Sleep and circadian rhythms: the effects of ketamine, caffeine and anthracyclines

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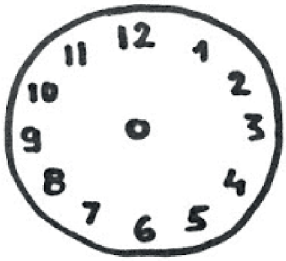
Comparison of sleep deprivation and a low dose of ketamine on sleep and the electroencephalogram of Brown Norway rats



Yumeng Wang, Marije Melgers, Johanna H Meijer, Tom Deboer. Comparison of sleep deprivation and a low dose of ketamine on sleep and the electroencephalogram of Brown Norway rats. *J Sleep Res. 2023 Feb 20:e13863.*

Abstract:

Ketamine is known for its antidepressive effects, but the mechanism underlying this effect remains largely unclear. In contrast to most antidepressive drugs, the action of ketamine is rapid, suggesting a different mode of action. A rapid antidepressive effect is also observed following sleep deprivation. Here we aim to evaluate the effect of a six-hour sleep deprivation (SD) and acute ketamine treatment on vigilance states, locomotor activity and electroencephalogram (EEG) power density spectra in Brown Norway rats under constant condition over two recording days. After SD and after the initial waking period induced by ketamine, both treatments induced a similar increase in non-rapid eye movement (NREM) sleep and EEG slow wave activity in NREM sleep. Rapid eye movement (REM) sleep was reduced immediately after both treatments, but was recovered later only after the sleep deprivation. The effects on the waking EEG differed between the treatments, with a faster theta peak during and after sleep deprivation, and no change in the waking spectrum after ketamine. In conclusion, sleep deprivation and ketamine both lead to an acute increment in NREM sleep slow wave activity as well as in a reduction in REM sleep. The results suggest that selective suppression of REM sleep, combined with enhancement of slow wave activity during NREM may be effective in the treatment of depression.

Key Words: sleep, sleep deprivation, ketamine, EEG, power spectrum, slow-wave

1. Introduction

Sleep quality is thought to be important in learning and memory, cognitive functioning, and mood, and disturbed sleep is a well-known risk factor in mood disorders [1,2]. Sleep abnormalities are commonly associated with psychiatric disorders such as major depressive disorder and bipolar depression disorder, and increasing evidence shows that chronic insufficient sleep may increase the risk of developing depression [2,3]. Mood disorders like depression are associated with altered sleep architecture [3]. For example, depression is related to a shorter sleep latency, increased rapid-eye movement (REM) sleep duration and REM density and also decreased non-REM (NREM) sleep [4,5]. NREM sleep and the electroencephalographic (EEG) slow-waves (EEG frequencies below 5 Hz) occurring during that sleep are considered to reflect homeostatic sleep pressure [6-8]. It has been suggested that impaired homeostatic regulation of SWA is associated with emotional dysregulation [9]. Although the mechanism of the bi-directional link between sleep and emotions is complex and not well understood, it still renders sleep a therapeutic target of antidepressant treatment.

Sleep deprivation (SD) and low dosages of the N-methyl-D-aspartate receptor (NMDAR) antagonist ketamine are two rapid-acting antidepressant treatment strategies that also influence sleep and the sleep EEG. However, little is known about the effects of ketamine on sleep regulation [10]. Ketamine has a short plasma elimination half-life but can relieve depression symptoms in humans both acutely and on the following days [11]. Total sleep deprivation is another rapid acting therapy for depression, which alleviates symptoms of depression in nearly 60% of depressed patients within hours [12]. Preclinical studies showed that ketamine induced a fast antidepressant effect in rats, 24 or 1 h prior to a forced swim test [13]. Similar to the effect of ketamine, mice and rats showed decreased immobility in the forced swim test after 12 or 24 hours of sleep deprivation [14,15]. Although the positive effect of sleep deprivation on depression is short-lasting, the rapid antidepressant effect can still be explored and compared with other rapid antidepressants to help us understand the mechanism mediating this action.

To our surprise, the effect of sleep, which showed the most pronounced changes during SD and ketamine treatment, is ignored in 80% of the papers on ketamine, SD, and mood [10]. The aim of this study was to assess to what extent ketamine and SD affect sleep, in both the acute sleep disrupted period

and in the long term (2 days), to establish similarities which may be relevant for the antidepressant effect in these two treatments. We observe similar changes in sleep and SWA in NREM sleep immediately after both treatments, and that the main differences between the two treatments lie in their effects on waking behavior and the EEG power density spectrum during waking in the initial phase of the two treatments. We also observed a long-lasting effect of the EEG power spectrum on the second recovery day in both treatments, and that REM sleep lost in the initial phase is only recovered following SD, but not after ketamine.

2. Methods

2.1 Animals

Twenty twelve-weeks old male Brown Norway rats (Charles River) were used in this study. Rats were group-housed under 12 h:12 h light-dark (LD) condition (Lights on 8:00 lights off 20:00) with food and water ad libitum in a temperature-controlled room (21–22 °C). During the experiments the animals were in constant darkness, while other conditions (food, water, temperature) remained the same. All animal experiments were approved by the Central Committee on Animals Research (CCD, the Netherlands) and were carried out in accordance with the EU Directive 2010/63/EU on the protection of animals used for scientific purposes.

2.2 Surgical procedure and EEG recordings

At a body weight of approximately 300 grams (16 weeks of age), the animals were put under deep anesthesia with ketamine (Aescoket, Boxtel, the Netherlands; 65 mg/kg) and Xylazine (Rompun, Bayer AG, Leverkusen, Germany; 13.3 mg/kg) [16]. The EEG/EMG surgery techniques were used as described previously [16-18]. EEG electrodes (Plastics One) were screwed through the skull on the dura over the right parietal cortex (2.0 mm lateral to the midline, 3.5 mm posterior to bregma) and the cerebellum (at the midline, 1.5 mm posterior to lambda). Two wires with suture patches (Plastics One) were inserted between the skin and the neck muscle tissue for EMG recordings. The wire branches of all electrodes were set in a plastic pedestal (Plastics One, Roanoke, VA) which was fixed to the skull with dental cement and three additional support screws. The rats were single housed after surgery and were allowed to recover for at least seven days in home cage under

12h:12h LD condition. After full recovery, the animals were connected to the recording system by a flexible cable and a counterbalanced swivel system, and they remained on the cable under constant darkness in the recording chamber for at least one week which can let the animal familiar with the environment and used to sleep with the cable before the start of the recording. The experiments were performed under constant dark conditions because ketamine may influence the circadian clock [19], and we wanted to make sure that we would not miss this effect if it occurred. The recording chamber was equipped with a passive infrared (PIR) sensor to record locomotor activity and a sensor to record drinking behavior [16-18]. The animals' locomotor activity and drinking activity were recorded continuously to obtain an estimate of the circadian phase (Supplemental Figure S1). From this, onset and offset of rest and activity were determined and an F-periodogram analysis provided an estimate of the circadian period [20] (Supplemental Figure S1 c). This enabled us to determine the time of SD and ketamine treatment for the next recording day and to determine whether SD or ketamine influenced the circadian timing of behavior.

2.3 Experimental design

The animals were divided into two groups, one group (n = 10) received a 6-h SD, starting at the onset of the rest period, and the other group (n =10) received ketamine and saline within one hour after the start of the rest phase in a randomized cross-over design experiment. This allowed us to get rid of the confounded results from SD and ketamine/saline treatment in the same animal.

