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Restraint increases prolactin and REM sleep in C57BL/6J mice but not in BALB/cJ mice

PETER MEERLO, AMY EASTON, BERNARD M. BERGMANN, AND FRED W. TUREK
Department of Neurobiology and Physiology, Northwestern University, Evanston, Illinois 60208

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Meerlo, Peter, Amy Easton, Bernard M. Bergmann, and Fred W. Turek. Restraint increases prolactin and REM sleep in C57BL/6J mice but not in BALB/cJ mice. *Am J Physiol Regulatory Integrative Comp Physiol* 281: R846–R854, 2001.—Sleep is generally considered to be a recovery from prior wakefulness. The architecture of sleep not only depends on the duration of wakefulness but also on its quality in terms of specific experiences. In the present experiment, we studied the effects of restraint stress on sleep architecture and sleep electroencephalography (EEG) in different strains of mice (C57BL/6J and BALB/cJ). One objective was to determine if the rapid eye movement (REM) sleep-promoting effects of restraint stress previously reported for rats would also occur in mice. In addition, we examined whether the effects of restraint stress on sleep are different from effects of social defeat stress, which was found to have a non-REM (NREM) sleep-promoting effect. We further measured corticosterone and prolactin levels as possible mediators of restraint stress-induced changes in sleep. Adult male C57BL/6J and BALB/cJ mice were subjected to 1 h of restraint stress in the middle of the light phase. To control for possible effects of sleep loss per se, the animals were also kept awake for 1 h by gentle handling. Restraint stress resulted in a mild increase in NREM sleep compared with baseline, but, overall, this effect was not significantly different from sleep deprivation by gentle handling. In contrast, restraint stress caused a significant increase in REM sleep compared with handling in the C57BL/6J mice but not in BALB/cJ mice. Corticosterone levels were significantly and similarly elevated after restraint in both strains, but prolactin was increased only in the C57BL/6J mice. In conclusion, this study shows that the restraint stress-induced increase in REM sleep in mice is strongly strain dependent. The concomitant increases in prolactin and REM sleep in the C57BL/6J mice, but not in BALB/cJ mice, suggest prolactin may be involved in the mechanism underlying restraint stress-induced REM sleep. Furthermore, this study confirms that different stressors differentially affect NREM and REM sleep. Whereas restraint stress promotes REM sleep in C57BL/6J mice, we previously found that in the same strain, social defeat stress promotes NREM sleep. As such, studying the consequences of specific stressful stimuli may be an important tool to unravel both the mechanism and function of different sleep stages.

rapid eye movement sleep, paradoxical sleep; immobilization stress; sleep deprivation; corticosterone

STRESS CAN BE DEFINED as a nonspecific physiological response to any kind of demand that an organism is facing (25). Because stress is a state of physiological activation and arousal, by definition, it inhibits sleep. Yet, animal studies have shown that on removal of a stressor this inhibitory effect is rapidly overcome and stress may actually promote and increase sleep during subsequent recovery (17, 23). However, a review of the literature suggests that different stressors may have different effects on non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep (17, 22, 23). Clearly, the final effect of a stressful stimulus on sleep not only depends on the nonspecific arousal common to all stressors, but also on the specific aspects that are characteristic for a given stimulus.

The differential effects of specific stressors on sleep can be illustrated by comparing social defeat stress and immobilization or restraint stress, two commonly used stress models in animal research. Both social defeat and restraint are very potent stressors in terms of classical nonspecific indicators, such as catecholamines and corticosterone (13). However, whereas social defeat stress stimulates NREM sleep, it appears that restraint stress mainly increases REM sleep. In rats, social defeat was found to increase slow and high-amplitude waves in the electroencephalogram (EEG) during subsequent NREM sleep (17). Because slow-wave activity (SWA; spectral power in the 1- to 4-Hz or delta frequency range) is generally considered as an indicator of sleep intensity, this finding suggested more intense sleep after acute social stress. In mice, social defeat not only increased NREM sleep SWA but also increased NREM sleep time. REM sleep, on the other hand, was strongly suppressed, followed by a rebound that barely made up for the REM sleep that was lost (18). In contrast, restraint stress in rats was reported to have only marginal effects on NREM sleep duration but caused a specific increase in REM sleep (23), a finding that has been replicated many times since (e.g., 5, 6, 8, 15). Some of the later studies have also reported a mild increase in NREM sleep, but generally that increase was small and most studies did not control for sleep loss per se. That is, at least part of the increase in NREM sleep may have been normal

Address for reprint requests and other correspondence: P. Meerlo, Dept. of Neurobiology and Physiology, 2153 North Campus Dr., Evanston, IL 60208 (E-mail: p-meerlo@northwestern.edu).

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recovery from sleep deprivation rather than a specific response to restraint stress. However, a caveat in the present state of knowledge is that most studies on restraint stress did not apply EEG spectral analysis and, therefore, the effects on NREM sleep SWA are uncertain. One study suggested an increase in SWA after restraint, but it is not clear how much of the SWA increase was due to sleep loss per se rather than to stress, because a sleep-deprivation control was not included (8). Thus the available data concerning the effects of restraint stress on NREM sleep are inconclusive.

In the present study we examined the effects of restraint stress in two strains of mice, including the strain that was recently used for our study on social defeat stress (18). The aim of this experiment was, first, to confirm the REM sleep-promoting effect of restraint stress and to establish whether it is a general phenomenon that also occurs in mice. Second, we examined whether or not restraint indeed differs from social defeat stress, which was found to increase NREM sleep time and NREM sleep SWA. Third, we compared the effects of restraint stress in two different strains of mice, C57BL/6J and BALB/cJ, to investigate how important genetic background is in terms of sensitivity to stress-induced alterations in sleep. There is some evidence that BALB/c mice are more sensitive to certain stressors than C57BL mice, at least in terms of corticosterone response (26). Fourth, we measured prolactin and corticosterone levels as potential mediators of restraint stress-induced changes in sleep and differences between the two strains herein. Plasma levels of both hormones strongly increase in response to restraint stress (12, 14), and both hormones are known to affect sleep (9, 24). In particular, the relationship between restraint stress and prolactin is of interest, because elevated prolactin has been associated with an increase in REM sleep (4, 24). Glucocorticoids, on the other hand, have been found to stimulate NREM sleep but suppress REM sleep (9).

