Sleep disturbance by pramipexole is modified by Meis1 in mice

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SUMMARY

Meis homeobox 1 (Meis1) is a transcription factor functioning in the development of the nervous system and the cardiovascular system. Both common and rare variants within the gene have been associated with restless legs syndrome (RLS), while its association with symptoms of insomnia has also been discovered recently. RLS is associated with sleep disturbances, and while Meis1 haploinsufficiency is one of the most promising strategies for an RLS animal model, sleep phenotyping of *Meis1* knockout mice has never been conducted. We report a detailed sleep analysis of heterozygous *Meis1* knockout mice and challenge it with pramipexole, a dopamine agonist used in the treatment of RLS. At baseline, the *Meis1*-haploinsufficient mice had a trend towards lower delta power in the electroencephalogram (EEG) during sleep compared to the wild-type littermates, possibly indicating reduced sleep quality, but not sleep fragmentation. Pramipexole had a sleep disrupting effect in both genotype groups. In addition, it exerted differential effects on the EEG power spectra of the two mouse lines, remarkably elevating the theta power of the mutant mice during recovery more than that of the wild-types. In conclusion, *Meis1* haploinsufficiency seems to have only a modest effect on sleep, but the gene may interact with the sleep-disrupting effect of dopamine agonists.

INTRODUCTION

Meis homeobox 1 (Meis1) is a three-amino acid loop extension (TALE) homeobox transcription factor, known to play an important role in the development of the nervous system (Barber *et al.*, 2013; Spieler *et al.*, 2014), the proximodistal limb axes (Mercader *et al.*, 1999) and various other organs, such as the heart (Mahmoud *et al.*, 2013). Pathologically, MEIS1 is associated consistently with restless legs syndrome (RLS) (Winkelmann *et al.*, 2007, 2011) and was recently also found to be associated with the symptoms of insomnia (Lane *et al.*, 2016). RLS is a neurological movement disorder affecting up to 10% of the population (Garcia-Borreguero *et al.*, 2006). RLS manifests as an urge to move the legs in the evening or during the night, forcing the affected person to stand up and walk (Trenkwalder and Paulus, 2010). During drowsiness and shallow non-rapid eye movement (NREM) sleep, most RLS patients have rhythmical leg jerks, periodic leg movements during sleep (PLMS), causing intermittent arousals from sleep (Ferri *et al.*, 2007). This may lead to sleep fragmentation or reduced sleep efficiency with an overall reduction of NREM sleep (Hornyak *et al.*, 2007; Montplaisir *et al.*, 1997).

The *MEIS1* intronic RLS risk haplotype is associated with decreased MEIS1 expression (Xiong *et al.*, 2009). The same signal is associated with the symptoms of insomnia (Lane *et al.*, 2016). Therefore, a haploinsufficiency model of *Meis1* is a potential animal model for RLS and insomnia. A heterozygous *Meis1* knockout mouse (Meis1^{tm1Mtor}) (Azcoitia *et al.*, 2005), where Meis1 has been inactivated by knocking in a modified ERT2 domain, has been shown to display a hyperactive phenotype (Spieler *et al.*, 2014), compatible with symptoms also seen in human RLS. Sleep disturbance, one of the key hallmarks of human RLS, could be regarded as a

potential readout to investigate RLS in an animal model (Manconi *et al*., 2007b). Sleep of the heterozygous Meis1 knockout mice has never been investigated.

In clinical practice, RLS is treated most commonly with dopamine agonists such as pramipexole (Trenkwalder and Paulus, 2010), also used frequently in the treatment of Parkinson's disease (PD). Pramipexole has a property of preferential binding to the D_3 dopamine receptor subtype. In patients with RLS, while pramipexole suppresses PLMS, it may even increase sleep fragmentation (Garcia-Borreguero *et al*., 2014). However, in the absence of PLMS, the spectral electroencephalography (EEG) profile is normal in RLS during sleep (Hornyak *et al*., 2005). Alternatively, pergolide, an ergoline-based dopamine agonist, reduces the delta and sigma power in EEG while increasing the amount of Stage 2 sleep (Tagaya *et al*., 2002), suggesting a possible interference by dopamine with the gamma-aminobutyric acid (GABA)-mediated thalamocortical generation of EEG delta oscillation (Zhang *et al*., 2009). In rats, pramipexole has also been demonstrated to have a moderate sleep-destabilizing effect during the first 2 h after administration (Lagos *et al*., 1998).

In this study, aiming to model RLS in a mouse model, we investigated the effect of Meis1 deficiency on sleep architecture and sleep responses to dopaminergic treatment in rodents. A detailed sleep phenotyping analysis is an integral part of the validation process to consider *Meis1* knockout mice as an RLS animal model. At the same time, we provide a detailed analysis on the effect of pramipexole on sleep in mice.

MATERIALS AND METHODS

All animal experiments carried out here were in accordance with the guidance of the European Community Council Directive, and the protocol for the ethical treatment of animals was approved by the local commission for the Care and Use of Laboratory Animals of the Regierung von Oberbayern (Government of Upper Bavaria).

