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Caffeine During Sleep Deprivation: Sleep Tendency and Dynamics of Recovery Sleep in Rats

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WURTS, S. W. AND D. M. EDGAR. *Caffeine during sleep deprivation: Sleep tendency and dynamics of recovery sleep in rats.* PHARMACOL BIOCHEM BEHAV **65**(1) 155–162, 2000.—The adenosine antagonist caffeine disrupts sleep, but whether caffeine promotes wakefulness by interfering with the expression of sleep or by attenuating sleepiness is unknown. The ability of caffeine to reduce sleep tendency in rats was directly tested by quantifying the number of stimuli needed to maintain wakefulness during sleep deprivation for 6 h after systemic caffeine treatment. In addition, the influence of caffeine on the dynamics between nonrapid-eye-movement (NREM) and rapid-eye-movement (REM) sleep was investigated by comparing the magnitude and time course of the compensatory sleep responses for 42 h postsleep deprivation. Caffeine significantly reduced the attempts to sleep during sleep deprivation, $F(1, 9)$ 8.83, $p = 0.0157$; 44.9% of vehicle), but did not change compensatory slow-wave activity during recovery sleep. During the initial recovery phase, caffeine suppressed compensatory REM sleep and reduced, but did not block, compensatory NREM sleep duration and continuity. By 42 h postsleep deprivation, the amount of NREM recovered (70.0% of deficit) did not differ from vehicle. In contrast, the REM sleep deficit recovered after caffeine (100%) was more than after vehicle (43.9%). Thus, caffeine slowed the rate of compensatory sleep after sleep deprivation, as indexed by the duration of sleep states and sleep continuity. © 1999 Elsevier Science Inc.

Adenosine Sleep homeostasis Circadian rhythm NREM sleep REM sleep sleep–wake cycle

CAFFEINE is the most widely used stimulant to combat inappropriately timed sleepiness. Acute delivery of caffeine prior to sleep prolongs sleep latency, reduces total sleep time, and increases sleep fragmentation in humans (7,14,20) and laboratory rodents (9,24,25,28,29,33), and the effects are dose dependent (12,37). Although the sleep disrupting effects of caffeine are culturally familiar and well documented (1,19), the mechanism of caffeine-induced wakefulness, and the interaction of the drug with the homeostatic mechanisms governing the expression of nonrapid-eye-movement sleep (NREM, or slow-wave sleep) and rapid eye movement (REM, or paradoxical sleep), are not yet fully understood.

Caffeine is a trimethylxanthine that competitively antagonizes the depressant effects of adenosine with high affinity at A1 receptors throughout the brain (1,6,19). The sleep-promoting effects of adenosine receptor agonists (24,33) may reflect a neuropharmacological component of sleep homeostasis in brain regions that directly (3,4) or indirectly modulate cortical activation, including the preoptic area of the hypothalamus (30), and the cholinergic neurons of the mesopontine tegmentum and basal forebrain (22,26). Consistent with this hypothesis, caffeine has been reported to decrease NREM sleep, especially the deeper stages of NREM (20,33,37) and the associated slow-wave activity (SWA), or spectral power in the delta band of the EEG that serves as a marker of homestatic sleep drive (5,14,15,28).

Whether caffeine promotes wakefulness by interfering with the expression of sleep or by attenuating homeostatic sleep drive is unclear. Morning caffeine reduces nighttime sleep efficiency, duration, and low-frequency power density in human NREM sleep, even when salivary caffeine levels are below that previously reported to influence alertness (15).

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However, caffeine only partially mimics the EEG spectral signs of the reduction in sleep pressure that are observed over the normal course of human sleep (14). In rats, wakefulness induced by 8 mg/kg does not elicit compensatory NREM sleep or SWA (29), suggesting that caffeine can disrupt sleep homeostasis. Wakefulness induced by a larger caffeine dose (15 mg/kg) also fails to invoke compensatory increases in NREM sleep duration, although SWA is elevated for several hours posttreatment (28). In one study, caffeine administered concurrently with sleep deprivation reduced the elevation of SWA that is normally observed during recovery from nonpharmacological sleep deprivation (28). Thus, caffeine may decrease sleepiness that accrues during wakefulness (28). The interpretation of those findings was limited, however, by the lack of an assessment of the attempts to sleep during sleep deprivation, the efficiency of the sleep deprivation as a function of drug or vehicle treatment, and prolonged recovery sleep, complicating the interpretation of caffeine effects on sleepiness during and after sleep deprivation.