METHODS

The study was performed with male C57BL/6J mice and male BALB/cJ mice, 3 to 4 mo of age, purchased from Jackson Laboratory. The animals were individually housed under a 12:12-h light-dark cycle, with lights on from 0600 to 1800. The ambient temperature was kept constant at 21 ± 1°C, and food and water were provided ad libitum throughout the experiments. The mice were allowed at least 3 wk of adaptation before the start of the experiments. In a first group of mice we studied the effects of restraint stress on sleep, and in a second group of animals we studied its effects on prolactin and corticosterone levels.

Restraint stress and gentle handling. The mice were subjected to 1 h of restraint stress during the sixth hour of the light phase. Restraint stress was achieved by enclosing the animals in a plastic tube with a diameter of 3 cm. The length of the tube was adjusted to the size of the animal to ensure complete immobilization. The tubes had openings at both ends for tail and nose. In addition to restraint stress, we included a 1-h sleep-deprivation control procedure to differentiate sleep changes induced by restraint from sleep changes induced by a period of wakefulness. The sleep-deprivation procedure that we will refer to as "gentle handling" consisted of keeping the animals awake with as little disturbance as possible by tapping on the cage, gently shaking the cage, and, if necessary, gently touching the animals.

Sleep recordings. In a first series of mice, we measured the effects of gentle handling and restraint stress on sleep architecture and sleep EEG (n = 8 for each strain). Permanent electrodes to record cortical EEG and neck muscle electromyogram (EMG) were implanted under metofane anesthesia. Two screws through the skull (1 mm diameter) 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 animals were allowed at least 2 wk of recovery from surgery. After recovery, the animals were hooked up to the recording equipment via a cable and swivel that allowed free movement throughout the cage. After at least 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, Astromed, West Warwick, RI). 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 5,000 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-compatible computer system with specialized software for acquiring and processing sleep data in rodents (Multisleep; Actimetrics, Evanston, IL). EEG and EMG were measured for 2 consecutive days, a baseline day and an experiment day, starting at lights-on. On the second day, the animals were subjected to gentle handling or restraint stress during the sixth hour of the light phase. The remaining 18 h of the experiment day were considered the recovery period (the second half of the light phase and the following dark phase). The mice were subjected to the gentle handling and restraint stress procedures in random order, with at least 1 wk in between. A new baseline recording preceded each procedure.

Analysis EEG/EMG data. By visual inspection of the EEG and EMG signals, 10-s epochs were classified as wakefulness, NREM sleep, or REM sleep (18). 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 were normalized by expressing them relative to each animal's baseline. The average NREM sleep delta power per time block was expressed as percentage of the average 24-h baseline NREM sleep delta power and is referred to here and in the figures as SWA. The accumulated NREM sleep delta power per time block 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, because restraint not only kept the animals awake during the 1-h experiment but also changed sleep time afterward. Therefore, the average SWA per time block during recovery not only depended on the experimental manipulation but also on the sleep-wakefulness ratio afterward. By calculating the SWE the mice accumulated during the recovery period it was

possible to establish whether an increase in SWA after restraint was due to increased additional wakefulness (i.e., the quantity of wakefulness) or due to an additional effect independent of the time awake (i.e., 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 blocks, for 6-h blocks, and for the total 18-h recovery period. The 1-h blocks were used for detailed illustration of the sleep patterns and the acute effects of the experimental manipulations. The 6- and 18-h values were less variable, gave a clearer indication of the overall effects, and were used for statistical analysis. The two strains of mice differed in their sleep architecture under baseline conditions and after sleep deprivation by gentle handling. Therefore, to be able to make a strain comparison for the specific effects of restraint stress on the various sleep parameters, we calculated and compared the deviations from the posthandling values.

Prolactin and corticosterone levels. In a second experiment, we measured the effects of restraint stress and gentle handling on plasma prolactin and corticosterone levels. Both hormones increase in response to restraint stress and are also known to affect sleep. We therefore considered prolactin and corticosterone as potential candidates to explain the effects of our experimental procedures on sleep. Male C57BL/6J and BALB/cJ mice were subjected to handling and restraint as described above ($n = 10$ for each manipulation). After 1 h of handling or restraint stress, the animals were rapidly 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 2,600 *g*, and the supernatant was stored at −80°C for later analysis. Prolactin and corticosterone concentrations were determined by RIA. The prolactin was NHPP mouse prolactin RIA provided by Dr. A. F. Parlow (Torrance, CA); detection limit was 1.0 ng/ml; intraassay coefficient of variation was 15.5%. The corticosterone was from double antibody ^{125}I RIA kit, ICN Biomedicals (Costa Mesa, CA); detection limit was 0.2 μg/100 ml; intraassay coefficient of variation for low pool was 11.9% and for high pool was 6.5%.

Statistics. Statistical analysis of the prolactin and corticosterone data was performed with two-way ANOVA with factors "strain" (C57BL/6J and BALB/cJ) and "treatment" (handling and restraint). For the sleep data, ANOVA with factor treatment was applied for each of the two strains separately to determine the effects of restraint stress and gentle handling relative to baseline. To determine if the two strains differed in restraint stress-specific changes in sleep (i.e., postrestraint values of the various sleep parameters expressed as deviation from the posthandling values), we applied an ANOVA with factor strain. For all the sleep data per 6-h block, ANOVA included a repeated-measures factor. When the overall ANOVA revealed a significant effect of treatment or strain, the different groups and consecutive 6-h blocks were analyzed separately with *t*-tests to determine at which blocks the differences occurred.

