormalities are a common early feature in patients with Huntington's disease. Curr. Neurol. Neurosci. Rep. 11, 211–217. https://doi.org/10.1007/s11910-010-0163-x.

Handley, R.R., Reid, S.J., Patassini, S., Rudiger, S.R., Obolonkin, V., McLaughlan, C.J., Jacobsen, J.C., Gusella, J.F., MacDonald, M.E., Waldvogel, H.J., Bawden, C.S., Faull, R.L.M., Snell, R.G., 2016. Metabolic disruption identified in the Huntington's disease transgenic sheep model. Sci. Rep. 11, 20681. https://doi.org/10.1038/srep20681.

Handley, R.R., Reid, S.J., Brauning, R., MacLean, P., Mears, E.R., Fourie, I., Patassini, S., Cooper, G.J.S., Rudiger, S.R., McLaughlan, C.J., Verma, P.J., Gusella, J.F., MacDonald, M.E., Waldvogel, H.J., Bawden, C.S., Faull, R.L.M., Snell, R.G., 2017. Brain urea increase is an early Huntington's disease pathogenic event observed in a prodromal transgenic sheep model and HD cases. Proc. Natl. Acad. Sci. U. S. A. 114, E11293–E11302. https://doi.org/10.1073/pnas.1711243115.

Hansotia, P., Wall, R., Berendes, J., 1985. Sleep disturbances and severity of Huntington's disease. Neurology 35, 1672–1674. https://doi.org/10.1212/wnl.35.11.1672.

Hauglund, N.L., Pavan, C., Nedergaard, M., 2020. Cleaning the sleeping brain – the potential restorative function of the glymphatic system. Curr. Opin. Physiol. 15, 1–6. https://doi.org/10.1016/j.cophys.2019.10.020.

Hines, D.J., Schmitt, L.I., Hines, R.M., Moss, S.J., Haydon, P.G., 2013. Antidepressant effects of sleep deprivation require astrocyte-dependent adenosine mediated signaling. Transl. Psychiatry 3, e212. https://doi.org/10.1038/tp.2012.136.

Hsieh, K.-C., Robinson, E.L., Fuller, C.A., 2008. Sleep architecture in unrestrained rhesus monkeys (*Macaca mulatta*) synchronized to 24-hour light-dark cycles. Sleep 31, 1239–1250.

Hughes, R.J., Badia, P., 1997. Sleep-promoting and hypothermic effects of daytime melatonin administration in humans. Sleep 20, 124–131. https://doi.org/10.1093/sleep/20.2.124.

Jacobsen, J.C., Bawden, C.S., Rudiger, S.R., McLaughlan, C.J., Reid, S.J., Waldvogel, H. J., MacDonald, M.E., Gusella, J.F., Walker, S.K., Kelly, J.M., Webb, G.C., Faull, R.L. M., Rees, M.I., Snell, R.G., 2010. An ovine transgenic Huntington's disease model. Hum. Mol. Genet. 19, 1873–1882. https://doi.org/10.1093/hmg/ddq063.

Kantor, S., Szabo, L., Varga, J., Cuesta, M., Morton, A.J., 2013. Progressive sleep and electroencephalogram changes in mice carrying the Huntington's disease mutation. Brain 136, 2147–2158. https://doi.org/10.1093/brain/awt128.

Kassubek, J., Landwehrmeyer, G.B., Ecker, D., Juengling, F.D., Muche, R., Schuller, S., Weindl, A., Peinemann, A., 2004. Global cerebral atrophy in early stages of Huntington's disease: quantitative MRI study. Neuroreport 15, 363–365. https://doi.org/10.1097/00001756-200402090-00030.

Killgore, W.D.S., 2010. Effects of sleep deprivation on cognition. Prog. Brain Res. 185, 105–129. https://doi.org/10.1016/B978-0-444-53702-7.00007-5.

Lazar, A.S., Panin, F., Goodman, A.O.G., Lazic, S.E., Lazar, Z.I., Mason, S.L., Rogers, L., Murgatroyd, P.R., Watson, L.P.E., Singh, P., Borowsky, B., Shneerson, J.M., Barker, R.A., 2015. Sleep deficits but no metabolic deficits in premanifest Huntington's disease. Ann. Neurol. 78, 630–648. https://doi.org/10.1002/ana.24495.

