Chronic social stress leads to altered sleep homeostasis in mice

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ABSTRACT

Disturbed sleep and altered sleep homeostasis are core features of many psychiatric disorders such as depression. Chronic uncontrollable stress is considered an important factor in the development of depression, but little is known on how chronic stress affects sleep regulation and sleep homeostasis. We therefore examined the effects of *chronic social stress* (CSS) on sleep regulation in mice. Adult male C57BL/6 mice were implanted for electrocortical recordings (ECoG) and underwent either a 10-day CSS protocol or control handling (CON). Subsequently, ECoG was assessed across a 24-h post-stress baseline, followed by a 4-h sleep deprivation, and then a 20-h recovery period. After sleep deprivation, CSS mice showed a blunted increase in sleep pressure compared to CON mice, as measured using slow wave activity (SWA, electroencephalographic power between 1 - 4 Hz) during non-rapid eye movement (NREM) sleep. Vigilance states did not differ between CSS and CON mice during post-stress baseline, sleep deprivation or recovery, with the exception of CSS mice exhibiting increased REM sleep during recovery sleep. Behavior during sleep deprivation was not affected by CSS. Our data provide evidence that CSS alters the homeostatic regulation of sleep SWA in mice. In contrast to acute social stress, which results in a faster SWA build-up, CSS decelerates the homeostatic build up. These findings are discussed in relation to the causal contribution of stress-induced sleep disturbance to depression.

46 Keywords: slow wave activity; sleep regulation; depression; chronic stress; REM

47 sleep

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1. INTRODUCTION

The need for sleep - sleep pressure - is regulated homeostatically, and in the electroencephalogram is best reflected by slow wave activity (SWA, 1-4.5 Hz) during NREM sleep (EEG) [1]. Sleep pressure increases across wakefulness, further increases during sleep deprivation [2, 3], and declines in the course of naps or nighttime sleep [4, 5]. Not only the duration but also the quality of waking, as well as the interaction with the environment, determine SWA.

In animals, including humans, the stress response to environmental stressors constitutes profound physiological changes to ensure survival and restore homeostasis [6, 7]. Environmental stressors are often acute social conflicts with conspecifics, which induce neuroendocrine and behavioral stress responses [8]. Acute social stress has been demonstrated to impact the build-up of SWA [9, 10], such that a short (1 hour) social conflict with an aggressive male conspecific leads to a doubling of SWA during NREM sleep [10]. Whether this marked increase in SWA induced by social stress reflects physiological sleep or rather an unrelated stress by-product process was investigated in a subsequent study [9]. Two groups of rats underwent a 6-hour sleep deprivation protocol, which was continuous by handling in one group and subdivided into an initial 1-hour of social conflict followed by a 5-hour sleep deprivation by handling in the other group. As expected, both groups revealed a strong effect of sleep deprivation by showing increased SWA during NREM sleep. Interestingly, the increase was significantly greater in the “social conflict with handling” group compared to the “handling only” group. Furthermore, because this

effect of increased SWA persisted throughout extended wakefulness, the authors concluded that acute social stress amplifies the homeostatic SWA build up. Wakefulness duration and quality affect subsequent SWA, with a positive correlation between the amount of exploratory behavior during sleep deprivation and the subsequent increase in SWA (72% of variability in SWA explained by time spent in exploratory behavior) [11]. Interestingly, both losers and winners of acute social conflicts exhibit increased SWA [12]. To date, to our knowledge, there have been no studies of the effects of chronic social stress (CSS) on SWA in subsequent sleep. It has been demonstrated that chronic social stress results in alterations of functional neural circuits [13] including resting state functional connectivity [14]. It is possible that CSS affects subsequent sleep, and in either a similar or indeed different manner to that reported for acute stress.

The aim of the present mouse study was to investigate the effects of CSS on sleep regulation in terms of SWA. The CSS protocol was a shortened version (10 days rather than 15 days) of that which has been demonstrated to induce depression-relevant changes in terms of resting-state functional connectivity [14], reduced motivation for reward [15], increased fear learning, helplessness and fatigability [16, 17], and physiological changes including increased peripheral and brain inflammation [16, 17]. Perhaps most relevant here, CSS mice exhibit increased activity during the light/inactive period, as measured in terms of drinking behavior [15]. Following the exposure of mice to either CSS or control handling (CON), effects on sleep were examined by means of electrocortical recordings to specifically

quantify vigilance states and SWA, during a 24-hour post-stress baseline, 4-hour sleep deprivation, and the subsequent 20-hour recovery period.

