Original Research Article

A single dose of hypnotic corrects sleep and EEG abnormalities in symptomatic Huntington's disease mice

Short title: Hypnotics normalize Huntington's disease mouse sleep and EEG

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Abstract

Sleep and electroencephalogram abnormalities are prominent early features of Huntington's disease (HD) that typically appear before the onset of characteristic motor symptoms. The changes in sleep and electroencephalogram seen in HD patients are largely recapitulated in mouse models of HD such as transgenic R6/2 lines. To test whether or not drugs with hypnotic properties can correct the sleep and electroencephalogram abnormalities seen in HD mice, we treated male wild-type (WT; N=7) and R6/2 mice (N=9) acutely with intraperitoneal injections of vehicle, zolpidem (5, 10 or 20 mg/kg) or amitriptyline (5, 10 or 20 mg/kg), and then monitored their sleep–wake behavior. In R6/2 mice, both zolpidem and amitriptyline suppressed the abnormally high REM sleep amount and electroencephalographic gamma (30-46 Hz) oscillations in a dose-dependent manner. Amitriptyline's effect on sleep was similar in both genotypes, whereas zolpidem showed significant genotype differences. Zolpidem exerted a strong hypnotic effect in WT mice by increasing electroencephalographic delta power, doubling the mean bout duration and the total amount of non-rapid eye movement sleep. However, no such effect was seen in R6/2 mice. Our study demonstrates that the pathophysiological changes seen in sleep and electroencephalogram are not 'hard-wired' in HD brain and can be reversed even at late stages of the disease. The diminished hypnotic effect of zolpidem suggests that the GABAergic control of sleep–wake states is impaired in HD mice. A better understanding of the neurochemical basis underlying these abnormalities should lead to more effective and rational therapies for HD.

Keywords: transgenic mice; hypnotic drugs; antidepressants; REM sleep; quantitative EEG; gamma oscillation

1. Introduction

The mutation causing Huntington's disease (HD) was discovered more than 20 years ago (1), yet an effective treatment to either prevent or slow the progression of the disease remains elusive. HD is best known as a movement disorder, but patients often show non-motor symptoms such as cognitive and psychiatric dysfunctions well before the onset of characteristic motor signs (2). Sleep and electroencephalogram (EEG) abnormalities are also prominent early features of HD that are often under-diagnosed (3). HD patients as well as pre-manifest gene carriers have shallow and fragmented night-time sleep, abnormal rapid eye movement (REM) sleep, and abnormally increased low-gamma oscillations in their sleep EEG (4-7). The changes in sleep and EEG seen in HD patients are largely recapitulated in mouse models of HD. For instance; we have shown previously that the R6/2 mouse, one of the best-characterized animal models of HD, has fragmented sleep-wake cycle and an increased propensity for REM sleep (8). In addition, EEG delta power decreases, whereas an abnormal low-gamma oscillation emerges in the sleep EEG of R6/2 mice well before the onset of any motor symptoms (8). Similar results have been found by other groups in R6/2 (9-11) and R6/1 transgenic mice (12-14), as well as in Q175 knock-in mouse models of HD (15-17).

Although there is a great demand for an effective therapy, only tetrabenazine is currently licensed as a treatment for excess chorea in HD (18). In clinical practice, on the other hand, many other medications are used for symptomatic treatment of HD, including a number of hypnotics and antidepressants (18). It is unknown whether or not such drugs have any effect on HD-specific sleep and EEG symptoms (3). The gamma-aminobutyric acid (GABA) A receptor modulator zolpidem and the tricyclic antidepressant amitriptyline are commonly prescribed for the treatment of sleep disturbances and depression in HD patients (18, 19). Both drugs display strong hypnotic properties in healthy humans and rodents (20-

23) but their effect on HD specific sleep and EEG symptoms has not yet been studied. To gain insight into the neurochemical basis underlying sleep and EEG abnormalities seen in HD as well as to test whether these abnormalities can be corrected by drugs, we treated R6/2 mice with zolpidem or amitriptyline at a symptomatic stage of the disease. We found that acute treatment with both of these drugs can normalize many of the sleep and EEG abnormalities present in R6/2 mice.

2. Materials and methods

2.1 Animals and housing conditions

All experiments were conducted under the authority of the United Kingdom Animals (Scientific Procedures) Act 1986. Male R6/2 (N = 9) and wild type (WT; N = 7) littermate mice were taken from a colony established at the University of Cambridge (CBAxC57/BL6 background). Genotyping and repeat length measurements were performed by Laragen (Los Angeles, CA). Genotyping was performed by PCR from tail snips taken at 3 weeks of age. CAG repeat lengths were measured by GeneMapper software (Life Technologies, NY). R6/2 mice had a mean CAG repeat length of 253 ± 2. We have shown that up to ~300 CAG repeats, the basic pathology and end-stage symptoms differ little from the 110 CAG repeats in the R6/2 mice. The main difference is the rate of progression of disease. Whereas 110 CAG repeat mice die at around 12-14 weeks of age, 250 CAG repeat R6/2 mice live until around 20-24 weeks (24-26). Before the end of study, one WT and one R6/2 mouse lost their EEG/EMG implants and were euthanized. In addition, one WT mouse died unexpectedly and two R6/2 mice died of their disease. Successful EEG/EMG recordings with all treatments were achieved in five out of seven WT mice and six out of nine R6/2 mice. Additional recordings were obtained in the remaining mice after some of the treatments (for further details see 2.3).

2.2 Surgery and EEG/EMG recordings

We implanted each mouse with EEG and EMG electrodes under isoflurane anesthesia (1.5-2%), as described previously (8). Briefly, we placed gold-plated stainless steel screw electrodes epidurally over the frontal (1.5 mm lateral and 1.0 mm anterior to bregma) and parietal (1.5 mm lateral and 1.0 mm anterior to lambda) cortex, above both left and right hemispheres for fronto-parietal EEG recordings. EMG signals were acquired by a pair of stainless steel spring wires (Plastics One Inc., Roanoke, VA) inserted into the neck extensor muscles (8). Before implantation, each screw was soldered to a soft insulated stainless steel wire, and all cables were connected to a single common eight-pin connector compatible with the data recording system (Pinnacle Technology, Lawrence, KS). Cables and the connector were all fixed to the skull with dental cement.

After surgery, we housed the mice in individual recording cages (with food and water available *ad libitum*) within sound-attenuating chambers with a 12:12 h light-dark cycle (30 lux daylight-type fluorescent tubes with lights on at 06:00), constant temperature (22 ± 1°C) and humidity (55 ± 10%). After a recovery period of 7-10 days, we connected the mice to recording cables. The recording cable was attached to a low torque electrical swivel above the cage that allowed free movement. After mice acclimated to the recording conditions for 3-4 days, we recorded EEG and EMG signals for 24h after each treatment. The mice remained connected to the recording cable throughout the study.

The EEG/EMG signals were amplified and filtered (EEG: 0.5-60 Hz, EMG: 10-100 Hz) by head-mounted preamplifiers and amplifiers (8202-DSL and 8206-SL, respectively; Pinnacle Technology, Lawrence, KS), and recorded on a computer (Vital Recorder, Kissei Comtec, Matsumoto, Japan) after analog-to-digital conversion.

2.3 Drug Administration

Zolpidem (Tocris, Bristol, UK) was dissolved in 10% dimethyl sulfoxide / 5 mM citric acid in saline (zolpidem vehicle). Amitriptyline (Sigma-Aldrich, Dorset, UK) was dissolved in saline (amitriptyline vehicle). Both zolpidem and amitriptyline display strong hypnotic properties (20-23). Thus to avoid a potential 'ceiling effect', where measured variables are at their maximum already under control conditions, we injected the mice just before dark onset and assessed the hypnotic potential of these drugs during the active dark period. The mice were given intraperitoneal (i.p.) injection in a volume of 10 ml/kg body weight. We treated the mice with three doses of zolpidem (5, 10, or 20 mg/kg), three doses of amitriptyline (5, 10, or 20 mg/kg), and their vehicles in a cross-over design with 2-3 days between the treatments. These doses were chosen based on the literature (20-23) and our pilot experiments. The different doses of a drug (zolpidem or amitriptyline) and its vehicle were given in a randomized order to the mice. The mice received zolpidem at 14-15 weeks of age and amitriptyline at 16-17 weeks of age. Five out of seven WT mice received all the treatments. The other two WT mice received all treatments apart from either the 5 mg/kg dose (one mouse) or the 10 mg/kg dose of amitriptyline (one mouse). Six out of nine R6/2 mice received all the treatments. Of the other three mice, two R6/2 mice received all doses of zolpidem and its vehicle, one R6/2 mouse received the 10 and 20 mg/kg doses of zolpidem and vehicle, but none of these mice received amitriptyline.

2.4 Data analysis and statistics

All signals were digitized at 256 Hz, digitally filtered (EEG: 0.5-60 Hz and EMG: 10-60 Hz), and semi-automatically scored as wake, non-REM (NREM) sleep, or REM sleep in 10 s epochs using SleepSign (Kissei Comtec, Matsumoto, Japan). Experienced scorers, blinded to treatment and genotype, visually inspected these preliminary scorings and made corrections

when appropriate. We then measured the duration of bouts, counted the number of bouts, and calculated the percentage of time spent in each behavioral state.

To reveal the changes in the frequency content of the recorded signal, we performed a spectral analysis of the EEG after the treatments. EEG power spectra were computed for consecutive 2 s epochs in the frequency range 0.5 to 49 Hz by fast Fourier transformation with a frequency resolution of 0.5 Hz. Before fast Fourier transformation, a window weighting function (Hanning) was applied. Epochs with movement-induced and other artifacts were discarded on the basis of the polygraph records. Data are presented in 1 Hz bins, where the bins were marked by their upper limits. The values of consecutive 2 s EEG epochs in wake, NREM and REM sleep, respectively, were averaged over 2 h after zolpidem and over 3 h after amitriptyline treatments.