During the 6-h SD, the animals (n=10) were observed with a dim red light in addition to the online EEG recording. Whenever the animals appeared drowsy or the EEG exhibited slow-waves, they were mildly disturbed by moderate noise, by the experimenter opening the cages and putting some novel objects inside, and, if necessary, by introducing fresh food, water, or nesting material into the cage [18,21]. The animals were never touched during the SD and were not disturbed during feeding and drinking.

Ketamine (Aescoket, Boxtel, the Netherlands) was dissolved in 0.9% saline (LUMC Pharmacy) at a concentration of 25mg/ml. The concentration was used previously in several studies and is known to induce an antidepressant-like effect accompanied by a moderate increase in activity in both naive and

stressed rats [13,22]. Under constant darkness at circadian time 1 (CT1), 1 hour after the predicted offset of locomotor activity, the animals (n = 10) received either ketamine (25mg/kg) or saline at a volume of 1ml/kg body weight in an intraperitoneal injection under a randomized cross-over design. At least three days of rest were given between injections.

2.4 EEG data acquisition and EEG power spectrum analysis

The EEG and EMG were simultaneously and continuously recorded for 48 h (after saline and ketamine treatment) and 72 h (before, during and after SD) as previously described [16-18]. The EEG and EMG signals were continuously recorded and amplified (amplification factor ~ 2000) and band-pass filtered (EEG: 0.5-30.0 Hz, −40 dB/decade; EMG 15.0-40.0 Hz, −40 dB/decade), digitized (sampling rate 100 Hz) in 10-sec epochs and automatically stored on hard disk (Spike2, Power1401, CED, Cambridge, UK). A fast Fourier transformation routine with a 10-sec window was performed offline (MATLAB, The MathWorks Inc., Natick, MA) to compute EEG power density spectra within the frequency range 0.1-25.0 Hz in 0.1 Hz resolution. All data were recorded simultaneously in ten-second epochs. Since, for the spectral analysis, it was necessary to re-calculate power density values relative to the first day of the saline control or baseline (first 24-h of the 72-h recording period of the SD experiment) condition, complete and clean recordings from all animals for all days are needed to enter the analysis. Due to problems with the quality of the recordings, two animals from the SD group and one animal from ketamine group did not contribute to the SWA and EEG spectral analysis data. The waking, NREM and REM sleep power spectrum were calculated relative to either the baseline (for the SD experiment) or saline (for the ketamine experiment) NREM sleep power spectrum.

2.5 Data analysis

Three vigilance states (waking, NREM and REM sleep) were scored offline in 10-sec epochs. The manual scoring of vigilance states based on the EEG and EMG recordings was performed according to standardized criteria for rats [16-18]. Vigilance states were determined, and artifacts were excluded for power spectral analysis. The average amount of the vigilance states (waking, NREM sleep, REM sleep and REM sleep per total sleep time) were analyzed in 1-h intervals and 6-h intervals over 48-h and 72-h. The locomotor activity was collected in ten-second intervals and was further averaged in 1-h and

6 - h intervals. To investigate the effect of SD and ketamine administration on sleep homeostasis, we analyzed the EEG power density in the slow-wave range (SWA, 1.0 – 4.0 Hz) in NREM sleep as described previously [16,17]. The 1-h and 6-h values of SWA in NREM sleep were expressed relative to the first 24-h of the saline or baseline day (=100%). The peak frequency and power density of theta in waking, REM sleep (6.0 – 9.0 Hz) and slow-waves in NREM sleep (1.0 – 4.0 Hz) was determined manually in the relative power density spectra in 0.1 Hz bins.

2.6 Statistics

For data analysis, GraphPad was used. A two-way repeated measures ANOVA was used to compare the effect of SD across time, EEG frequencies, and treatment conditions. Two-way ANOVA was used to compare the effect of drug across time, EEG frequencies, and treatment conditions. Two way ANOVA was used to compare the effect of drug and SD on EEG peak frequency and power density in this peak. In some cases, where missing values occurred in REM sleep / total sleep and SWA in NREM sleep, a mixed-effects model (REML) was used to compare the effect of SD or ketamine across circadian time. If the result was significant, we ran post hoc Bonferroni multiple comparisons corrected t-test, and the significant time points and EEG frequencies are reported in the results. The associated F-statistic is reported in all ANOVA tests. Values of $p < 0.05$ were considered statistically significant.

3 Results

3.1 Effect of sleep deprivation on vigilance states, slow-wave activity in NREM sleep, and locomotor activity.

The 6-h SD affected sleep and waking over the following 18-h. A clear decrease in waking and increases in NREM and REM sleep were observed (Fig.1 a - c, circadian time x SD interaction, $F_{71, 1278} = 4.942$, $F_{71, 1278} = 4.905$, $F_{71, 1278} = 3.623$ for waking, NREM sleep and REM sleep respectively, $p < 0.0001$). The first 5 - 6 hours after SD a clear decrease in waking and an increase in NREM sleep were seen (Fig. 1 a, $p_{CT11} = 0.0192$; Fig. 1 b, $p_{CT8} = 0.035$, $p_{CT11} = 0.0093$) and an increase in REM sleep was observed a few hours later (Fig 1 c, $p_{CT14} = 0.0481$). The decrease in waking and increase in NREM sleep were reflected in the first two 6-h values after SD (Fig 1 g, time x SD interaction, $F_{3, 54} = 207.0$, $p < 0.0001$, $p_{CT6-CT12} < 0.0001$, $p_{CT12-CT18} < 0.0001$, Fig.

1 h, $F_{3, 54} = 206.7$, $p < 0.0001$, $p_{CT6 - CT12} < 0.0001$, $p_{CT12 - CT18} < 0.0001$). The effects of SD on REM sleep started and ended later than the effects on NREM sleep and waking. The 6-h REM sleep values were increased 12-24 hours after the end of the SD (Fig.1 i, time x SD interaction, $F_{3, 54} = 109.0$, $p < 0.0001$, $p_{CT12 - CT18} < 0.0001$, $p_{CT18 - CT24} = 0.0015$). REM/total sleep in the first 6 hours after the SD was also lower compared to baseline (Fig. 1 j, SD, $F_{1, 36} = 61.38$, $p < 0.0001$, $p_{CT6 - CT12} < 0.0001$) which was due to the large increase in the amount of NREM sleep. The following active period after the SD (CT12 – CT24) showed increased REM/total sleep (Fig, 1 j, SD, $F_{1, 18} = 20.43$, $p = 0.0003$, time x SD interaction, $F_{3, 54} = 126.5$, $p < 0.0001$, $p_{CT12 - CT18} = 0.0004$, $p_{CT18 - CT24} = 0.0039$). PIR locomotor activity showed clear modulations across the day (Fig. 1 e, $F_{71, 1278} = 6.223$, $p < 0.0001$), but there was no difference in locomotor activity found after SD compared to baseline in the post hoc analysis.

SWA in NREM sleep showed a clear increase immediately after the SD, followed by a declining curve that continued for at least 6 hours (Fig. 1 f, n = 8, circadian time x SD interaction, F65, 887 = 8.324, $p < 0.0001$, $p_{CT7 - CT12} < 0.05$). Surprisingly, the animals showed an undershoot in SWA with an average of 79.83% on the second day after SD, compared with baseline condition with an average of 107.6% (Fig. 1 f, $p_{CT3} = 0.0101$ on recovery day; Figure S2, SD, $F_{3, 42} = 18,74$, $p < 0.0001$, $p_{CT0 - CT6} < 0.0001$). No effects of SD on the circadian regulation of locomotor activity, or the timing of vigilance states were found.

