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Research report

Effects of social stimuli on sleep in mice: non-rapid-eye-movement (NREM) sleep is promoted by aggressive interaction but not by sexual interaction

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Abstract

Sleep is generally considered to be a process of recovery from prior wakefulness. In addition to being affected by the duration of the waking period, sleep architecture and sleep EEG also depend on the quality of wakefulness. In the present experiment, we examined how sleep is affected by different social stimuli (social conflict and sexual interaction). Male C57BL/6J mice were placed in the cage of an aggressive dominant male or an estrous female for 1 h in the middle of the light phase. The conflict with an aggressive male had a pronounced NREM sleep-promoting effect. EEG slow wave activity, a measure of NREM sleep intensity, was increased for about 6 h and NREM sleep time was significantly increased for 12 h. REM sleep was strongly suppressed during the remainder of the light phase after the conflict, followed by a rebound later in the recovery phase. The sexual interaction, in contrast, had only mild effects. Both NREM sleep and REM sleep were somewhat suppressed shortly after the interaction. In a separate group of mice, blood samples were taken to measure prolactin and corticosterone. The results suggest that the temporary suppression of REM sleep following the social stimuli may be partly due to elevated corticosterone. The different effects of the social stimuli on NREM sleep are not easily explained by differences in the hormone responses. In conclusion, although both social conflict and sexual interaction induce a strong physiological activation, only social conflict has a strong stimulatory effect on NREM sleep mechanisms. © 2001 Published by Elsevier Science B.V.

Theme: Neural basis of behavior

Topic: Biological rhythms and sleep

Keywords: Stress; Social defeat; Sleep deprivation; EEG delta power; Slow wave sleep; Paradoxical sleep

1. Introduction

Sleep is a complex phenomenon that consists of two distinct stages, non-rapid-eye-movement (NREM) sleep and rapid-eye-movement (REM) sleep, each of which may have its own function. Traditionally, NREM sleep is thought of as a process during which the brain recovers from wakefulness but, in spite of many theories, the exact nature of this recovery process remains an unsolved mystery [1,8]. The intensity of the NREM sleep recovery process is thought to be reflected in the amount and amplitude of slow waves in the EEG [2]. Slow wave activity (SWA) during NREM sleep, that is, the spectral density in the delta frequency range (1–4 Hz), is a function of prior wakefulness, a relationship that has been established in many species including humans [5], rats [21], and mice [9]. The longer the prior period of wakefulness, the higher is SWA at the beginning of NREM sleep. Thereafter, SWA gradually decreases in the course of sleep.

In addition to being affected by the duration of the waking period, SWA during NREM sleep also depends on the quality of wakefulness. A recent study in male rats showed an increase in NREM sleep SWA after a social conflict with an aggressive conspecific, compared to animals that were kept awake for the same duration by gentle handling [16]. Since social defeat in rodents is a well-validated stress model [11,14], these data suggested that experiencing stress during wakefulness may accelerate the build up of sleep debt and increase the need for sleep [16]. However, the critical aspect of the social conflict

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procedure that causes the increase in SWA remains to be determined.

Since little is known about the effects of different waking experiences on sleep, it is an important question whether the increase in NREM sleep SWA after a conflict is due to certain specific elements of the response to social defeat, or whether it is the consequence of non-specific physiological activation and arousal that may also occur in response to other stimuli. In the present experiment, we examined whether the increase in NREM sleep SWA after an aggressive social conflict in rats is a general phenomenon, which also occurs in other species (mice) and also occurs after social stimuli from a different nature (sex). We compared the effects of a social conflict with an aggressive conspecific with the effects of sexual interaction with an estrous female. Both kinds of social interaction are highly arousing and induce a strong non-specific physiological activation. Increased activity of the sympathetic nervous system and the hypothalamus–pituitary–adrenal axis are found both after social conflict [6,20] and sexual interaction [3,4]. However, certain brain systems that are activated are different as is reflected in, for instance, different patterns of c-fos expression [10,13]. Also the emotional processes that are involved and the specific behavioral responses elicited are different. An aggressive dominant male is an aversive stimulus that mice will try to avoid by running away but an estrous female is a stimulus that will attract male mice. Together, the comparison between an aggressive and a sexual interaction seemed a valid approach to the question whether the increase in NREM sleep SWA after a conflict is due to general physiological activation or to some specific aspect of social defeat.

In a first group of mice we measured the effects of social conflict and sexual interaction on sleep architecture and sleep EEG. In a second group of animals, blood samples were taken at the end of the experimental manipulations for analysis of prolactin and corticosterone as indicators of physiological activation. Both of these hormones increase in response to social defeat as well as sexual interaction [3,6]. Moreover, both hormones are known to affect sleep. Prolactin is often associated with an increase in REM sleep [19] whereas glucocorticoids have been found to suppress REM sleep but stimulate NREM sleep [7]. Therefore, we used prolactin and corticosterone as indicators of arousal that perhaps could explain some of the changes in sleep after the social interactions.