2. MATERIALS AND METHODS

2.1 Animals and maintenance

C57BL/6J mice were bred in-house and male offspring were weaned at age 3 weeks and housed in groups of three. Eight littermate trios were included in this study. Mice were aged 9-14 weeks at study onset and weighed 24.0-34.0 g. The aggressive male CD-1 mice (Janvier, France, www.janvier-europe.com) were aged 8 months, were ex-breeders, and housed singly at study onset. Mice were maintained on a reversed 12:12 h light-dark cycle (lights off 07:00-19:00 h) in an individually ventilated caging system (IVC), at 20-22 °C and 50-60% humidity. Cages were type 2L and contained woodchips, a sleep igloo and tissue bedding. Complete-pellet diet (Provimi, Kliba Ltd, Kaiseraugst, Switzerland) and water were available ad libitum. The study was conducted under a permit (110/2009) for animal experimentation issued by the Veterinary Office, Zurich, Switzerland. All efforts were made to minimize the number of animals used and any unnecessary stress.

2.2 Study design

The study design is illustrated in Figure 1. Each cage contained a littermate trio of C57BL/6J mice. Electrocortical surgery was conducted with two mice per trio. The

mouse that did not undergo surgery remained in the cage and was assigned to the naive (NV) group. The two implanted mice per trio were assigned to either the chronic social stress (CSS) or the control (CON) group. This design was used to reduce inter-group differences in baseline EEG measures. A week after surgery, CSS mice were transferred to the cage of a CD-1 mouse in order to conduct the CSS protocol (details below), whilst the NV and CON mice remained together as pairs. Following the 10-day protocol, all mice were transferred to an electrocorticogram (ECoG) laboratory. After one adaptation day, ECoG was recorded for two consecutive days. The first day served as post-stress baseline, and was followed by a 4-h sleep deprivation (SD) and a 20-h recovery period. Because 4-h and e.g. 6-h SD induce similar SWA effects in C57BL/6 mice [18], we chose the duration of 4-h SD. Whilst in the ECoG laboratory, CON and NV mice remained together in the same cage but separated by a perforated Plexiglas divider. CSS and CD-1 mice were maintained in the same cage (separated by the divider) until the end of SD, and were then separated during the recovery period. The time between the last CSS attack period (see 2.4) and the start of the baseline recording was 74 - 77 h.

(Figure 1 about here)

2.3 Surgical procedure

Surgery for ECoG recording was conducted according to a standard procedure [19]. Under sevoflurane (nose mask, 3-6 %; 1:4 O₂-to-air ratio), mice were implanted on the dura with three gold-plated miniature screws ($\varnothing$ = 0.9 mm) using the following coordinates: right hemisphere, frontal, 1.5 mm anterior to bregma, 2 mm lateral to midline; right hemisphere, parietal, 2 mm anterior to lambda, 2 mm lateral to midline;

midline, reference, above cerebellum, 2 mm posterior to lambda. Two gold wires ($\varnothing$ = 0.2 mm) were inserted bilaterally into the neck muscles for electromyogram (EMG) recording. Electrodes were attached to stainless steel wires connected to the skull with dental acrylic cement. Mice received one pre- and two post-operative doses of analgesic (0.1 mg/kg buprenorphine, s.c.). Mice regained pre-surgery weight within 2-3 days and were given a 7-day recovery period before the CSS protocol started (Fig. 1). At the end of the experiment all brains were inspected for potential infections from electrodes and observed to be free of cortical damage.

