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# Effects of $N^6$-cyclopentyladenosine and caffeine on sleep regulation in the rat

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**Abstract**

To study the role of adenosine in sleep regulation, the adenosine $A_1$ receptor agonist $N^6$-cyclopentyladenosine (CPA) and the antagonist caffeine were administered to rats. Intraperitoneal (i.p.) CPA 1 mg/kg but not 0.1 mg/kg, suppressed rapid-eye-movement (REM) sleep and enhanced electroencephalographic (EEG) slow-wave activity (power density 0.75–4.0 Hz) in non-REM sleep. The latter effect was remarkably similar to the response to 6-h sleep deprivation. The effects persisted when CPA-induced hypothermia was prevented. Caffeine (10 and 15 mg/kg i.p.) elicited a dose-dependent increase in waking followed by a prolonged increase of slow-wave activity in non-REM sleep. The combination of caffeine (15 mg/kg) and sleep deprivation caused less increase in slow-wave activity than sleep deprivation alone, indicating that caffeine may reduce the buildup of sleep pressure during waking. The results are consistent with the involvement of adenosine in the regulation of non-REM sleep.

*Keywords:* Caffeine; Adenosine; Sleep regulation; Sleep EEG (electroencephalogram); (Rat)

## 1. Introduction

The term 'sleep homeostasis' refers to the tendency of the sleep regulating system to maintain a constant mean level of sleep. Sleep loss elicits a compensatory response which consists in an increase in sleep intensity and sometimes also in a prolongation of sleep. One of the main markers of the intensity of non-rapid-eye-movement (non-REM) sleep is EEG slow-wave activity which can be defined as the spectral power density in the 0.75–4.0 Hz band. Slow-wave activity typically declines in the course of the sleep episode and is enhanced after prolonged waking (Borbély et al., 1981; Borbély et al., 1984). That slow-wave activity is an index of non-REM sleep intensity in the rat is evident from its correlation with both sleep continuity (as measured by brief awakenings; Franken et al., 1991; Tobler and Borbély, 1986) and the arousal threshold (Neckelmann and Ursin, 1993).

Benington and Heller (1995) have recently proposed an interesting hypothesis on the mechanisms underlying sleep homeostasis. In their view, a main function of sleep is the replenishment of cerebral glycogen stores that have been progressively depleted during waking. Depletion of these stores is assumed to give rise to the release of adenosine which augments the sleep drive and potentiates EEG slow-wave activity in non-REM sleep. The sleep state would allow the resynthesis of cerebral glycogen. By its action on postsynaptic adenosine $A_1$ receptors, adenosine increases $K^+$ conductance ($gK^+$) (reviewed by Green and Haas, 1991) and thereby promotes the tonic hyperpolarization of thalamocortical neurons. In addition to the enhancement of $gK^+$, also presynaptic adenosine $A_1$ receptors as well as $A_2$ receptors may mediate the effects of adenosine (see Benington and Heller, 1995 for references).

The role of adenosine in sleep regulation is supported by the findings that adenosine itself as well as adenosine receptor agonists and a selective nucleoside transport inhibitor promote sleep, and particularly 'deep' non-REM sleep (Haulica et al., 1973; Radulovacki et al., 1982, 1984; Wauquier et al., 1989; Ticho and Radulovacki, 1991), and that adenosine inhibits mesopontine cholinergic neurons implicated in the control of arousal (Rainnie et al., 1994). The selective adenosine $A_1$ agonist $N^6$-cyclopentyladenosine (CPA) was recently shown to produce a dose-dependent increase of slow-wave activity in the rat after both systemic and intracerebroventricular administration (Be-

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nington et al., 1995). To counteract possible non-specific effects of the hypothermia elicited by CPA, experiments were also performed at an ambient temperature of 32°C. However, the effects on body or brain temperature were not reported.

The main objective of the present study was to further explore the role of adenosinergic mechanisms in sleep regulation by studying the effects of CPA, and comparing its effect on the sleep EEG with that of sleep deprivation. In addition to stimulating adenosine $A_1$ receptors, adenosine receptors were blocked by caffeine and the after-effects of its sleep inhibiting action were compared to those of prolonged waking without drugs. We were particularly interested to see whether the presence of caffeine attenuates the buildup of a homeostatic sleep drive, an effect that seemed to be present in a recent human sleep study (Landolt et al., 1995).

## 2. Materials and methods

### 2.1. Animals

The experiments were performed in male adult rats of the Sprague-Dawley SIVZ strain. The animals had a mean body weight of 352.1 ± 7.8 g on the day before the first drug administration. They were kept individually in Macrolon cages (36 × 20 × 35 cm) within sound-attenuated recording boxes. The animals were maintained on a 12 h light-12 h dark cycle (light from 08:00–20:00 h; daylight-type fluorescent tubes, 18 W, approximately 300 Lux) with food and water available ad libitum. During the light period the ambient temperature inside the cages was 22.5 ± 0.1°C. The animals were adapted to these conditions for at least 14 days prior to the recordings.