Experimental animals

The original Meis1^{tm1Mtor} mice used in the experiments were generated in Dr Torres's laboratory in Madrid, Spain (Azcoitia *et al*., 2005) on a C57BL/6JOlaHsd background. The mouse line harbours an in-frame insertion of the ERT2 domain within the coding region of *Meis1*, resulting in a non-functional Meis1 protein in the absence of tamoxifen. We obtained these mice to breed heterozygous mice and their wild-type littermates in Helmholtz Zentrum München, Munich, Germany, housed under a standard 12-h light–dark cycle in a pathogen-free environment. The transgenic line was maintained by backcrossing it to wild-type C57BL/6JOlaHsd every generation for 9–10 generations. Two weeks before being subjected to surgical operation, the mice were transferred to the breeding facility of the Max Planck Institute of Psychiatry, Munich, Germany, and prior to surgery they were housed individually in a custom-made Plexiglas cage located in a sound-attenuated recording chamber (light intensity 100 l×, temperature 24 ± 1°C, lights on at 09:00 hours). During the entire time, the mice had *ad-libitum* access to feed and water.

A cohort of 10 male heterozygous Meis1 knockout mice and 10 male wild-type littermates was used for the sleep recordings. In order to prevent a batch effect, the mice were monitored simultaneously together with their corresponding wild-type littermates.

Surgery

The mice underwent surgery at the age of 18–20 weeks with inhalation anaesthesia using an isoflurane/oxygen mixture (isoflurane; DeltaSelect, Dreieich, Germany). Fixed with a stereotaxic frame, they were implanted chronically with electrodes to achieve polysomnographic analysis, as described earlier (Kumar *et al*., 2015; Romanowski *et al*., 2010). Four gold wires (0.25–0.30 mm diameter) to record EEG were inserted through the skull and placed epidurally over the frontal and parietal cortices (coordinates, A 1.5 and 3 mm, L ± 1.7 mm each). An additional two gold wires were implanted into the cervical part of the trapezoid muscles to record neck electromyogram (EMG). All electrodes were soldered to a micro-socket, which was fixed to the skull with dental acrylic resin. The animals received atropine sulphate subcutaneously (0.025 mg kg^{-1}, atropine; Braun Melsungen, Melsungen, Germany) to stabilize circulation, as well as meloxicam (1.0 mg kg^{-1}; Metacam, Braun Melsungen) as post-operative analgesic. After surgery, the mice were allowed to spend 2 weeks for recovery in their respective home cage before baseline recordings.

Sleep recordings

The implant of EEG and EMG electrodes was connected through a flexible tether to an electric swivel whose weight was counterbalanced by a mechanical device above each cage. Therefore, the mice could be acclimated to the recording setup during the recovery period and move almost without restriction. EEG and EMG signals were amplified (×10 000); while the EEG signal was filtered (0.25–64 Hz), filtered EMG signals (175–1000 Hz) underwent root mean square rectification. Both signals were digitized by a high-speed analogue-to-digital converter (NI-USB-6343-X-series; National Instruments, Austin, TX, USA) at a sampling rate of 128 Hz. The signals were then processed with a LabVIEW (National Instruments)-based software designed specifically for mouse sleep EEG/EMG analysis (EGEraVigilanz; S.E.A., Cologne, Germany). The data were stored on a personal computer (PC), and analysis was performed offline.

Experimental protocol

A single dose of saline as vehicle, either alone or containing a low or high dose (0.3 and 3 mg kg^{-1}, respectively) of

pramipexole (pramipexole dihydrochloride; Sigma Aldrich, St Louis, MO, USA) was injected subcutaneously (volume 5 μL g^{-1} body weight) at the beginning of the inactive period of the animals [09:00 hours, Zeitgeber time (ZT) 0]. Any potential failure in injection was recorded. After injection, the animals were replaced into the cage immediately for recording.

Animals were divided into two groups: half the animals received saline injection before low-dose PPX, whereas the other half received low-dose PPX first. All animals received the high dose of PPX last after an additional saline injection. At least 2 days of recovery was always allowed between each injection, while injections with the low and high doses were separated for more than 6 days. All animals were killed after the high-dose PPX recordings to reconfirm their genotype.

Data analysis

The polygraphic data were processed with LabVIEW-based acquisition software. A fast-Fourier transform (FFT) algorithm was used for the power spectrum analysis of EEG across various frequency ranges, i.e. the delta (0.5–5 Hz), theta (6–9 Hz), sigma (10–15 Hz), beta (16–32 Hz) and gamma (32-64 Hz) bands. Epochs with poor signal quality or artefacts were eliminated from spectral analysis (< 2%). The occurrence of epochs containing artefacts particularly depends upon neither genotype nor activity of the animals. With an adapted FFT algorithm (Louis *et al.*, 2004), semi-automatic classification of vigilance states as wakefulness, rapid eye movement (REM) or non-REM (NREM) sleep was performed in 4-s epochs, as described elsewhere (Kimura *et al.*, 2010). The defined vigilance states were confirmed further visually and corrected by an experienced sleep scorer. To conduct the power spectrum comparison between two genotypes and its differences in response to drug administration, the mean values per 0.25 Hz bin were calculated for each vigilance state and normalized to the individual average of the total EEG power from all vigilance states across all frequency bins within each epoch (Jakubcakova *et al.*, 2011; Kumar *et al.*, 2015). Sleep–wake architecture was analysed to determine if the entry frequency of each vigilance state (bout number) and the duration of each episode (bout length) were different between two genotypes during both baseline and post-pramipexole conditions. The criteria used to define sleep–wake architecture were based on previous reports (Hu *et al.*, 2011; Opp, 1997), by which we determined the entry and the termination of all vigilance states when two consecutive 4-s epochs were scored as the same state (Jakubcakova *et al.*, 2011). Sleep latency was analysed by measuring time spent after injection until the first observation of a solid NREM sleep episode that lasted for at least 15 epochs, with up to six times brief interruptions (maximum two epochs) according to criteria reported previously (Jakubcakova *et al.*, 2011; Winsky-Sommerer *et al.*, 2007). REM sleep latency was determined by counting epochs until the first appearance of REM sleep after injection.