2. Materials and methods

The study was performed with 4-month-old male C57BL/6J mice purchased from Jackson Laboratory (Bar Harbor, ME, USA). The animals were individually housed under a 12-h light/12-h dark cycle with lights on from 06:00 to 18:00 h. Food and water were provided ad libitum throughout the experiments. The mice were allowed at least 3 weeks of adaptation before the start of the experiments. In a first experiment we examined the effects of social conflict and sexual interaction on sleep, and in a second experiment blood samples were taken to measure prolactin and corticosterone levels.

2.1. Social interactions

Male mice were subjected to a social conflict with an aggressive male or an interaction with a female during the sixth hour of the light phase. At that time basal prolactin and corticosterone levels are low and relatively stable, making it easier to establish experimentally induced increases in the hormones. For the 1-h social conflict, the experimental animals were placed in the cage of an aggressive conspecific from the same strain (C57BL/6J). The aggressors were older and heavier animals that had been trained to fight and protect their home cage territory by confronting them with young intruders on a regular basis. All experimental animals were attacked and defeated, as indicated by fleeing and freezing behavior. After the 1-h confrontation, the experimental mice were returned to their home cage and the sleep recording chamber and had no further contact with the aggressive dominant animal [15].

For the sexual interaction, the experimental animals were placed in the cage of a female mouse of the same strain (C57BL/6J). The females had been treated with estradiol and progesterone injections to induce behavioral estrus at the time of the interaction, thereby minimizing variation in the interaction due to the estrus cycle as much as possible [17]. The experimental animals were clearly attracted by the females, followed them through the cage, and frequently mounted them to copulate. After 1 h the experimental animals were returned to their home cage and had no further contact with the female.

In addition to the social interactions, we included a 1-h sleep deprivation control procedure to differentiate any effects induced by the social stimuli from normal recovery after sleep loss. The sleep deprivation procedure consisted of keeping the mice awake with as little disturbance as possible by tapping on the cage, gently shaking the cage, and if necessary gently touching the animals. This sleep deprivation procedure is referred to as handling or gentle handling.

2.2. Sleep recordings

For sleep recordings, eight male mice were implanted with permanent electrodes to record cortical EEG and neck muscle EMG. Surgery was performed under deep metofane anesthesia. Two screws through the skull (diameter 1 mm) served as EEG electrodes. One screw was placed above the right hemisphere, 2 mm from the midline and 1 mm anterior of bregma. The other screw was placed

on the left hemisphere, 3 mm from the midline and 1 mm anterior of lambda. Two insulated stainless-steel wires served as EMG electrodes and were inserted under the neck muscles. The EEG and EMG electrodes were attached to a connector that was cemented on the skull with dental acrylic. The mice were allowed at least 2 weeks of recovery from surgery. After recovery, the animals were hooked up to the recording equipment via a recording cable and a swivel, which allowed free movement throughout the cage. After 3 days of habituation to the recording tether, EEG and EMG signals were recorded and fed into an amplifier (Grass model 12; Grass Instrument Division, Astro-med, West Warwick, RI, USA). The EEG signal was amplified 10 000 times, high-pass filtered at 1 Hz (−6 dB, 6 dB/octave) and low-pass filtered at 30 Hz (−6 dB, 6 dB/octave). The EMG signal was amplified 5000 times, high-pass filtered at 3 Hz and low-pass filtered at 100 Hz. The signals were then converted to digital format and stored at 102.4 Hz resolution. The signals were collected and stored on an IBM computer system with specialized software for acquiring and processing sleep data in rodents (Multisleep; Actimetrics, Evanston, IL, USA). EEG and EMG were measured for two consecutive days, a baseline day and an experiment day, starting at lights-on. On the second day, the animals were subjected to either gentle handling, sexual interaction, or social conflict during the sixth hour of the light phase. The remaining 18 h of that day, i.e., the second half of the light phase and the following dark phase, were considered the recovery period. All mice were subjected to the three different treatments in random order with at least 1 week in between.