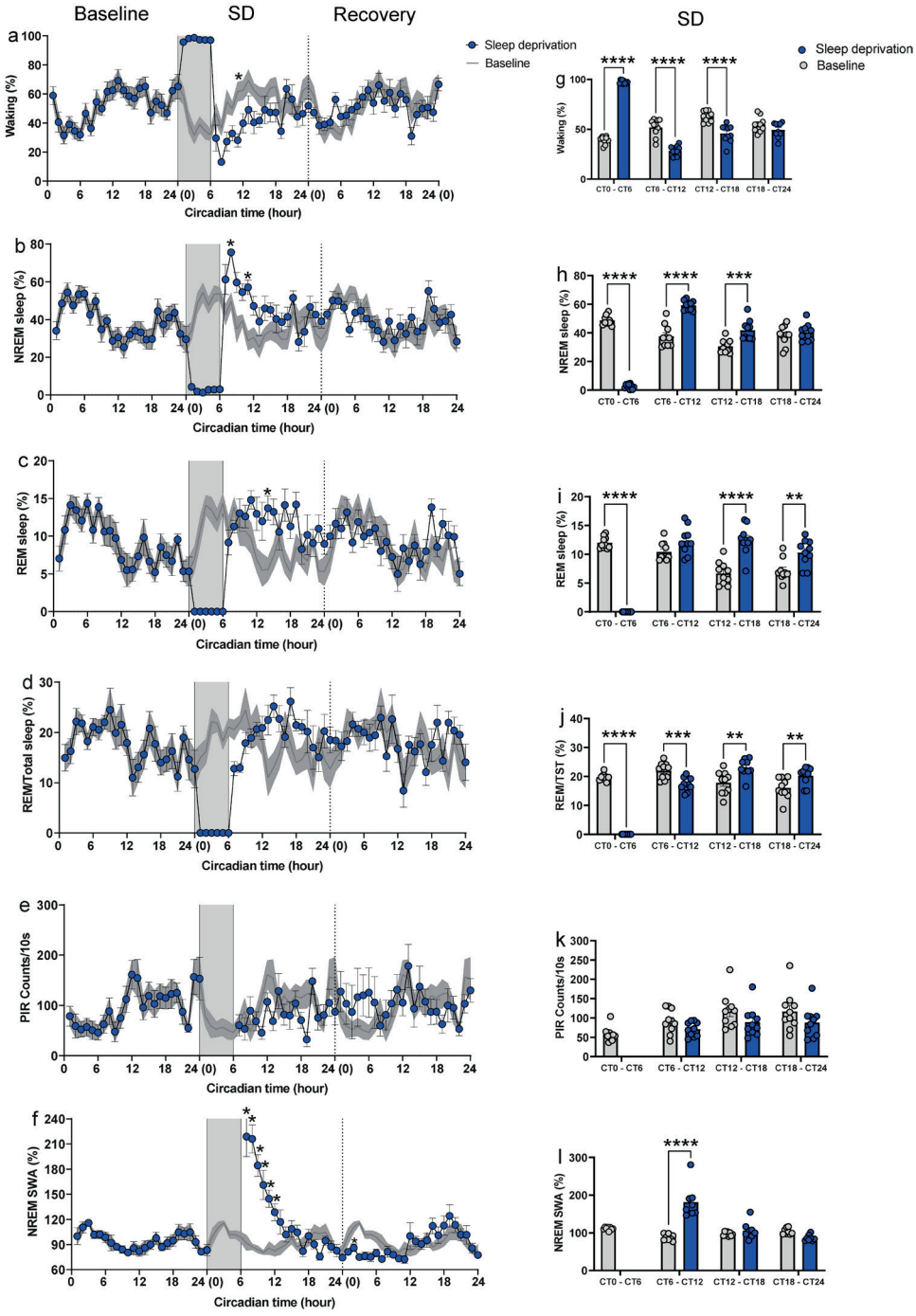


Figure 1. Vigilant stages, locomotor activity and slow-wave activity in NREM sleep of sleep deprivation over 72 hours. a – f. Time course of waking, NREM sleep, REM sleep, REM sleep/ Total sleep time, locomotor activity, and slow-wave activity (SWA) in NREM

sleep in 1-h values for sleep deprivation (n = 10 animals, n=8 for SWA) in 72 hours. The first 24-h was considered as baseline day, the second day was the sleep deprivation day, the third day was the recovery day. The baseline is repeated in the background in dark grey. The light grey area indicates the sleep deprivation period. SWA in NREM sleep is expressed relative to the average NREM sleep SWA during the first 24 hours (=100%). * $p < 0.05$. g – i. Six-hour values of waking, NREM sleep, REM sleep, REM sleep/ total sleep, locomotor activity, and SWA in NREM sleep for the sleep deprivation (blue, n = 10, n = 8 animals for SWA analysis) and baseline (grey, n = 10, n = 8 animals for SWA analysis) condition over 24 hours. ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Data is shown as mean ± SEM.

3.2 Effect of acute ketamine on vigilance states, slow-wave activity in NREM sleep and locomotor activity.

Ketamine compared with vehicle, acutely increased waking and reduced NREM and REM sleep for approximately 2 hours (Fig. 2 a, drug, $F_{1, 864} = 8.780$, $p = 0.0031$; circadian time x drug interaction, $F_{47, 864} = 2.012$, $p < 0.0001$; $p_{CT2} = 0.0046$, $p_{CT3} < 0.0001$;Fig.2 b, circadian time x drug interaction, $F_{47, 864} = 2.220$, $p < 0.0001$, $p_{CT2} = 0.0014$, $p_{CT3} < 0.0001$;Fig.2 c, circadian time x drug interaction, $F_{47, 864} = 1.505$, $p = 0.0172$; $p_{CT3} = 0.0018$; Fig. 2 d, circadian time x drug interaction, $F_{47, 844} = 1.580$, $p = 0.0088$). All vigilance state 1-h values subsequently returned to baseline and no differences with vehicle were observed thereafter in any of the vigilance states (Fig 2 a-d).

The total amount of NREM sleep over the first 6 hours of the day of ketamine treatment did not differ significantly from vehicle (Fig. 2 h, time x drug interaction, $F_{3, 72} = 2.710$, $p = 0.0513$), but REM sleep was significantly reduced (Fig. 2 i, time x drug interaction, $F_{3, 72} = 4.721$, $p = 0.0046$, $p_{CT0 - CT6} = 0.0003$), and waking was enhanced (Fig. 2 g, time x drug interaction, $F_{3, 72} = 4.043$, $p = 0.0103$, $p_{CT0\text{-}CT6} = 0.0052$). As can be seen in the hourly values, the acute waking effect of ketamine lasts for two hours after which sleep is initiated. Although the first 6 hours of NREM sleep did not show notable differences between ketamine and saline, NREM sleep was significantly increased (62.24 ± 2.58 %) compared with saline administration (46.61 ± 2.12 %) during the second half of that six hours (CT4-CT6, paired t-test, $p = 0.0013$), when the main increase in sleep and SWA was found. Because of the reduction in REM sleep, REM/total sleep was also lower in the first 6-h interval after ketamine compared with vehicle (Fig.2 j, drug, F1, 72 = 4.010, p=0.0490, pCT0 – CT6 = 0.0084). In contrast to NREM sleep, REM sleep did not show big differences (9.44 ± 0.93 %) compared with saline administration (11.56 ± 0.83 %) during CT 4 – CT 6 (paired t-test, $p = 0.0551$), but due to

the increase of NREM sleep, REM/TST was decreased significantly (13.84 ± 1.4 %) compared with saline (18.66 ± 1.3 %) during CT 4 – CT 6 (paired t -test, $p = 0.0145$). REM sleep and REM/total sleep did not show a rebound in the following hours. Locomotor activity was increased for approximately one hour after ketamine administration compared with vehicle (Fig.2 e, circadian time x drug interaction, $F_{47, 694} = 1.927$, $p = 0.0003$, $p_{CT2} < 0.0001$).