During the scoring phase, two recordings (one low-dose PPX and one high-dose PPX recording) were discarded from further analysis due to insufficient signal quality. The remaining recordings were all used in the final analysis.

Statistical analysis

At baseline, differences in time–course changes in the amount of each vigilance state, expressed as hourly percentage, and sleep architecture, e.g. bout counts and duration, between genotypes were analysed with two-way analysis of variance (ANOVA) with factors genotype and time, with an interaction term between the two. *Post-hoc* comparison at single time-points was performed using linear regression. Regarding wake-bout counts and duration analysis, a two-tailed *t*-test was also used to evaluate single time-points during the first inactive period every 2-h time bin. NREM and REM sleep latencies were analysed with one-way ANOVA, with genotype as the factor.

Baseline genotype effects on EEG spectra (< 20 Hz) were analysed with one-way ANOVA in each frequency bin of 0.25 Hz, with genotype as a factor. Multiple testing correction was introduced in the EEG spectral analysis by estimating the number of effective tests by a combined simulation/permutation procedure (n = 10 000) and found to be close to a number of 11 (for a total of 80 frequency bins considered due to the high correlation between neighbouring frequency bins). Each test was corrected with this number of 11 tests as well as the number of time bins analysed.

The interaction between the effects of pramipexole and the genotype effects on vigilance states was analysed using linear model, with genotype, time bin, pramipexole dose and all interaction terms as factors. The genotype effect in single 6- and 1-h time bins and single doses was analysed *post-hoc* using a one-way ANOVA with genotype as factor. A linear mixed model was used to analyse the interaction between the effects of pramipexole and the genotype effects on sleep bout duration and counts. Time bin, pramipexole dose and genotype were used as variables. *Post-hoc* comparison at single time bins was performed using an ANOVA on a linear model with factors genotype and pramipexole, including the interaction term. The dose–genotype interaction effect on NREM and REM sleep latencies was estimated using an ANOVA on a linear model with factors genotype, pramipexole dose and the interaction term.

A P-value of < 0.05 was considered statistically significant. All statistical analyses were performed using GraphPad Prism software (version 5.04; GraphPad Software, Inc., La Jolla, CA, USA) or R statistical platform (https://www.r-project.org/).

RESULTS

Effects of reduced Meis1 on 24-h sleep–wake behaviour

Both wild-type and Meis1 heterozygous mice displayed circadian-driven and nocturnally active dynamics in sleep–wake activity as typical behaviour of nocturnal rodents

(Fig. 1a–c). When compared to wildtypes, Meis1 haploinsufficiency had no significant impact on the 24-h time–course of either wakefulness (Fig. 1a, $P = 0.785$), NREM sleep (Fig. 1b, $P = 0.730$) or REM sleep (Fig. 1c, $P = 0.928$) at baseline. Similarly, any genotype effects were not found after saline injection on the amount of each vigilance state (Fig. 1d–i, Supporting information, Table S1), although the injection itself elicited wakefulness in both genotypes for 1-h post-injection time (Fig. 1d,g), with longer sleep latency in wild-types than in heterozygous mice (Table 1).

Next, we injected two different doses (0.3 or 3 mg kg^{-1}) at light onset to examine the effects of this dopaminergic agonist on sleep during their inactive phase. No genotype effect was observed on the pramipexole response when analysed across all time bins and doses. The interaction effect of pramipexole dose and genotype on wakefulness ($P = 0.687$), NREM sleep ($P = 0.740$) and REM sleep ($P = 0.272$) remained subsignificant. However, the two genotypes had a differential effect on the pramipexole effect on NREM sleep latency ($P = 0.021$). The effect on REM sleep latency remained similar for both genotypes ($P = 0.502$). The sleep responses of each injection are shown in Fig 1d–i.