2.3. Analysis of vigilance states

By visual inspection of the EEG and EMG signals, 10-s epochs were classified as wakefulness, NREM sleep or REM sleep. Wakefulness was characterized by a low amplitude EEG with mixed frequency components and a relatively high, often irregular EMG activity. During NREM sleep the EEG had a high amplitude and was dominated by 1–4 Hz slow frequency waves, while EMG activity was low. REM sleep was characterized by a low amplitude EEG dominated by 6–9 Hz theta waves and low EMG activity. In addition, the EEG signal was subjected to spectral analysis by fast Fourier transformation and, for all NREM sleep epochs, the EEG power in the delta or slow wave range (1–4 Hz) was calculated. To correct for interindividual differences in strength of the EEG signal, the delta power values of all animals were normalized by expressing them relative to their own baseline delta power. The average NREM sleep delta power per time interval was expressed as percentage of the average 24-h baseline NREM sleep delta power and is referred to in the text and figures as slow wave activity (SWA). The accumulated NREM sleep delta power per time interval was expressed as percentage of the total 24-h baseline NREM sleep delta power and is referred to as cumulative slow wave energy (SWE). The cumulative SWE was calculated to take into account the actual time the animals were asleep and to correct for differences in sleep patterns that occurred after the various experimental manipulations. The social interactions not only kept the animals awake during the 1-h experiment but also changed sleep time afterwards. Therefore, the average SWA per time interval during recovery not only depended on the experimental manipulation but also on the sleep–wakefulness ratio afterwards. By calculating the accumulated SWE during the recovery period it was possible to measure whether an increase in SWA was due to increased intermittent wakefulness (the quantity of wakefulness) or due to an additional effect of the social interactions (the quality of wakefulness). For presentation and statistical analysis of the data, NREM and REM sleep time, NREM sleep SWA, and cumulative NREM sleep SWE were calculated for 1-h intervals and for three consecutive 6-h intervals during the recovery period. The 1-h intervals were used for detailed illustration of the sleep patterns and the acute effects of the experimental manipulations. The 6-h values, which were less variable, give a clearer indication of the overall effects and were used for statistical analysis.

2.4. Prolactin and corticosterone

In a separate experiment using different animals, we measured the effects of the three experimental manipulations on plasma prolactin and corticosterone levels. These hormones were chosen because they are known to increase in response to arousing stimuli and both hormones have been reported to affect sleep. Groups of male C57BL/6J mice were subjected to either gentle handling, sexual interaction, or social conflict as described above ($n=10$ for each manipulation). After 1 h, the animals were decapitated and trunk blood was collected in chilled centrifuge tubes (0°C) containing EDTA. The blood was centrifuged at 4°C for 15 min at $2600\times g$ and the supernatant was stored at −80°C for later analysis. Prolactin and corticosterone concentrations were determined by radioimmunoassay.

2.5. Statistics

Statistical analysis of the prolactin and corticosterone data was performed with one-way ANOVA with factor condition (gentle handling, sexual interaction, or social conflict). When ANOVA showed a significant condition effect, the different experimental manipulations were tested pair-wise with two-sample *t*-tests. For the sleep data, statistical analysis was performed with repeated measures ANOVA with a factor condition (baseline and recovery from handling, sexual interaction, or social conflict) and a factor time interval (consecutive 6-h blocks). Since the baselines that preceded each of the experimental manipula-

tions did not differ from each other, the average of the three was used for the ANOVA. When the overall ANOVA revealed a significant effect of condition or a significant condition×time interaction, paired *t*-tests were applied to determine between which conditions and at which 6-h intervals the differences occurred.

3. Results

3.1. NREM sleep

The detailed pattern of NREM sleep per hour is shown in Fig. 1 and the averages for consecutive 6-h blocks are given in Fig. 2. Repeated measures ANOVA revealed a significant condition effect ($F_{3,28}=4.62$, $P=0.010$) and a significant condition×6-h interval interaction ($F_{6,56}=4.37$, $P=0.001$). From the three experimental manipulations, the social conflict had by far the largest impact on NREM sleep. Whereas the sexual interaction with a female and the gentle handling procedure only had small effects, the social conflict caused a pronounced increase in subsequent NREM sleep time. Overall, the amount of NREM sleep after the conflict was significantly elevated above baseline levels during the remainder of the light phase and first half of the dark phase (Fig. 2, first and second 6-h block). During this 12-h period, the amount of NREM sleep after the conflict also was significantly higher than the levels after the sexual interaction, and during the first half of the dark phase it was significantly higher than after sleep deprivation by gentle handling. In contrast to the aggressive interaction with a male, the sexual interaction with a female resulted in a small decrease in the amount of NREM sleep below baseline levels (Fig. 2, first 6-h block).