2.4 Chronic social stress (CSS)

The study protocol was adapted from that which we have developed for the study of neural, behavioral and physiological effects of CSS in mice (e.g., [14, 16]). The protocol is usually applied for 15 days. In the present study we were concerned that the daily attack sessions could result in damage to the ECoG cap. Accordingly, we reduced the number of daily attack sessions to 10 per CSS mouse (Fig. 1). On day 1 of the CSS protocol, each CSS mouse was placed in the cage of an aggressive CD-1 mouse so that bouts of attack occurred. The mice remained in the same cage for a maximum of either 60 sec of attack or 10 min. A transparent Plexiglas divider was then used to separate the mice in the same cage. The divider was perforated to allow sensory communication while preventing physical attacks. After 24 h each CSS mouse was placed in the cage of a novel CD-1 aggressor mouse, and the divider was removed for the 10-min attack period. Each day mouse pairs were interchanged

in this way and the procedure repeated across 10 consecutive days. Physical attack sessions were conducted between 14 h and 17 h. Overall, the shortest daily cumulative attack duration was 26 s and the 60 s maximum duration occurred frequently. Across the eight CSS mice the mean daily cumulative duration of attack time was consistent: from 42.1 ± 4.2 s to 46.9 ± 4.5 s (Mean $\pm$ SE). Bite wounds were prevented by trimming the incisor teeth of the CD-1 mice every third day using rodent tooth-cutting forceps. Mice did not obtain major visible injuries. Based on subsequent poor signal quality, the ECoG cap of one CSS mouse was damaged (see Results). CON and NV mice were handled daily. All mice were weighed daily: CSS typically induces an increase in the day-to-day change in body weight (Δ BW) [14, 16, 17]. We calculated Δ BW for 5-day time blocks of pre-CSS (day -4 to 1), CSS day 2 to 6 and CSS day 7 to 11 as Δ BW = (abs (BW day n – BW day n-1)) / (BW day n-1)*100.

2.5 Electrocortical recordings (ECoG)

During ECoG recordings, a divider separated CSS mice from aggressor CD-1 mice, and likewise a divider separated CON mice from NV littermates. The ECoG cap was attached to a connector soldered to a thin cable attached to a swivel. The light-dark cycle was maintained throughout the experiment (light on 19:00-07:00 h), and food and water were available ad libitum. Before each recording, EEG and EMG channels were calibrated with a 10 Hz, 300- μ V peak-to-peak sine wave. ECoG and EMG signals were amplified (amplification factor $\sim$ 2000), filtered (high-pass filter: -3 dB at 0.016 Hz; low-pass filter: -3 dB at 40 Hz), sampled with 512 Hz, digitally filtered (ECoG: low-pass finite impulse response (FIR) filter, 25 Hz; EMG: band-pass FIR

filter, 20-50 Hz)), and stored with a resolution of 128 Hz. ECoG power spectra were computed for 4 s epochs by a fast Fourier transform routine. Adjacent 0.25 Hz frequency bins were averaged into 0.5 Hz (0.25 - 5 Hz) and 1.0 Hz (5.25 - 25 Hz) bins. SWA was computed within 1.0 - 4.0 Hz.

The vigilance states NREM sleep, REM sleep and Wake were determined visually off-line from the ECoG and EMG signals, according to standard methods [19]. The percentage of epochs containing artefacts were: for NREM sleep: 6.9 ± 3.1 % in CON, 1.5 ± 0.4 % in CSS during post-stress baseline, 3.7 ± 2.8 % in CON, 1.6 ± 0.5 % in CSS during recovery sleep; for REM sleep: 1.7 ± 0.6 % in CON, 0.5 ± 0.2 % in CSS during post-stress baseline, 0.9 ± 0.6 % in CON, 0.6 ± 0.2 % in CSS during recovery sleep; and for wake: 31.6 ± 4.1 % in CON, 30.4 ± 3.7 % in CSS during post-stress baseline, 37.7 ± 3.9 % in CON, 33.9 ± 3.4 % in CSS during recovery sleep ($M \pm SE$). Epochs containing artefacts were excluded from spectral analysis.