The effects of caffeine on sleep may be further complicated by dynamic interactions between the drives for different arousal states. For example, NREM and REM sleep homeostatic mechanisms can be independently engaged, yet influence the expression of each other, as evidenced by selective SWA or REM sleep deprivation studies (2,11). Therefore, differential effects of caffeine on these homeostatic mechanisms may alter sleep architecture after sleep deprivation. Acute caffeine treatment in rats increases the latency to REM sleep onset and reduces REM sleep duration (28,33,37), even when administered after REM sleep deprivation when the homeostatic drive to compensate for the lost REM sleep is high (25). After REM sleep deprivation, caffeine delays the appearance of compensatory REM sleep, but does not affect the total amount recovered (25). Local administration of adenosine agonists into specific brain regions can selectively increase REM sleep (17,21,23). Thus, caffeine may exert distinct effects on REM sleep expression, that could in turn, influence NREM sleep, in addition to the direct effects of caffeine on NREM sleep.

In the present study, the interactions of caffeine with the dynamics between sleepiness, sleep deprivation, and the expression of NREM and REM sleep were investigated in rats. The ability of caffeine to attenuate sleepiness was directly tested by quantifying the number of stimuli needed to maintain wakefulness during acute sleep deprivation after systemic caffeine treatment. The differential influence of caffeine on NREM and REM sleep homeostasis was assessed by the magnitude and time course of the compensatory sleep responses (duration of sleep states, SWA, and length of sleep bouts) after release from sleep deprivation.

METHODS

Surgery

Ten male Wistar rats (Charles River Laboratories, Wilmington, MA) aged 2–3 months, with a mean weight of 334 ± 0.015 g, were surgically prepared for chronic EEG and EMG recording (10). The rats were sedated with diazepam (1.6 mg/kg, IM) and anesthetized with isofluorane (3% in medical grade oxygen) before placement in a stereotaxic frame (Kopf Instruments, Tajunga, CA). The electrode implants were gas sterilized prior to surgery, and consisted of six stainless steel wires that had been soldered to a miniature gold and Teflon connector (Microtech Inc., Boothwyn, PA). Four EEG leads were secured to the skull with stainless steel screws inserted 2.0 mm anterior to bregma and ± 2.0 mm from midline, and 6.4 mm posterior to bregma, and ± 3.0 mm from midline. Holes for the screws were drilled (Dremel bit size HP-1) through the skull, but care was taken not to puncture the dura. Two EMG electrodes were positioned under the nuchal trapezoid muscles. The implant was attached to the skull with cyanoacrylate and dental cement. Postoperative care included pain management with nalbuphine (2 mg/kg, IM) and buprenorphine (0.03 mg/kg, IP). Prophylactic control of infection was provided pre- and postoperatively with chloramphenicol (10 mg in 0.1 ml, IP) and topical antibiotics as needed. Surgical recuperation was permitted for a minimum of 3 weeks.

Sleep Deprivation and Data Collection

Rats were housed individually in sleep-deprivation chambers for the duration of the experiment. Each chamber consisted of a perforated stainless steel cylinder (39.7 cm diameter by 32.1 cm length) that was positioned horizontally inside a Plexiglas frame (637.2 cm^2 floor space). Access to food and water was provided *ad lib* in a 24-h light–dark cycle (LD 12:12) with lights-on (zeitgeber time, ZT-0) at 0800 h. The temperature of the sound-attenuated recording room was 24.1 ± 0.1 °C. The cranial electrode implants were connected to rotating commutators (Biela Engineering, Irvine, CA) by flexible cables, allowing the animals unimpeded movement throughout the chambers. At least 2 weeks were allowed for recording cable and chamber adaptation.

EEG and EMG were sampled in 5-s epochs using SCORE™, a PC-based, automated sleep–wake data collection system (10,32). This system identified vigilance states as NREM, REM, wake, or theta-dominated wake in real time based on the match of the epoch content to individual vigilance state templates that were constructed for each animal. Data quality was monitored by frequent inspection of the signals, and was corrected by replacement of recording cables or adjustment of the scoring templates for individual rats as needed. The digitized EEG and integrated EMG were collected as raw data and allowed for off-line verification of vigilance state scoring and data quality control.

An automated method of total sleep deprivation was employed in this study. When SCORE™ detected an epoch of NREM or REM sleep, the program activated a motor to rotate the cylindrical chamber for 5 s at a rate of 3.5 rpm. This motion initiated the righting reflex of the rats to disrupt their sleep. Therefore, each 5-s epoch in which a rotation occurred was defined as an attempt to sleep.

Drug Administration

Caffeine (Sigma, St. Louis, MO) was dissolved in sterile 0.25% methylcellulose in distilled water and injected IP at a dose and volume of 12.5 mg/ml/kg. Vehicle control injections (0.2 ml, IP) were made from the same methylcellulose stock. All rats were drug naïve at the time of treatment.

Study Design and Data Analysis

In a pseudorandomized crossover design (Fig. 1), rats were injected with caffeine or vehicle at ZT-0, immediately before sleep deprivation. All rats completed both treatments and served as their own controls. Vigilance state data were collected continuously across baseline (48 h prior to treatment), sleep deprivation (6 h after injection), and recovery (42 h after release from sleep deprivation). At least 4 days separated each trial.