To reveal the changes in higher frequency bands after the treatments, in addition to analyzing the entire EEG spectrum (1-49 Hz), we also compared the discrete changes in the low-gamma range (30-46 Hz) of the EEG. Finally, to reveal the differences between the groups, we normalized the EEG power spectral values of R6/2 mice to the mean power spectral values of vehicle-treated WT mice of the same age.

To compare statistically the raw EEG data and vigilance state parameters, we used multivariate analysis of variance with repeated measures and Bonferroni test for *post hoc* comparisons (Statistica 12, Statsoft, Tulsa, OK). The results were considered statistically significant at $P < 0.05$. All results are expressed as means ± SEM.

3. Results

3.1 Zolpidem is less potent hypnotic in HD mice than in WT mice

At 14-15 weeks of age, R6/2 mice already have an increased propensity for REM sleep and a fragmented sleep-wake pattern comparted to WT mice (8, 10). The effect of zolpidem (20

mg/kg) on vigilance states was seen only in the first 2-3 hours after treatment (Fig. 1). This is consistent with the short half-life of the drug (27). In R6/2 mice, zolpidem (10 and 20 mg/kg) reduced the abnormally increased REM sleep amount by half [drug x genotype interaction: $F_{(3,39)} = 3.27$, $P < 0.05$, Fig. 2C], but had no significant effect on REM sleep in WT mice (Fig. 2C). Furthermore, zolpidem decreased the time spent awake and increased the amount of NREM sleep in a dose-dependent manner in both WT and R6/2 mice during the first 3 h post-treatment [drug effects: $F_{(3,39)} = 25.66$, $P < 0.01$ and $F_{(3,39)} = 29.84$, $P < 0.01$, respectively; Fig. 2A and B]. Compared to vehicle treatment, zolpidem doubled the amount of NREM sleep at its highest dose (20 mg/kg) and it was hypnotic even at its lowest dose (5 mg/kg) in WT mice (Fig. 2B). By contrast, zolpidem was less hypnotic in R6/2 mice as even at its highest dose (20 mg/kg) it increased NREM sleep amount only by about 40% (Fig. 2B). The difference in the hypnotic effect of zolpidem in WT and R6/2 mice was even more obvious when we compared the mean duration of sleep-wake bouts. In WT mice, zolpidem (20 mg/kg) consolidated NREM sleep by doubling the mean duration of NREM sleep bouts [drug effect: $F_{(3,39)} = 8.31$, $P < 0.01$; Table 1]. Zolpidem (20 mg/kg) also reduced the mean duration of awakenings in WT mice by almost half during this time [drug effect: $F_{(3,39)} = 9.30$, $P < 0.01$; Table 1]. By contrast, the mean duration of sleep-wake bouts did not change significantly in R6/2 mice after zolpidem treatment.

3.2 Zolpidem suppresses the abnormal low-gamma EEG oscillations during NREM sleep

By 14-15 weeks of age, R6/2 mice had twice as much low-gamma activity (with a peak at 39-40 Hz) in their NREM sleep EEG as did WT mice under control conditions (Fig. 3D). Zolpidem decreased these abnormal low-gamma oscillations in NREM sleep EEG in a dose-dependent manner during the first two hours after the treatment. The 20 mg/kg dose of zolpidem reduced these abnormal low-gamma oscillations by about half in R6/2 mice in the

first 2 h after treatment [drug x frequency interaction: $F_{(48,336)} = 5.53$, $P < 0.01$; Fig. 3B' and D]. The higher doses of zolpidem (10 and 20 mg/kg) also decreased EEG theta power (5-10 Hz) during NREM sleep in both WT and R6/2 mice [drug x frequency interactions: $F_{(144,864)} = 2.21$, $P < 0.01$ and $F_{(144,1008)} = 4.03$, $P < 0.01$, respectively; Fig. 3A and B]. The lowest dose of zolpidem (5 mg/kg) increased delta power (1-4 Hz) during NREM sleep in WT mice [drug x frequency interactions: $F_{(144,864)} = 2.21$, $P < 0.01$; Fig. 3A and C] but not in R6/2 mice. The changes in REM sleep EEG in R6/2 mice were not significant (Fig. 4).

3.3 Amitriptyline reduces REM sleep amount and consolidates NREM sleep

Despite the fact that under control conditions R6/2 mice had four times as much REM sleep as was seen in WT mice, amitriptyline abolished REM sleep to the same extent in both WT and R6/2 mice. The effect lasted for several hours (Fig. 5), and was dose-dependent (Fig. 6). In the first 6 h after the treatment, amitriptyline (20 mg/kg) decreased the amount of REM sleep by about 90% in both WT and R6/2 mice compared to vehicle treatment [drug effect: $F_{(3,27)} = 28.18$, $P < 0.01$; drug x genotype interaction: $F_{(3,27)} = 11.95$, $P < 0.01$; Fig. 6]. This was achieved primarily by suppressing the initiation of REM sleep as shown by the reduction in bout numbers. After vehicle treatment, WT mice had five to six bouts while R6/2 mice had 20-21 bouts of REM sleep on average. By contrast, WT mice entered into REM sleep only once, or not at all after amitriptyline (20 mg/kg) treatment. Similarly, R6/2 mice entered into REM sleep fewer than four times within a 6 h period after the drug. That is more than 80% fewer REM sleep bouts after amitriptyline (20 mg/kg) than was seen under control condition in both genotypes [drug x genotype interaction: $F_{(3,27)} = 7.65$, $P < 0.01$; Table 2]. Interestingly, while the propensity for initiating REM sleep was suppressed, the maintenance of REM sleep was not affected, as the mean duration of REM sleep bouts did not change significantly in either genotype after amitriptyline treatment (Table 2).

The hypnotic effect of amitriptyline was dose-dependent in both WT and R6/2 mice. At the 20 mg/kg dose, amitriptyline nearly doubled the amount of NREM sleep in WT mice and increased it by about 60% in R6/2 mice during the first 6 h after the treatment [drug effect: $F_{(3,27)} = 34.64$, $P < 0.01$; Fig. 6]. NREM sleep also became less fragmented in R6/2 mice, with NREM sleep bouts being more than twice as long after amitriptyline (20 mg/kg) treatment than they were under the control condition [drug x genotype interaction: $F(3,27) = 5.33$, $P < 0.01$; Table 2]. At the same time, the amount of wakefulness was reduced by more than half in both WT and R6/2 mice after the 20 mg/kg dose of amitriptyline [drug effect: $F_{(3,27)} = 23.44$, $P < 0.01$; Fig. 6]. This reduction in total wake amount was due to the shortening of wake bouts (WT mice: -71.2 % and R6/2 mice: -45.2%) since the number of wake bouts did not change significantly (Table 2). The inability of mice to maintain long periods of wakefulness together with the increased amount of NREM sleep indicate strong sedative-hypnotic properties of the drug at the highest dose tested (20 mg/kg).

3.4 Amitriptyline corrects abnormal low-gamma EEG oscillations in HD mice

Since amitriptyline virtually abolished REM sleep in both WT and R6/2 mice for several hours (Fig. 5), we could only analyze the changes in EEG power spectra during NREM sleep after the treatment. After analyzing the time course of changes, we found that amitriptyline dose-dependently decreased the abnormal low-gamma oscillations in NREM sleep EEG in R6/2 mice for 3 h [drug x frequency interaction: $F_{(48,240)} = 5.62$, $P < 0.01$; Fig. 7B']. Under control conditions, 16-17 weeks old R6/2 mice had a fourfold increase in EEG low-gamma activity compared to WT mice, with a peak frequency at 36-37 Hz (Fig. 7D). The highest dose (20 mg/kg) of amitriptyline decreased these abnormal low-gamma oscillations in R6/2 mice close to the level of control treated WT mice (Fig. 7D). Compared to vehicle, the 20 mg/kg dose of amitriptyline also shifted nearly the entire EEG spectrum down, with

differences reaching significance at 8 Hz in WT mice and 7-9 Hz in R6/2 mice [drug x frequency interactions: $F_{(144,576)} = 5.58$, $P < 0.01$ and $F_{(144,720)} = 4.12$, $P < 0.01$, respectively; Fig. 7A and B]. In contrast to the 20 mg/kg dose, lower doses of amitriptyline (5 and 10 mg/kg) increased low frequency oscillations in NREM sleep EEG at 1-5 Hz in WT mice and at 3-7 Hz in R6/2 mice compared to vehicle (Fig. 7A and B). These opposite changes in the EEG spectrum suggest that high doses of amitriptyline produce non-specific as well as specific effects, although the effect of amitriptyline on abnormal low-gamma oscillations was consistent across all doses in R6/2 mice.

4. Discussion

We show for the first time that the sleep and EEG abnormalities found in HD mice can be reversed after a single treatment with zolpidem or amitriptyline. In R6/2 mice, zolpidem reduced the abnormally increased REM sleep amount and the abnormal low-gamma oscillations in NREM sleep EEG by half. Amitriptyline abolished REM sleep as well as the abnormal low-gamma EEG oscillations in R6/2 mice for several hours. Both drugs increased the amount of NREM sleep and decreased the time spent awake, in both WT and R6/2 mice.