2.6 Sleep deprivation

As reported in previous studies [18], sleep deprivation (SD) was performed for 4-h, starting at light onset (19:00 h) on the second day after transfer to the ECoG laboratory, by means of introducing new objects to the cage. Mice were videotaped during SD (Roline, RIC-45 IP Camera). Based on these video recordings behavior was then scored off-line from an ethogram, using instantaneous sampling with a 10-sec interval. The mutually exclusive behaviors scored were “no behavior”, “quietly awake”, “grooming”, “exploring”, “object exploring” and “social interaction at the

divider". "Number of rearings" during exploratory behavior was also identified [20]. All sensory stimulations undertaken by the experimenter to restrict mice from sleep, *i.e.*, "disturb", "object in", "object out", were also counted. The experimenter who scored behaviors as well as ECoG was blind to the experimental condition.

2.7 Statistical analysis

The frontal derivation was analysed. ECoG data analysis and statistics were performed using the MATLAB software package (MathWorks). Analysis of variance (ANOVA, mixed model) was conducted with the between-subject factor of 'group' (CSS, CON) and, depending on the dependent variable, a within-subject factor of 'time' e.g., day blocks for body weight, 2-h blocks for vigilance states and SWA. In the case of significant interaction effects, post hoc testing was carried out using the least significant difference (LSD) test. Statistical significance was set at $p < 0.05$ and $p < 0.1$ was used as a borderline non-significant trend effect. Data are expressed as mean $\pm$ standard deviation or standard error, as indicated.

3. RESULTS

The present study investigated the effects of a 10-day CSS protocol on vigilance states and on SWA as a marker of the homeostatic regulation of sleep in mice; the CSS protocol was adapted for ECoG recordings. In line with previous CSS studies, mean duration of total daily attack was 44.5 ± 1.9 sec per mouse. For absolute BW (Fig. 2A), there was a significant 'group' $\times$ 'time' interaction ($F(4, 40) = 4.883$, $p < 0.003$), but no significant group difference at any specific time point ($p \geq 0.06$); the

significant main effect of 'time' ($p < 0.0005$) reflected the increase in BW across time blocks. For day-to-day Δ BW (Fig. 2B), in accordance with previous studies, there was a significant 'group' x 'time' interaction ($F(4, 40) = 9.552$, $p < 0.0005$), with post hoc analysis showing increased Δ BW in CSS relative to CON and NV mice for days 2-6 ($p < 0.0005$) and in CSS relative to NV mice for days 7-11 ($p < 0.02$).

(Figure 2 about here)

3.1 Vigilance states

Following a one-day adaptation to the ECoG laboratory, sleep was assessed during a 24-h post-stress baseline, a 4-h SD and a 20-h recovery period. One CSS mouse was excluded from the study because of poor signal quality, resulting in $n=7$ for CSS and $n=8$ for CON. During post-stress baseline recording, CSS and CON mice exhibited vigilance states typical for light-dark conditions, with a predominance of Wake during the dark period and a predominance of NREM sleep during the light period (Fig. 3, $p < 0.0001$ for factor 'time'). As expected, SD markedly increased Wake ($99.4 \pm 0.3\%$ hours 1-2 and $98.4 \pm 0.7\%$ hours 3-4 in CON mice, $99.3 \pm 0.3\%$ hours 1-2 and $97.0 \pm 1.1\%$ hours 3-4 in CSS mice; Fig. 3A). There was no significant effect of CSS in post-stress baseline or recovery for Wake (Fig. 3A) or in post-stress baseline or recovery for NREM sleep (Fig. 3B) (all 'group' effects, $p = \text{n.s.}$). For REM sleep, while no group effect was found during post-stress baseline ($p = \text{n.s.}$), a difference was found in recovery, with CSS mice showing significantly increased REM sleep relative to CON mice ('group' $F(1,9) = 5.2$, $p < 0.05$; Fig. 3C). In order to

examine the effect of SD on vigilance states, we conducted a 2-way ANOVA with factors 'condition' (post-stress baseline vs. recovery) and 'time' (hour 5-6 to hour 22-24) and included CSS and CON mice. For NREM sleep (Fig. 3B), there was a main effect of 'condition' ($p < 0.05$) reflecting increased NREM sleep after SD that was sustained across the 18 h period. This was also the case for REM sleep, with a main effect of 'condition' ($p < 0.05$) (Fig. 3C).