Lebreton, F., Cayzac, S., Pietropaolo, S., Jeantet, Y., Cho, Y.H., 2015. Sleep physiology alterations precede plethoric phenotypic changes in R6/1 Huntington's disease mice. PLoS One 10, e0126972. https://doi.org/10.1371/journal.pone.0126972.

Leuchter, M.K., Donzis, E.J., Cepeda, C., Hunter, A.M., Estrada-Sánchez, A.M., Cook, I.A., Levine, M.S., Leuchter, A.F., 2017. Quantitative electroencephalographic biomarkers in preclinical and human studies of Huntington's disease: are they fit-for-purpose for treatment development? Front. Neurol. 8, 91. https://doi.org/10.3389/fneur.2017.00091.

MacDonald, M.E., Ambrose, C.M., Duyao, M.P., Myers, R.H., Lin, C., Srinidhi, L., Barnes, G., Taylor, S.A., James, M., Groot, N., MacFarlane, H., Jenkins, B., Anderson, M.A., Wexler, N.S., Gusella, J.F., Bates, G.P., Baxendale, S., Hummerich, H., Kirby, S., North, M., Youngman, S., Mott, R., Zehetner, G., Sedlacek, Z., Poustka, A., Frischauf, A.M., Lehrach, H., Buckler, A.J., Church, D., Doucette-Stamm, L., O'Donovan, M.C., Riba-Ramirez, L., Shah, M., Stanton, V.P., Strobel, S.A., Draths, K.M., Wales, J.L., Dervan, P., Housman, D.E., Altherr, M., Shiang, R., Thompson, L., Fielder, T., Wasmuth, J.J., Tagle, D., Valdes, J., Elmer, L., Allard, M., Castilla, L., Swaroop, M., Blanchard, K., Collins, F.S., Snell, R., Holloway, T., Gillespie, K., Datson, N., Shaw, D., Harper, P.S., 1993. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 72, 971–983. https://doi.org/10.1016/0092-8674(93)90585-E.

Morton, A.J., 2013. Circadian and sleep disorder in Huntington's disease. Exp. Neurol. https://doi.org/10.1016/j.expneurol.2012.10.014.

Morton, A.J., Howland, D.S., 2013. Large genetic animal models of huntington's disease. J. Huntingtons. Dis. 2, 3–19. https://doi.org/10.3233/JHD-130050.

Morton, A.J., Rudiger, S.R., Wood, N.I., Sawiak, S.J., Brown, G.C., Mclaughlan, C.J., Kuchel, T.R., Snell, R.G., Faull, R.L.M., Simon Bawden, C., 2014. Early and progressive circadian abnormalities in Huntington's disease sheep are unmasked by social environment. Hum. Mol. Genet. 23, 3375–3383. https://doi.org/10.1093/hmg/ddu047.

Morton, A.J., Middleton, B., Rudiger, S., Bawden, C.S., Kuchel, T.R., Skene, D.J., 2020. Increased plasma melatonin in presymptomatic Huntington disease sheep (*Ovis aries*): compensatory neuroprotection in a neurodegenerative disease? J. Pineal Res. 68, e12624 https://doi.org/10.1111/jpi.12624.

Nguyen, L., Bradshaw, J.L., Stout, J.C., Croft, R.J., Georgiou-Karistianis, N., 2010. Electrophysiological measures as potential biomarkers in Huntington's disease: review and future directions. Brain Res. Rev. 64, 177–194. https://doi.org/10.1016/j.brainresrev.2010.03.004.

Nicol, A.U., Morton, A.J., 2020. Characteristic patterns of EEG oscillations in sheep (*Ovis aries*) induced by ketamine may explain the psychotropic effects seen in humans. Sci. Rep. 10, 9440. https://doi.org/10.1038/s41598-020-66023-8.

Painold, A., Anderer, P., Holl, A.K., Letmaier, M., Saletu-Zyhlarz, G.M., Saletu, B., Bonelli, R.M., 2010. Comparative EEG mapping studies in Huntington's disease patients and controls. J. Neural Transm. 117, 1307–1318. https://doi.org/10.1007/s00702-010-0491-7.

Painold, A., Anderer, P., Holl, A.K., Letmaier, M., Saletu-Zyhlarz, G.M., Saletu, B., Bonelli, R.M., 2011. EEG low-resolution brain electromagnetic tomography (LORETA) in Huntington's disease. J. Neurol. 258, 840–854. https://doi.org/10.1007/s00415-010-5852-5.