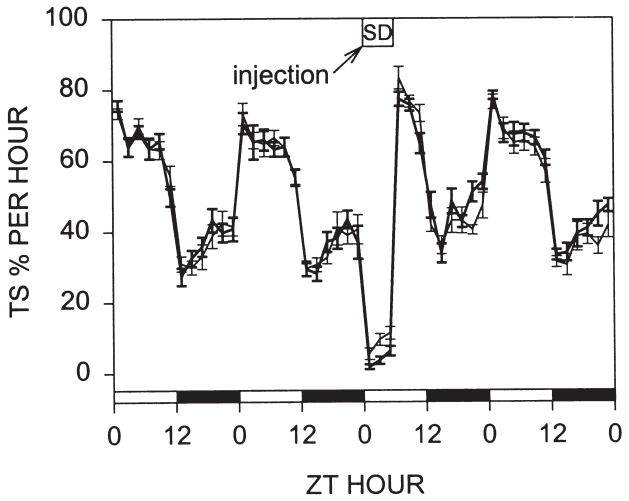

FIG. 1. Total sleep (TS) duration 48 h prior to 12.5 mg/kg caffeine (heavy line) or vehicle (thin line) injection (arrow), during 6-h sleep deprivation (SD box), and 42 h postsleep deprivation in the rat. Light/dark bars indicate lights on/off. TS data are plotted as the mean ± SE. Crossover $n = 10$.

Principle variables measured were the percent per hour of NREM, REM, and wake (including theta-dominated wake). Total sleep (TS) was calculated as the sum of NREM and REM per hour. The number of sleep attempts per hour during sleep deprivation (sleep tendency) was determined by the number of NREM and REM epochs that triggered deprivation stimuli (cylinder rotations). Total sleep-bout length mean (SBL_{mean}) and maximum (SBL_{max}) and wake-bout length mean (WBL_{mean}) and maximum (WBL_{max}) were calculated in minutes per hour. Bouts were defined as a minimum of three consecutive, 10-s epochs of qualifying states and terminated with at least three contiguous epochs of nonqualifying states. Intervening epochs of nonqualifying states that did not reach the bout termination criteria did not contribute to the bout-length calculation.

Spectral analysis was performed on the raw digitized (100 Hz) EEG waveforms across the entire 4 days of recording. Epochs of EEG containing artifact were screened with an algorithm, confirmed visually, and eliminated from further analysis. The power spectra for each epoch was determined using Hartley's modification of the fast Fourier transform (5a). Slow-wave activity (SWA) per hour was defined as the mean power in the delta band (0.1–4.0 Hz) during NREM, and was normalized as a percent of the daily baseline mean SWA for each animal.

Statistics were calculated using SAS 6.04 (SAS Institute, Cary, NC). Comparison of the 48-h baselines between the caffeine and vehicle conditions were made with two-way repeated-measures analysis of variance (RM-ANOVA) for NREM, REM, TS, wake, SBL_{max}, SBL_{mean}, WBL_{max}, WBL_{mean}, and SWA in 6-h bins. Because no statistical differences were found between baseline conditions, the caffeine and vehicle baseline data were combined by taking the mean per hour for each variable. Two-way RM-ANOVA was also used to detect differences between baseline, vehicle, and caffeine conditions at each hour of sleep deprivation and at each 6-h bin during the 42-h recovery period. In the presence of a significant main effect, Tukey's Studentized Range test was used to contrast the conditions at different times of day ($\alpha = 0.05$). Paired *t*-tests were used to compare vehicle and caffeine conditions on the total amount of TS, NREM, and REM lost from sleep deprivation and recovered by 42 h after release from sleep deprivation.

RESULTS

Baseline

During the 48 h prior to caffeine and vehicle treatment, rats displayed a strong circadian rhythm in TS that persisted through the recovery period after sleep deprivation (Fig. 1). The sleep history before treatment was equivalent in the caffeine and vehicle conditions.

Caffeine Effects During Sleep Deprivation

Caffeine significantly decreased sleep tendency during sleep deprivation. The number of attempts to enter sleep during each hour of sleep deprivation after caffeine or vehicle injection are depicted in the top panel of Fig. 2. Regardless of treatment condition, the rats attempted to sleep more as the sleep deprivation progressed, $F(5,45) = 14.70$, $p = 0.0001$.