RESULTS

NREM sleep. Whereas gentle handling did not have any major effects on the amount of NREM sleep, restraint stress caused an initial reduction for ~1 h afterward followed by an increase during the first half of the dark phase (Fig. 1). When consecutive 6-h blocks during recovery from restraint and handling and the corresponding 6-h blocks during baseline were compared for the amount of NREM sleep, repeated-measures ANOVA revealed a significant treatment effect and a significant treatment × 6-h block interaction for both strains (C57BL/6J: $F_{2,21} = 3.47$, $P = 0.050$ and $F_{4,42} = 5.41$, $P = 0.001$; BALB/cJ: $F_{2,21} = 6.42$, $P = 0.007$ and $F_{4,42} = 5.73$, $P = 0.001$; Fig. 2). The short-lasting reduction in NREM sleep after restraint stress significantly lowered the total amount of NREM sleep in the remainder of the light phase only in the C57BL mice but not in the BALB mice (Fig. 2, first 6-h block). In both strains, the amount of NREM sleep during the first half of the dark phase after restraint stress was significantly higher compared with baseline and handling (Fig. 2, second 6-h block). This may represent, in part, a compensation for the NREM sleep that was lost immediately after restraint stress. Comparing the two strains for the specific effects of restraint stress on NREM sleep time during the three consecutive 6-h blocks of recovery revealed a significant strain × 6-h block interaction ($F_{2,28} = 3.73$, $P = 0.037$). However, when tested separately, none of the three 6-h blocks significantly differed between the strains.

The total amount of NREM sleep during the entire 18-h recovery period was significantly affected by the treatment in both the C57BL/6J mice ($F_{2,21} = 3.47$, $P = 0.050$) and the BALB/cJ mice ($F_{2,21} = 6.42$, $P = 0.007$). In both strains, the total amount of NREM sleep after restraint stress was significantly higher compared with baseline but not significantly higher than the amount of NREM sleep after handling (Fig. 3). The total amount of NREM sleep during the 18-h recovery period after restraint stress, expressed as deviation from the NREM sleep time after gentle handling, was not different between the strains (Fig. 3). Thus, although the C57BL/6J mice and BALB/cJ mice slightly differed in the temporal pattern of NREM sleep during recovery, the total changes relative to the posthandling values were similar in both strains.

NREM sleep SWA. The NREM sleep that was lost due to the experimental manipulation led to a temporary increase in NREM sleep SWA after the manipulations, which gradually disappeared in the course of the remainder of the light phase (Fig. 1). For NREM sleep SWA in 6-h blocks during recovery from restraint, recovery from handling, and during baseline, there was a significant treatment × 6-h block interaction for both the C57BL/6J mice and the BALB/cJ mice ($F_{4,42} = 3.94$, $P = 0.008$ and $F_{4,42} = 3.50$, $P = 0.015$, respectively; Fig. 2). SWA during the remaining 6 h of the light phase was significantly elevated after both restraint and handling compared with baseline in the C57BL/6J mice and after restraint compared with baseline and handling in the BALB/cJ mice (Fig. 2, first 6-h block). In the BALB/cJ mice, NREM sleep SWA dropped below baseline levels during the second half of the dark phase after restraint (Fig. 2, third 6-h block). However, the two strains did not significantly differ in the specific effects of restraint stress relative to handling. Thus, although the within-strain comparison indicated slight differences in the 6-h pattern of SWA, there were no significant differences between the C57BL/6J and BALB/cJ mice for the specific effects of

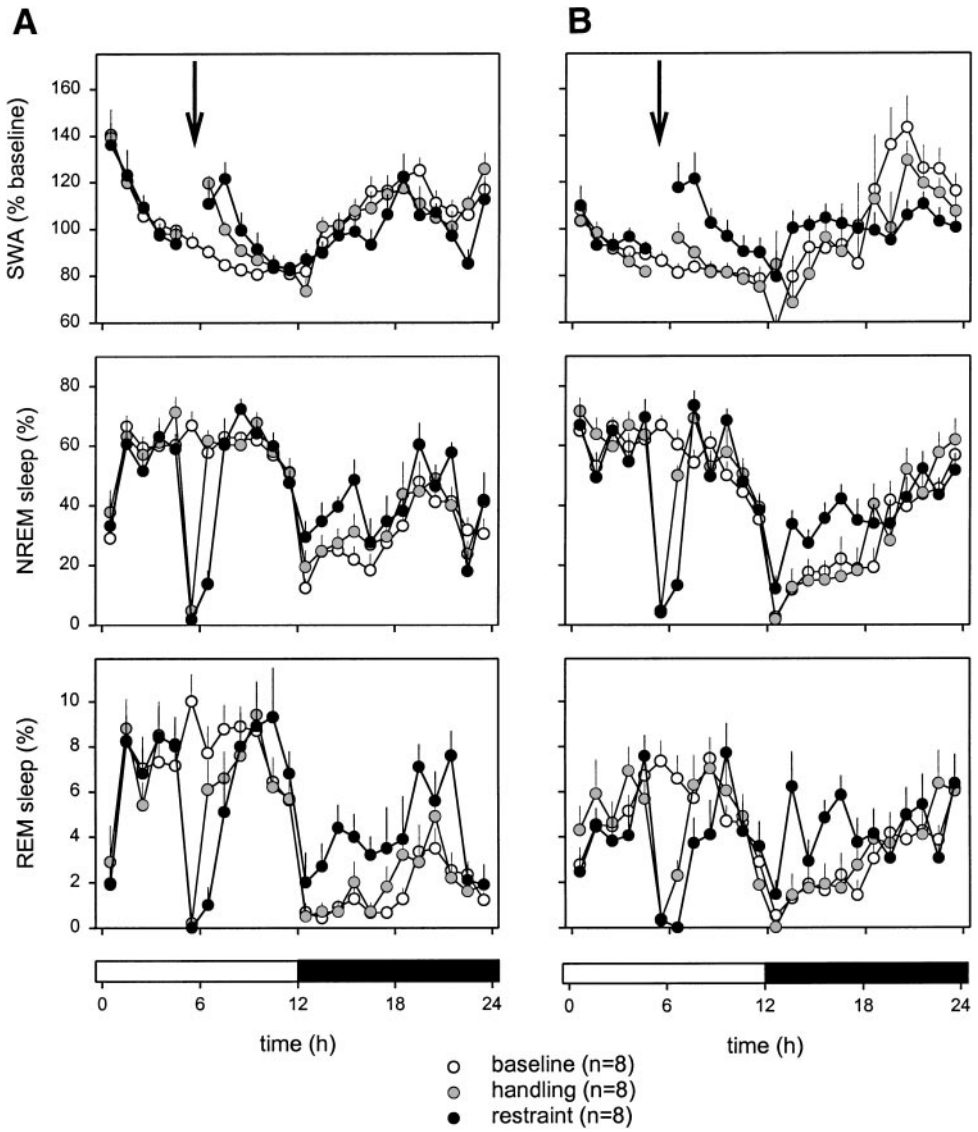

Fig. 1. Non-rapid eye movement (NREM) sleep slow-wave activity (SWA; % 24-h baseline), NREM sleep time (%), and REM sleep time (%) in C57BL/6J mice (*A*) and BALB/cJ mice (*B*) subjected to 1 h of gentle handling or 1 h restraint stress during the 6th h of the light phase. Arrows, time of the experimental manipulation. Light/dark cycle is indicated below the graphs.