The difference in NREM sleep latency hinted at an immediate, possibly short-lasting, genotype effect on the

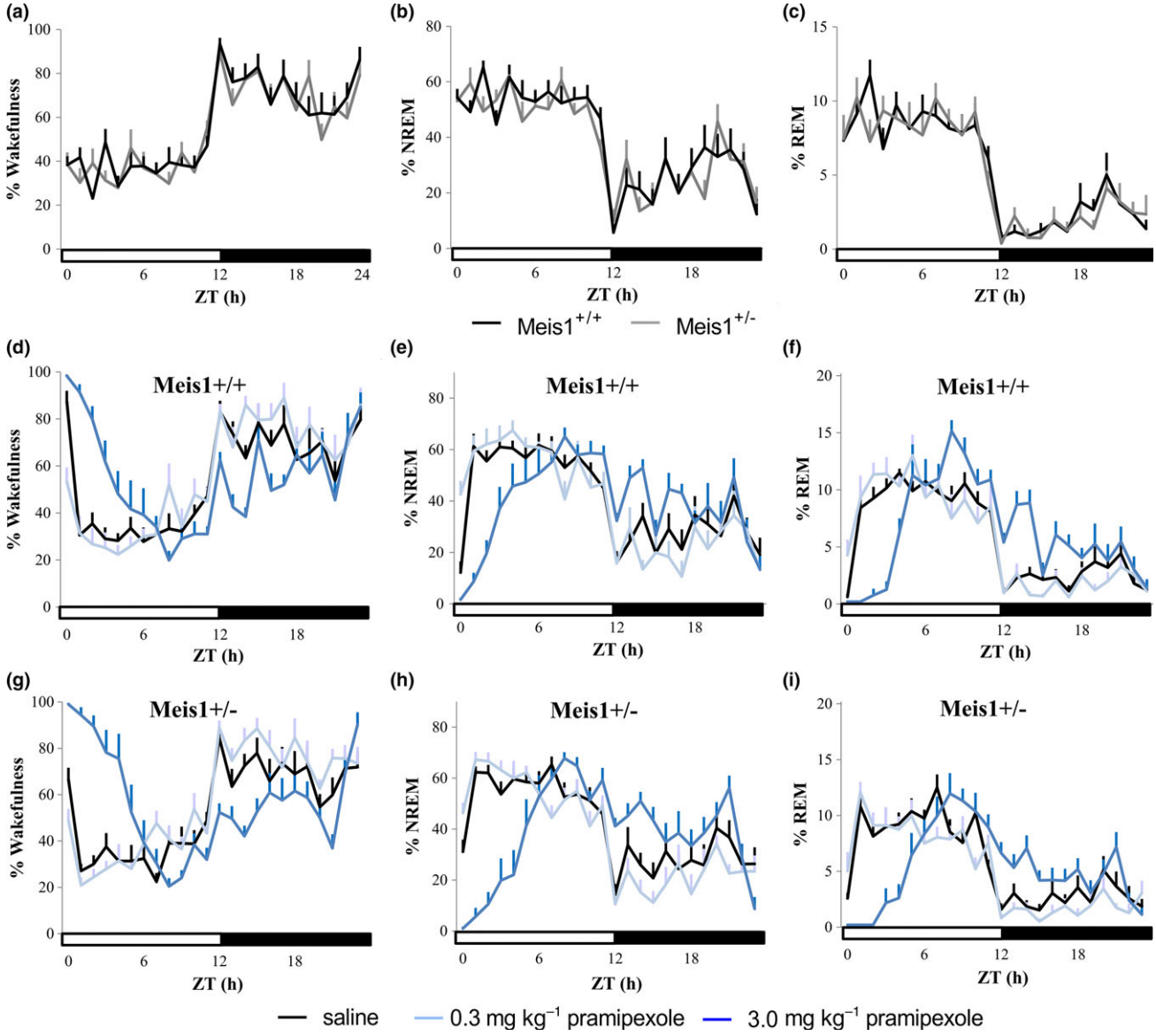

Figure 1. The effects of the genotype and pramipexole interventions on different vigilance states. The upper panels show the baseline effect of Meis1 haploinsufficiency on the percentage of wakefulness (a), non-rapid eye movement (NREM) sleep (b) and REM sleep (c), respectively. The middle and lower panels show the effects of pramipexole on the hourly percentage of each vigilance state in wild-types (d–f) and heterozygous knockout animals (g–i). The administration of pramipexole was performed at light onset, Zeitgeber time (ZT) 0. The open and filled horizontal bars attached on the *x*-axis indicate the light and dark periods, respectively.

Table 1 Non-rapid eye movement (NREM) and REM sleep latencies in minutes, after the control (saline) as well as low (0.3 mg kg^{-1}) and high (3.0 mg kg^{-1}) dose pramipexole injections in both genotype groups

		Latency to NREM sleep (min)			Latency to REM sleep (min)		
Genotype	*n*	Saline	Pramipexole 0.3 mg kg^{-1}	Pramipexole 3.0 mg kg^{-1}	Saline	Pramipexole 0.3 mg kg^{-1}	Pramipexole 3.0 mg kg^{-1}
$Meis1^{+/+}$	9	55.5 ± 5.9	20.4 ± 2.5	129.5 ± 20.3*	78.6 ± 7.5	60.4 ± 8.9	274.1 ± 23.0*
$Meis1^{+/-}$	9	35.5 ± 6.0	16.7 ± 2.3	189.2 ± 27.9*	55.8 ± 5.3	36.8 ± 5.2	281.0 ± 24.0*
P-value		0.031	0.287	0.103	0.025	0.036	0.837

*Statistical significance ($P < 0.01$) in sleep latencies affected by pramipexole within the same genotype (one-way analysis of variance). NREM: non-rapid eye movement.

pramipexole response. Therefore, we analysed the vigilance states in shorter, 6-h time bins separately for each pramipexole dose (Supporting information, Table S1). The saline and low-dose pramipexole injections did not cause a significant change in sleep–wake behaviour in either genotype. After injection of the higher dose of pramipexole, however, sleep was suppressed significantly in both genotypes in the first 6-h time bin ($P < 0.01$), and consequently increased at the beginning of the dark phase ($P < 0.01$). The Meis1 mutants had more wakefulness (252.7 ± 15.2 min $Meis1^{+/+}$ versus 293.3 ± 13.6 min $Meis1^{+/-}$, $P = 0.048$) and less NREM sleep (96.1 ± 13.1 min $Meis1^{+/+}$ versus 60.3 ± 12.0 min $Meis1^{+/-}$, $P = 0.046$) compared to the wild-type controls. Further dissection of the first 6 h of recording after the high-dose injection into 1-h time bins did not reveal significant genotype effects.