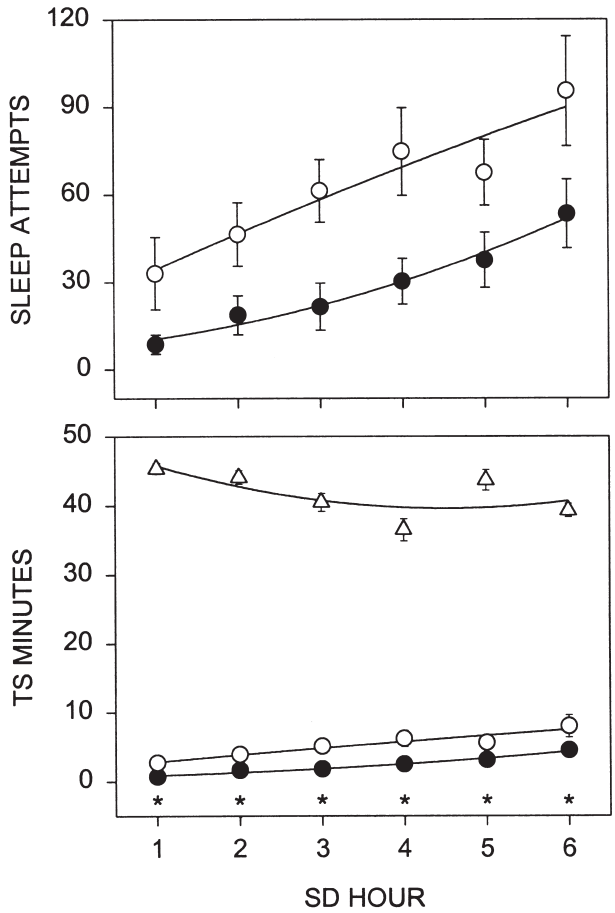

FIG. 2. Number of sleep attempts (top) and minutes of total sleep (TS; bottom) per hour during sleep deprivation (SD) in caffeine (solid circles) or vehicle (open circles)-treated rats. Caffeine (12.5 mg/kg) was administered at the beginning of SD. Baseline TS is shown by open triangles. Data are plotted as the mean ± SE (crossover $n = 10$) and fitted with second-order regressions. *$p < 0.05$ caffeine and vehicle vs. baseline; Tukey's Studentized Range Test.

TABLE 1

SLEEP LOST DURING 6-HOUR SLEEP DEPRIVATION (SD) AND TOTAL RECOVERED (MEAN ± SE) 42 HOURS POST-SD IN CAFFEINE AND VEHICLE-TREATED RATS

Variable	Condition		
	Caffeine	Vehicle	Paired *t*-Test
TS			
Lost (minutes relative to baseline)	−235 ± 6.9	−218 ± 8.1	−2.97 $p < 0.0157$
Rec. (minutes relative to baseline)	173 ± 18.3	120 ± 13.8	2.43 $p < 0.0380$
% of baseline lost	94.2 ± 1.4	87.2 ± 2.5	2.87 $p < 0.0184$
% of loss recovered	74.1 ± 8.8	55.0 ± 5.9	1.67 $p < 0.1290$
NREM			
Lost (minutes relative to baseline)	−203 ± 5.4	−186 ± 7.4	−2.82 $p < 0.0199$
Rec. (minutes relative to baseline)	141 ± 19.4	103 ± 9.9	1.69 $p < 0.1254$
% of baseline lost	93.8 ± 1.4	86.2 ± 2.8	2.77 $p < 0.0217$
% of loss recovered	70.0 ± 10.4	56.2 ± 5.7	1.01 $p < 0.3372$
REM			
Lost (minutes relative to baseline)	−32.8 ± 2.7	−31.8 ± 3.0	−1.71 $p < 0.1209$
Rec. (minutes relative to baseline)	31.8 ± 4.2	17.3 ± 6.7	2.50 $p < 0.0339$
% of baseline lost	96.3 ± 1.8	92.5 ± 4.1	1.43 $p < 0.1855$
% of loss recovered	100 ± 13.9	43.9 ± 24.1	2.29 $p < 0.0480$

Caffeine reduced sleep attempts below vehicle levels at all hours of sleep deprivation, $F(1, 9) = 8.83$, $p = 0.0157$, but the effect of caffeine on sleep tendency did not depend on the duration of sleep deprivation, $F(5, 45) = 0.75$, NS). The attempts to sleep after caffeine treatment were reduced to 44.9% of the vehicle level over the entire sleep deprivation period.

TS was similar in caffeine- and vehicle-treated rats during sleep deprivation. Figure 2 shows that at every hour of sleep deprivation, the TS minutes in caffeine and vehicle conditions were equivalent and below baseline levels, $F(10, 90) = 11.65$, $p = 0.0001$. However, when TS was assessed in minutes relative to baseline or as a percent of baseline TS lost, over the entire sleep deprivation period, caffeine treatment resulted in slightly more TS loss than the vehicle treatment (Table 1). Similar assessments in Table 1 revealed rats lost 17 more min of NREM during the 6-h sleep deprivation after caffeine than they did after vehicle, but REM amounts were the same between conditions.