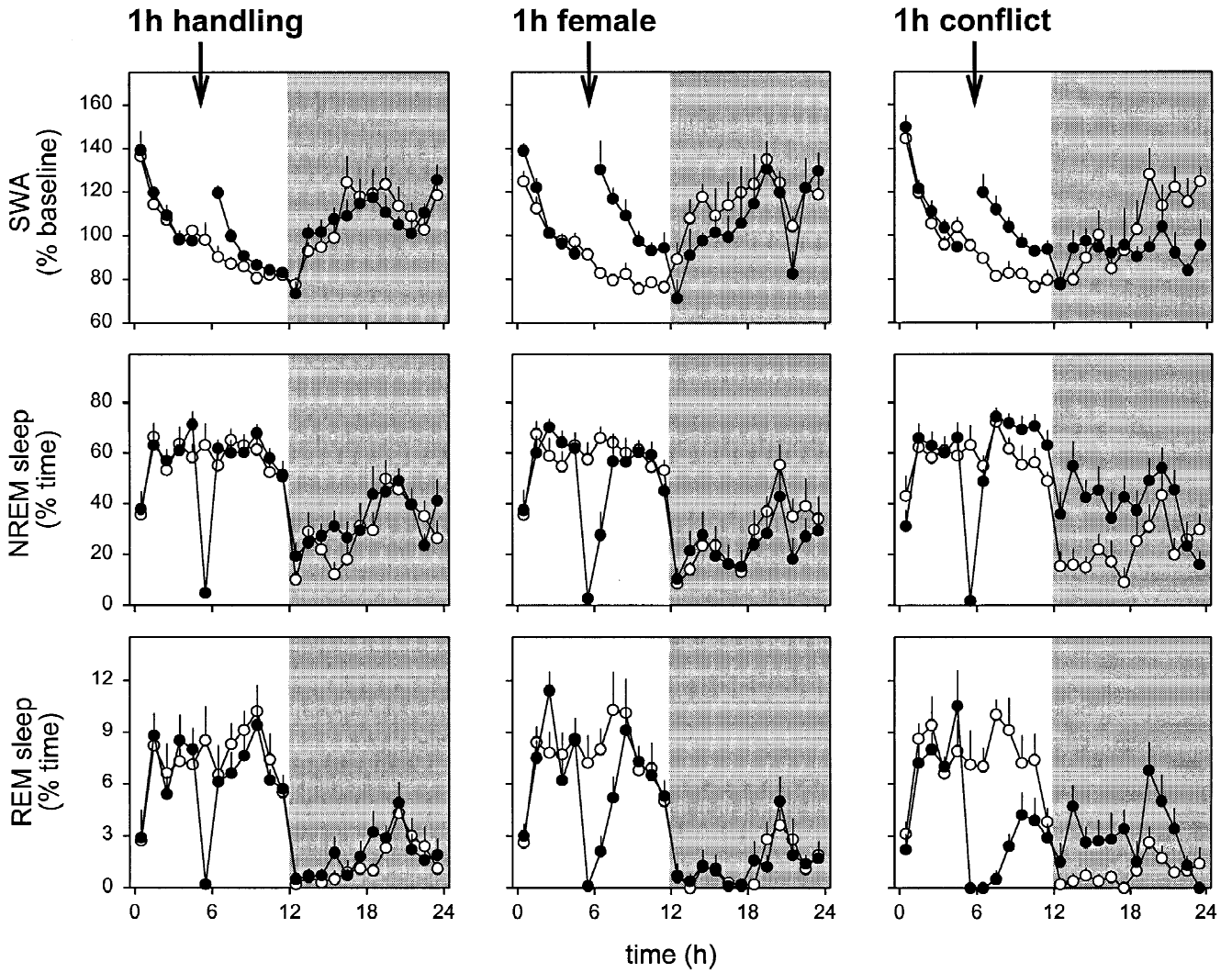

Fig. 1. Hourly values of REM sleep, NREM sleep, and NREM sleep SWA in male C57BL mice subjected to 1 h of gentle handling, a 1-h sexual interaction with an estrous female, or a 1-h aggressive interaction with a dominant male ($n=8$ for each manipulation). Experimental manipulations took place in the middle of the light phase. Shaded areas indicate the dark phase. Open symbols represent the baseline recording (○) and the closed symbols are the experimental day (●).