Amitriptyline and zolpidem are both prescribed to HD patients for the treatment of depression and sleep disturbances (18, 19). HD patients as well as pre-manifest gene carriers have shallow and fragmented night-time sleep, and abnormal REM sleep (4-7). R6/2 mice also have a fragmented sleep–wake pattern and an abnormal increase in REM sleep amount during their active dark period (8, 10). Though amitriptyline had a more robust and longer lasting effect than zolpidem, both drugs reduced the amount of REM sleep and wakefulness, and increased the time spent in NREM sleep in both WT and R6/2 mice in our study. Similar results have been found by others in both humans and rodents after acute treatment with zolpidem or amitriptyline (20-23). Although, repeated administration of these drugs can

result different effect. For instance, most of antidepressants suppress REM sleep early in treatment, but this effect gradually diminishes after their repeated administration (28, 29). The ability of zolpidem and amitriptyline to suppress REM sleep in R6/2 mice is particularly interesting, since R6/2 mice had more than twice as much REM sleep during the active dark period as WT mice. REM sleep is generated by neurons primarily located in the brain stem (30, 31), although other brain sites such as the ventrolateral periaqueductal grey matter or the lateral hypothalamic area may also be relevant (32, 33). We have suggested previously that the incomplete suppression of REM sleep in HD mice during the active period may be a result of an abnormally functioning orexin system (8). The changes in REM sleep induced by zolpidem and amitriptyline in R6/2 mice suggest that although the primary REM sleep regulatory circuits cannot efficiently suppress the initiation of REM sleep during the active period they are still functional and can be modulated by drugs in HD mice.

Although zolpidem efficiently reduced REM sleep in both genotypes, it proved to be less hypnotic in R6/2 mice than it was in WT mice. The zolpidem-induced increase in NREM sleep lasted for 3 h in WT mice but it was limited to the first hour post-treatment in R6/2 mice. Also, whereas zolpidem consolidated sleep in WT mice by lengthening NREM sleep episodes, shortening awakenings and increasing EEG delta power during NREM sleep, no such effect was seen in R6/2 mice. The reduced efficacy of zolpidem in R6/2 mice suggests impairment in the GABAergic control of sleep-wakefulness. In accordance with this idea, decreased GABAergic neurotransmission is found in the frontal and parietal cortices, as well as in various parts of the basal ganglia such as the globus pallidus (GP) of symptomatic R6/2 mice (34). GABAergic neurons of GP externus (GPe) project directly to layer V neurons of the neocortex (35-38), which seems to play a critical role in slow wave sleep generation (39). Since GP neurons are most active when the neocortex is activated, such as during wakefulness and REM sleep (40), the corticopetal GPe neurons are likely to promote sleep by

suppressing cortical activity and inhibiting fast cortical oscillations (41). Indeed, optogenetic-stimulation of GPe neurons increased sleep and EEG delta power (42), while lesion of GP decreased sleep and increased sleep-wake fragmentation in laboratory rats (43). GP neurons receive inhibitory inputs from the striatum, and although the concentration of GABA is normal in R6/2 mouse striatum (44), striatal neurons are hyperactive in symptomatic R6/2 mice (45). Thus, based on the above, it seems very likely that an increased striatal inhibition of pallidocortical projections in R6/2 mice could lead to a disinhibition of layer V cortical neurons (Fig. 8). As a result, increased cortical activity would occur in R6/2 mice regardless of vigilance state. This could account for the fragmented sleep, decreased EEG delta power, and increased REM sleep amount seen in R6/2 mice, and would also explain the reduced hypnotic efficacy of zolpidem in these mice. Insufficient inhibition of cortical activity by cortical-projecting GP neurons could also account for at least some of the EEG irregularities seen in R6/2 mice, such as the increased low-gamma oscillations during sleep.

Gamma oscillations are primarily generated by networks of glutamatergic and GABAergic interneurons of the cortex, and controlled by cortically projecting GABAergic neurons of the basal forebrain (46). These gamma oscillations normally occur during states of activated neocortex such as wakefulness and REM sleep (47, 48). In schizophrenic patients, abnormal EEG gamma oscillations can be seen during periods of psychosis and sleep (49). The fact that abnormal EEG gamma oscillations occur in R6/2 mice during synchronized or deactivated state of neocortex (8, 10, 11) also supports the idea of an insufficient inhibition of cortical activity in HD. The striatum, which receives many of its inputs from the neocortex (Fig. 8), also shows abnormal low-gamma oscillations in R6/2 mice during quiet rest (9, 50). Furthermore, abnormally increased low-gamma oscillations have been found recently in the sleep EEG of early HD patients (7). Gamma oscillations are associated with a number of cognitive processes, including perceptual and associative learning, object representation and

selective attention (51-54). These cognitive processes are often disrupted in HD patients (55-62). This could be a consequence of abnormal brain oscillations seen in HD, including the increased gamma activity. In our study, a single treatment with either zolpidem or amitriptyline led to an immediate suppression of the abnormal low-gamma EEG oscillations in R6/2 mice during NREM sleep. Zolpidem reduced the abnormal low-gamma EEG oscillations by half while amitriptyline almost completely abolished them in R6/2 mice. Zolpidem enhances the $GABA_A$ receptor function by binding selectively to the omega-1 receptor subtype (63). Therefore, it seems most likely that zolpidem decreased the abnormal low-gamma EEG oscillations in R6/2 mice by increasing $GABA_A$-mediated inhibition of cortical activity. Whether this is achieved by acting specifically on layer V cortical neurons or just by increasing the overall GABAergic tone in the brain needs to be determined. Amitriptyline is primarily a serotonin-noradrenaline reuptake inhibitor, but it also acts on multiple neurochemical systems via serotonergic, adrenergic, histaminic, muscarinic, and σ_1 receptors as well as glutamatergic NMDA receptors (64). Although the precise mechanism through which amitriptyline suppresses the abnormal EEG gamma oscillations in HD mouse brain is not known, it is likely that NMDA and/or muscarinic receptors are involved in this process. This is supported by the observation that in addition to GABA and glutamate, acetylcholine also plays an important role in gamma modulation through its muscarinic receptors (65). We predict that a complete and sustained suppression of abnormal brain oscillations might improve HD symptoms other than disrupted sleep. Consistent with this suggestion, it has been shown that motor function improved in an N171-82Q mouse model of HD after six weeks by amitriptyline treatment, started at the pre-symptomatic stage of the disease (66). Although, there are no clinical trials comparing the use of these drugs in HD patients, it is notable that amitriptyline (1 mg/kg/day), in combination with tetrabenazine (50 mg/kg/day), eliminated psychotic symptoms in an atypical juvenile form of HD just after few

weeks of treatment (67). HD patients are treated with drugs with the preconception that the drugs will have the same effect in the HD patient as they do in the normal subject. Our data suggests that this may not be the case. A more thorough examination of the effect of hypnotics and antidepressants on HD brain function would clearly be of value.

In summary, we show for the first time that sleep and EEG abnormalities specific to HD can be reversed in a mouse model of the disease after acute treatment with hypnotic drugs. Both zolpidem and amitriptyline normalized the amount of REM sleep and led to an immediate suppression of abnormal low-gamma EEG activity during NREM sleep in R6/2 mice. Furthermore, our data demonstrate that sleep and EEG measures are useful indicators of drug-induced changes in mouse models of HD. Since HD mice, including the R6/2 mice studied here, largely recapitulate the changes in sleep and EEG seen in HD patients, they may be useful to evaluate the therapeutic potential of both existing and future treatment options. Critically, these results also provide evidence that at least some of the pathophysiological changes are not 'hard-wired' in HD mouse brain and can be modulated by drugs even at a late stage of the disease.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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References

1. The Huntington's Disease Collaborative Research Group (1993): A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell* 72: 971-983.
2. Huntington Study Group (1996): Unified Huntington's Disease Rating Scale: reliability and consistency. *Mov Disord* 11: 136-142.
3. Morton AJ (2013): Circadian and sleep disorder in Huntington's disease. *Exp Neurol* 243: 34-44.
4. Silvestri R, Raffaele M, De Domenico P, Tisano A, Mento G, Casella C, et al. (1995): Sleep features in Tourette's syndrome, neuroacanthocytosis and Huntington's chorea. *Neurophysiol Clin* 25: 66-77.
5. Arnulf I, Nielsen J, Lohmann E, Schiefer J, Wild E, Jennum P, et al. (2008): Rapid eye movement sleep disturbances in Huntington disease. *Arch Neurol* 65: 482-488.
6. Goodman AO, Rogers L, Pilsworth S, McAllister CJ, Shneerson JM, Morton AJ, et al. (2011): Asymptomatic sleep abnormalities are a common early feature in patients with Huntington's disease. *Curr Neurol Neurosci Rep* 11: 211-217.
7. Lazar AS, Panin F, Goodman AO, Lazic SE, Lazar ZI, Mason SL, et al. (2015): Sleep, but no metabolic, deficits in pre-manifest huntington's disease. *Ann Neurol*.
8. Kantor S, Szabo L, Varga J, Cuesta M, Morton AJ (2013): Progressive sleep and electroencephalogram changes in mice carrying the Huntington's disease mutation. *Brain* 136: 2147-2158.
9. Hong SL, Cossyleon D, Hussain WA, Walker LJ, Barton SJ, Rebec GV (2012): Dysfunctional behavioral modulation of corticostriatal communication in the R6/2 mouse model of Huntington's disease. *PLoS One* 7: e47026.