Electrodes for recording the electroencephalogram (EEG) and the electromyogram (EMG), and a thermistor for recording cortical temperature ($T_{CRT}$) were implanted under deep pentobarbital anesthesia as described previously (Franken et al., 1991). Gold-plated, round-tipped miniature screws served as EEG-electrodes and were placed on the dura over the right parietal cortex (2.0 mm lateral to the midline, 3.5 mm posterior to the bregma) and the cerebellum (1.5 mm posterior to the lambda). A third screw served to improve the fixation of the assembly to the skull. The EMG was recorded with two gold wires (diam. = 0.2 mm) inserted into the neck muscles. $T_{CRT}$ was recorded with a thermistor (Thermometrics, P20, R(25°C) = 1 kΩ, max. diam. = 0.5 mm, accuracy ±0.05°C) which had been inserted through a hole over the left frontal cortex with the tip resting on the dura over the parietal cortex. The four electrodes and the thermistor were soldered to a connector which was fixed to the skull with dental cement. After surgery the animals were allowed to recover for at least 6 days. One day before the experiment they were connected to the cable for 12 h.

### 2.2. Data acquisition

The EEG and EMG signals were amplified (amplification factor 2000, band-pass filter 0.016–40 Hz, −3 dB points, 24 dB/octave) and on-line digitized (sampling rate 256 Hz). The EEG was digitally low-pass filtered at 25 Hz and stored with a sampling rate of 128 Hz. Consecutive 4-s epochs were subjected to a Fast Fourier transform routine and EEG power density values were computed for 4-s epochs in the frequency range of 0.25–25.0 Hz. Between 0.25 and 5.0 Hz the values were averaged to yield 0.5-Hz bins, and between 5.25 and 25.0 Hz to yield 1-Hz bins. The EMG was full-wave rectified and integrated over 4-s epochs, and $T_{CRT}$ and ambient temperature inside the cage were sampled at 4-s intervals. The vigilance states non-REM sleep, REM sleep and waking were determined off-line by visual inspection of the EEG and EMG recordings, the EEG power density in the δ band (0.75–4.0 Hz), and the integrated EMG (Franken et al., 1991). EEG slow-wave activity (mean EEG power density in the 0.75–4.0 Hz range) was determined for each epoch scored as non-REM sleep. Slow-wave activity was expressed as a percentage of the individual mean baseline value to reduce the interindividual variation due to differences in the absolute slow-wave activity level. Epochs containing EEG artefacts were visually identified and omitted from further analysis of the power spectra (mean percentage of artefacts over the entire experiment: 4% ± 0.4).

Sleep onset latency was defined as the first 2 min of sleep which contained not more than five 4-s epochs of waking and which was followed by a 30-min period containing at least 50% sleep.

### 2.3. Experimental protocol

CPA and caffeine were dissolved in 0.9% saline. All drugs were injected intraperitoneally in a volume of 1 ml/kg. Before control recordings the rats received 1 ml/kg of saline. All treatments were administered at light onset (08:00 h) and recordings were obtained throughout the 12-h light period, and in one experiment also in the first 6 h of the dark period.

#### 2.3.1. CPA

Based on previous results (Benington and Heller, 1995; Benington et al., 1995) two doses of CPA were investigated. The rats were injected with CPA 0.1 mg/kg, 1.0 mg/kg and saline (three rats received the low dose only, seven rats the high dose only, and one animal both doses). At least 7 days elapsed between CPA treatments, and at least 3 days elapsed after a saline injection. The order of treatment was saline-CPA ($n = 8$), CPA-saline ($n = 2$), and saline-CPA-saline ($n = 1$).

In an additional experiment, seven rats were injected with either saline or CPA 1.0 mg/kg. Saline preceded CPA in three animals. Approximately 30–75 min before