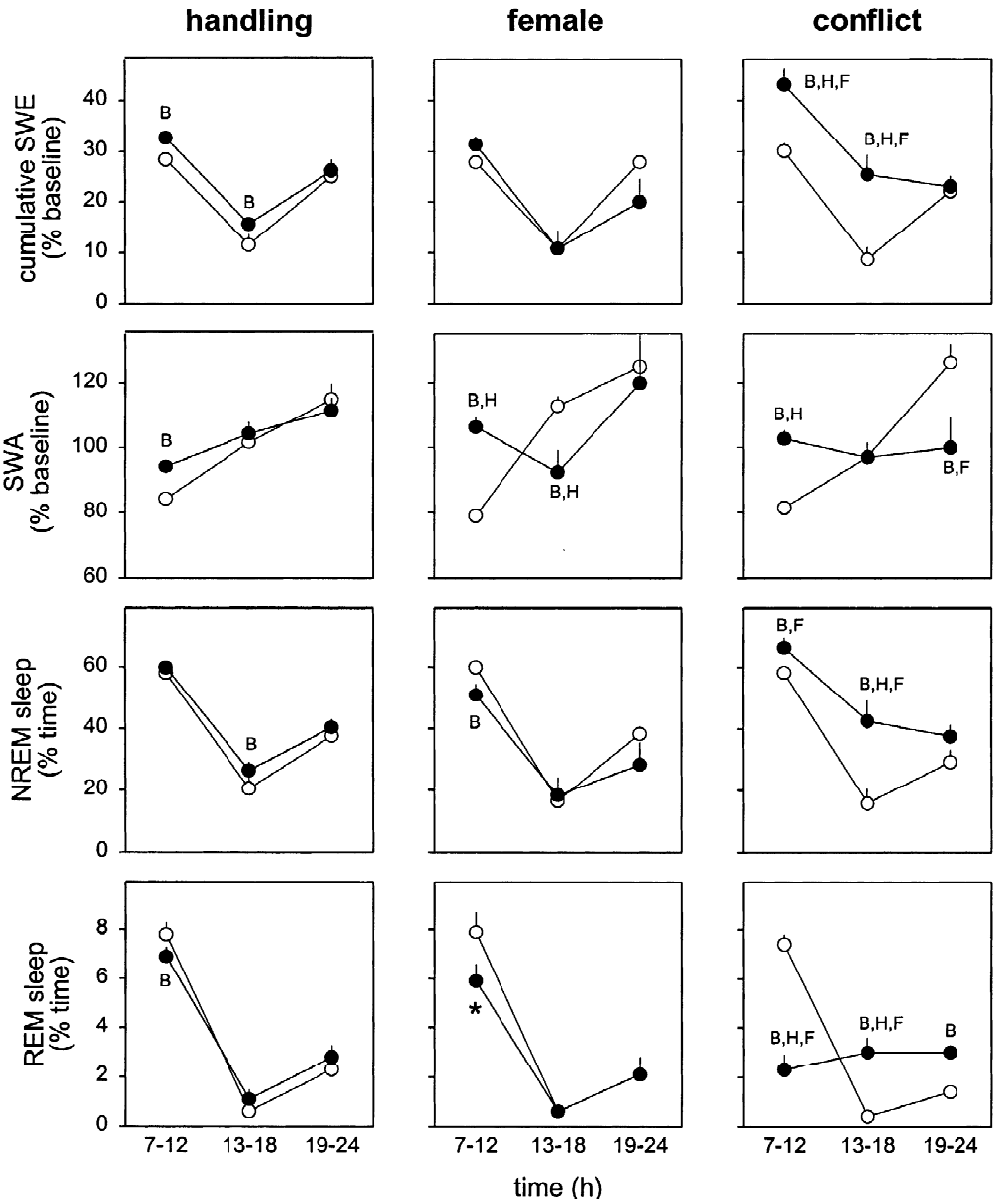

Fig. 2. REM sleep, NREM sleep, NREM sleep SWA, and cumulative NREM sleep SWE per 6-h interval in male mice following 1 h of gentle handling, a 1-h sexual interaction with an estrous female, or a 1-h aggressive interaction with a dominant male. The first 6-h interval is the second half of the light phase (hours 7–12 in Fig. 1); the second 6-h interval is the first half of the dark phase (hours 13–18 in Fig. 1); and the third 6-h interval is the second half of the dark phase (hours 19–24 in Fig. 1). Open symbols (○), baseline recording; closed symbols (●), experimental day. Data are expressed as averages (±S.E.M.) and were subjected to analysis of variance (see text). Only when ANOVA revealed a significant effect of treatment or a treatment×6-h interval interaction, were successive 6-h intervals compared separately using *t*-tests. Significant differences: B, relative to baseline; H, relative to handling; F, relative to female (two-tailed paired *t*-test, $P<0.05$; *indicates a trend with $P=0.054$).

The more detailed illustration of NREM sleep patterns in Fig. 1 suggests that this effect mainly consisted of an acute and short-lasting suppression of NREM sleep immediately following the interaction. Also the gentle handling procedure did not have a major effect on subsequent NREM sleep time, except for a very small but significant increase during the first half of the dark phase (Fig. 2, second 6-h block).

3.2. NREM sleep SWA

As expected, the NREM sleep that was lost during the experimental manipulations induced a compensatory increase in subsequent NREM sleep SWA, in line with the concept that higher SWA reflects more intense sleep. After all the experimental manipulations, the increase in NREM sleep SWA gradually disappeared in the course of recovery

sleep. However, the increase in SWA, appeared to persist longer after the social interactions than after the gentle handling procedure (Fig. 1). When repeated measures ANOVA was applied for the 6-h values of SWA, there was a significant condition×6-h interval interaction ($F_{6,56}$= 3.77, P=0.003). Compared to baseline, SWA was significantly elevated after all three experimental manipulations but more so after the social stimuli than after gentle handling (Fig. 2, first 6-h block). However, SWA levels dropped below baseline levels early in the dark phase after the sexual interaction (Fig. 2, second 6-h block) and late in the dark phase after the conflict (Fig. 2, third 6-h block).