10. Fisher SP, Black SW, Schwartz MD, Wilk AJ, Chen TM, Lincoln WU, et al. (2013): Longitudinal analysis of the electroencephalogram and sleep phenotype in the R6/2 mouse model of Huntington's disease. *Brain* 136: 2159-2172.
11. Callahan JW, Abercrombie ED (2015): Relationship between subthalamic nucleus neuronal activity and electrocorticogram is altered in the R6/2 mouse model of Huntington's disease. *J Physiol* 593: 3727-3738.
12. Pignatelli M, Lebreton F, Cho YH, 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* 48: 409-417.
13. Jeantet Y, Cayzac S, Cho YH (2013): beta oscillation during slow wave sleep and rapid eye movement sleep in the electroencephalogram of a transgenic mouse model of Huntington's disease. *PLoS One* 8: e79509.
14. Lebreton F, Cayzac S, Pietropaolo S, Jeantet Y, Cho YH (2015): Sleep Physiology Alterations Precede Plethoric Phenotypic Changes in R6/1 Huntington's Disease Mice. *PLoS One* 10: e0126972.
15. Loh DH, Kudo T, Truong D, Wu Y, Colwell CS (2013): The Q175 mouse model of Huntington's disease shows gene dosage- and age-related decline in circadian rhythms of activity and sleep. *PLoS One* 8: e69993.
16. Nagy D, Tingley FD, 3rd, Stoiljkovic M, Hajos M (2015): Application of neurophysiological biomarkers for Huntington's disease: evaluating a phosphodiesterase 9A inhibitor. *Exp Neurol* 263: 122-131.
17. Fisher SP, Schwartz MD, Wurts-Black S, Thomas AM, Chen TM, Miller MA, et al. (2015): Quantitative Electroencephalographic Analysis Provides an Early-Stage Indicator of Disease Onset and Progression in the zQ175 Knock-In Mouse Model of Huntington Disease. *Sleep*.

18. Ross CA, Tabrizi SJ (2011): Huntington's disease: from molecular pathogenesis to clinical treatment. *Lancet Neurol* 10: 83-98.
19. Adam OR, Jankovic J (2008): Symptomatic treatment of Huntington disease. *Neurotherapeutics* 5: 181-197.
20. Brunner DP, Dijk DJ, Munch M, Borbely AA (1991): Effect of zolpidem on sleep and sleep EEG spectra in healthy young men. *Psychopharmacology (Berl)* 104: 1-5.
21. Kopp C, Rudolph U, Tobler I (2004): Sleep EEG changes after zolpidem in mice. *Neuroreport* 15: 2299-2302.
22. Doerr JP, Spiegelhalder K, Petzold F, Feige B, Hirscher V, Kaufmann R, et al. (2010): Impact of escitalopram on nocturnal sleep, day-time sleepiness and performance compared to amitriptyline: a randomized, double-blind, placebo-controlled study in healthy male subjects. *Pharmacopsychiatry* 43: 166-173.
23. Obal F, Jr., Benedek G, Lelkes Z, Obal F (1985): Effects of acute and chronic treatment with amitryptyline on the sleep-wake activity of rats. *Neuropharmacology* 24: 223-229.
24. Morton AJ, Glynn D, Leavens W, Zheng Z, Faull RL, Skepper JN, et al. (2009): Paradoxical delay in the onset of disease caused by super-long CAG repeat expansions in R6/2 mice. *Neurobiol Dis* 33: 331-341.
25. Dragatsis I, Goldowitz D, Del Mar N, Deng YP, Meade CA, Liu L, et al. (2009): CAG repeat lengths > or =335 attenuate the phenotype in the R6/2 Huntington's disease transgenic mouse. *Neurobiol Dis* 33: 315-330.
26. Cummings DM, Alaghband Y, Hickey MA, Joshi PR, Hong SC, Zhu C, et al. (2012): A critical window of CAG repeat-length correlates with phenotype severity in the R6/2 mouse model of Huntington's disease. *J Neurophysiol* 107: 677-691.
27. Nutt DJ, Stahl SM (2010): Searching for perfect sleep: the continuing evolution of GABAA receptor modulators as hypnotics. *J Psychopharmacol* 24: 1601-1612.

28. Wilson S, Argyropoulos S (2005): Antidepressants and sleep: a qualitative review of the literature. *Drugs* 65: 927-947.

29. Staner L, Kerkhofs M, Detroux D, Leyman S, Linkowski P, Mendlewicz J (1995): Acute, subchronic and withdrawal sleep EEG changes during treatment with paroxetine and amitriptyline: a double-blind randomized trial in major depression. *Sleep* 18: 470-477.

30. Brown RE, Basheer R, McKenna JT, Strecker RE, McCarley RW (2012): Control of sleep and wakefulness. *Physiol Rev* 92: 1087-1187.

31. Weber F, Chung S, Beier KT, Xu M, Luo L, Dan Y (2015): Control of REM sleep by ventral medulla GABAergic neurons. *Nature* 526: 435-438.

32. Lu J, Sherman D, Devor M, Saper CB (2006): A putative flip-flop switch for control of REM sleep. *Nature* 441: 589-594.

33. Luppi PH, Clement O, Fort P (2013): Paradoxical (REM) sleep genesis by the brainstem is under hypothalamic control. *Curr Opin Neurobiol* 23: 786-792.

34. Gourfinkel-An I, Parain K, Hartmann A, Mangiarini L, Brice A, Bates G, et al. (2003): Changes in GAD67 mRNA expression evidenced by in situ hybridization in the brain of R6/2 transgenic mice. *J Neurochem* 86: 1369-1378.

35. Gritti I, Mainville L, Mancia M, Jones BE (1997): GABAergic and other noncholinergic basal forebrain neurons, together with cholinergic neurons, project to the mesocortex and isocortex in the rat. *J Comp Neurol* 383: 163-177.

36. Sarter M, Bruno JP (2002): The neglected constituent of the basal forebrain corticopetal projection system: GABAergic projections. *Eur J Neurosci* 15: 1867-1873.

37. Furuta T, Koyano K, Tomioka R, Yanagawa Y, Kaneko T (2004): GABAergic basal forebrain neurons that express receptor for neurokinin B and send axons to the cerebral cortex. *J Comp Neurol* 473: 43-58.

38. Hur EE, Zaborszky L (2005): Vglut2 afferents to the medial prefrontal and primary somatosensory cortices: a combined retrograde tracing in situ hybridization study [corrected]. *J Comp Neurol* 483: 351-373.

39. Sanchez-Vives MV, McCormick DA (2000): Cellular and network mechanisms of rhythmic recurrent activity in neocortex. *Nat Neurosci* 3: 1027-1034.

40. Urbain N, Gervasoni D, Souliere F, Lobo L, Rentero N, Windels F, et al. (2000): Unrelated course of subthalamic nucleus and globus pallidus neuronal activities across vigilance states in the rat. *Eur J Neurosci* 12: 3361-3374.

41. Vetrivelan R, Qiu MH, Chang C, Lu J (2010): Role of Basal Ganglia in sleep-wake regulation: neural circuitry and clinical significance. *Front Neuroanat* 4: 145.

42. Qiu MH, Yao QL, Vetrivelan R, Chen MC, Lu J (2014): Nigrostriatal Dopamine Acting on Globus Pallidus Regulates Sleep. *Cereb Cortex*.

43. Qiu MH, Vetrivelan R, Fuller PM, Lu J (2010): Basal ganglia control of sleep-wake behavior and cortical activation. *Eur J Neurosci* 31: 499-507.

44. Reynolds GP, Dalton CF, Tillery CL, Mangiarini L, Davies SW, Bates GP (1999): Brain neurotransmitter deficits in mice transgenic for the Huntington's disease mutation. *J Neurochem* 72: 1773-1776.

45. Rebec GV, Conroy SK, Barton SJ (2006): Hyperactive striatal neurons in symptomatic Huntington R6/2 mice: variations with behavioral state and repeated ascorbate treatment. *Neuroscience* 137: 327-336.

46. Kim T, Thankachan S, McKenna JT, McNally JM, Yang C, Choi JH, et al. (2015): Cortically projecting basal forebrain parvalbumin neurons regulate cortical gamma band oscillations. *Proc Natl Acad Sci U S A* 112: 3535-3540.

47. Franken P, Dijk DJ, Tobler I, Borbely AA (1994): High-frequency components of the rat electrocorticogram are modulated by the vigilance states. *Neurosci Lett* 167: 89-92.

48. Steriade M, Amzica F, Contreras D (1996): Synchronization of fast (30-40 Hz) spontaneous cortical rhythms during brain activation. *J Neurosci* 16: 392-417.
49. Herrmann CS, Demiralp T (2005): Human EEG gamma oscillations in neuropsychiatric disorders. *Clin Neurophysiol* 116: 2719-2733.
50. Miller BR, Walker AG, Barton SJ, Rebec GV (2011): Dysregulated Neuronal Activity Patterns Implicate Corticostriatal Circuit Dysfunction in Multiple Rodent Models of Huntington's Disease. *Front Syst Neurosci* 5: 26.
51. Engel AK, Fries P, Singer W (2001): Dynamic predictions: oscillations and synchrony in top-down processing. *Nat Rev Neurosci* 2: 704-716.
52. Gruber T, Muller MM, Keil A (2002): Modulation of induced gamma band responses in a perceptual learning task in the human EEG. *J Cogn Neurosci* 14: 732-744.
53. Fell J, Fernandez G, Klaver P, Elger CE, Fries P (2003): Is synchronized neuronal gamma activity relevant for selective attention? *Brain Res Brain Res Rev* 42: 265-272.
54. Buzsaki G, Draguhn A (2004): Neuronal oscillations in cortical networks. *Science* 304: 1926-1929.
55. Ho AK, Sahakian BJ, Brown RG, Barker RA, Hodges JR, Ane MN, et al. (2003): Profile of cognitive progression in early Huntington's disease. *Neurology* 61: 1702-1706.
56. Sprengelmeyer R, Young AW, Calder AJ, Karnat A, Lange H, Homberg V, et al. (1996): Loss of disgust. Perception of faces and emotions in Huntington's disease. *Brain* 119 (Pt 5): 1647-1665.
57. Hennenlotter A, Schroeder U, Erhard P, Haslinger B, Stahl R, Weindl A, et al. (2004): Neural correlates associated with impaired disgust processing in pre-symptomatic Huntington's disease. *Brain* 127: 1446-1453.