Painold, A., Milz, P., Faber, P.L., Anderer, P., Kapfhammer, H.-P., Kochi, K., Lehmann, D., 2012. P-793 - reduced intracortical functional connectivity in huntington's disease. Eur. Psychiatry 27 (Suppl. 1), S1. https://doi.org/10.1016/s0924-9338(12)74960-x.

Paksarian, D., Rudolph, K.E., Stapp, E.K., Dunster, G.P., He, J., Mennitt, D., Hattar, S., Casey, J.A., James, P., Merikangas, K.R., 2020. Association of outdoor artificial light at night with mental disorders and sleep patterns among US adolescents. JAMA Psychiatry. https://doi.org/10.1001/jamapsychiatry.2020.1935 e201935.

Pallier, P.N., Morton, A.J., 2009. Management of sleep/wake cycles improves cognitive function in a transgenic mouse model of Huntington's disease. Brain Res. 1279, 90–98. https://doi.org/10.1016/j.brainres.2009.03.072.

Perentos, N., Martins, A.Q., Watson, T.C., Bartsch, U., Mitchell, N.L., Palmer, D.N., Jones, M.W., Jennifer Morton, A., 2015. Translational neurophysiology in sheep: measuring sleep and neurological dysfunction in CLN5 batten disease affected sheep. Brain 138, 862–874. https://doi.org/10.1093/brain/awv026.

Perentos, N., Martins, A.Q., Cumming, R.J.M., Mitchell, N.L., Palmer, D.N., Sawiak, S.J., Morton, A.J., 2016. An EEG investigation of sleep homeostasis in healthy and CLN5 batten disease affected sheep. J. Neurosci. 36, 8238–8249. https://doi.org/10.1523/JNEUROSCI.4295-15.2016.

Perentos, N., Nicol, A.U., Martins, A.Q., Stewart, J.E., Taylor, P., Morton, A.J., 2017. Techniques for chronic monitoring of brain activity in freely moving sheep using wireless EEG recording. J. Neurosci. Methods 279, 87–100. https://doi.org/10.1016/j.jneumeth.2016.11.010.

Piano, C., Imperatori, C., Losurdo, A., Bentivoglio, A.R., Cortelli, P., Della Marca, G., 2017a. Sleep-related modifications of EEG connectivity in the sensory-motor networks in Huntington disease: an eLORETA study and review of the literature. Clin. Neurophysiol. 128, 1354–1363. https://doi.org/10.1016/j.clinph.2016.11.019.

Piano, C., Mazzucchi, E., Bentivoglio, A.R., Losurdo, A., Buonaura, G.C., Imperatori, C., Cortelli, P., Marca, G. Della, 2017b. Wake and sleep EEG in patients with Huntington disease an eLORETA study and review of the literature. Clin. EEG Neurosci. 48, 60–71. https://doi.org/10.1177/1550059416632413.

Pignatelli, M., Lebreton, F., Cho, Y.H., Leinekugel, X., 2012. "Ectopic" theta oscillations and interictal activity during slow-wave state in the R6/1 mouse model of Huntington's disease. Neurobiol. Dis. https://doi.org/10.1016/j.nbd.2012.07.015.

Ponomareva, N., Klyushnikov, S., Abramycheva, N., Malina, D., Scheglova, N., Fokin, V., Ivanova-Smolenskaia, I., Illarioshkin, S., 2014. Alpha-theta border EEG abnormalities in preclinical Huntington's disease. J. Neurol. Sci. 344, 114–120. https://doi.org/10.1016/j.jns.2014.06.035.

Reid, S.J., Patassini, S., Handley, R.R., Rudiger, S.R., McLaughlan, C.J., Osmand, A., Jacobsen, J.C., Jennifer Morton, A., Weiss, A., Waldvogel, H.J., MacDonald, M.E., Gusella, J.F., Simon Bawden, C., Faull, R.L.M., Snell, R.G., 2013. Further molecular characterisation of the OVT73 transgenic sheep model of huntington's disease identifies cortical aggregates. J. Huntingtons. Dis. 2, 279–295. https://doi.org/10.3233/JHD-130067.

Rosas, H.D., Salat, D.H., Lee, S.Y., Zaleta, A.K., Pappu, V., Fischl, B., Greve, D., Hevelone, N., Hersch, S.M., 2008. Cerebral cortex and the clinical expression of Huntington's disease: complexity and heterogeneity. Brain 131, 1057–1068. https://doi.org/10.1093/brain/awn025.