3.3. Cumulative NREM sleep SWE

The interpretation of changes in SWA after the social interactions compared to the gentle handling procedure is complicated by the fact that the interactions not only kept the animals awake during the encounter but also changed the amount of NREM sleep afterwards. Therefore, the SWA not only depended on the experimental manipulations but also on the sleep–wake ratio afterwards. To take into account the actual amount of sleep time, we calculated the accumulation of NREM sleep slow wave energy. The accumulated NREM sleep SWE per 6-h interval is shown in Fig. 2. Repeated measures ANOVA revealed a significant effect of condition ($F_{3,28}$=5.46, P=0.004) and a significant condition×6-h interval interaction ($F_{6,56}$=6.32, P<0.001). The accumulated SWE during the first 12 h after the social conflict was significantly higher than under all other conditions (Fig. 2, first and second 6-h block). During the remainder of the light phase after the conflict (first 6-h block), the increase in NREM sleep SWE was due to both elevated SWA (compared to baseline and handling) and increased NREM sleep time (compared to baseline and sexual interaction). During the first half of the dark phase (second 6-h block), although SWA had returned to baseline levels, the animals that had been subjected to a social conflict continued to accumulate more SWE due to an increase in NREM sleep time (compared to all other conditions). The analysis further shows that, despite the lower SWA during the second half of the dark phase, the accumulation of SWE during that time did not differ from baseline or from the other treatments (Fig. 2, third 6-h block). In other words, due to increased levels of NREM sleep, it required less SWA to accumulate the same amount of SWE. In contrast to the social conflict, the sexual interaction had no major effect on the accumulation of SWE. After sexual interaction, the SWE was not significantly different from SWE during baseline or SWE after sleep deprivation by gentle handling. These data suggest that the prolonged increase in SWA after the sexual interaction compared to sleep deprivation by gentle handling can be explained by the additional wakefulness that occurred afterwards. The 1-h sleep deprivation by gentle handling itself resulted in a small but significant increase in SWE compared to SWE levels during baseline (Fig. 2, first and second 6-h block).

3.4. REM sleep

The amount of REM sleep per hour is depicted in Fig. 1 and the 6-h averages are given in Fig. 2. Repeated measures ANOVA showed a significant condition×6-h interval interaction ($F_{6,56}$=25.11, P<0.001). From the three treatments, especially the interaction with an aggressive conspecific had a strong effect on subsequent REM sleep. The conflict with the dominant male induced an acute and pronounced suppression of REM sleep. The amount of REM sleep was significantly lower than under all other conditions during the remainder of the light phase immediately after the conflict (Fig. 2, first 6-h block). This temporary decrease in REM sleep was followed by a rebound later in the recovery period. During the first half of the dark phase the amount of REM sleep was significantly higher than under all other conditions (Fig. 2, second 6-h block) and during the second half of the dark phase it was still elevated above baseline levels (Fig. 2, third 6-h block). At the end of the dark phase the animals had almost made up for the REM sleep that was lost immediately after the conflict (2.8±0.3% REM sleep during the 18-h period following conflict versus 3.1±0.2% during the same 18-h baseline period; not significantly different). Also after the sexual interaction with an estrous female there was a trend for REM sleep to be suppressed (Fig. 2, first 6-h block; P=0.054 compared to baseline). This effect, however, was much smaller than after that of the conflict and it was it was not followed by a clear rebound. The amount of REM sleep during the dark phase after the sexual interaction was not different from baseline. Sleep deprivation by gentle handling only caused a very small decrease of REM sleep during the first 6 h afterwards (Fig. 2, first 6-h block).

3.5. Prolactin and corticosterone levels

The prolactin and corticosterone levels at the end of the 1-h experimental manipulations are shown in Fig. 3. The prolactin levels at the end of the social interactions were quite variable but there was clearly no difference between the aggressive interaction with a male and the sexual interaction with a female. On average, the prolactin levels after both social interactions appeared to be higher than after 1 h gentle handling but, due to the variability, especially in the conflict group, the overall effect of the treatment did not reach statistical significance (ANOVA: $F_{2,27}$=2.51, P<0.099). The corticosterone levels were less variable and ANOVA revealed a clear overall effect of the treatment ($F_{2,27}$=52.69, P<0.001). The corticosterone levels after both the conflict and the sexual interaction were higher than after gentle handling. In addition, the corticosterone levels after the conflict were slightly but