Caffeine Effects During Recovery Sleep

Figure 3 illustrates the time course of vigilance state variables (TS, NREM, REM, SWA, SBL_{max}, SBL_{mean}, WBL_{max}, and WBL_{mean}) in 6-h blocks over the 42-h recovery period after sleep deprivation and caffeine or vehicle treatment vs. baseline. Compensatory responses to sleep deprivation were assessed as statistical differences between baseline and treatment values. Table 1 lists the amount of TS, NREM, and REM recovered in minutes relative to baseline or normalized as the percent of the sleep loss that was recovered over the total 42-h recovery period.

Total sleep. The time course of compensatory TS (Fig. 3A) after sleep deprivation differed between caffeine and vehicle treatments, $F(12, 108) = 8.85$, $p = 0.0001$. In the vehicle condition, TS duration exceeded baseline levels for 12 h after release from sleep deprivation. This compensatory sleep terminated during the last half of the dark phase. In the caffeine condition, TS also exceeded baseline, but significantly less than after vehicle treatment during the first 6 h postsleep deprivation. However, by 13–18 h after release from sleep deprivation, the compensatory sleep after caffeine treatment began to surpass the TS levels in the vehicle condition. The TS in the caffeine condition returned to baseline levels 19–30 h postsleep deprivation (light phase), but the compensatory sleep resumed during hours 31–42 (dark phase). Over the entire 42-h recovery period, caffeine-treated rats showed more compensatory TS relative to baseline than after vehicle treatment. In both conditions, however, the rats recovered a similar percentage of their lost sleep (Table 1).

NREM. The time course of NREM compensatory sleep after caffeine or vehicle treatment (Fig. 3B) mirrored the TS pattern, $F(12, 108) = 8.25$, p = 0.0001. During the first 6 h of recovery from sleep deprivation, rats in the caffeine condition had more NREM sleep than baseline, but significantly less than they did after vehicle treatment. The level of NREM sleep of vehicle-treated rats returned to baseline by 12-h postsleep deprivation. Caffeine-treated rats, on the other hand, showed elevated NREM sleep for 18 h after release from sleep deprivation. The NREM sleep then declined to baseline levels for 19–30 h (during the following light phase) and resumed above baseline during the next dark phase. The total amount of compensatory NREM sleep in the 42-h recovery period did not statistically differ between treatment conditions (Table 1).

REM. The temporal pattern of compensatory REM after sleep deprivation (Fig. 3C) differed between caffeine and vehicle treatments, $F(12, 108) = 6.46$, $p = 0.0001$. REM recovery in the vehicle condition resembled the pattern observed for TS and NREM—there was an immediate elevation above baseline after release from sleep deprivation that lasted for 12 h until the middle of the dark period. By contrast, after caffeine treatment, compensatory REM did not begin until 7–12 h after sleep deprivation ended and continued until the end of the dark period. A very small increase in REM above vehicle, but not baseline levels, reemerged 36–42 h after sleep deprivation. Of all the sleep variables, REM in the caffeine condition was the only measure that did not show a change from baseline immediately after release from sleep deprivation. Although caffeine and vehicle treatments during sleep deprivation produced the same amount of REM sleep loss, rats in the