58. Harrington DL, Smith MM, Zhang Y, Carlozzi NE, Paulsen JS, Group P-HIotHS (2012): Cognitive domains that predict time to diagnosis in prodromal Huntington disease. *J Neurol Neurosurg Psychiatry* 83: 612-619.

59. Stout JC, Paulsen JS, Queller S, Solomon AC, Whitlock KB, Campbell JC, et al. (2011): Neurocognitive signs in prodromal Huntington disease. *Neuropsychology* 25: 1-14.

60. Finke K, Bublak P, Dose M, Muller HJ, Schneider WX (2006): Parameter-based assessment of spatial and non-spatial attentional deficits in Huntington's disease. *Brain* 129: 1137-1151.

61. Lemiere J, Decruyenaere M, Evers-Kiebooms G, Vandenbussche E, Dom R (2004): Cognitive changes in patients with Huntington's disease (HD) and asymptomatic carriers of the HD mutation--a longitudinal follow-up study. *J Neurol* 251: 935-942.

62. Lawrence AD, Watkins LH, Sahakian BJ, Hodges JR, Robbins TW (2000): Visual object and visuospatial cognition in Huntington's disease: implications for information processing in corticostriatal circuits. *Brain* 123 (Pt 7): 1349-1364.

63. Dang A, Garg A, Rataboli PV (2011): Role of zolpidem in the management of insomnia. *CNS Neurosci Ther* 17: 387-397.

64. Sanchez C, Hyttel J (1999): Comparison of the effects of antidepressants and their metabolites on reuptake of biogenic amines and on receptor binding. *Cell Mol Neurobiol* 19: 467-489.

65. Rodriguez R, Kallenbach U, Singer W, Munk MH (2004): Short- and long-term effects of cholinergic modulation on gamma oscillations and response synchronization in the visual cortex. *J Neurosci* 24: 10369-10378.

66. Cong WN, Chadwick W, Wang R, Daimon CM, Cai H, Amma J, et al. (2015): Amitriptyline improves motor function via enhanced neurotrophin signaling and

mitochondrial functions in the murine N171-82Q Huntington disease model. *J Biol Chem* 290: 2728-2743.

67. Jardri R, Medjkane F, Cuisset JM, Vallee L, Delion P, Goeb JL (2007): Huntington's disease presenting as a depressive disorder with psychotic features. *J Am Acad Child Adolesc Psychiatry* 46: 307-308.

Figure legends

Fig. 1. Zolpidem had a transient hypnotic effect in Huntington's disease mice. The hourly amounts of wake (**A**, **D**), NREM sleep (**B**, **E**), and REM sleep (**C**, **F**) are shown in 14-15 weeks old WT (**A**, **B**, **C**) and R6/2 (**D**, **E**, **F**) mice after a single treatment (arrow) with vehicle or zolpidem (20 mg/kg; i.p.) at dark onset (18:00). Data are presented as mean ± SEM. *P < 0.05 compared to vehicle.

Fig. 2. Abnormal increase in REM sleep was partially suppressed by zolpidem in HD mice. Changes in the percentage of wake (**A**), NREM sleep (**B**) and REM sleep (**C**) are shown in WT and R6/2 mice during the 3 h period after treatment with vehicle or zolpidem (5, 10 and 20 mg/kg; i.p.) at dark onset (18:00). Data are presented as mean ± SEM. *P < 0.05 compared to vehicle.

Fig. 3. Zolpidem reduced the abnormal low-gamma EEG oscillations in R6/2 mice by half during NREM sleep. Changes in absolute (**A**, **B**) and relative (**C**, **D**) power values of EEG spectra during NREM sleep as shown in WT (**A**, **C**) and R6/2 (**B**, **D**) mice during the 2 h period after vehicle or zolpidem (5, 10 and 20 mg/kg; i.p.) treatment. Enlarged images of absolute EEG power values in the low-gamma band outlined by the box in **A** and **B** are shown in the insets (**A'**, **B'**). The EEG power spectral values of WT and R6/2 mice were normalized after zolpidem treatment to the mean power spectral values (100 %) of vehicle-treated WT mice (**C**, **D**). Data are shown as mean ± SEM in 1 Hz bins. *P < 0.05 compared to vehicle.

Fig. 4. Zolpidem had no significant effect on REM sleep EEG in Huntington's disease mice. Changes in absolute power values of EEG spectra during REM sleep as shown in R6/2

mice during the 2 h period after vehicle or zolpidem (5, 10 and 20 mg/kg; i.p.) treatment. Enlarged image of absolute EEG power values in the low-gamma band outlined by the box is shown in the inset (**A'**). Data are shown as mean ± SEM in 1 Hz bins.

Fig. 5. Amitriptyline induced NREM sleep and abolished REM sleep for several hours. The hourly amounts of wake (**A**, **D**), NREM sleep (**B**, **E**), and REM sleep (**C**, **F**) are shown in 16-17 weeks old WT (**A**, **B**, **C**) and R6/2 (**D**, **E**, **F**) mice after a single treatment (arrow) with vehicle or amitriptyline (20 mg/kg; i.p.) at dark onset (18:00). The dark period is shown as shaded area. Data are presented as mean ± SEM. **P* < 0.05 compared to vehicle.

Fig. 6. Amitriptyline increased NREM sleep and reduced REM sleep in a dose dependent manner. Changes in the percentage of wake (**A**), NREM sleep (**B**) and REM sleep (**C**) are shown in WT and R6/2 mice during the 6 h period after treatment with vehicle or amitriptyline (5, 10 and 20 mg/kg; i.p.) at dark onset (18:00). Data are presented as mean ± SEM. **P* < 0.05 compared to vehicle.

Fig. 7. Amitriptyline normalized the irregular low-gamma EEG oscillations seen in R6/2 mice. Changes in absolute (**A**, **B**) and relative (**C**, **D**) power values of EEG spectra during NREM sleep as shown in WT (**A**, **C**) and R6/2 (**B**, **D**) mice during the 3 h period after vehicle or amitriptyline (5, 10 and 20 mg/kg; i.p.) treatment. Enlarged images of absolute EEG power values in the low-gamma band outlined by the box in **A** and **B** are shown in the insets (**A'**, **B'**). The EEG power spectral values of WT and R6/2 mice were normalized after amitriptyline treatment to the mean power spectral values (100 %) of vehicle-treated WT mice (**C**, **D**). Data are shown as mean ± SEM in 1 Hz bins. **P* < 0.05 compared to vehicle.

Fig. 8. **Putative changes in sleep-regulatory neural circuitry in R6/2 mice.** (**A**) Globus pallidus (GP) neurons that are most active during wake and REM sleep receive inhibitory inputs from the striatum. GABAergic neurons in the external segment of GP (GPe) project directly to the cortex. (**B**) In symptomatic R6/2 mice, striatal neurons are hyperactive and GABAergic neurotransmission is decreased in GP. As a result, increased cortical activity occurs in R6/2 mice regardless of vigilance state that could account for the sleep and EEG abnormalities seen in these mice (see text for further details). GPi, internal globus pallidus.

Table 1. Vigilance state parameters in R6/2 and WT mice after vehicle or zolpidem treatment.

Genotype	WT				R6/2 250			
Dose	Veh (n = 7)	5 mg/kg (n = 7)	10 mg/kg (n = 7)	20 mg/kg (n = 7)	Veh (n = 9)	5 mg/kg (n = 7)	10 mg/kg (n = 9)	20 mg/kg (n = 8)
Mean bout duration (min)								
WAKE	6.1 ± 1.1	4.7 ± 1.3	3.7 ± 0.6	3.2 ± 0.6[a]	5.0 ± 0.5	4.1 ± 0.4	3.0 ± 0.2	3.1 ± 0.4
NREM sleep	2.6 ± 0.5	3.5 ± 0.4	3.9 ± 0.3	5.5 ± 0.9[a]	3.0 ± 0.2	4.0 ± 0.5	4.2 ± 0.4	4.3 ± 0.4
REM sleep	1.3 ± 0.4	0.9 ± 0.1	1.0 ± 0.4	0.5 ± 0.2	1.3 ± 0.1	1.5 ± 0.1	1.0 ± 0.1	1.1 ± 0.1
Number of bouts								
WAKE	21.7 ± 2.2	25.6 ± 3.8	24.4 ± 2.4	22.9 ± 3.0	19.8 ± 1.4	19.9 ± 2.4	22.8 ± 1.5	22.2 ± 1.3
NREM sleep	22.4 ± 2.5	25.9 ± 3.7	25.0 ± 2.4	23.3 ± 2.9	24.8 ± 1.4	24.5 ± 2.4	26.1 ± 1.5	24.9 ± 1.4
REM sleep	1.7 ± 0.6	1.3 ± 0.5	1.0 ± 0.4	1.3 ± 0.6	9.2 ± 1.2	7.8 ± 1.1	7.2 ± 1.0	6.1 ± 1.0

Mean duration and number of bouts in each state during the first 3 hours after vehicle or zolpidem treatment. Results shown as mean ± SEM. [a]$P < 0.05$ compared to vehicle (Veh) treatment of the same genotype.

Table 2. Vigilance state parameters in R6/2 and WT mice after vehicle or amitriptyline treatment.