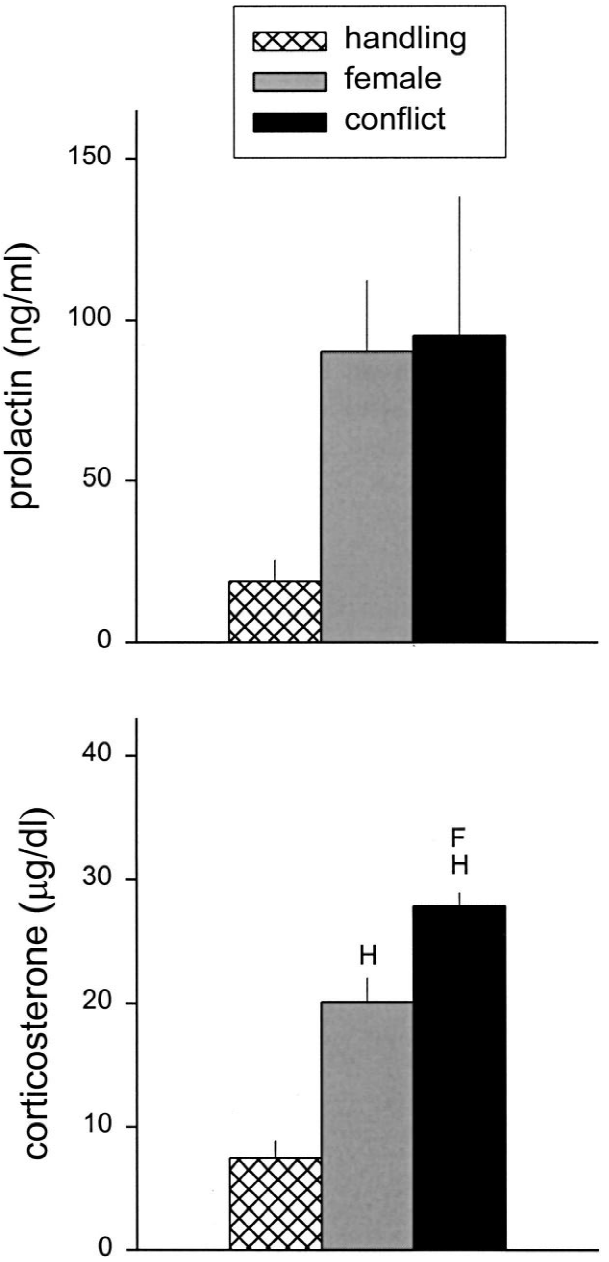

Fig. 3. Prolactin and corticosterone levels in male C57BL mice after 1 h of gentle handling, after a 1-h sexual interaction with an estrous female, or after a 1-h aggressive interaction with a dominant male. Data are expressed as averages (±S.E.M.) and were subjected to analysis of variance (see text). Only when ANOVA revealed a significant overall effect of treatment, were groups tested pair-wise. Significant differences: H, relative to handling; F, relative to female (two-tailed two-sample *t*-test, $P<0.05$).

significantly higher compared to levels after the sexual interaction (Fig. 3).

4. Discussion

A social conflict has a strong NREM sleep-promoting effect in male mice. An encounter with an aggressive and dominant conspecific for 1 h induced an increase in subsequent NREM sleep SWA for about 6 h and an increase in the amount of NREM sleep duration for about 12 h. In addition, there was a pronounced suppression of REM sleep during the first 6 h after the interaction, followed by a rebound later in the recovery period that largely made up for the REM sleep that was lost. The stimulation of NREM sleep appears to be at least partly specific for social defeat stress and does not seem to be the consequence of general physiological activation. Sexual interaction with a female did not cause the pronounced changes in sleep seen after a conflict, even though sexual interaction is known to cause a strong activation of the sympathetic nervous system and the HPA axis as well [3,4]. Even if the non-specific physiological activation in response to sexual interaction was somewhat lower, as suggested by our own corticosterone measurements, one might still expect changes in sleep to go in a quantitatively similar direction. However, this was not the case. In strong contrast to the pronounced increase of NREM sleep time after the conflict, the sexual interaction resulted in a short-lasting suppression of NREM sleep. Although NREM sleep SWA was elevated after the sexual interaction compared to SWA after sleep deprivation by gentle handling, this can be explained by the increase in wakefulness after the interaction, whereas the conflict clearly had an additional effect. In other words, whereas the sexual interaction increased SWA by increasing the duration of wakefulness, the social conflict increased SWA by amplifying the effect of wakefulness. The only apparent similarity between the consequences of an aggressive conflict and a sexual interaction was the temporary suppression of REM sleep. However, in the case of the sexual interaction this decrease in REM sleep was much smaller and partly non-specific, as it occurred in parallel with a decrease of NREM sleep. Overall, the effects of the sexual interaction were not dramatically different from sleep deprivation by gentle handling. Also the gentle handling procedure itself had only minor effects on subsequent sleep. A recent study has shown that after 4–6 h of sleep deprivation C57BL mice have a more pronounced increases in NREM sleep SWA and NREM sleep time [9]. Comparing the present results with the effects of 4–6 h sleep deprivation in that study underscores the potent NREM sleep-promoting action of a social conflict. Changes in NREM sleep time and SWA after 1 h social conflict are more similar to the effects of 4–6 h of sleep deprivation than to the effects of 1 h sleep deprivation.