(Figure 3 about here)

3.2 Slow-wave activity

We next investigated CSS effects on the homeostatic regulation of sleep in terms of SWA. Under post-stress baseline conditions, SWA followed a similar time course in both groups (Fig. 4A, $p = \text{n.s.}$ for factor 'group'). As expected, SD induced a SWA increase in both groups (comparison with post-stress baseline, $p < 0.05$, strongest effect initially). For 'group' x 'time' ANOVA of the recovery period there was a significant interaction effect on SWA ($F(1,9) = 2.3$, $p < 0.05$; Fig. 5B). Post hoc testing identified that the SWA increase was smaller in CSS mice relative to CON mice at the 2-h time intervals R1 (trend-level, $p = 0.06$), R2 (trend-level, $p = 0.09$) and R3 ($p < 0.05$). At each of these time intervals we then conducted an *a posteriori* power spectral analysis of CSS effects. In a 'group' x 'frequency' ANOVA, there was a significant main effect of 'group' for R1 ($p < 0.007$) and significant interaction effects for R2 ($p < 0.00001$) and R3 ($p < 0.00001$). For each time interval, *post hoc* LSD tests revealed reduced EEG power in CSS mice compared with CON mice specific to low frequencies i.e., the SWA frequency band, but extending up to 8 Hz in interval R1

(Fig. 5A-C). Therefore, power spectral analysis confirmed that the group effect was most prominent when sleep pressure was high. Because SWA depends on wake quality [11], we then examined for associations with the scores for behaviors measured during SD. CSS and CON mice exhibited no differences on any scored behavior ($p \geq 0.15$). The number of interventions during SD did not differ between groups, as quantified by 'number of disturbances' (CSS: 3.1 ± 2.1 , CON: 5.8 ± 9.6 , $p = \text{n.s.}$) and 'times object in/out' (CSS: 3.6 ± 3.4 , CON: 6.9 ± 10.4 , $p = \text{n.s.}$). These data indicate that the changes in SWA do not appear to be explicable in terms of a difference in the quality of waking during SD.

(Figure 4 about here)

(Figure 5 about here)

4. DISCUSSION

This study investigated the effects of chronic social stress on the homeostatic regulation of sleep in mice. While CSS did not change SWA during a 24-h post-stress baseline, 4h of SD elicited a blunted homeostatic SWA increase during subsequent sleep and briefly increased REM sleep. These findings show an effect of CSS on sleep homeostasis specific to the condition of enforced wakefulness, with no CSS effect apparent under baseline conditions. Our data show a SWA increase in CSS and CON mice following sleep deprivation. However, this SWA increase was significantly smaller in CSS relative to CON mice in a 6 h period following SD. That CSS mice did not show attenuated SWA at the beginning of post-stress baseline

sleep indicating that a sleep challenge is needed to uncover effects of CSS. Therefore, chronic stress together with the additional pressure of waking results in altered sleep physiology. We cannot exclude the possibility that a longer stress exposure protocol, including the 15-day protocol that we have used in other studies e.g. [14], would also impact on SWA in baseline sleep. Interestingly, CSS mice also showed increased REM sleep during recovery sleep, supporting an association between SWA in NREM sleep and features of REM sleep, as reported previously [21].

Previous research revealed that acute (1 h) social conflict in rats leads to increased SWA in subsequent sleep [10], whereas the current study provides evidence that chronic (10 day) social stress leads to an attenuated increase in SWA following SD. Therefore, the effects of social stress on sleep homeostasis would appear to depend on the duration of social stress. CSS may induce specific changes at a neurophysiological level that are reflected in altered SWA. Similar to our results, in rats, Mrdalj et al. demonstrated that chronic postnatal stress flattened the sleep homeostasis curve in a long-lasting manner [22]. Together, these studies suggest that stress affects the most established marker of sleep homeostasis, SWA, and that the direction of the effect is dependent on the duration of stress exposure. A further finding is that whereas acute stress effects were reported to be specific to SWA [10], CSS affects an extended frequency range up to 8 Hz and the study of chronic stress in rats also reported this [22]. When comparing the current findings with the existing literature, it is important to consider that the present study did not investigate the response to social conflict per se, but rather investigated the effects of chronic social

stress in terms of brief conflict encounters with and otherwise continuous distal exposure to dominant, aggressive mice.