Effects of Meis1 and pramipexole on sleep–wake architecture

To evaluate any further influence of reduced Meis1 on sleep parameters, as a next step sleep architecture (bout duration and bout counts) was analysed in 2-h time bins. The analysis revealed no baseline differences between the two genotype groups (Fig. 2a,b). Meanwhile, saline injection reduced the count of wake bouts significantly but prolonged the wake bout duration more in the wild-type group compared to the mutant group during the first 2 h (Fig. 2, $P = 0.028$ by *post-hoc* analysis); this might have been caused by a type of injection stress, which appeared more strongly in wild types. In either case, Meis1 haploinsufficiency by itself did not cause sleep fragmentation.

When analysed through all genotypes and time bins, the Meis1 genotype groups reacted differently to the injection of pramipexole. An interaction effect of genotype and pramipexole dose on bout count was not observed ($P = 0.123$). However, the interaction effect was present on bout duration ($P = 0.007$).

When dissected into 2-h time bins, although saline injection itself reduced the wake bout counts, the low dose of pramipexole increased the number of wakefulness bouts during the first 2 h of the inactive period, suggesting increased sleep fragmentation in both groups. During the rest of the inactive period, as well as the entire active period, the sleep/wake bout counts were unchanged. On the contrary, the high dose of pramipexole extended the wake bout duration drastically, and in return decreased the sleep/wake bout counts during the first part of the inactive period in both genotype groups, suggesting dramatically solid sleep suppression. Although the pramipexole effect on wakefulness per bout counts was not significantly dependent upon genotype in any time bin (Fig. 2c,e), the effect of pramipexole on wake-bout duration appeared more pronouncedly in the Meis1 mutant group during the first two time bins (Fig. 2d,f, $P = 0.0028$ in ZT 0–2, $P = 0.0052$ in ZT 2–4).

Meis1 haploinsufficiency affects EEG power spectra

Meis1 knockout animals had a tendency to show lower EEG power in the low-frequency bands (< 4 Hz) during NREM sleep and wakefulness (Fig. 3a,b, representative EEG power spectra during ZT4–6). Interestingly, this was accompanied with slightly increased power in the theta activity (5–7 Hz) in the mutant mice. The trend was observed throughout the entire 24-h recording. However, after controlling for multiple testing, none of the time bins showed statistical significance at baseline during wakefulness, NREM sleep or REM sleep (Fig. 3a–c).

No time bin during wakefulness or sleep showed a statistically significant genotype effect after the saline injection either, and the spectra were not different from the baseline recording. The low dose of pramipexole had no marked effects on the EEG spectra during NREM and REM sleep in either genotype group (Fig. 3d for NREM sleep). During wakefulness, the low dose increased the EEG power in the delta and theta bands immediately after the injection (Supporting information, Fig. S1). No marked effect was seen in later time bins during the inactive period. No genotype-dependent differences in the drug effect were observed at this dose during sleep or wakefulness.

In contrast, the higher dose of pramipexole showed much more prominent, genotype-dependent effects on the EEG spectra. During inactive period wakefulness, the peak of the spectrum was shifted towards higher frequencies in both