SWA in NREM sleep after ketamine administration was increased considerably and significantly during CT4 and CT5 (Fig. 2 f, n = 9, drug, $F_{1, 721} = 18.96$, $p < 0.0001$, time x drug interaction, $F_{46, 721} = 1,543$, $p = 0.0135$, $p_{CT4} = 0.0006$, $p_{CT5} < 0.0001$). This was followed by a gradual decline in SWA, similar to the decline seen after SD. However, the undershoot in SWA we observed on the second day after the SD did not occur after ketamine treatment. There was no significant difference on the second day after the ketamine treatment in vigilance states, locomotor activity and SWA compared with vehicle (Fig. 2 a - f). No effects of ketamine on the circadian regulation of locomotor activity, or the timing of vigilance states were found.

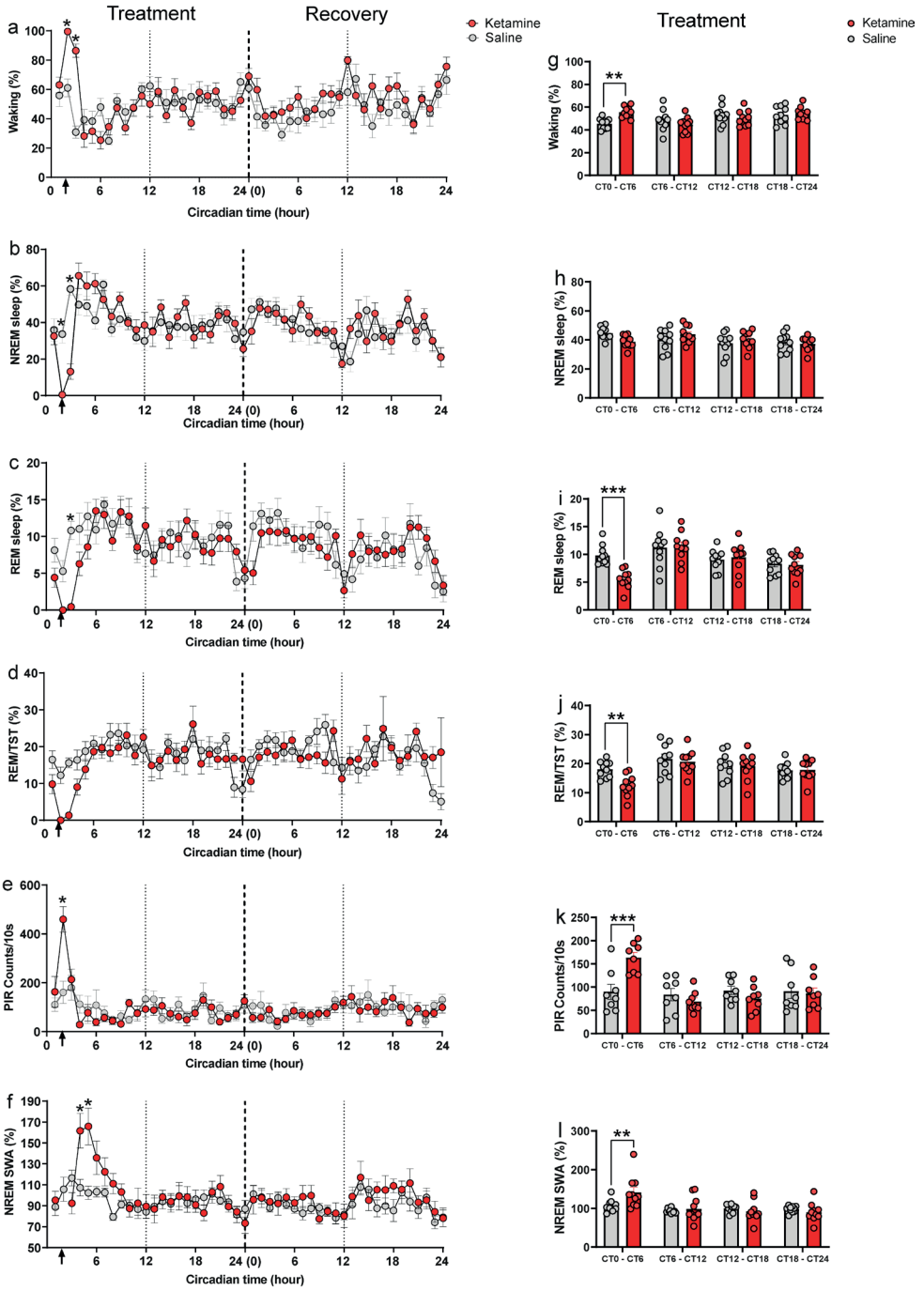


Figure 2. Vigilant stages, locomotor activity and slow-wave activity in NREM sleep after ketamine and saline treatment. a – f. Time course of waking, NREM sleep, REM sleep, REM sleep/ Total sleep time, locomotor activity and slow-wave activity (SWA) in NREM

sleep in 1-h values for ketamine and saline administration (n = 10 animals, n=9 for SWA) in 48 hours. The first 24-h was considered as treatment or control day, the second day was the recovery day. SWA in NREM sleep is expressed relative to the average NREM sleep SWA during the first 24 hours of the saline control day (=100%). * $p < 0.05$. g – i. Six-hour values of waking, NREM sleep, REM sleep, REM sleep/ total sleep, locomotor activity and SWA in NREM sleep for ketamine administration (red, n = 10, n = 9 for SWA) and saline (grey, n = 10, n = 9 for SWA) administration on the treatment day. The arrow indicates the injection time (CT1). ** $p < 0.01$, *** $p < 0.001$. Data is shown as mean ± SEM.