Genotype	WT				R6/2 250			
Dose	Veh (n = 7)	5 mg/kg (n = 6)	10 mg/kg (n = 6)	20 mg/kg (n = 7)	Veh (n = 6)	5 mg/kg (n = 6)	10 mg/kg (n = 6)	20 mg/kg (n = 6)
Mean bout duration (min)								
WAKE	5.2 ± 0.7	4.9 ± 0.9	3.0 ± 0.4	1.5 ± 0.3[a]	3.1 ± 0.4	3.9 ± 0.3	2.3 ± 0.4	1.7 ± 0.2
NREM sleep	3.3 ± 0.2	4.8 ± 0.4	3.9 ± 0.4	4.6 ± 0.5	2.8 ± 0.4	3.9 ± 0.3	4.7 ± 0.6	6.4 ± 0.7[a]
REM sleep	1.1 ± 0.2	0.9 ± 0.2	1.2 ± 0.5	0.3 ± 0.2	1.5 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	1.1 ± 0.2
Number of bouts								
WAKE	42.9 ± 4.0	36.3 ± 3.0	52.5 ± 3.2	61.4 ± 5.6	55.8 ± 9.3	38.2 ± 3.8	46.2 ± 4.6	43.8 ± 4.3
NREM sleep	45.3 ± 3.7	38.5 ± 2.4	53.3 ± 3.6	61.6 ± 5.4	66.0 ± 8.4	49.5 ± 2.8	53.2 ± 4.2	47.0 ± 4.7
REM sleep	5.6 ± 1.2	3.5 ± 1.3	1.7 ± 0.7	0.6 ± 0.3	20.8 ± 2.6	16.8 ± 1.5	11.7 ± 1.9[a]	3.3 ± 0.8[a]

Mean duration and number of bouts in each state during the first 6 hours after vehicle or amitriptyline treatment. Results shown as mean ± SEM. [a]$P < 0.05$ compared to vehicle (Veh) treatment of the same genotype.

Fig 1

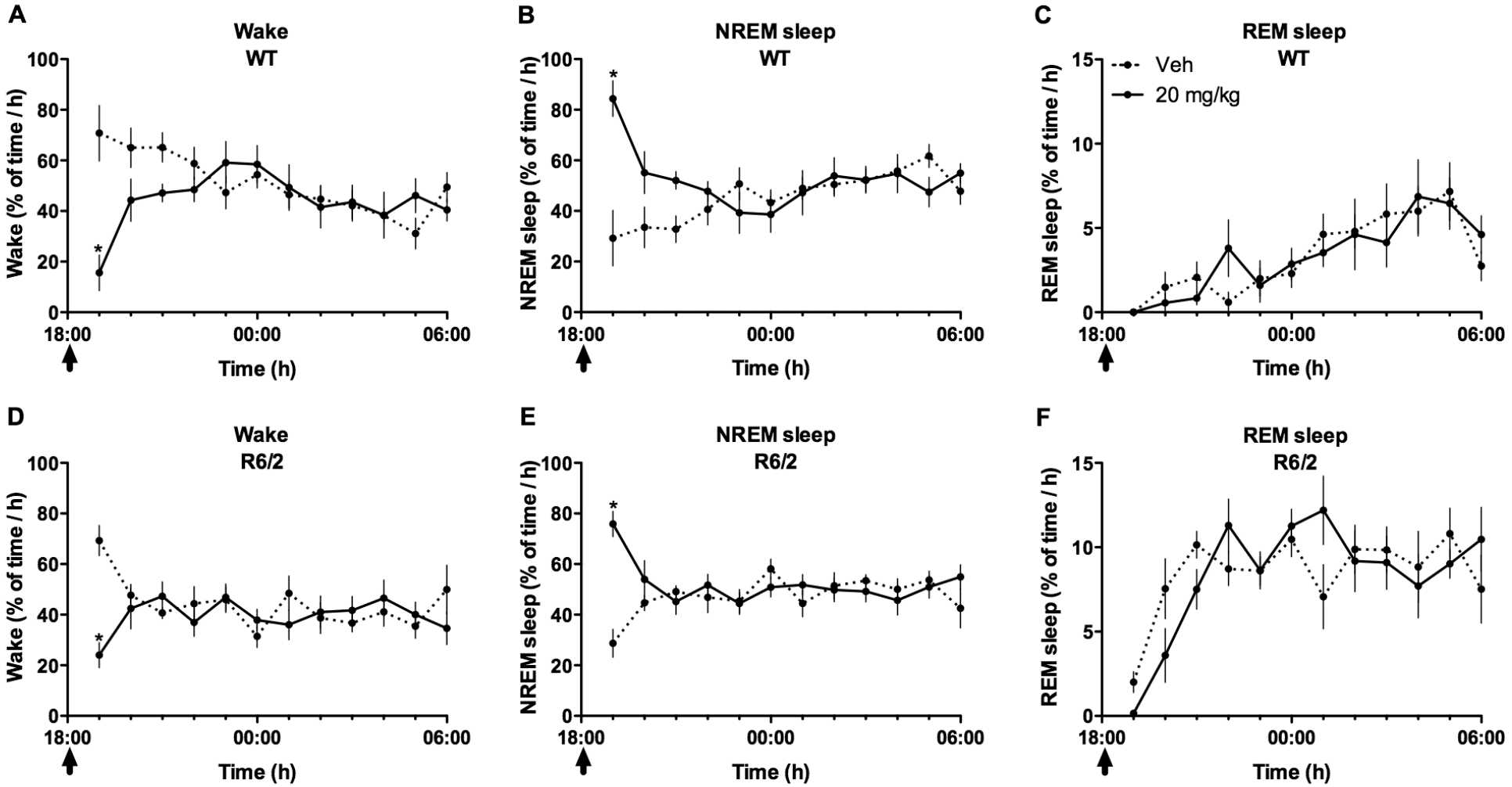

A
Wake
WT
Wake (% of time / h)
Time (h)
18:00
00:00
06:00
B
NREM sleep
WT
NREM sleep (% of time / h)
C
REM sleep
WT
Veh
20 mg/kg
REM sleep (% of time / h)
D
Wake
R6/2
E
NREM sleep
R6/2
F
REM sleep
R6/2

Fig 2

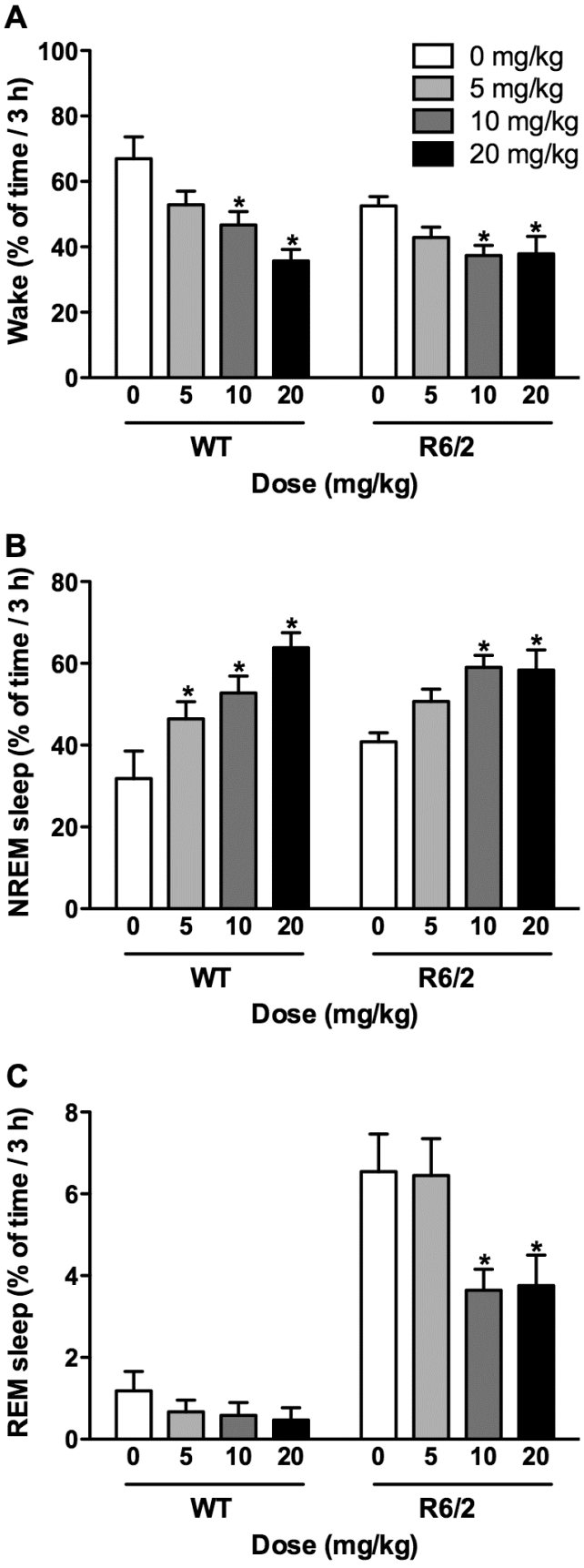

A
Wake (% of time / 3 h)
100
80
60
40
20
0
0 mg/kg
5 mg/kg
10 mg/kg
20 mg/kg
*
0 5 10 20 0 5 10 20
WT
R6/2
Dose (mg/kg)
B
NREM sleep (% of time / 3 h)
80
60
40
20
0
*
0 5 10 20 0 5 10 20
WT
R6/2
Dose (mg/kg)
C
REM sleep (% of time / 3 h)
8
6
4
2
0
*
0 5 10 20 0 5 10 20
WT
R6/2
Dose (mg/kg)