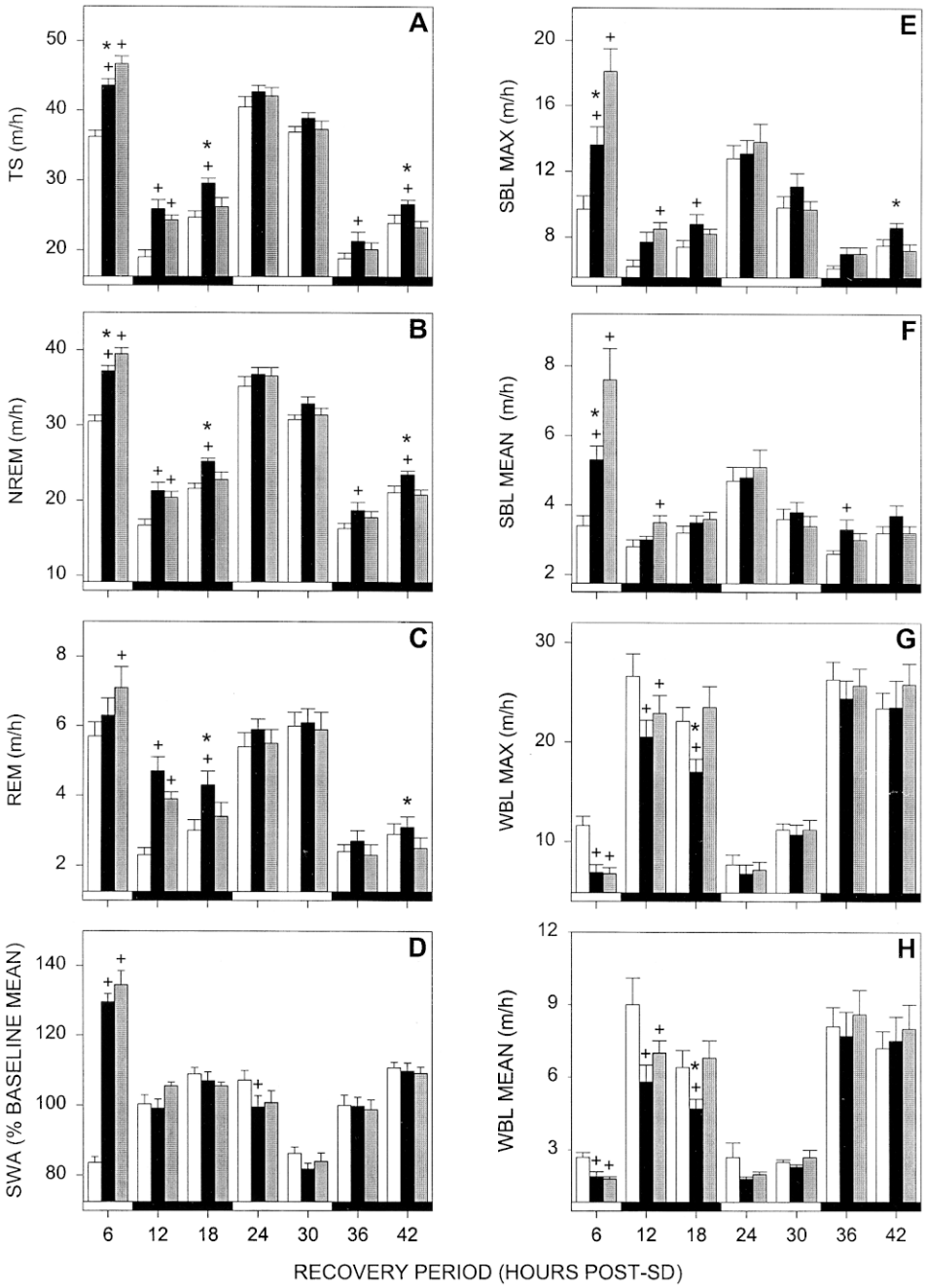

FIG. 3. Vigilance state variables (TS, NREM, REM, SWA, SBLmax, SBLmean, WBLmax, WBLmean) over the 42-h recovery period following sleep deprivation (SD) and 12.5 mg/kg caffeine (black bars) or vehicle (gray bars) treatment vs. baseline (white bars). Light/dark bars along the x-axis indicate lights on/off. Data are plotted as the mean ± SE (crossover $n = 10$) and blocked in 6-h bins. $^{*}p < 0.05$ vs. vehicle, $^{+}p < 0.05$ vs. baseline, Tukey's Studentized Range Test.

caffeine condition recovered all of the lost REM, while rats in the vehicle condition only recovered about half the amount lost.

SWA. Figure 3D shows sleep deprivation induced compensatory SWA after caffeine and vehicle treatment, $F(12, 108) = 70.41$, $p = 0.0001$. Both treatment conditions resulted in equally large increases in SWA over baseline for 6 h after release from sleep deprivation. SWA was slightly reduced 19–24 h after sleep deprivation ended in the caffeine condition. Unlike the measures of sleep duration, SWA during recovery did not differ between treatment conditions at any time point, nor was any delayed rebound observed.

SBL. Caffeine affected sleep continuity during recovery from sleep deprivation, whether indexed by SBL_{max}, $F(12,$

108) = 9.89, $p = 0.0001$, or SBL_{mean}, $F(12, 108) = 10.74$, $p = 0.0001$. Figure 3E and F shows that after vehicle treatment, sleep bouts were longer than baseline for the first 12 h postsleep deprivation. In the caffeine condition, sleep bouts were longer than baseline for the first 6 h after sleep deprivation, but the response was attenuated compared to the vehicle condition. The lengthening of sleep bouts reemerged in caffeine-treated rats 13–18 h and 31–42 h after sleep-deprivation termination.

WBL. The compensatory responses to sleep deprivation of increased sleep duration, SWA, and SBL were reflected by a concomitant decrease in the length of wake bouts. Figure 3G and H shows that wake bouts change in length over time during recovery from sleep deprivation after caffeine or vehicle treatment, as measured by WBL_{max}, $F(12, 108) = 3.22$, $p = 0.0006$, or WBL_{mean}, $F(12, 108) = 2.84$, $p = 0.002$. Wake bouts were shorter than baseline after both caffeine and vehicle treatments during the first 12 h postsleep deprivation termination. By 13–18 h after release from sleep deprivation, WBL continued to be shorter than baseline in the caffeine, but not vehicle, condition.