3.3 Effect of ketamine and sleep deprivation on the waking EEG power density spectrum

To compare the effect of ketamine and SD on the waking EEG power density spectrum, we analyzed the EEG power spectrum of the most active two hours (CT1 – CT3) after ketamine administration, which were mainly spend in waking, and the corresponding two hours during the SD. The waking power spectrum was examined to determine similarities and differences between waking induced brain activity during SD and waking induced brain activity immediately after ketamine treatment. Interestingly, the waking spectrum differed considerably between the two. Power density was increased around 7.7 Hz during the SD but not during waking after the ketamine administration (Fig.3 a, n = 8, frequency x SD interaction, $F_{249, 1750} = 1.468$, $p < 0.0001$, $p_{0.3 – 0.4\ Hz} < 0.0006$, $p_{7.4 – 7.9\ Hz} < 0.05$; Fig. 3 b, n = 9, drug, $F_{1, 3500} = 74.39$, $p < 0.0001$, frequency x drug interaction, $F_{249, 3500} = 1.253$, $p = 0.0056$). The peak frequencies differ significantly between SD baseline and saline control, which is probably due to the handling of the animals in the saline condition, which did not happen in the SD baseline condition. Concentrating on the theta peak of waking, we found that the peak frequency during SD was faster (7.5 ± 0.1 Hz) compared to baseline (6.1 ± 0.1 Hz), but that the power density, in the theta peak, when corrected for peak frequency, was not significantly different. Theta peak frequency and amplitude during waking after ketamine administration (6.9 ± 0.2 Hz), did not differ from control (6.7 ± 0.2 Hz) (Fig.3 c, treatment x group interaction, $F_{1, 30} = 21.82$, $p < 0.0001$, $p_{baseline\ vs.\ SD} < 0.0001$, $p_{baseline\ vs.\ saline} = 0.0273$, $p_{SD\ vs.\ ketamine} = 0.008$; Fig.3 d, treatment x group interaction, $F_{1, 30} = 0.9840$, ns).

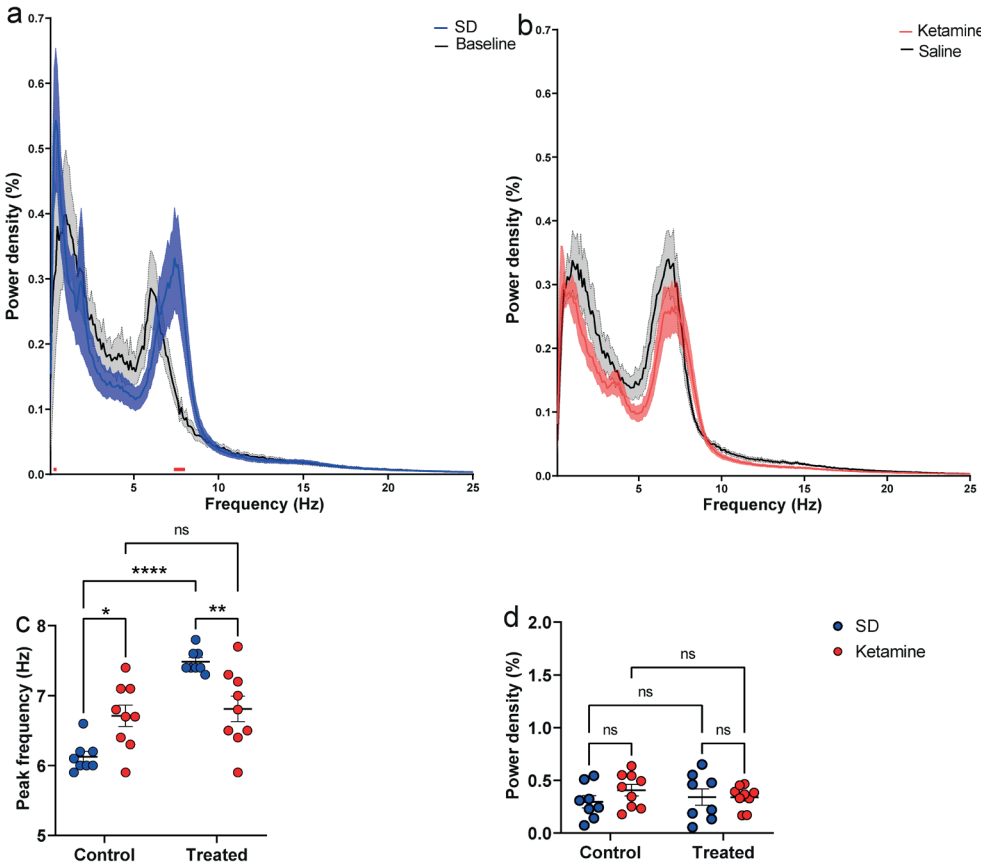


Figure 3. Acute effect of sleep deprivation and ketamine administration on the power density spectrum of the waking EEG. a – b. Relative EEG power spectrum in waking during CT 1 – CT 3 for sleep deprivation (blue, n = 8) and baseline (black, n=8) and after ketamine (red, n = 9) administration or saline (black, n = 9). The red bar indicates significant differences between sleep deprivation and baseline ($p < 0.05$). c. Peak frequency between 6.0 – 9.0 Hz in waking during CT 1 – CT 3 for sleep deprivation (blue, n = 8) and ketamine (red, n = 9) administration. d. Power density of the peak frequency from C in waking during CT 1 – CT 3 for sleep deprivation (blue, n = 8) and ketamine (red, n = 9) administration. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$, ns: not significant. Data is shown as mean ± SEM.

3.4 Effect of ketamine and sleep deprivation on EEG sleep power spectrum during the early recovery period

Six hours of SD resulted in an increase in sleep and similar results were obtained after the initial 2-h waking period (CT1-CT3) induced by the ketamine administration. To further compare these two treatments, cortical EEG power density spectra were compared between CT6 – CT9 of the baseline

and SD condition, and CT3 – CT6 of vehicle and ketamine administration, which were the times where both treatments gave the highest amount of NREM sleep and the highest SWA in NREM sleep values after treatment. The power spectra in NREM sleep after SD and ketamine both showed the typical increase in EEG SWA (Fig. 4 a, frequency x SD interaction, $F_{249, 1750}$ = 212.9, p < 0,0001, SD, F1, 17500 = 6763, p < 0.0001, $p_{0.6 - 4.8\ Hz}$ < 0.05; Fig. 4 d, frequency x drug interaction, $F_{249, 4000}$ = 13.22, p < 0.0001, drug, $F_{1, 4000}$ = 455.4, p < 0.0001, $p_{0.6 - 3.2\ Hz}$ < 0.05).

Concentrating on the peak frequency and power density of the slow-wave activity peak in NREM sleep, we found that slow-wave power density was increased in the first hours after the SD (7.60 ± 0.31 %) compared with baseline (3.33 ± 0.13 %) (Fig. 4 j, treatment x group interaction, $F_{1, 30}$ = 16.37, p = 0.0003, pbaseline vs. SD < 0.0001), but also that the slow-waves were faster compared to baseline (Fig. 4 g, SD: 1.8 ± 0.1 Hz, baseline: 1.5 ± 0.1 Hz, treatment x group interaction, $F_{1, 30}$ = 5.678, p = 0.0104, , pbaseline vs. SD = 0.0237). Compared to the ketamine treatment the slow-wave peak frequency after SD was also faster and had a larger power density (Fig. 4 g, $p_{SD\ vs.\ ketamine}$ = 0.0187; Fig. 4 j, $p_{SD\ vs.\ ketamine}$ < 0.0001). As a control, we compared the spectra between the baseline day of SD and the saline control day and found no significant differences between those two (Fig. 4 g and j).