The daily attacks that form part of CSS took place at the same time each day (*i.e.*, 14 - 17 h) across the 10 days. Predictability can influence the perception of the stressor and modulate the direction of the effects of stress on sleep [23]. Predictable stressors may be perceived as less severe, which may contribute to the absence of group differences in the post-stress baseline. The efficacy of our protocol compared to the 15-day protocol which leads to depression-relevant effects on physiology, brain and behavior (*e.g.*, [15, 16, 24]), was demonstrated by the consistent biomarker of increased body weight delta in CSS compared with CON mice. Quantifying the neurotransmitter and neuromodulators related to stress in addition to sleep (*e.g.*, monoamines, hypocretin/orexin, corticotropin releasing hormone, inflammatory factors) would provide a more mechanistic underpinning of the physiological effects of CSS. Indeed, transcriptomic studies of cortical and limbic brain regions have identified altered expression of a number of genes relevant to dopamine and serotonin neurotransmission in 15-day CSS mice [16].

Chronic psychosocial stress is a major aetiological factor in depression [25, 26]. The S-deficiency hypothesis of Borbély et al. [27] proposes a slower build-up of homeostatic sleep pressure during wakefulness in depressed patients, including lower SWA at sleep onset. Indeed, depressed humans show a slower accumulation of SWA [28-30], and the current findings are in line with this. CSS induces adrenal gland and spleen hypertrophy, and increased levels of pro-inflammatory factors in the

periphery and brain [16, 17], indicating an impact of CSS on stress-responsive systems that, in turn, are associated with altered sleep [31].

As expected, effects of SD on vigilance states included an increase in NREM and REM sleep, and in CSS and CON mice. Changes in vigilance states could have accounted for the post-SD blunted SWA increase in CSS mice. However, with the exception of increased REM sleep during the recovery period, vigilance states did not differ in CSS compared to CON mice. We thus conclude that CSS in mice induces specific changes in the best-established marker of sleep regulation, namely SWA. Interestingly, in a recent study we observed that CSS mice (15-day protocol) exhibit increased light-phase activity compared with CON mice [15]. SWA is determined by the quality as well as the quantity of wakefulness, e.g. [11], such that differences in behavior and therefore wakefulness quality during SD could have accounted for the observed CSS-effect. However, results showed no behavioral differences between CSS and CON mice during the SD, suggesting that neither alterations in vigilance nor behavioral differences during SD contributed to the blunted SWA increase in CSS mice.

Using 4-day stress exposure in the form of repeated fear conditioning of an auditory tone to footshock, Sanford et al. report a subsequent reduction in REM sleep, and re-exposure to the tone several days later again reduced REM sleep [32]. Accordingly, physical and social stressors, as well as the stress duration, would appear to contribute to effects on REM. ECoG-assessments will be needed to measure CSS-dependent changes in vigilance states long-term. Additionally, future studies should investigate topographical differences in the effect of CSS exposure on

sleep regulation by also analysing EEG from the parietal derivation. Future studies may also examine whether chronic stress affects specific frequency ranges in waking and in REM sleep.

The study of neuronal correlates of SWA might well provide mechanistic insight into impaired homeostatic regulation in CSS. SWA closely reflects synchronized activity of cortical neurons [33] and synaptic plasticity [34]. The blunted build-up of SWA in CSS mice might thus indicate adaptations to chronic stress by means of impaired synchronisation via changes in synaptic mechanisms. Synaptic plasticity is linked to stress via endocrine and immune pathways [16, 35-37]. It is possible that CSS is associated with altered pyramidal cell excitability and inhibitory activity [37], via stress-related changes of postsynaptic AMPA, NMDA and glutamate receptors in the medial prefrontal cortex. Interestingly, CSS results in increased functional connectivity within prefrontal cortex [14] and in changes in transcriptome expression [16].

In summary, our findings show that CSS alters the homeostatic SWA response, and does so in a direction opposite to that induced by acute stress. It will be important to further increase the understanding of interactions between chronic social stress, sleep regulation, and physiology-behavior links. While quality of waking can alter sleep regulation and vigilance states, quality of sleep may increment the development of a depressive phenotype [38], suggesting a bi-directional and probably self-intensifying cycle.