DISCUSSION

Understanding the interactions between caffeine pharmacology, sleep loss, and circadian timing is relevant to individuals whose alert wakefulness is challenged by shift work or sustained operations. The degree of sleepiness resulting from prolonged wakefulness can be inferred from increases in the amount of time spent asleep, the length of uninterrupted bouts of sleep, and the depth of sleep as measured by delta power in the NREM EEG (5,8,31). Although direct assessment of sleepiness during wakefulness can be measured in humans by changes in the latency to sleep onset, this index is rarely used in animal studies. Using automated sleep deprivation technology for the direct measurement of sleepiness in the rat, this study shows that caffeine reduced sleep tendency during sleep deprivation and induced specific temporal and arousal state dynamics during compensatory sleep.

Caffeine Interaction With Sleep Tendency

Caffeine reduced sleep tendency during sustained wakefulness, but did not eliminate sleep drive. At every hour of sleep deprivation, rats made fewer attempts to sleep after caffeine than vehicle treatment (Fig. 2). Nevertheless, caffeine did not block the increase in sleep attempts as the sleep deprivation progressed. Whether this cumulative increase in sleep attempts is the result of 1) the disinhibition of sleep tendency as caffeine is metabolized, 2) sleep drive building high enough to surpass the caffeine inhibition of sleep tendency, or 3) some combination thereof, is difficult to ascertain without direct measures of CNS caffeine levels and binding affinity.

The lack of an interaction between the duration of sleep deprivation and treatment condition was unexpected, and could be due to disproportionate effects of sleep deprivation and caffeine on alertness. For example, an interaction may occur with a longer sleep deprivation duration or a smaller dose of caffeine. However, a moderate dose of caffeine (15 mg/kg) can induce spontaneous waking equivalent to 4 h of sleep deprivation (28); thus, an interaction was predicted given the 12.5 mg/kg dose administered before 6 h of sleep deprivation in the present study. The lack of an interaction effect between caffeine and sleepiness in rats is supported by several human MSLT studies (13,16,27,34), suggesting that caffeine pharmacodynamics act on sleep homeostatic mechanisms in an additive way. This additive effect probably lasted throughout the 6-h sleep deprivation in the present study, given the half-life of caffeine is approximately 7 h in rats (28).

The differences in compensatory sleep between caffeine and vehicle conditions, both during the sleep deprivation and recovery periods, cannot be explained by differences in baseline levels of sleepiness. In this crossover study, the rats had similar sleep histories prior to treatment (Fig. 1) and lost roughly the same amount of sleep during the deprivation period. Although the ANOVA on TS per hour of sleep deprivation between caffeine, vehicle, and baseline conditions (Fig. 2) yielded no difference in TS lost between treatments, the cumulative values for the amount of TS lost did statistically differ (Table 1). Caffeine-treated rats lost 17 more minutes of TS over the 6 h of sleep deprivation than vehicle-treated rats, but this difference is negligible considering the same rats slept for 250 min during baseline. Thus, changes in compensatory sleep after caffeine treatment are more likely due to drug effects and not to differences in the amount of sleep lost during sleep deprivation.

Caffeine Interaction With Recovery Sleep

Caffeine did not block the recovery of sleep lost after 6 h of sleep deprivation in rats, but rather slowed the rate of recovery of most sleep variables. Compensatory sleep responses were observed in TS, NREM and REM duration, SWA, and sleep continuity. Moreover, caffeine-treated rats recovered as much NREM in 42 h as they did after vehicle treatment. Therefore, caffeine did not erase the accumulation of a sleep deficit accrued during sustained wakefulness. The time course of compensatory sleep was influenced by caffeine treatment during sleep deprivation. All variables except SWA showed a more gradual and delayed recovery of lost sleep in the caffeine condition compared to vehicle treatment. Interestingly, small compensatory responses in TS, NREM, and sleep continuity occurred 30–42 h postsleep deprivation, suggesting that sleep compensation was carried over multiple sleep cycles, well after the caffeine had been metabolized.

Caffeine did not appear to alter the rate or extent of compensatory sleep as indexed by SWA. In each treatment condition, SWA was elevated to the same extent above baseline levels for the first 6 h after release from sleep deprivation (Fig. 3D). These findings are not consistent with a prior report that caffeine (15 mg/kg) administered at ZT-0 before a 6-h sleep deprivation moderately reduced SWA relative to vehicle controls for 6 h during recovery sleep (28). This apparent inconsistency between studies may be explained by differences in caffeine dose or in the amount of sleep loss between treatment conditions during sleep deprivation—an important measure that was not reported in that study (28). SWA decreased slightly below baseline 24 h after the caffeine treatment, consistent with a report that caffeine can mildly reduce the lowest frequencies of SWA in humans 16–24 h after caffeine ingestion (15). Compared with other measures of compensatory sleep, SWA showed an especially large increase and rapid return to baseline in the first 6 h postsleep deprivation. Thus, SWA may be preferentially recovered relative to other sleep variables (11).