In addition, we compared the power density spectra in waking and REM sleep between the two treatments. Notably, after SD, during waking, the animals still displayed higher power density around 7.2 Hz (Fig. 4 b, SD, $F_{1, 1750}$ = 50.48, p < 0.0001, $p_{6.6 - 7.9\ Hz}$ < 0.05). The statistical analysis showed a main effect of drug (Fig. 4 e, drug, $F_{1, 4000}$ = 15.51, p < 0.0001) on the waking spectrum after ketamine administration, but the post hoc analysis did not show large differences in individual frequency bins. When comparing theta peak frequency and power density, no significant differences between ketamine and SD were found for theta peak power density during waking after the animals initiated sleep (Fig. 4 k, treatment x group interaction, F1,30 = 0.6253, ns). In the waking power spectrum, after SD, the theta peak frequency was faster compared to baseline (Fig. 4 h, Baseline: 6.3 ± 0.1 Hz, SD: 7.1 ± 0.1 Hz, treatment x group interaction, $F_{1, 30}$ = 25.16, p < 0.0001, pbaseline vs. SD < 0.0001). Ketamine treatment did not change the waking peak frequency, and the waking theta peak after SD was faster compared to that after ketamine administration (Fig. 4 h, $p_{SD\ vs.\ ketamine}$ < 0.0001).

There was a significant effect of SD on the REM sleep EEG power spectrum (Fig. 4 c, frequency x SD interaction, $F_{249,1750} = 2.031$, $p < 0.0001$, $p_{6.1-7.7\ Hz} < 0.05$), and we also found a main effect of ketamine on the REM sleep power spectrum (Fig. 4 f, drug, $F_{1,4000} = 7.660$, $p = 0.0057$), similar to waking. No significant differences between ketamine and SD were found for both theta peak frequency and theta power density in the REM sleep EEG (Fig. 4 i and l).

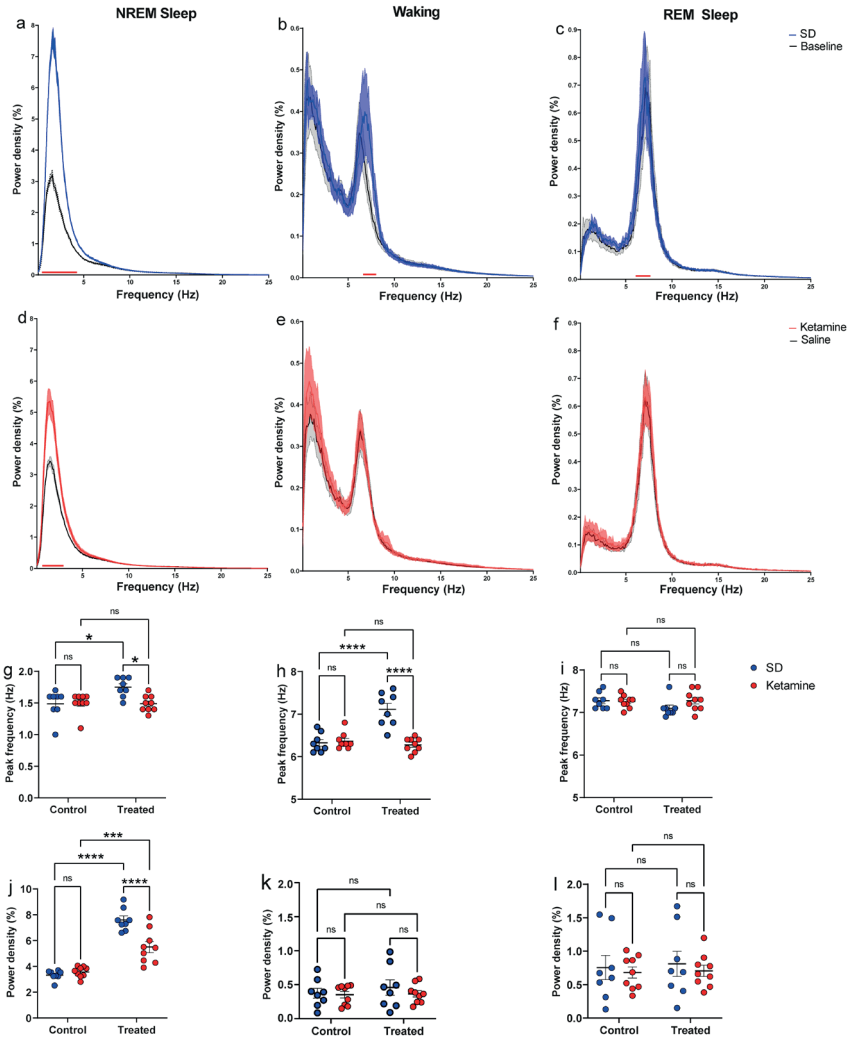


Figure 4. Effect of sleep deprivation and ketamine administration on the EEG power spectrum during the early recovery phase. a – c. Relative EEG power spectrum in NREM sleep, waking, REM sleep during CT 6 – CT 9 for sleep deprivation (blue, n = 8) and baseline (black, n = 8). Red bar indicates significant differences between sleep deprivation and baseline conditions ($p < 0.05$. d – f. Relative EEG power spectrum in NREM sleep, waking,

REM sleep during CT 3 – CT 6 for saline and ketamine administration (red, n = 9) and saline administration (black, n = 9). Red bar indicates significant differences between ketamine and saline conditions ($p < 0.05$). g – i . Peak frequency in the slow-wave range (1 - 4 Hz) for NREM sleep, and the theta range (6 - 9 Hz) for waking and REM sleep for sleep deprivation (blue, n = 8) and ketamine (red, n = 9) administration. j – l. EEG power density of the peak frequency in the slow-wave range for NREM sleep, and theta range for waking and REM sleep for sleep deprivation (blue, n = 8) and ketamine (red, n = 9) administration. * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$, ns: not significant. Data is shown as mean ± SEM.

3.5 Effect of ketamine and sleep deprivation on EEG sleep power spectrum on the second recovery day

As discussed in the introduction, ketamine has an antidepressant effect that lasts longer than the acute effect it has on the vigilance states and the EEG. Therefore we analyzed the 24 hours EEG power density spectra on the second day after the SD and ketamine administration (Fig. 5). We only observed main effects on the REM sleep power density spectra after ketamine administration on the second day and no effect on the waking power density spectra (Fig. 5f, drug, REM, $F_{1, 4000} = 4.632$, $p = 0.0314$; Fig. 5d, drug, waking, $F_{1, 4000} = 1.823$, ns). Similar to ketamine, we found a main effect of SD on the REM sleep power spectrum on the second day (Fig. 5c, SD, $F_{1, 1750} = 10.30$, $p = 0.0014$, $p_{6.5\ Hz}=0.0189$, $p_{7.4\ Hz}=0.0003$, $p_{7.6\ Hz}=0.0081$). The effect of SD on the waking power spectrum was small, but still significant (Fig. 5 b, SD, $F_{1, 1750} = 4.923$, $p = 0.0266$). On the second day after the SD, we observed an undershoot in SWA (Figure S2), and we anticipated to see a similar effect in the power spectrum. Indeed, we observed between 1.2 – 2.1 Hz, a decrease in power density the second day after SD, which reflects the undershoot we observed in SWA in Supplemental Figure S21 (Fig. 5 a, SD, $F_{1, 1750} = 51.94$, $p < 0.0001$, $p_{1.2 – 2.3\ Hz} < 0.05$). For the second day of ketamine administration, we noticed a similar effect as after SD in the NREM sleep spectrum, although we did not observe the effect on the hourly values on the second day (Fig. 5 d, frequency x drug interaction, $F_{249, 4000} = 1.371$, $p = 0.0002$, $p_{1.2 – 2.5\ Hz} < 0.05$). Despite the acute sleep disturbance, ketamine administration and SD were not associated with long-term changes in sleep-wake architecture, however, both SD and ketamine were associated with long-term changes in the EEG power density spectra, especially for NREM and REM sleep.