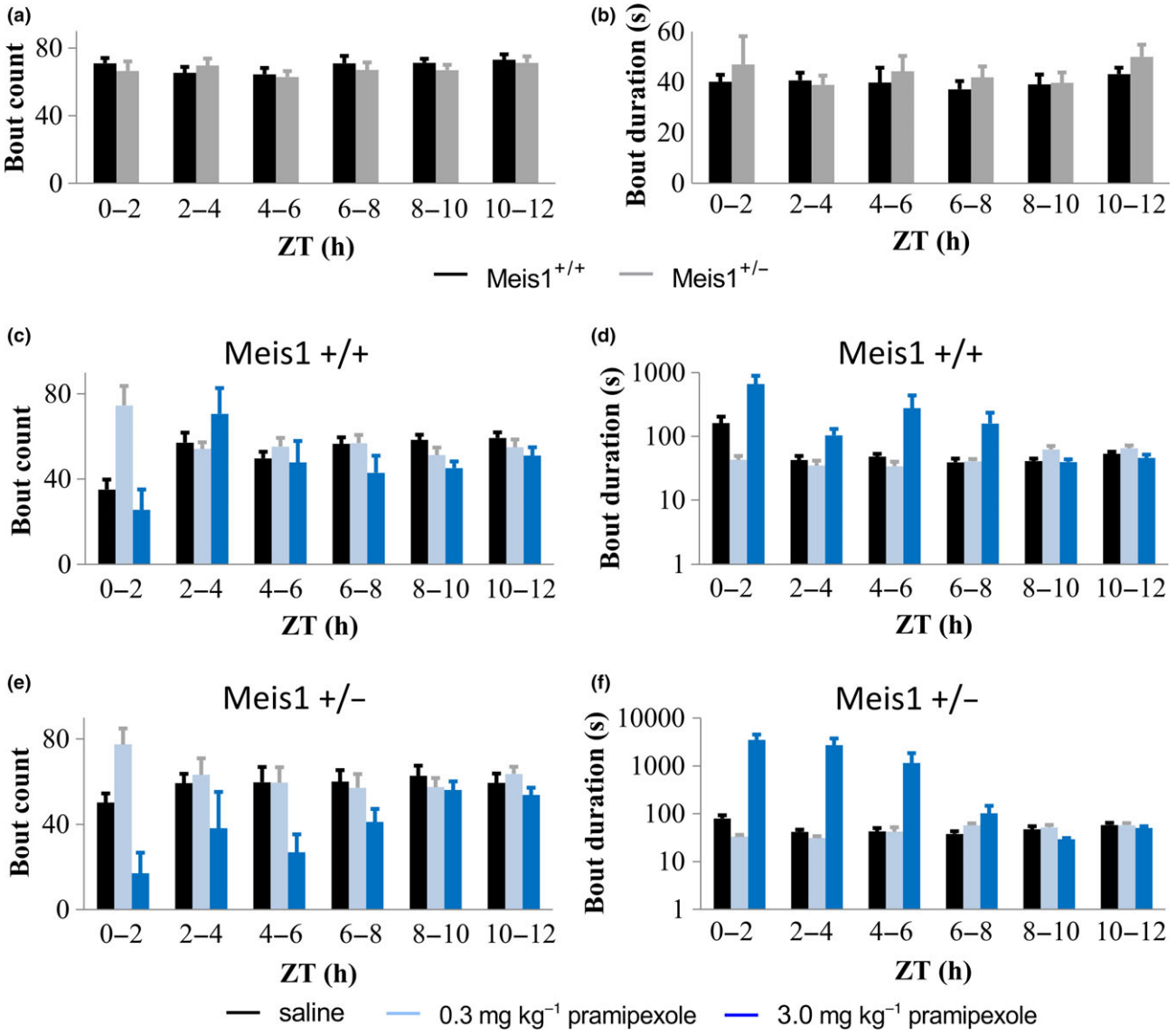

Figure 2. The count and duration of wakefulness bouts during the inactive period at baseline (a,b) and after pramipexole injections (c–f). Black bars represent wild-type controls, whereas grey bars represent heterozygous animals (a,b). The response to pramipexole (c–f; low dose in light blue, high dose in darker blue, saline in black bars) differed between the two genotypes in the first two time bins of the bout duration analysis (ZT 0–2, $P = 0.0028$, ZT 2–4, $P = 0.0052$). ZT: Zeitgeber time, presented in 2-h time bins from the start of the recording.

genotype groups, with higher power in the theta band (Supporting information, Fig. S1). During NREM sleep, the peak tended to shift even more prominently towards higher frequencies, with increased power across the theta and sigma bands (Fig. 3d, especially EEG power spectra during ZT4–6). This effect appeared more obviously in the mutant group compared to wild-types (Fig. 3d), as the EEG power was higher than in the wild-type animals in two time bins (ZT 2–4 at 8.75 Hz and ZT 4–6 within the frequency range 7.5–9.25 Hz, $P < 0.05$ after correction for multiple testing). This indicates that Meis1 interacts with the dopaminergic agonist to influence the EEG synchronization activity. No genotype-dependent drug effects were observed in the spectra during wakefulness (Supporting information, Fig. S1) or REM sleep (data not shown).

DISCUSSION

This study revealed, for the first time, the impact of haploinsufficiency of Meis1, the highest-confidence RLS-associated gene in humans, on the regulation of sleep and EEG activity in rodents. In addition, we present a detailed analysis on the dose-dependent effect of pramipexole on quantitative and qualitative sleep. The results demonstrate that pramipexole at a low dose shortens sleep latency but fragments sleep, while a higher dose disrupts sleep in addition to producing qualitative changes in the EEG spectra during post-treatment NREM sleep. The latter qualitative effect of pramipexole on sleep is modified by Meis1 haploinsufficiency.

Meis1 haploinsufficiency produced no particular baseline effects either on the amount or the architecture of sleep.