Differential Effects of Caffeine on NREM and REM Sleep

Caffeine produced distinct effects on the time course of NREM and REM recovery sleep after sleep deprivation. The

compensatory response in NREM duration in the caffeine condition began immediately after release from sleep deprivation, and was expressed predominantly in the initial 18 h posttreatment (Fig. 3B). REM sleep compensation after caffeine treatment, by contrast, did not begin until approximately 6 h after release from sleep deprivation, and was predominantly expressed in the subsequent 12 h (Fig. 3C). The difference between the time courses of NREM and REM compensatory sleep may reflect preferential NREM recovery and consequent displacement of REM sleep in the first 6 h postsleep deprivation, or could even reflect an interaction of caffeine with the circadian system (see below). In either case, these findings are consistent with a previous report that the onset of compensatory REM sleep shifts after caffeine treatment (25).

The suppression of compensatory REM sleep in the first 6 h postsleep deprivation did not appear to be secondary to residual caffeine-induced wakefulness, but rather, caffeine may have directly interfered with REM sleep regulation. During this time block, wake bouts were shorter than baseline, and TS was elevated above baseline, indicating reduced wakefulness despite any residual caffeine. Furthermore, NREM and REM recovery sleep levels were proportionately elevated above baseline in the vehicle condition (roughly 130%), but this relationship was not maintained in the caffeine condition. Interestingly, the elevated levels of SWA—an indicator of heightened NREM homeostatic sleep drive—were not different between treatment conditions, and thus could not account for disproportionate amounts of REM sleep recovered in the first 6 h postsleep deprivation. Moreover, the initial suppression of REM sleep by caffeine may have permitted the large compensatory SWA response, due to the mutual inhibition between REM sleep drive and SWA (11). Taken together, these findings support a putative role for adenosine in REM sleep control (17,21,23).

In addition to the differential effects on the timing of compensatory NREM and REM sleep, caffeine disproportionately changed the total amount sleep recovered in both sleep states. By 42 h postsleep deprivation, caffeine treated rats recovered only a portion of their lost NREM sleep, which did not statistically differ from vehicle treatment (Table 1). REM sleep loss due to sleep deprivation in the caffeine condition, however, was compensated for 100%—about twice as much as the vehicle level (Table 1). In a previous report, caffeine did not alter the amount of REM sleep recovered in 36 h following 1 d of selective REM sleep deprivation (25). The difference between studies is unclear, but because NREM homeostasis was probably challenged more in our total sleep deprivation protocol than in the REM deprivation study, the difference may reflect a complex interaction between caffeine and the drives for NREM and REM sleep. Nevertheless, the findings of the present study suggest that the homeostatic mechanisms that regulate the quantity of sleep recovered after sleep deprivation include distinctive NREM and REM sleep components that respond differently to caffeine.

Interaction of Caffeine With Time of Day

Following the robust compensatory sleep responses evidenced in NREM and SWA in the first 6 h postsleep deprivation, more subtle, but significant changes in NREM, REM, and bout lengths were observed exclusively in the subsequent dark periods, up to 42 h postsleep deprivation. No significant increases in any sleep variable were observed in the light phase 19–30 h postsleep deprivation. This delayed compensatory sleep may have been most apparent at times of day when rats were normally active because at those times, recovery sleep was compared against the baseline circadian minimum for sleep variables. According to this logic of "floor" and "ceiling" effects on circadian minima and maxima for vigilance states (18), the compensatory sleep may not have been evident during the light period because it would not be possible for rats to sleep more than their physiological ceiling permits. This explanation seems unlikely, however, because the compensatory sleep variables in the vehicle condition were markedly greater during the first 6 h postsleep deprivation (lights on) than during the subsequent light phase from 19–30 h postdeprivation. Thus, the lack of compensatory sleep responses during this light phase appeared to reflect time of day interactions, rather than ceiling effects. SWA was also the only variable that did not show a delayed recovery or a time of day interaction. Unlike measures of sleep duration and continuity, however, SWA is not measured in time units, and thus is not subject to the same time-delimited ceiling effects.

Compensatory REM sleep in the caffeine condition was greatest during the initial dark phase following sleep deprivation. Although this timing may have been due to the coincidence of the light/dark schedule and the pharmacokinetics of caffeine, circadian regulatory factors may have been involved. Preliminary data suggests that REM sleep is actively under circadian control during the light phase, and is not inhibited during the dark phase in the rat (35). Therefore, the dark phase may constitute a permissive window for compensatory REM sleep expression after sleep deprivation. Additional sleep deprivation and stimulant interaction studies are needed at different circadian phases to definitively establish circadian interactions with compensatory REM sleep.

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