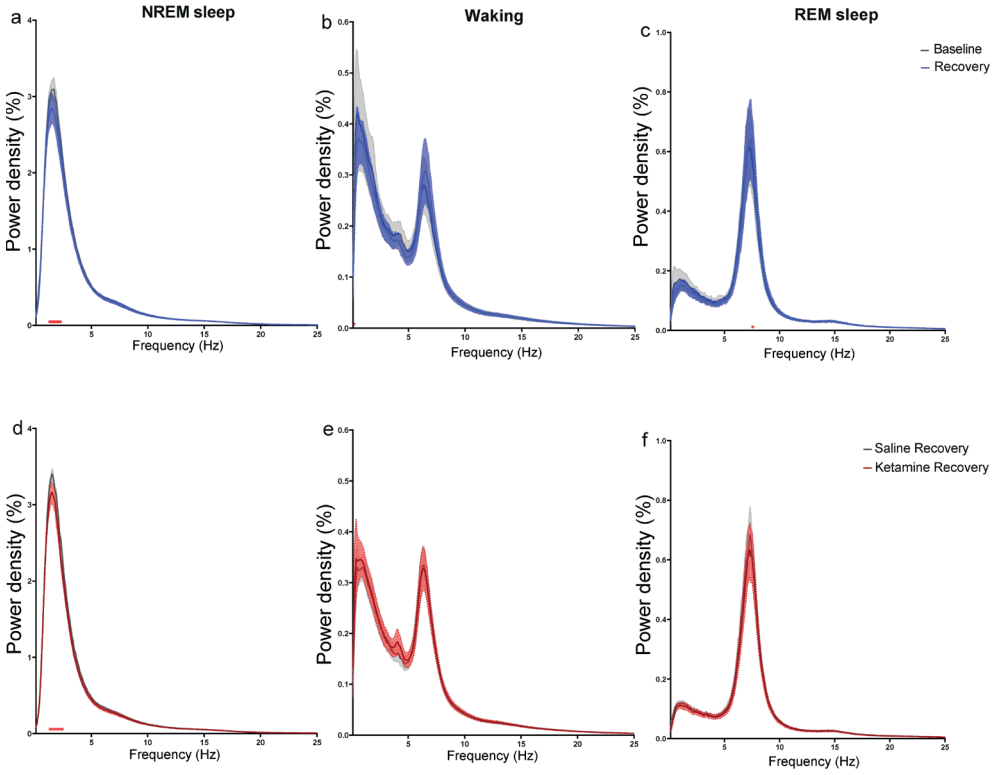


Figure 5. Effect of sleep deprivation and ketamine administration on the EEG power spectrum on the second recovery day. a - c. Relative EEG power spectrum in NREM sleep, waking, REM sleep for sleep deprivation (blue, n = 8) and baseline (black, n = 8). Red bar indicates significant differences between sleep deprivation and baseline conditions ($p < 0.05$). d - f. Relative EEG power spectrum in NREM sleep, waking, REM sleep for saline and ketamine administration (red, n = 9) and saline administration (black, n = 9). Red bar indicates significant differences between ketamine and saline conditions. Data is shown as mean ± SEM.

4. Discussion

4. 1 Effects of SD and ketamine on the sleep-wake architecture and locomotor activity

As found previously, SD induced an increase in NREM sleep and no changes in REM sleep in the first 6 hours following the SD [21,23]. The increase in NREM sleep lasted for 12 hours, and REM sleep started to increase after the

increase in NREM sleep started to diminish. It has been shown previously after SD that NREM sleep is more immediately recovered compared to REM sleep, however not after a relatively short SD of only 6 hours. Ketamine, an NMDAR antagonist, initially led to increased waking and activity which has been previously reported in rodents [24]. This increase lasted approximately 2-3 hours which corresponds well with the reported half live of 1.3 hours of ketamine in the rat [25]. Correspondingly, NMDAR antagonists are known to promote neurotransmitter release which influences brain areas that control arousal [26], but the induction of waking and suppression of sleep is still not completely understood. In this study, ketamine was expected to induce waking, however, we did not see a change in the amount of NREM sleep during the subsequent sleep period. Acute ketamine reduced REM sleep which is in line with some of the previous studies, but results tend to vary between studies [24]. This may be due to differences in light schedules, drug concentration or species. REM sleep suppression is common with many traditional antidepressants, and in some studies, there exists a relationship between the amount of REM sleep and emotional function [27,28]. Preclinical studies show that an SD lasting 24-h can increase serotonin (5 - HT) levels in the hippocampus in rats and depletion of 5 - HT actually blocks the antidepressant of ketamine in rats [13,29]. This may explain the change of sleep-wake architecture in the context of rapid changes in monoamine levels [30]. We observed a reduction in REM sleep for several hours in both treatments. REM sleep eventually recovered after SD, but not after ketamine. In view of the difference in the duration of the antidepressant effect of SD and ketamine and the difference in the effects on REM sleep between the two treatments, decreased REM sleep and the timing of REM sleep recovery may be relevant for the duration of the antidepressant therapeutic mechanism of the two treatments.

In rodents, ketamine administration can generate a behavioral syndrome characterized by hyperlocomotion, ataxia signs, and stereotypies [31]. Our recordings were obtained in a restricted condition, and the experimenters could not stay in the recording room and observe the animal after the injection. Increased locomotion, after ketamine was detected from the PIR recording, but did not allow to identify other psychotic-like behaviors. This type of behavior was also not observed in another study in rats using the same dosage, after which the animals were observed [13]. We therefore cannot exclude that this type of behavior occurred, but think that it is unlikely.

4.2 Acute effect on power spectrum and sleep homeostasis

To investigate this further, we compared the changes in EEG power density spectra during the initial phase of induced waking, the subsequent early recovery phase, and recovery on the second day, and determined similarities and differences which may elucidate the overlaps in their antidepressant action.

We show that the EEG power density spectrum of the waking period, induced in the first 2 hours after ketamine treatment, is different from that during SD, which may indicate that the fast antidepressant effect does not result from the type of brain activity during the initial waking period. Remarkably, the increase in locomotor activity after ketamine treatment was not accompanied by a change in theta EEG peak frequency as was the case during SD, where theta peak frequency became faster. Previous studies showed mixed results in rodents, with some studies showing an increase in theta activity in the first 30 min after 30 mg/kg ketamine administration, while other studies showed decreased power density within the range of 2.5–21 Hz [32,33]. The third group of studies found no effect on theta activity [24]. Recently, a study in humans showed an increase in theta activity after the ketamine administration in healthy subjects [34]. All these contradicting findings may be due to differences in recording location of the EEG, preceding sleep-wake history, circadian time of day, the drug concentration and even the species. In the present experiment we show that theta frequency became faster during SD, but when corrected for this change, there is no change in peak power density. The difference in theta frequency between SD and ketamine treatment suggests that the rapid antidepressant effect of ketamine and SD is probably not due to the quality of the initial waking period induced by the treatments.

After SD or the initial wake period induced by ketamine, both treatments showed an increase in the activity in the slow-wave range in the NREM sleep EEG. SWA is thought to reflect the build-up of sleep pressure during waking, which decreases during sleep [6]. More and more studies show that increased SWA is associated with increased BDNF, increased synaptic strength, and enhanced synaptic plasticity [35]. We performed our recording for 72- h in constant darkness, and noticed that the increase in SWA after SD was similar to that of a 24-h SD in rats under LD condition [36]. This may indicate that sleep pressure increases faster during waking under constant dark conditions. Further, we noticed an interaction between ketamine and circadian time in

SWA over 48 hours in our data. It is also possible that the effects of ketamine on sleep homeostasis depend on the circadian time. Considering the similarities in the changes in SWA after SD and ketamine treatment, it may be that SWA in NREM sleep serves as a reflection of an increased neuronal synaptic strength and plasticity, and may be related to the mechanism underlying the rapid antidepressant effects of the two treatments [37].