restraint expressed as deviation from the handling effects. When the 18-h recovery period was taken as a whole, there was no significant overall effect of handling or restraint stress on the average SWA in either of the two strains (Fig. 3).

Cumulative NREM sleep SWE. The relative differences in the temporal pattern of NREM sleep SWA between treatments and strains may have been partly due to the fact that restraint stress not only kept the animals awake during the experimental hour but also changed the amount of sleep afterward. In other words, the SWA not only depended on the manipulation itself but also on the sleep-wake ratio afterward. Therefore, in addition to average SWA values for each time block, we calculated the accumulated NREM sleep SWE. In C57BL/6J mice, comparing the cumulative NREM sleep SWE for three 6-h blocks during recovery from restraint, recovery from handling, and during baseline, there was a significant effect of treatment ($F_{2,21} = 6.01$, $P = 0.009$). In the BALB/cJ mice, there was a significant effect of treatment ($F_{2,21} = 5.73$, $P = 0.010$) and a significant treatment × 6-h block interaction ($F_{4,42} = 6.51$, $P = 0.000$; Fig. 2). There were small differences between the treatments, the main effect being an increase in accumulated NREM sleep SWE during the first half of the dark phase after restraint stress in the BALB/cJ mice, followed by a decrease in the second half of the dark phase (Fig. 2, second and third 6-h block). When the two strains were compared for the specific effects of restraint stress relative to handling, there was a significant treatment × 6-h block interaction ($F_{2,28} = 6.51$, $P = 0.005$), but when the 6-h blocks were tested separately, there were no significant differences between the strains.

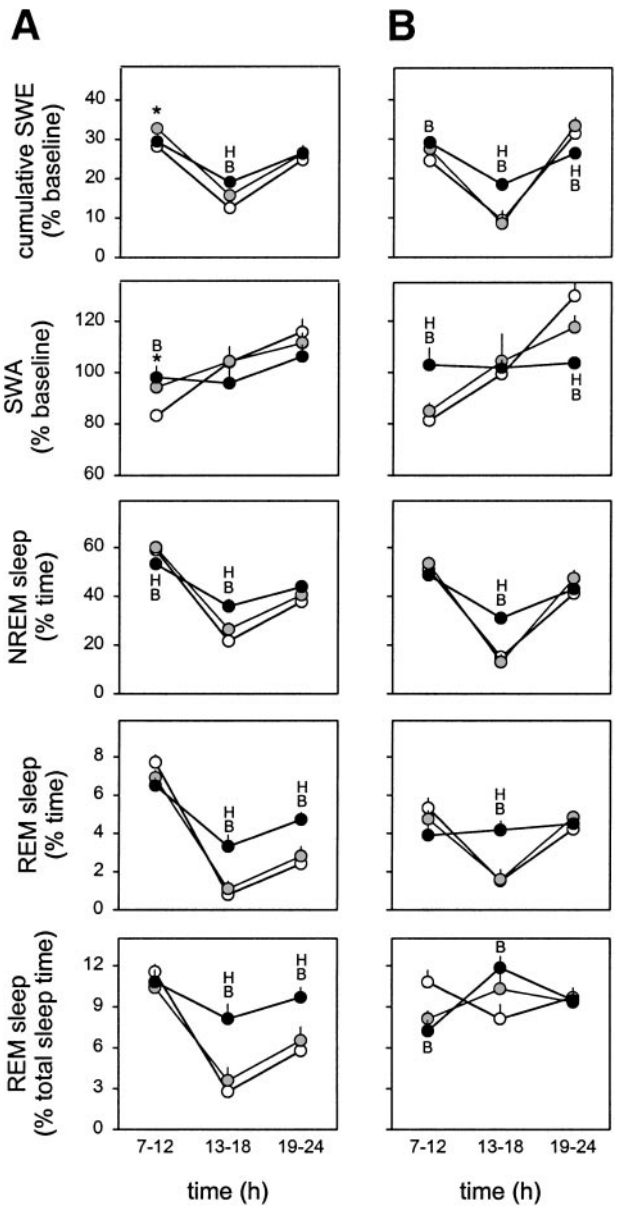

Fig. 2. Cumulative NREM sleep slow-wave energy (SWE; % total 24-h baseline), NREM sleep SWA (% average 24-h baseline), NREM sleep time (%), REM sleep time (%), and REM sleep time relative to total sleep time (%) in 6-h blocks for baseline (○), gentle handling (shaded circles), and restraint stress (●) in C57BL/6J mice (*A*) and BALB/cJ mice (*B*). The first 6-h block is the second half of the light phase (*hours 7–12* in Fig. 1); the second 6-h block is the first half of the dark phase (*hours 13–18* in Fig. 1); and the third 6-h block is the second half of the dark phase (*hours 19–24* in Fig. 1). Data are expressed as averages (±SE) and were subjected to ANOVA (see RESULTS). Only when ANOVA revealed a significant effect of treatment were the successive 6-h blocks compared separately with *t*-tests. Significant differences: *handling relative to baseline; B, restraint relative to baseline; H, restraint relative to handling (2-tailed *t*-test, $P < 0.05$).