The finding of an increase in NREM sleep SWA after a social conflict that is larger than that after sleep deprivation of similar duration confirms an earlier study in rats [16]. However, in rats there was no major increase in NREM sleep duration. After a conflict, the rats slept as much as under baseline condition but not more. Also the acute suppression of REM sleep was much larger in the present experiment than in the rat study. In rats, there was only a

mild reduction of REM sleep for 1–2 h immediately after the conflict, which was not followed by a clear rebound. The more pronounced effects of social defeat on sleep architecture in the present study may be a difference between rats and mice or it may be related to the time of day at which the conflict took place. In the rat study, the animals were subjected to a social conflict in the middle of the dark phase, whereas in the present study mice were exposed to a conflict in the middle of the light phase.

The physiological mechanisms that are responsible for promoting NREM sleep after a social conflict remain to be determined. We measured corticosterone levels at the end of the experimental manipulations, because, (1) a social conflict induces a pronounced increase in corticosterone levels [6,20], and (2) it has been reported that glucocorticoids stimulate NREM sleep [7]. However, although corticosterone may be partly involved in the NREM sleep-stimulating effect of a conflict, it is not likely the main responsible factor. Corticosterone levels after the conflict were strongly elevated compared to gentle handling but they were only slightly higher than after the sexual interaction. Therefore, if corticosterone would be an important NREM sleep-promoting substance one might have expected an increase in NREM sleep after the sexual interaction as well. Yet, if anything, the sexual interaction had opposite effects on NREM sleep. Clearly, the endocrine data have to be treated with some caution since the measurements were limited to a single point at the end of the experimental manipulations and we did not measure the temporal patterns in the course of the interaction and during the recovery afterwards. Nevertheless, the most plausible interpretation of the data is that factors other than corticosterone are involved in the NREM sleep-promoting effect of a social conflict. The fact that a social conflict induces a stronger increase in NREM sleep than sleep deprivation by gentle handling or wakefulness induced by sexual interaction makes it an interesting comparative model to study the mechanisms and function of this sleep stage.

The temporary suppression of REM sleep after a social conflict may be partly due to an increase in NREM sleep pressure. However, sleep deprivation of 4–6 h that induces similar increases in NREM sleep as we found after a social conflict, does not result in the same pronounced REM sleep suppression [9]. Thus, a social conflict may have a REM sleep suppressing effect independent from its effects on NREM sleep. The finding of a REM sleep suppression is intriguing in light of several reports that restraining or immobilizing animals causes an increase in REM sleep (e.g. Refs. [12,18]). Both social defeat and immobilization are commonly used stress models in animal research but apparently these stressors have different effects on the regulation of REM sleep. Since both social conflict and immobilization stress induce pronounced increases in corticosterone [12,20], it may seem unlikely that the latter can be responsible for the REM sleep suppression in the present study. However, a recent study suggested that the increase in REM sleep after immobilization occurs *despite* the increase in corticosterone and that corticosterone may actually suppress the occurrence of REM sleep after immobilization stress [12]. In line with this is the finding that administration of exogenous glucocorticoids indeed induces a profound reduction in REM sleep [7]. Moreover, the differences in corticosterone levels at the end of the social interactions in the present study to a certain degree parallel the magnitude of REM sleep suppression (a mild suppression after the sexual interaction and a strong suppression after the social conflict). Thus, our data, together with other studies, suggest that the suppression of REM sleep after a social conflict may be partly mediated by corticosterone. The suppression of REM sleep after the social interactions are not likely due to prolactin. First of all, prolactin is often associated with an increase in REM sleep, not a decrease [19]. Second, prolactin levels after the conflict and the sexual interaction were not different, whereas the decrease in REM sleep was much stronger after the conflict than after the sexual interaction.

5. Concluding remarks

A social conflict has complex and dual effects on sleep. A conflict elicits a very strong physiological response in terms of classical indicators of stress such as an activation of the sympathetic nervous system and the HPA axis, and an increase in heart rate, blood pressure, and metabolism. The function of this stress response is to help an animal in appropriately dealing with the acute threat, and the concomitant arousal inevitably inhibits sleep. Needless to say, the animals do not sleep during the conflict situation. However, upon return to the home cage, rats and mice immediately sleep as much as they normally do under baseline conditions, even during the first hours after the confrontation. The sleep-inhibiting arousal state caused by a social conflict is surprisingly rapidly overcome or overruled. In fact, the animals are not only capable of falling asleep but actually have more NREM sleep and also have higher SWA suggesting they sleep deeper. In other words, a social conflict inhibits sleep for as long as it lasts but promotes sleep afterwards. Importantly, whereas the temporary inhibition of sleep may be due to general or non-specific arousal, the NREM sleep promotion later on appears to be at least partly specific for a social conflict and does not necessarily occur after other arousing stimuli.

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