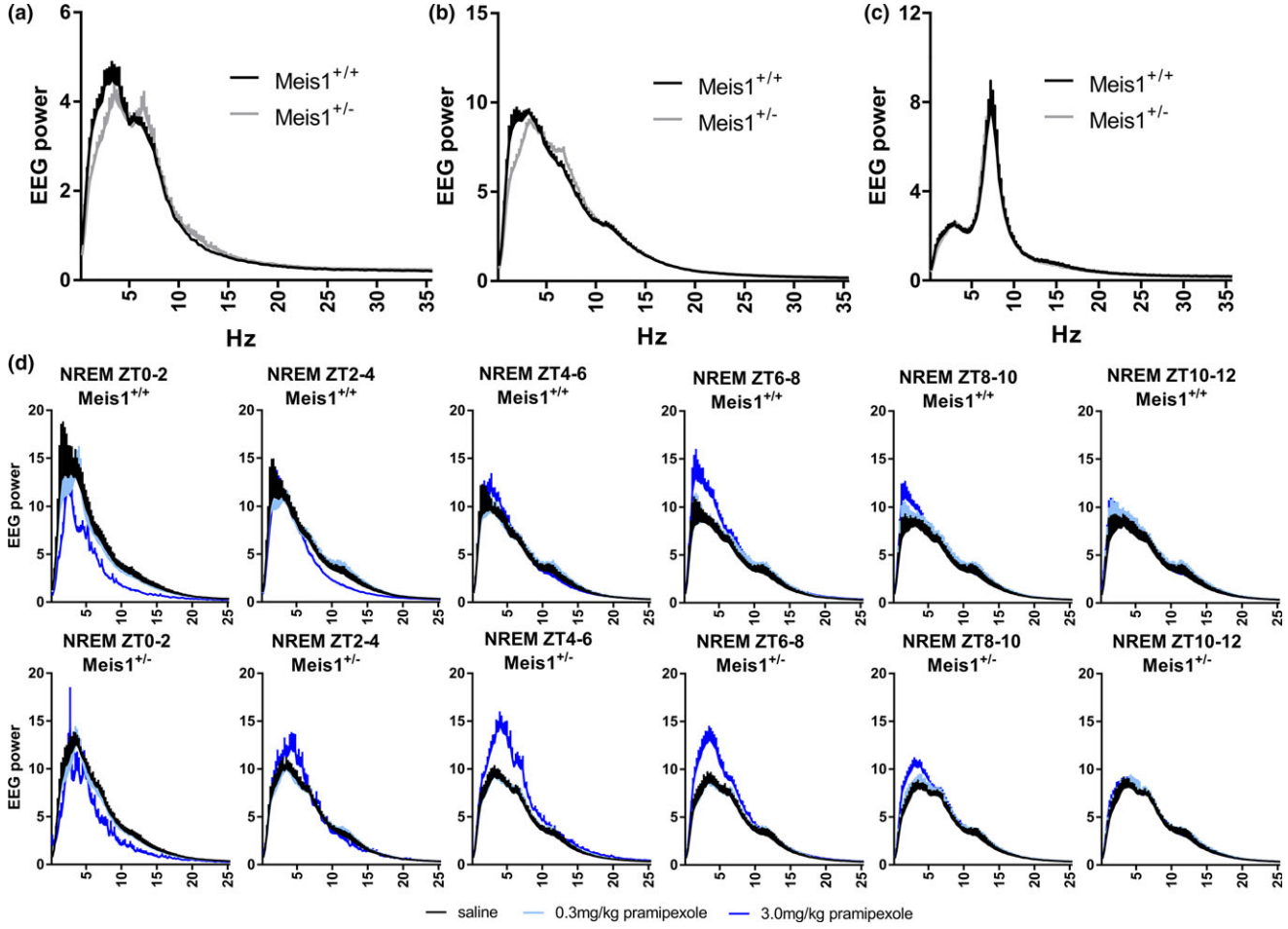

Figure 3. Example of electrocephalography (EEG) spectra from heterozygous Meis1 knockout and wild-type mice. During the baseline recording [Zeitgeber time (ZT) 4–6], heterozygous animals showed reduced power in the delta band of the EEG spectrum during wakefulness (a) and non-rapid eye movement (NREM) sleep (b), but no differences during REM sleep (c), although the differences did not reach statistical significance after correction for multiple testing. The low dose of pramipexole had no effect on EEG spectrum in either line during NREM sleep (d). The effects of the high dose on NREM sleep EEG differed in heterozygous animals compared to wild-types (d, genotype effect $P < 0.001$ in first three 2-h time bins, $P < 0.05$ in the fourth and fifth bin after the injection).

Consequently, Meis1 seems not to convey a primary role in causing sleep disturbances, such as prolonged sleep onset and frequent nocturnal awakenings, associated with human RLS (Hornyak *et al.*, 2007; Montplaisir *et al.*, 1997). Of note, all human RLS sleep studies in RLS patients have been performed so far in a very heterogeneous patient population, not stratified by their Meis1 genotype. Therefore, it remains possible that documented sleep problems in RLS patients are driven by multiple genetic factors rather than just Meis1, in combination with environmental, non-genetic factors.

In addition to RLS, *MEIS1* variants have been associated recently with symptoms of insomnia (Lane *et al.*, 2016). The same variant identified in the genome-wide association study (GWAS) investigating symptoms of insomnia is associated with reduced Meis1 expression (Xiong *et al.*, 2009), also suggesting a possible contribution of Meis1 deficiency in insomnia. Our results do not support this finding, demonstrating no baseline effect of Meis1 haploinsufficiency on sleep architecture in mice. Further genetic and functional studies are required to examine what exactly is the role of *MEIS1* in insomnia, or if the GWAS finding was confounded by a large number of RLS cases in the study population.