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393 All authors declare no competing financial interests.

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FIGURE LEGENDS

Figure 1: Overview of the study design. Groups are represented by shaded backgrounds (black: chronic social stress (CSS); grey: control (CON); white: naive (NV)). Experiment refers to CSS protocol (CSS group) or handling (CON and NV groups); ECoG: electrocortical recording. Numbers of animals included in analysis are indicated for each group.

Figure 2: Body weight from immediately prior to until the end of the chronic social stress period. (A) Absolute body weight. (B) Day-to-day body weight delta. In mixed-model ANOVA there was a 'group' x 'time' interaction with *post hoc* tests identifying increased ΔBW in CSS mice vs CON and NV mice at days 2-6 (**** $p < 0.0005$) and in CSS mice vs NV mice at days 7-11 (* $p < 0.02$).

Figure 3: Vigilance states during post-stress baseline (24 h), sleep deprivation (4 h) and recovery (20 h). White and black bars represent 12-h light and 12-h dark periods, respectively. (A) Wake, (B) non-rapid eye movement (NREM), and (C) rapid eye movement (REM) sleep are shown for chronic social stress (CSS) and control (CON) groups. Data are averaged across 2-h time intervals. Values are expressed as a percentage of entire recording time. A mixed model ANOVA was performed for each panel with between-factor 'group' (CON vs. CSS) and within-factor 'time' (12 x 2 h for Post-Stress Baseline, 10 x 2 h for Recovery). REM sleep in recovery was

significantly increased in CSS relative to CON mice (mixed model ANOVA and post hoc LSD tests are indicated as text in the figure).

Figure 4: Slow wave activity (SWA, 1-4.5 Hz) in chronic social stress (CSS) and control (CON) groups during (A) post-stress baseline and (B) recovery. White and black bars represent 12-h light and 12-h dark periods, respectively. SWA is averaged across 2-h time intervals. Values are expressed as a percentage of the mean of 24-h post-stress baseline SWA. Asterisks (gray box) indicate time intervals with significant or trend-level increases in SWA relative to the post-stress baseline condition for CON (black) and CSS (red) mice (“*” $p < 0.05$; “°” $p < 0.1$; *i.e.*, 6 h post-stress baseline vs. 6 h recovery etc). For post-stress baseline and recovery separately, ANOVA was performed with ‘group’ (CSS vs. CON) as between-subject factor and ‘time’ (2-h intervals) as within-subject factor. For post-stress baseline there was a significant effect of ‘time’ ($F(1,11) = 2.7$, $p < 0.005$) and no ‘group’ or interaction effect (both $p = n.s.$). For recovery, there was a significant ‘group’ x ‘time’ interaction ($F(1,9) = 2.3$, $p < 0.05$), reflecting group differences in SWA. Post-hoc LSD tests identified lower SWA in CSS compared to CON mice in the first three 2 h intervals post-SD (indicated as R1, R2, R3).

Figure 5: Power spectral analysis of electrocortical recordings (ECoG) after sleep deprivation. Power spectra are shown for chronic social stress (CSS, red) and control (CON, black) mice for (A) hours 1-2 (R1), (B) hours 3-4 (R2), and (C) hours 5-6 (R3)

post-sleep deprivation, expressed as a percentage of 24-h post-stress baselines (M + SE). Mixed-model ANOVA with between-subject factor 'group' and within-subject factor 'frequency' revealed for R1: 'group' $F(1,29) = 10.3$, $p = 0.007$, 'interaction' $F(1,29) = 1.0$, $p = \text{n.s.}$; for R2 'group' $F(1,29) = 50.4$, $p < 0.00001$, 'interaction' $F(1,29) = 11.7$, $p < 0.00001$; and for R3: 'group' $F(1,29) = 41.2$, $p = 0.00002$, 'interaction' $F(1,29) = 27.4$, $p < 0.00001$. Post hoc LSD tests identified frequency-specific group effects (* $p < 0.05$). “

554 FIGURES

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