Ketamine did not change the REM sleep EEG power density spectrum. In contrast, we notice a change in the theta power density during REM sleep after SD. Thus, it seems that similar effects of SD and ketamine on the EEG during the early recovery period are only found in the NREM sleep EEG.

4.3 Long-term effect on power spectrum and sleep homeostasis

Analysis of the second recovery day after SD and ketamine showed a reduction of SWA in the NREM sleep EEG power spectrum and a main effect on the REM sleep power spectrum. This may indicate that both SD and ketamine influence sleep homeostasis for a longer time period under DD. This is surprising, as the plasma elimination half-life of ketamine (1.3 – 1.5 hours in rats) is short [25], and nevertheless, the changes we observed remained for at least 24 hours after administration. As we observed an undershoot in SWA on the second day which is not seen in other rat studies with such a short (6h) SD [21]. Undershoots in SWA, or negative rebounds as they are also called, have been observed before after SD in rats, but then after a 24-hour SD [36]. The strong undershoot we observed may be related to the delayed compensation in REM sleep which may suppress SWA in those hours. In summary, it seems that the effects of SD on NREM sleep SWA and REM sleep in our experiment are relatively strong when compared to other studies with a 6-h SD in rats. In contrast to the strong acute sleep disturbance, ketamine administration and SD were not associated with long-term changes in sleep-wake architecture, however, both SD and ketamine were associated with long-term changes in the EEG power density spectrum, especially in the NREM and REM sleep EEG.

There is a strong link between sleep and major depression. However, the causal relationship remains unclear. It is, for instance, unknown whether changes in depression symptoms precede or follow changes in sleep, and whether a longer or shorter sleep duration is related to improvements of depression core symptoms [6,38]. Investigating this may further explain how and when

an acute sleep deprivation exerts its rapid antidepressant effect and why it typically relapses after subsequent sleep occurs. It also needs to be researched how, with for instance light therapy, the antidepressant effect can be extended [39-42]. Other questions that remain are whether it suffices to concentrate on NREM sleep or total sleep deprivation [6], as in the past also successful treatments were achieved with REM sleep deprivation [43].

One of the limitations of this study are that we did not sample the EEG frequency over 25 Hz. It has been shown in other studies that, low dosages of ketamine increase EEG power density of gamma frequencies (30 – 100 Hz) in both human and rodents [44-46]. This enhanced gamma activity was associated with higher arousal or alertness [47]. A second limitation is that we did not use an animal model that shows behaviors characteristic of depression, but only report on healthy animals. Future studies should also include the analysis of the coherence of other cortex regions and EEG bands that have been shown to be involved in responding to ketamine or SD, such as gamma oscillations and should include animal models of depression when possible.

Taken together, the common actions of SD and ketamine on sleep consist of an acute increase in NREM sleep SWA and a reduction in REM sleep. This suggests that these two effects may be related to the rapid antidepressant effect seen after both. The finding that REM sleep eventually recovers after SD, but not after ketamine is in accordance with the assumption that reducing the amount of REM sleep and increasing REM sleep latency is antidepressant in both of these treatments, and fits with previous findings showing that the effect of SD on depressive symptoms is transient and after ketamine longer lasting [11,48]. The latter suggests that reducing REM sleep may be an important feature of a sustainable positive effect on depressive symptoms after ketamine treatment. The acute application of SD and ketamine appear to induce similar cortical changes during NREM and REM sleep and may further influence long-lasting effects in the brain. Therefore, our data suggest that a previously unappreciated targeting of NREM sleep SWA, and REM sleep may help to improve antidepressant therapies.

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Supporting information

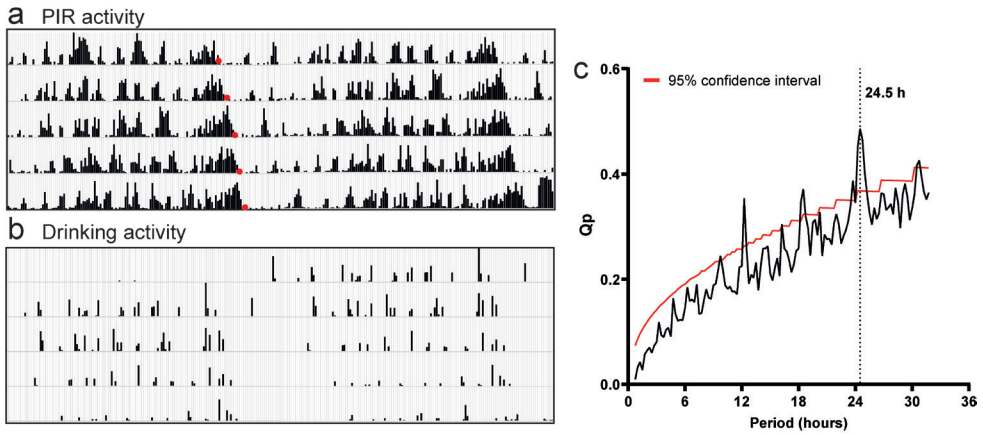

Figure. S1. Representative double plotted actogram. a. Representative double plotted actogram of PIR activity under constant darkness. Black areas indicate PIR activity. Red dot indicates the activity offset. Bin size = 10 min. b. Representative double plotted actogram of drinking activity under constant darkness. Black areas indicate drinking activity. c. The tau of the PIR activity was determined as the highest peak in the F-periodogram.

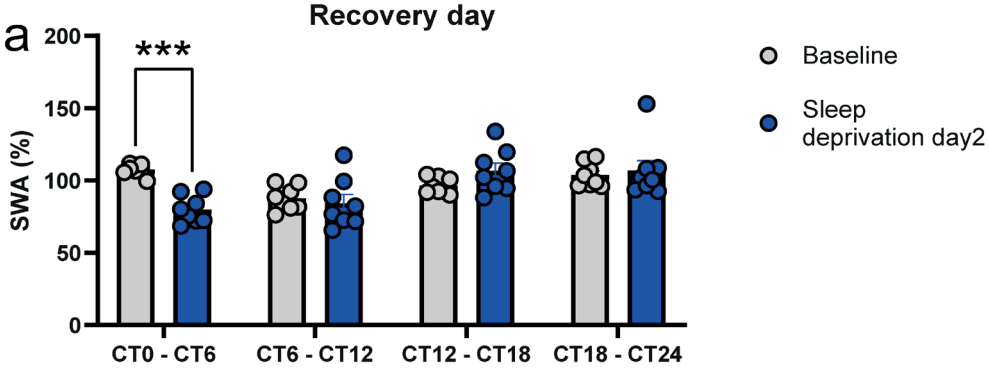

Figure. S2. Slow wave activity in NREM sleep and locomotor activity of sleep deprivation on the recovery day 2. Six-hour values of SWA in NREM sleep for the sleep deprivation (blue, n = 8) and baseline (grey, n = 8) condition on the second day after sleep deprivation. Data is shown as mean ± SEM.