In this study, spectral analysis of the EEG during wakefulness and NREM sleep revealed a tendency towards slightly decreased delta activity, but increased theta power in the Meis1 haploinsufficient mice. However, the effect did not survive correction for multiple testing, possibly indicating that the power of our study was not sufficient to detect the subtle changes in EEG power spectra. During wakefulness, the potentially abnormal EEG spectrum could be explained by the increased locomotor activity of the animals (Spieler *et al.*, 2014), which might be reflected in their EEG profile. In contrast, during NREM sleep reduced delta power could represent a lower sleep quality caused by reduced Meis1 expression. If confirmed in future studies, these changes may indicate an involvement of Meis1 in the regulation of sleep homeostasis.

In addition to the genotype effects, we analysed the effect of pramipexole, a D_3-preferential dopamine agonist, on sleep

in rodents. Pramipexole exerted a modest sleep-fragmenting effect at the lower dose and a sleep-suppressing effect at the higher dose, regardless of Meis1 genotype. This is in line with previous findings in humans as well as in mice. Pramipexole suppresses PLMS and RLS symptoms effectively in RLS patients (Manconi *et al*., 2007a), but either does not affect (Manconi *et al*., 2012) or may even increase sleep fragmentation (Garcia-Borreguero *et al*., 2014). In mice, where pramipexole has not been studied in detail previously, pergolide is reported to reduce the amount of NREM sleep similarly on larger doses (Laloux *et al*., 2008), although pergolide is a D_2-preferential dopamine agonist. Conversely, levodopa, a dopamine precursor, does not affect sleep even at higher doses (Laloux *et al*., 2008). Levodopa, once metabolized into dopamine, activates both D_1- and D_2-like families of dopamine receptors. This might indicate that the sleep-suppressing effect is more specific to the D_2-like receptor family, when activated pharmacologically by the dopamine agonists.

The effects of pramipexole on sleep appeared in a slightly different manner between the two genotype groups, pointing towards Meis1 affecting dopaminergic manipulation of sleep. In the Meis1 knockout group, the high dose of pramipexole resulted in a more increased power of the theta and sigma bands compared to the control group during the rebound of sleep after strong sleep suppression, indicating unnatural and impaired quality of recovery sleep. Therefore, Meis1 seems to interact with the dopaminergic system, promoting the sleep-disrupting effect of high doses of pramipexole. The role of Meis1 in the dopaminergic system has been hinted at previously, based on expression of Meis1 in dopaminergic regions in the brain (Xiong *et al*., 2009), but this is the first time such an interaction has been reported at a behavioural level. This could have implications for personalizing RLS medication according to the patient's Meis1 genotype. If Meis1 down-regulation also enhances the sleep-disturbing effect of dopaminergic therapy in humans, patients with *Meis1* allele responsible for the disease should be directed towards other types of therapy.

Our data show no sleep fragmentation in the Meis1 mouse model at baseline, possibly suggesting a lack of PLMS in these mice. Most RLS patients display PLMS in sleep recordings (Hornyak *et al*., 2007). PLMS are accompanied by cortical arousals in humans and are highly responsive to dopaminergic therapy (Manconi *et al*., 2007a). Therefore, PLMS are one of the most potential RLS biomarkers for modelling the disease in animals (Manconi *et al*., 2007b). Normative data exist of mouse tibialis anterior EMG, a technique used to monitor PLMS in humans (Silvani *et al*., 2015), but the method has not yet been used to study RLS animal models. Unlike our Meis1-deficient mice, knockout mouse models of other RLS genes *Btbd9* (Deandrade *et al*., 2012) and *Ptprd* (Drgonova *et al*., 2015) show disturbed sleep. This could mean that, in future studies, the Meis1 knockout mouse model might not be the best model to explore RLS-related sleep phenotypes such as PLMS in rodents, but this remains speculative. However, more disease-specific Meis1 models, such as mouse lines harbouring a causal RLS point mutation, remain a potential approach (Allen *et al*., 2017).

There are some technical limitations to our study. First, a replication cohort has not been investigated in our study design and our results need to be replicated in independent mouse populations. Secondly, no tibial EMG monitoring was performed in our study, limiting the analysis of all potential sources of sleep disturbance in RLS. Finally, only male mice were used in our measurements.

In conclusion, our data demonstrate that Meis1 haploinsufficiency has no direct major effect on sleep architecture, but may have an effect on qualitative sleep in mice. This suggests that Meis1 does not play an imperative role in RLS-related hyperarousal or sleep fragmentation. However, the interaction of Meis1 with the dopaminergic system on the behavioural level was revealed, which suggests important implications in the development of personalized medicine for RLS in the future.

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AUTHOR CONTRIBUTIONS

AVS contributed to mouse breeding, data analysis and manuscript writing. BS contributed to experimental planning and mouse breeding. CF contributed to performing the experiments. MT generated the mouse line and contributed to manuscript writing. BMM contributed to statistical analysis. MK contributed to experimental planning, performing the experiments, data analysis and manuscript writing. JW contributed to experimental planning and manuscript writing.

CONFLICT OF INTEREST

The authors have no conflicts of interest to report.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Complete results of the electrocephalography **(**EEG) spectral analysis during wakefulness in Meis1 knock-out mice and age- and sex-matched wild-type controls.

Table S1. Full sleep stage amounts [in minutes, after the control (saline)] as well as low- (0.3 mg kg^{-1}) and high- (3.0 mg kg^{-1}) dose pramipexole injections, presented in 6- and 24-h bins.