For the total accumulated NREM sleep SWE during the entire 18-h recovery period, there was a significant treatment effect in both the C7BL/6J mice ($F_{2,21} = 5.97$, $P = 0.009$) and the BALB/cJ mice ($F_{2,21} = 5.78$, $P = 0.010$). In both strains, the accumulated NREM sleep SWE after restraint stress was higher than under baseline conditions. However, the accumulated SWE after restraint did not differ from the total SWE after gentle handling, suggesting that the increase in SWE was mainly a compensation for the sleep loss per se, rather than a specific effect of restraint (Fig. 3). Also, there was no difference between the two strains for the total 18 h changes in accumulated NREM sleep SWE after restraint stress relative to handling (Fig. 3). Thus, although there were slight differences in the temporal pattern, the total amount of accumulated NREM sleep SWE during the 18-h recovery after restraint stress was not different between the strains.

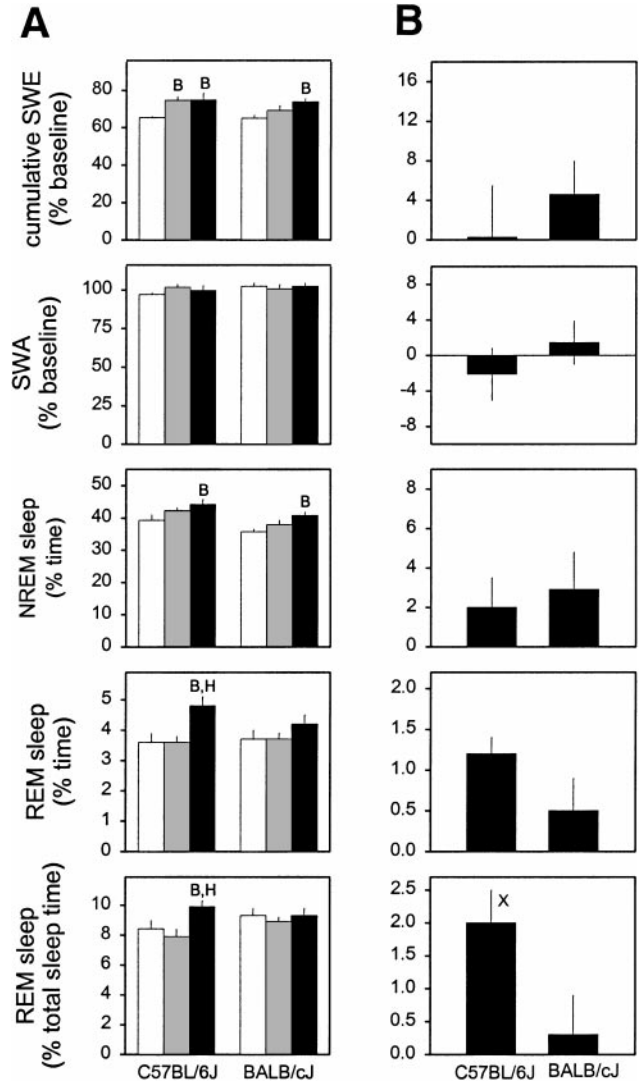

Fig. 3. Cumulative NREM sleep SWE (%total 24-h baseline), NREM sleep SWA (%average 24-h baseline), NREM sleep time (%), REM sleep time (%), and REM sleep time relative to total sleep time (%) for the entire 18-h recovery period after gentle handling and restraint stress. Open bars, baseline; shaded bars, handling; solid bars, restraint. *A*, absolute values; *B*, values after restraint stress as deviation from the values after gentle handling. Data are expressed as averages (±SE) and were subjected to ANOVA (see RESULTS). When ANOVA revealed a significant effect, the different treatments and strains were compared separately with *t*-tests. Significant differences between treatments within strains (*A*): B, relative to baseline; H, relative to handling. Significant differences between strains in restraint stress-specific effects (*B*): X (2-tailed *t*-test, $P < 0.05$).

REM sleep. Whereas gentle handling did not have major effects on the amount of REM sleep, except maybe for a slight initial suppression in the BALB/cJ mice, restraint stress caused a reduction in REM sleep for 2–3 h in both strains. The initial decrease was followed by an increase during the dark phase, which seemed to persist longer in the C57BL/6J mice (Fig. 1). Comparing the three consecutive 6-h blocks during recovery from restraint and handling and the corresponding 6-h blocks during baseline for the amount of REM sleep revealed a significant treatment effect ($F_{2,21} = 8.18$, $P = 0.002$) and a significant treatment × 6-h block interaction ($F_{4,42} = 5.64$, $P = 0.001$) for the C57BL/6J mice but only a significant treatment × 6-h block interaction ($F_{4,42} = 6.06$, $P = 0.001$) for the BALB/cJ mice (Fig. 2). The initial short-lasting suppression of REM sleep did not significantly lower the overall amount of REM sleep during the remainder of the light phase (Fig. 2, first 6-h block). In the C57BL/6J mice, REM sleep was increased throughout the dark phase after restraint stress compared with handling and under baseline conditions (Fig. 2, second and third 6-h block). In the BALB/cJ mice, REM sleep after restraint was only elevated during the first half of the dark phase (Fig. 2, second 6-h block). The differences between the two strains in the amount of REM sleep during the three consecutive 6-h blocks of recovery after restraint stress, expressed as deviation from the posthandling values, did not reach statistical significance.

The total amount of REM sleep during the entire 18-h recovery period was significantly affected by the treatment in the C57BL mice ($F_{2,21} = 8.17$, $P = 0.002$) but not in the BALB mice (Fig. 3). In the C57BL/6J mice, the total amount of REM sleep after restraint was significantly higher than after handling or under baseline conditions. However, for the total amount of REM sleep after restraint stress expressed as deviation from the posthandling values, the difference between the two strains did not reach statistical significance (Fig. 3).

REM sleep/total sleep. Because under normal conditions REM sleep is preceded by NREM sleep, changes in REM sleep may in part be secondary to changes in NREM sleep. Therefore, to determine whether changes in REM sleep were specific or occurred in parallel to changes in NREM sleep, we expressed REM sleep time as percentage of total sleep time (REM/TS). For REM/TS there was a significant treatment effect ($F_{2,21} = 11.21$, $P = 0.000$) and a significant treatment × 6-h block interaction ($F_{4,42} = 4.30$, $P = 0.005$) in the C57BL/6J mice and a significant treatment × 6-h block interaction ($F_{4,42} = 2.88$, $P = 0.034$) for the BALB/cJ mice (Fig. 2). In the C57BL mice, REM/TS after restraint was significantly higher throughout the dark period, not only compared with baseline values, but also compared with posthandling values (Fig. 2, second and third 6-h block). In the BALB/c mice, however, REM/TS after restraint stress and handling were not different. The specific effects of restraint stress on REM/TS during the three consecutive 6-h blocks, expressed as deviation from the posthandling values, significantly differed between the strains ($F_{1,14} = 7.43$, $P = 0.016$), with the C57BL/6J mice having a stronger increase of REM/TS than BALB/cJ mice.