Roth, C., Achermann, P., Borbély, A.A., 1999. Frequency and state specific hemispheric asymmetries in the human sleep EEG. Neurosci. Lett. 271, 139–142. https://doi.org/10.1016/S0304-3940(99)00048-8.

Rothe, T., Deliano, M., Wójtowicz, A.M., Dvorzhak, A., Harnack, D., Paul, S., Vagner, T., Melnick, I., Stark, H., Grantyn, R., 2015. Pathological gamma oscillations, impaired dopamine release, synapse loss and reduced dynamic range of unitary glutamatergic synaptic transmission in the striatum of hypokinetic Q175 Huntington mice. Neuroscience 311, 519–538. https://doi.org/10.1016/j.neuroscience.2015.10.039.

Scammell, T.E., Arrigoni, E., Lipton, J.O., 2017. Neural circuitry of wakefulness and sleep. Neuron. https://doi.org/10.1016/j.neuron.2017.01.014.

Schneider, W.T., Vas, S., Nicol, A.U., Morton, A.J., 2020. Characterizing sleep spindles in sheep. eNeuro 7. https://doi.org/10.1523/ENEURO.0410-19.2020. ENEURO.0410-19.2020.

Silber, M.H., Ancoli-Israel, S., Bonnet, M.H., Chokroverty, S., Grigg-Damberger, M.M., Hirshkowitz, M., Kapen, S., Keenan, S.A., Kryger, M.H., Penzel, T., Pressman, M.R., Iber, C., 2007. The visual scoring of sleep in adults. J. Clin. Sleep Med. 3, 121–131. https://doi.org/10.5664/jcsm.26814.

Skene, D.J., Middleton, B., Fraser, C.K., Pennings, J.L.A., Kuchel, T.R., Rudiger, S.R., Bawden, C.S., Morton, A.J., 2017. Metabolic profiling of presymptomatic Huntington's disease sheep reveals novel biomarkers. Sci. Rep. 7, 43030. https://doi.org/10.1038/srep43030.

Steriade, M., Nunez, A., Amzica, F., 1993. Intracellular analysis of relations between the slow (< 1 Hz) neocortical oscillation and other sleep rhythms of the electroencephalogram. J. Neurosci. 13, 3266–3283. https://doi.org/10.1073/pnas.90.5.2078.

Thiruvady, D.R., Georgiou-Karistianis, N., Egan, G.F., Ray, S., Sritharan, A., Farrow, M., Churchyard, A., Chua, P., Bradshaw, J.L., Brawn, T.L., Cunnington, R., 2007. Functional connectivity of the prefrontal cortex in Huntington's disease. J. Neurol. Neurosurg. Psychiatry 78, 127–133. https://doi.org/10.1136/jnnp.2006.098368.

Tononi, G., Cirelli, C., 2006. Sleep function and synaptic homeostasis. Sleep Med. Rev. 10, 49–62. https://doi.org/10.1016/j.smrv.2005.05.002.

Vas, S., Casey, J.M., Schneider, W.T., Kalmar, L., Morton, A.J., 2020. Wake-promoting and EEG spectral effects of modafinil after acute or chronic administration in the R6/2 mouse model of Huntington's disease. Neurotherapeutics. https://doi.org/10.1007/s13311-020-00849-y.

Werth, E., Achermann, P., Borbély, A.A., 1996. Brain topography of the human sleep EEG: Antero-posterior shifts of spectral power. Neuroreport 8, 123–127. https://doi.org/10.1097/00001756-199612200-00025.

Wiegand, M., Möller, A.A., Lauer, C.J., Stolz, S., Schreiber, W., Dose, M., Krieg, J.C., 1991. Nocturnal sleep in huntington's disease. J. Neurol. 238, 203–208. https://doi.org/10.1007/BF00314781.

Wolf, E., Kuhn, M., Normann, C., Mainberger, F., Maier, J.G., Maywald, S., Bredl, A., Klöppel, S., Biber, K., van Calker, D., Riemann, D., Sterr, A., Nissen, C., 2016. Synaptic plasticity model of therapeutic sleep deprivation in major depression. Sleep Med. Rev. 30, 53–62. https://doi.org/10.1016/J.SMRV.2015.11.003.