Fig 3

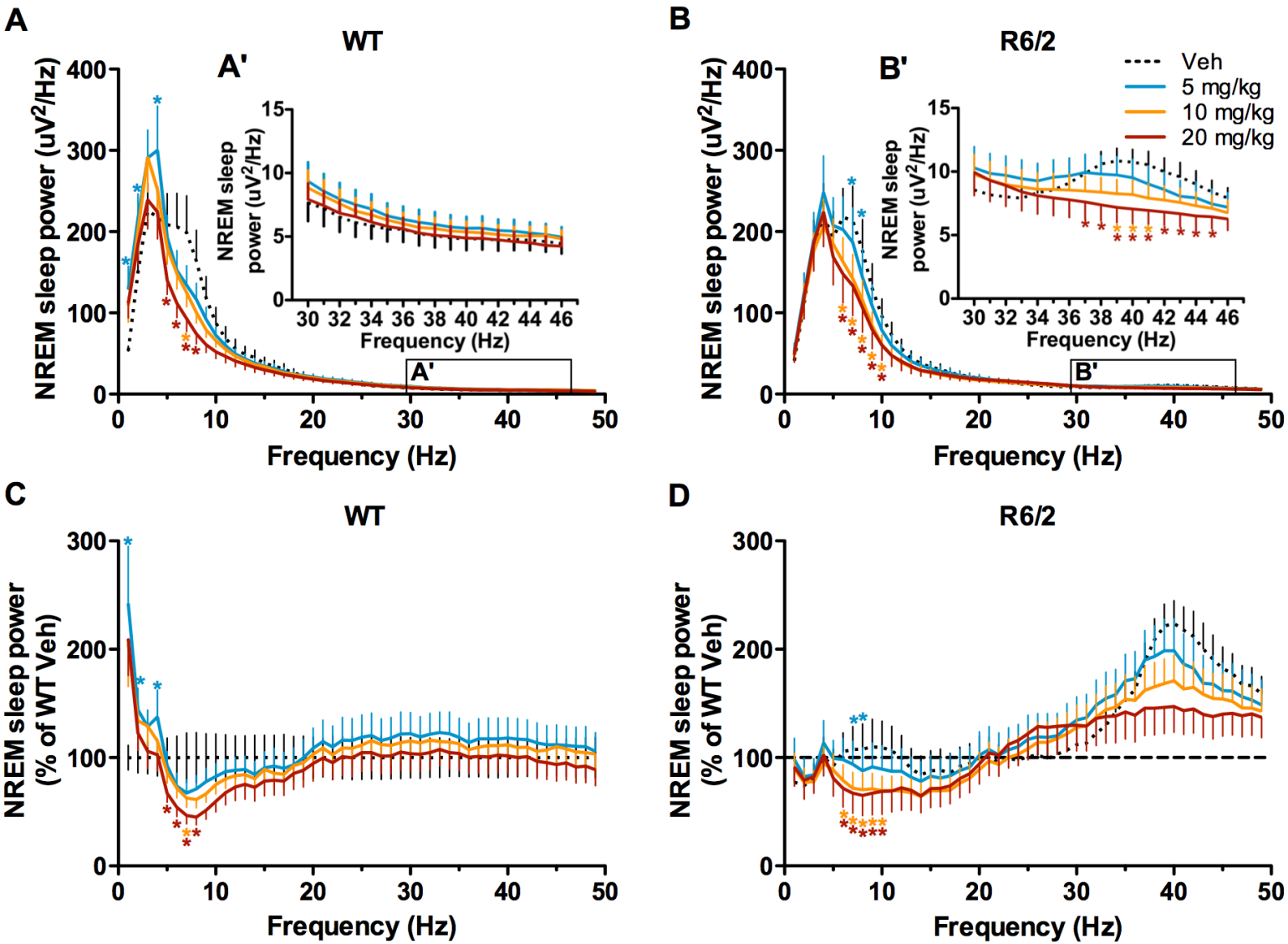

A
WT
A'
NREM sleep power (uV²/Hz)
Frequency (Hz)
NREM sleep power (uV²/Hz)
Frequency (Hz)
A'
B
R6/2
B'
Veh
5 mg/kg
10 mg/kg
20 mg/kg
NREM sleep power (uV²/Hz)
Frequency (Hz)
NREM sleep power (uV²/Hz)
Frequency (Hz)
B'
C
WT
NREM sleep power (% of WT Veh)
Frequency (Hz)
D
R6/2
NREM sleep power (% of WT Veh)
Frequency (Hz)

Fig 4

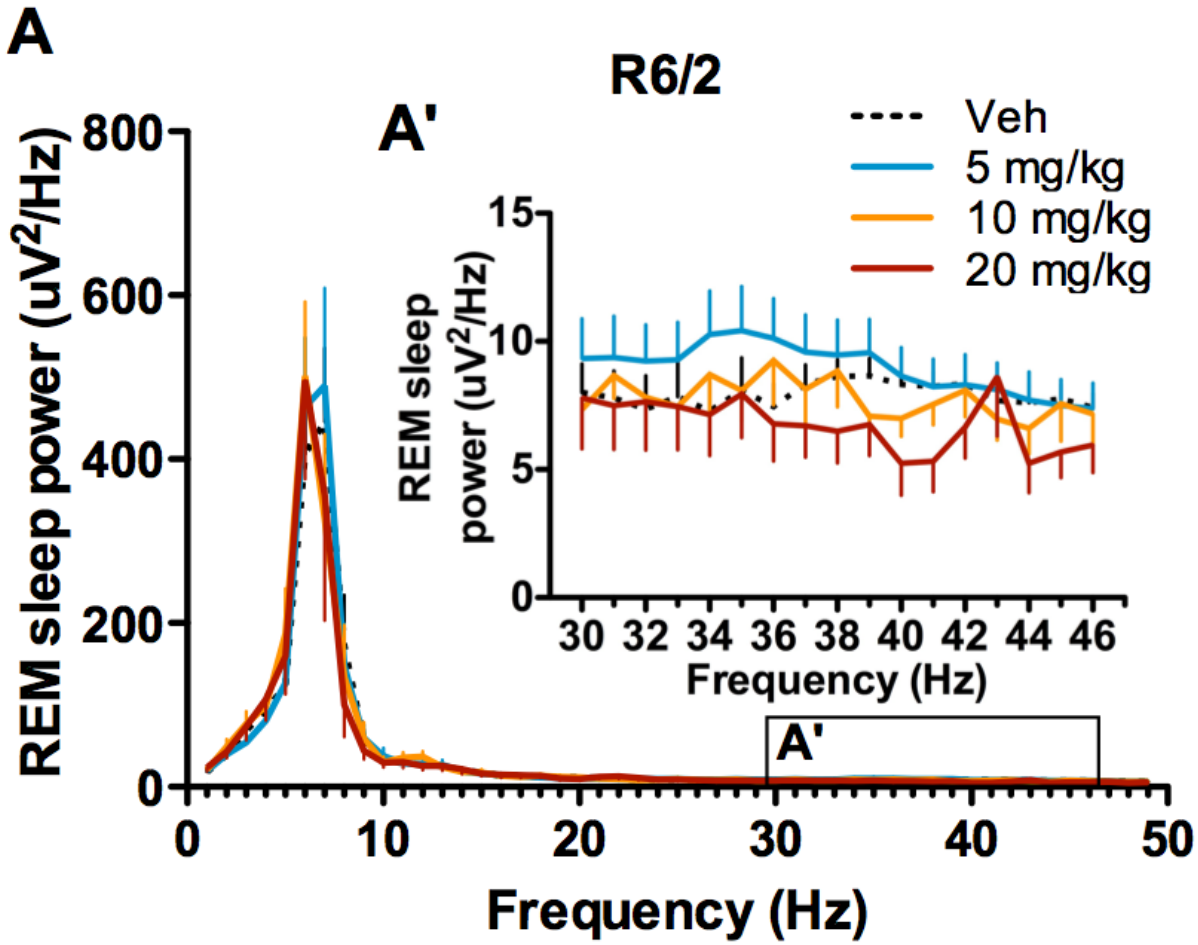

A
R6/2
A'
Veh
5 mg/kg
10 mg/kg
20 mg/kg
REM sleep power (uV²/Hz)
800
600
400
200
0
0
10
20
30
40
50
Frequency (Hz)
REM sleep power (uV²/Hz)
15
10
5
0
30 32 34 36 38 40 42 44 46
Frequency (Hz)
A'