REM/TS for the entire 18-h recovery period was as significantly affected by the treatment in the C57BL/6J mice ($F_{2,21} = 4.84$, $P = 0.019$) but not in the BALB/cJ mice. In the C57BL/6J mice, REM/TS was significantly higher after restraint stress than after handling or under baseline conditions (Fig. 3). Also, REM/TS after restraint stress expressed as deviation from the posthandling values significantly differed between the two strains ($F_{1,14} = 5.16$, $P = 0.039$). Together, the results indicate that restraint stress caused a specific increase in REM sleep in C57BL/6J mice but not in the BALB/cJ mice.

Prolactin and corticosterone. For prolactin levels, two-way ANOVA revealed a significant effect of strain ($F_{1,36} = 91.96$, $P < 0.001$), a significant effect of treatment ($F_{1,36} = 121.61$, $P < 0.001$), and a significant strain × treatment interaction ($F_{1,36} = 113.43$, $P < 0.001$). Prolactin levels after gentle handling were low and did not differ between the two strains. After restraint stress, prolactin levels in the C57BL/6J mice were elevated more than 10-fold compared with handling. Surprisingly, however, prolactin levels after restraint stress in BALB/cJ mice were still low and not distinguishable from levels after handling (Fig. 4). Corticosterone levels, on the other hand, were only affected by the treatment ($F_{1,36} = 155.70$, $P < 0.001$).

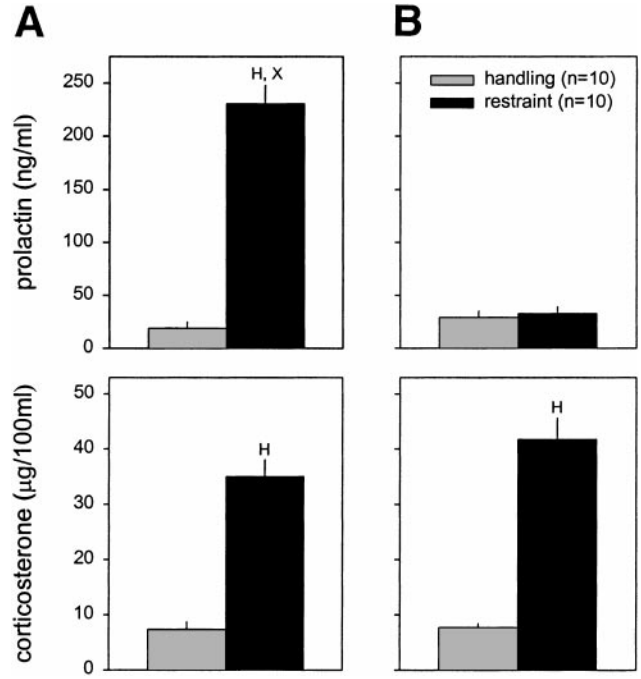

Fig. 4. Prolactin and corticosterone levels in male C57BL/6J (*A*) and BALB/cJ (*B*) mice after 1 h of gentle handling and 1 h restraint stress. Data are expressed as averages (±SE) and were subjected to ANOVA (see RESULTS). When ANOVA revealed a significant effect of treatment or strain, pairwise comparisons were performed with *t*-tests. Significant differences: H, restraint vs. handling; X, C57BL vs. BALB (2-tailed *t*-test, $P < 0.05$).

The data show that corticosterone levels were low after sleep deprivation by gentle handling and strongly elevated after restraint stress. In contrast to prolactin levels, there was no major difference in corticosterone levels between the two strains (Fig. 4).

DISCUSSION

The most striking result of this study was the effect of restraint stress on subsequent REM sleep and the differences between the two strains in this effect. For 2–3 h after restraint, REM sleep was suppressed, partly parallel with the decrease in NREM sleep. After this initial period of suppression, REM sleep increased beyond baseline levels. This increase was much more pronounced in the C57BL mice than it was in the BALB mice. In the C57BL mice, the REM sleep increase during the dark phase was much larger than the initial loss of REM sleep that occurred during and shortly after the stress. Also, the increase in REM sleep was disproportionally large even when the changes in NREM sleep were taken into account. In other words, the percentage of REM sleep was increased even when it was expressed relative to total sleep time. Importantly, there was no increase in REM sleep after sleep deprivation by gentle handling. Thus, in the C57BL mice, restraint stress caused a selective increase in REM sleep, which cannot be ascribed to any acute REM sleep loss or change in NREM sleep. In contrast, in the BALB mice the increase in REM sleep during the first half of the dark phase after restraint stress can be explained partly as a compensation for the initial loss of REM sleep and partly as a nonselective increase parallel to the increase in NREM sleep. In this strain, the overall amount of REM sleep during the entire recovery period was not significantly different from baseline or gentle handling. Especially when changes in the amount of NREM sleep are taken into account (i.e., REM sleep expressed as percentage of total sleep), the amount of REM sleep in BALB/cJ mice was similar under all conditions.

The effects of restraint stress on NREM sleep were less pronounced than the effects on REM sleep. Overall, the mice had significantly more NREM sleep during the 18-h recovery period after restraint stress than they had under baseline conditions. Also, compared with the handling treatment the mice had somewhat more NREM sleep after restraint stress, but this difference did not reach statistical significance. In other words, the overall increase in NREM sleep time after restraint stress was not clearly different from the normal compensatory increase after sleep deprivation by handling.

After restraint stress, NREM sleep SWA was significantly increased above baseline for several hours. In the C57BL mice, this elevation was not clearly different from the increase in SWA after gentle handling. Thus, although there was a clear difference in the quality of wakefulness, the increase in NREM sleep SWA mainly seemed to depend on the duration of wakefulness. In the BALB mice, the initial elevation in SWA was higher after restraint than it was after handling. However, the total amount of NREM sleep SWE that was accumulated was not different after handling and restraint stress, neither during the first 6 h after the experimental manipulation when SWA was elevated nor during the total 18-h recovery period. In other words, the difference in SWA was attributable to the additional wakefulness early during the recovery period after the restraint stress. Overall, there was a small increase in the accumulated NREM sleep SWE after both handling and restraint stress, presumably to compensate for the sleep that was lost during the experimental manipulation. Together, taking into account both NREM sleep time and NREM sleep SWA, the data do not provide convincing evidence for a specific NREM sleep-promoting effect of restraint stress beyond what occurs after sleep deprivation by gentle handling.

The present results obtained in mice are in line with reports on restraint stress-induced increases in REM sleep in rats (15, 23), except that in mice we found the typical and specific REM sleep increase in only one of the two strains tested. Also, in contrast to recent reports on the effects of social defeat stress (17, 18), restraint stress appears to have little or no stimulating effect on NREM sleep. On the other hand, in both rats and mice, the increase in REM sleep that occurs after restraint stress has not been found after social defeat. Thus, whereas restraint or immobilization appears to have a specific REM sleep-promoting effect, a social conflict appears to have a pronounced NREM sleep-promoting effect. Therefore, the changes in sleep in these commonly used stress models are not due to some sort of nonspecific stress response but, rather, are due to certain specific aspects of these stressors. In this respect, one cannot speak in general terms about the effects of stress on sleep but should refer to the effects of specific stimuli.

Plasma prolactin levels may provide an important lead to the mechanism of the specific increase in REM sleep after restraint stress. The differential effect of restraint stress on REM sleep in C57BL/6J and BALB/cJ mice was paralleled by the changes in prolactin in these strains. In the C57BL mice, prolactin levels after restraint stress were >10-fold higher than after gentle handling. In the BALB mice, on the other hand, prolactin levels after restraint stress were low and similar to those after gentle handling. Interestingly, both the high prolactin response in the C57BL mice (~230 ng/ml) and the low response in the BALB/c strain (~35 ng/ml) may be specific for restraint stress. When we recently measured prolactin levels after a number of other arousing stimuli (social defeat stress and sexual interaction with a female), both strains had similar elevations ranging from 70 to 100 ng/ml (Ref. 18 and unpublished data). This suggests that, compared with other activating stimuli, the C57BL/6J mice have an extraordinary high prolactin response to restraint, whereas the BALB/cJ mice have a very low response. It is noteworthy that the corticosterone responses to restraint stress and the other stimuli were

not dramatically different, emphasizing the specificity of the restraint stress effect on prolactin-regulating mechanisms. Also, in rats, there is some evidence that restraint stress may induce a stronger prolactin response than other stimuli. In one study, cold stress and restraint stress induced similar corticosterone responses, whereas prolactin levels were elevated 2-fold after cold stress and 10-fold after restraint stress (14).

There are different possible explanations for a parallel increase in prolactin and REM sleep after restraint stress. First, prolactin itself may be causally involved in a cascade of events that ultimately results in the increase in REM sleep. This hypothesis is supported by various studies showing that systemic or intracerebroventricular injection of prolactin increases REM sleep (24). Also, a recent study suggested prolactin as a mediator of an increase in REM sleep in rats that were exposed to ether stress, because the ether-induced increase in REM sleep was blocked by hypophysectomy and by intracerebroventricular administration of antiserum to prolactin (4). A second possibility is that the prolactin response and REM sleep increase are not directly associated but, instead, have a common causal factor. Restraint stress induces changes in a variety of factors that, in turn, are known to affect both prolactin levels and REM sleep. One upstream mediator of the restraint stress effects on prolactin and REM sleep may be corticotropin-releasing factor (CRF), a key regulator of the integrated stress response (2, 11). CRF not only stimulates prolactin release during stress (1, 20), but intracerebroventricular injection of CRF was found to amplify the REM sleep rebound after sleep deprivation as well (16). Most importantly, intracerebroventricular injection of a CRF-receptor antagonist before restraint stress in rats abolished the REM sleep increase normally seen after restraint (10). Another important factor may be dopamine. There are several anatomically and functionally distinct dopaminergic pathways in the brain, and, although the effects of stress depend on the system under study, there is some evidence that the hypothalamic dopaminergic activity after restraint stress is decreased (19). Because hypothalamic dopamine inhibits prolactin release, a reduced inhibition is thought to be partly responsible for the increase in prolactin during restraint stress (3, 19). In addition, because there is some evidence that dopamine also suppresses REM sleep (21, 27), a stress-induced decrease of dopaminergic activity might also be partly responsible for the increase in REM sleep after restraint stress. Even further upstream, serotonin is at least partly responsible for the restraint stress-induced decrease in hypothalamic dopaminergic activity and the increase in plasma prolactin levels (7). A serotonergic input from the dorsal raphe nuclei to the hypothalamus also has been implicated in the increase in REM sleep after restraint stress (5).

Clearly, the mechanism underlying the restraint stress-specific increase in REM sleep may very well consist of multiple components and, in a complex interplay, serotonin, dopamine, CRF, and prolactin may all be partly responsible for it. Further studies to unravel the exact mechanism will face the following intriguing questions. First, why is there only a restraint stress-specific increase in REM sleep in C57BL/6J mice and not in the BALB/cJ strain? Second, why is there an increase in REM sleep after restraint stress but not after other stressors such as social defeat? Because the stress-induced prolactin levels parallel the changes in REM recovery sleep, both in the comparison between strains of mice and the comparison between stressors, it provides an important lead for further studies.

In conclusion, the present data confirm that sleep architecture not only depends on the duration of wakefulness but also on its quality. In rats and mice, sleep is strongly affected by experiences that are commonly classified as "stress." However, it is clear that different stressors have different effects on sleep. Whereas restraint stress has a specific REM sleep-promoting effect, social defeat stress, for instance, has a prominent NREM sleep-promoting effect. As such, studying the different effects of specific stressful stimuli on sleep may be a useful approach to unravel the regulatory mechanisms and functions of specific sleep stages.