Fig 5

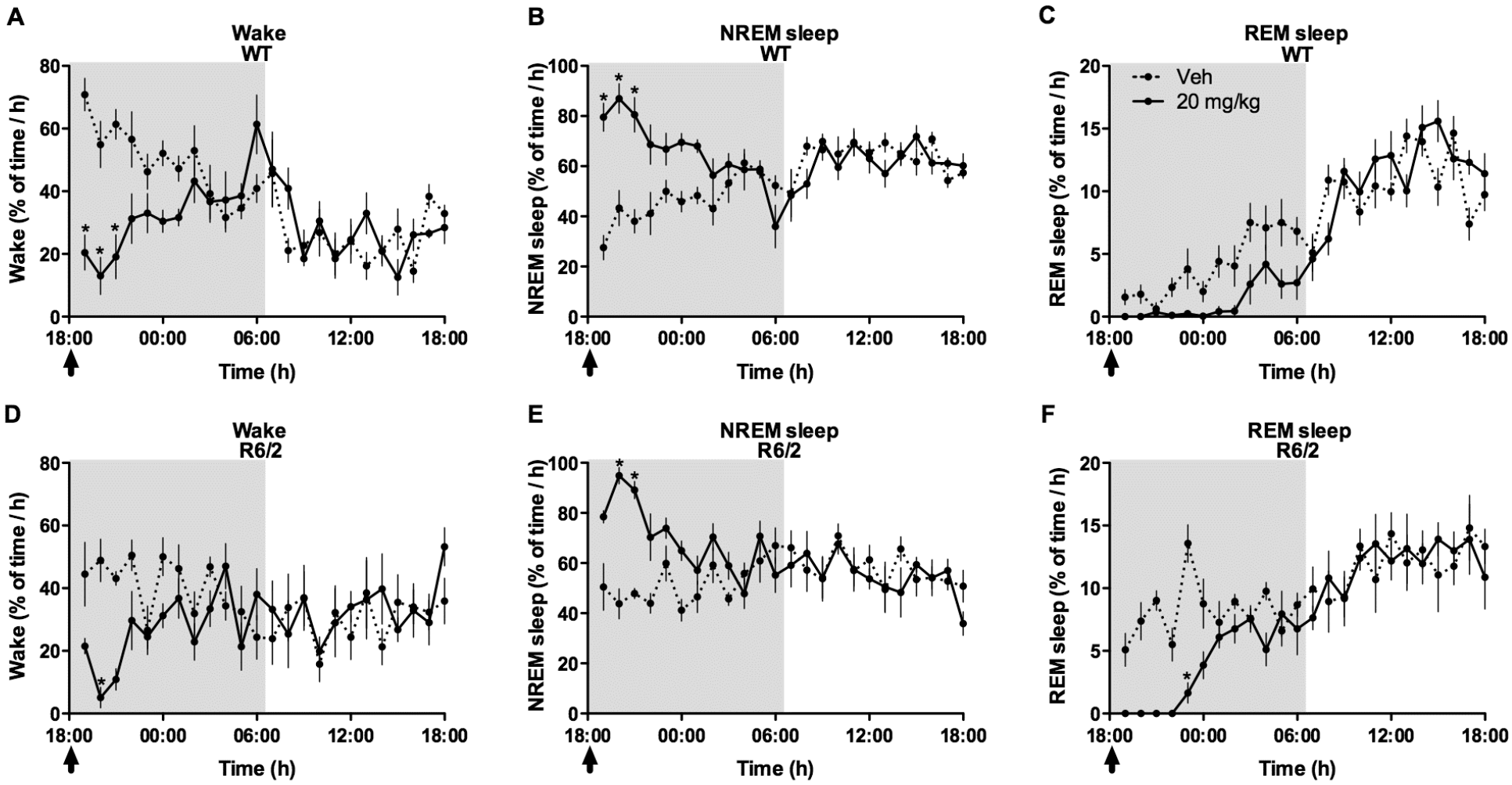
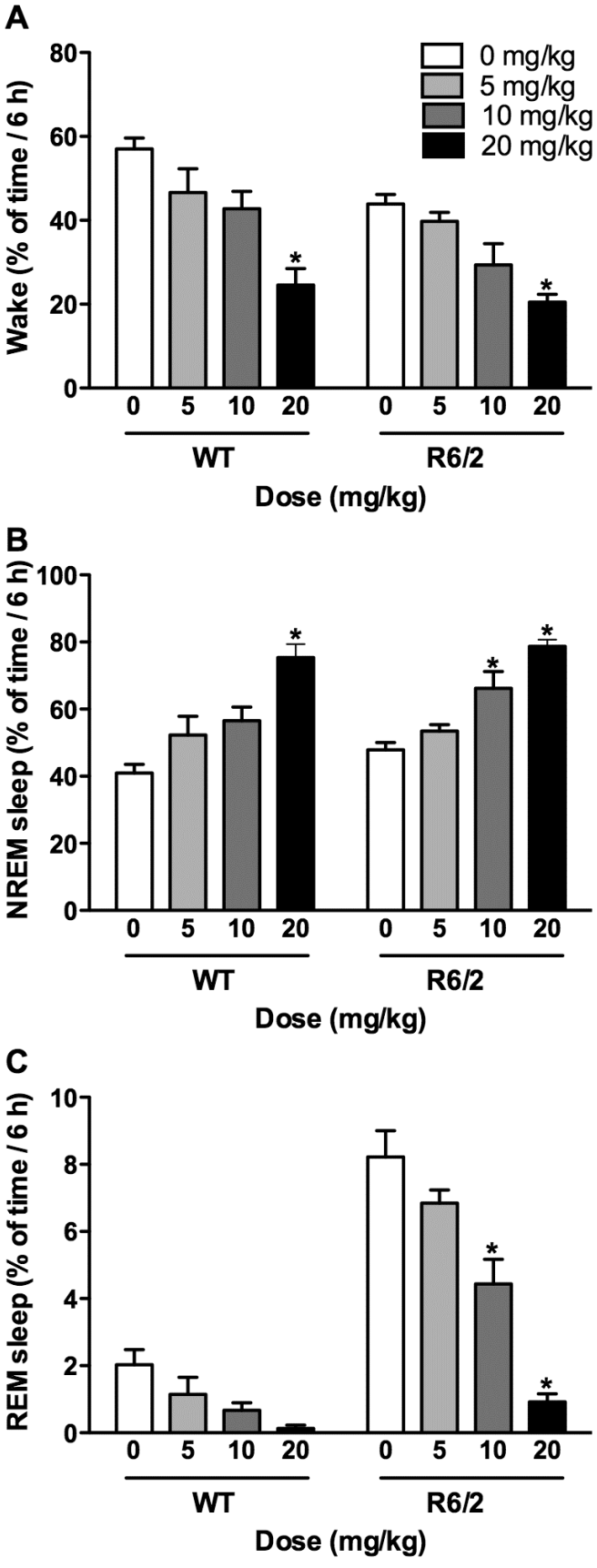

A
Wake
WT
Wake (% of time / h)
B
NREM sleep
WT
NREM sleep (% of time / h)
C
REM sleep
WT
REM sleep (% of time / h)
Veh
20 mg/kg
D
Wake
R6/2
Wake (% of time / h)
E
NREM sleep
R6/2
NREM sleep (% of time / h)
F
REM sleep
R6/2
REM sleep (% of time / h)
18:00
00:00
06:00
12:00
18:00
Time (h)

Fig 6


A
Wake (% of time / 6 h)
0 mg/kg
5 mg/kg
10 mg/kg
20 mg/kg
0
20
40
60
80
0 5 10 20 0 5 10 20
WT
R6/2
Dose (mg/kg)
B
NREM sleep (% of time / 6 h)
0
20
40
60
80
100
0 5 10 20 0 5 10 20
WT
R6/2
Dose (mg/kg)
C
REM sleep (% of time / 6 h)
0
2
4
6
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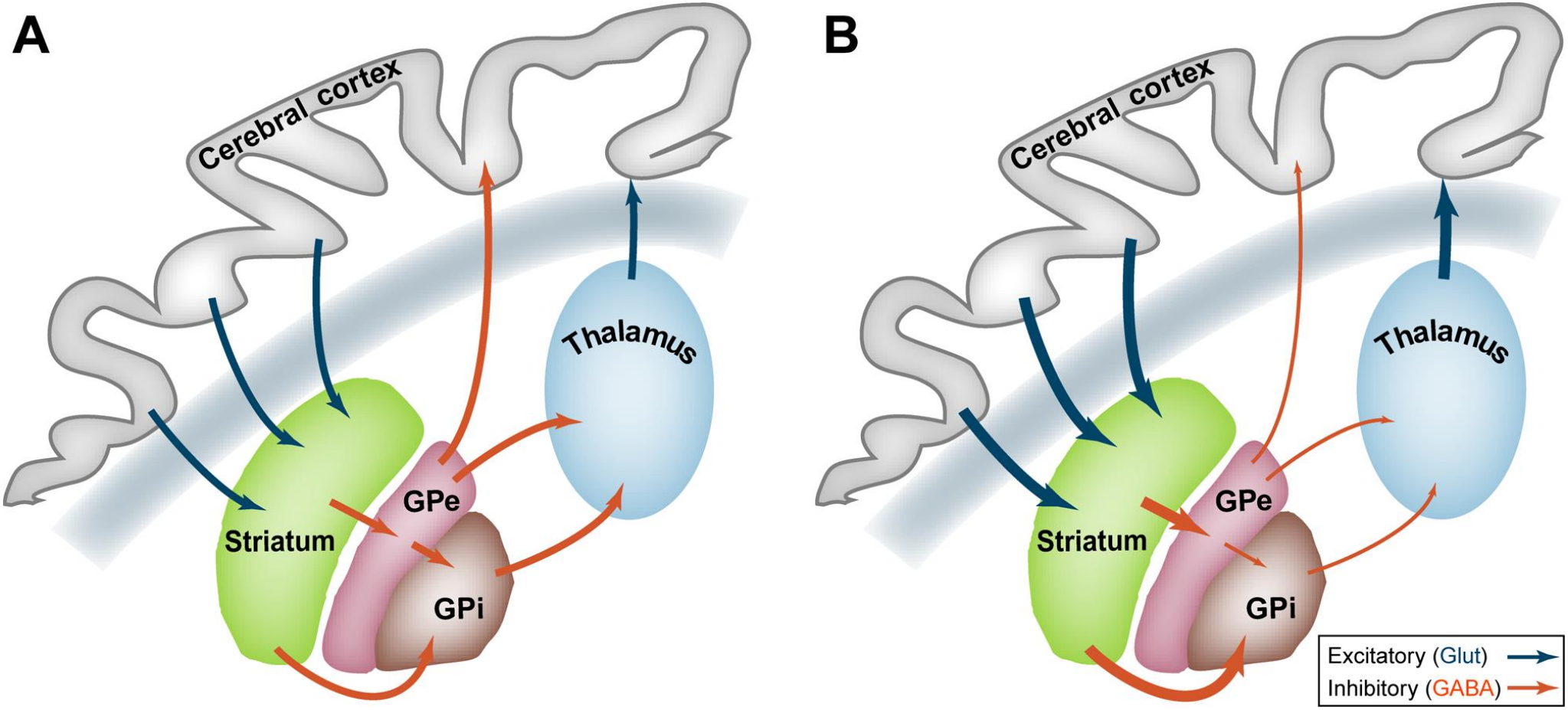
0 5 10 20 0 5 10 20
WT
R6/2
Dose (mg/kg)

Fig 7


A
Cerebral cortex
Thalamus
Striatum
GPe
GPi
B
Cerebral cortex
Thalamus
Striatum
GPe
GPi
Excitatory (Glut)
Inhibitory (GABA)