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REFERENCES

1. **Akema T, Chiba A, Oshida M, Kimura F, and Toyoda J.** Permissive role of corticotropin-releasing factor in the acute stress-induced prolactin release in female rats. *Neurosci Lett* 198: 146–148, 1995.
2. **Axelrod J and Reisine TD.** Stress hormones: their interaction and regulation. *Science* 224: 452–459, 1984.
3. **Ben-Jonathan N.** Dopamine: a prolactin-inhibiting hormone. *Endocr Rev* 6: 564–589, 1985.
4. **Bodosi B, Obál F, Gardi J, Komlodi J, Fang J, and Krueger JM.** An ether stressor increases REM sleep in rats: possible role of prolactin. *Am J Physiol Regulatory Integrative Comp Physiol* 279: R1590–R1598, 2000.
5. **Bonnet C, Leger L, Baubet V, Debilly G, and Cespuglio R.** Influence of a 1h immobilization stress on sleep states and corticotropin-like intermediate lobe peptide (CLIP or $ACTH_{18–39}$, Ph-$ACTH_{18–39}$) brain contents in the rat. *Brain Res* 751: 54–63, 1997.
6. **Bouyer JJ, Deminiere JM, Mayo W, and Le Moal M.** Inter-individual differences in the effects of acute stress on the sleep-wakefulness cycle in the rat. *Neurosci Lett* 225: 193–196, 1997.
7. **Demarest KT, Moore KE, and Riegle GD.** Acute restraint stress decreases dopamine synthesis and turn-over in the median eminence: a model for the study of the inhibitory neuronal influences on tuberoinfundibular dopaminergic neurons. *Neuroendocrinology* 41: 437–444, 1985.
8. **Dugovic C, Maccari S, Weibel L, Turek FW, and Van Reeth O.** High corticosterone levels in prenatally stressed rats predict persistent paradoxical sleep alterations. *J Neurosci* 19: 8656–8664, 1999.
9. **Friess E, Wiedemann K, Steiger A, and Holsboer F.** The hypothalamic-pituitary-adrenocortical system and sleep in man. *Adv Neuroimmunol* 5: 111–125, 1995.
10. **Gonzalez M and Valatx JL.** Effect of intracerebroventricular administration of α-helical CRH (9–41) on the sleep/waking

cycle in rats under normal conditions or after subjection to an acute stressful stimulus. *J Sleep Res* 6: 164–170, 1997.
11. **Johnson EO, Kamilaris TC, Chrousos GP, and Gold PW.** Mechanisms of stress: a dynamic overview of hormonal and behavioral homeostasis. *Neurosci Biobehav Rev* 16: 115–130, 1992.
12. **Kant GJ, Mougrey EH, and Meyerhoff JL.** Diurnal variation in neuroendocrine response to stress in rats: plasma ACTH, B-endorphin, B-LPH, corticosterone, prolactin and pituitary cyclic AMP responses. *Neuroendocrinology* 43: 383–390, 1986.
13. **Koolhaas JM, De Boer SF, De Ruiter AJH, Meerlo P, and Sgoifo A.** Social stress in rats and mice. *Acta Physiol Scand* 161, *Suppl* 640: 69–72, 1997.
14. **Lenox RH, Kant GJ, Sessions GR, Pennington LL, Mougey EH, and Meye JL.** Specific hormonal and neurochemical responses to different stressors. *Neuroendocrinology* 30: 300–308, 1980.
15. **Marinesco S, Bonnet C, and Cespuglio R.** Influence of stress duration on sleep rebound induced by immobilization in the rat: a possible role for corticosterone. *Neuroscience* 92: 921–933, 1999.
16. **Marrosu F, Gessa GL, Giagheddu M, and Fratta W.** Corticotropin-releasing factor (CRF) increases paradoxical sleep (PS) rebound in PS-deprived rats. *Brain Res* 515: 315–318, 1990.
17. **Meerlo P, Pragt BJ, and Daan S.** Social stress induces high intensity sleep in rats. *Neurosci Lett* 225: 41–44, 1997.
18. **Meerlo P and Turek FW.** Effects of social stimuli on sleep in mice: non-rapid-eye movement (NREM) sleep is promoted by aggressive interaction but not by sexual interaction. *Brain Res* 907: 84–92, 2001.
19. **Moore KE, Demarest KT, and Lookingland KJ.** Stress, prolactin and hypothalamic dopaminergic neurons. *Neuropharmacology* 26: 801–808, 1987.
20. **Morel G, Enjalberg A, Proulx L, Pelletier G, Bardes N, Grossard F, and Dubois PM.** Effects of corticotropin-releasing factor on the release and synthesis of prolactin. *Neuroendocrinology* 49: 669–675, 1989.
21. **Ongini E, Bonizzoni E, Ferri N, Milani S, and Trampus M.** Differential effects of dopamine D-1 and D-2 receptor antagonist antipsychotics on sleep-wake patterns in the rat. *J Pharmacol Exp Ther* 266: 726–731, 1993.
22. **Palma BD, Suchecki D, and Tufik S.** Differential effects of acute cold and footshock on the sleep of rats. *Brain Res* 861: 97–104, 2000.
23. **Rampin C, Cespuglio R, Chastrette N, and Jouvet M.** Immobilization stress induces a paradoxical sleep rebound in the rat. *Neurosci Lett* 126: 113–118, 1991.
24. **Roky R, Obal F, Valatx JL, Bredow S, Fang J, Pagano LP, and Krueger JM.** Prolactin and rapid eye movement sleep regulation. *Sleep* 18: 536–542, 1995.
25. **Selye H.** A syndrome produced by diverse nocuous agents. *Nature* 138: 32, 1936.
26. **Shanks N, Griffiths J, Zalcman S, Zacharko RM, and Anisman H.** Mouse strain differences in plasma corticosterone following uncontrollable footshock. *Pharmacol Biochem Behav* 36: 515–519, 1990.
27. **Trampus M, Ferri N, Monopoli A, and Ongini E.** The dopamine D1 receptor is involved in the regulation of REM sleep in the rat. *Eur J Pharmacol* 194: 189–194, 1991.