Table 1
Mean 12-h values of vigilance states, slow-wave activity (SWA; mean EEG power density 0.75–4.0 Hz) in NREM sleep and cortical temperature ($T_{CRT}$) after saline and CPA

| | CPA 0.1 mg/kg ($n = 4$) | | CPA 1.0 mg/kg ($n = 8$) | | | CPA 1.0 mg/kg and warming ($n = 7$) | | |
|---|---|---|---|---|---|---|---|---|
| | NaCl | CPA | NaCl | CPA | | NaCl | CPA | |
| Waking | 36.5 (2.7) | 32.3 (2.0) | 36.4 (2.2) | 45.9 [a] | (3.0) | 35.4 (1.3) | 36.2 | (1.2) |
| NREMS | 49.3 (3.0) | 52.5 (2.0) | 50.3 (2.0) | 46.2 | (3.0) | 52.2 (0.9) | 54.8 | (1.8) |
| REMS | 14.2 (0.8) | 15.2 (1.6) | 13.3 (0.9) | 7.9 [a] | (1.2) | 12.4 (0.7) | 9.0 | (1.2) |
| SWA in NREMS | 100 | 110.5 (9.0) | 100 | 135.9 [b] | (12.2) | 100 | 138.7 [a] | (12.6) |
| $T_{CRT}$ (°C) | 36.9 (0.1) | 36.8 (0.1) | 36.9 (0.1) | 35.7 [a] | (0.1) | 36.5 (0.1) | 37.0 [a] | (0.1) |

The vigilance states are expressed as a percentage of 12-h recording time. SWA in NREM sleep is expressed relative to the 12-h mean value in saline (= 100%). The values are means and S.E.M. in brackets, $n$ = number of animals. Significant differences between saline and treatments [a] $P < 0.01$, [b] $P < 0.05$; 2-tailed paired $t$-test.

the CPA injection ambient temperature was raised by a heating spiral placed under the cage. The warming procedure increased ambient temperature from 22.0 ± 0.4°C to 28.2 ± 0.2°C.

### 2.3.2. Caffeine

The dose of caffeine was chosen to maintain wakefulness for approximately 4 h. A non-pharmacological sleep deprivation had been shown to affect the sleep EEG only after it had been maintained for at least 3 h (Tobler and Borbély, 1986; Tobler and Borbély, 1990). Rats were administered caffeine 10 mg/kg ($n = 6$) or 15 mg/kg i.p. ($n = 8$), and saline (eleven rats received saline first). Three days after caffeine (15 mg/kg) administration the animals were subjected to sleep deprivation.

The duration of sleep deprivation was defined individually to match the duration of the caffeine-induced wakefulness. Sleep was prevented by introducing objects into the home cage and, if necessary, by acoustic stimuli (gentle tapping on the cage).

### 2.3.3. Combination of caffeine and sleep deprivation

A group of six rats was injected with caffeine (15 mg/kg) or saline at light onset and then kept awake for 6 h (caffeine was the first injection in two rats). At least 3 days were allowed between the two treatments. Recordings were obtained during the 6-h sleep deprivation and the subsequent 12-h recovery period.

### 2.4. Caffeine concentration in plasma

Separate groups of rats were injected with 15 mg/kg caffeine i.p. and blood was collected after decapitation at various time points after the injection (0.5, 1, 2, 3, 4, 6, 8

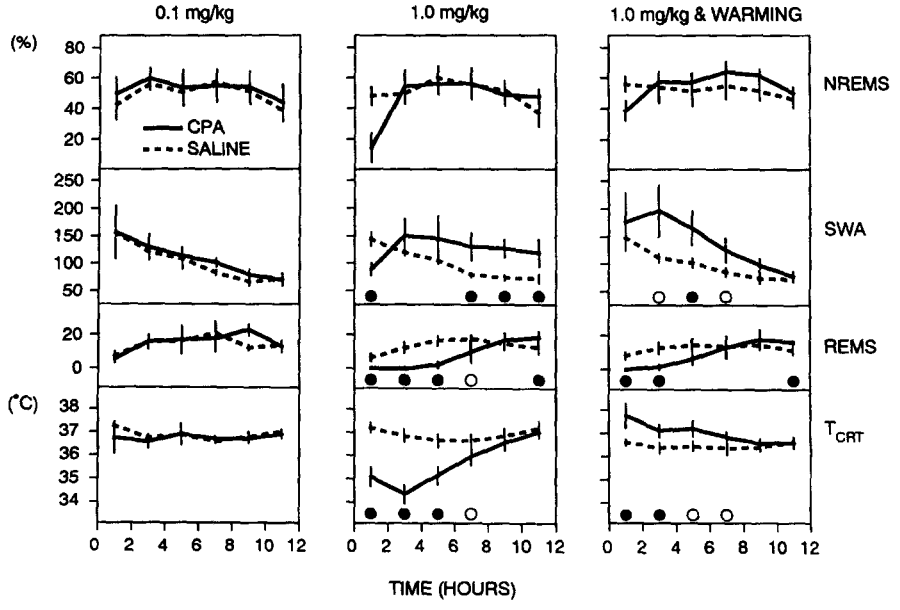


Fig. 1. Vigilance states, slow-wave activity (SWA; EEG power density 0.75–4.0 Hz) and cortical temperature ($T_{CRT}$) for saline, CPA 0.1 mg/kg and 1.0 mg/kg at 23°C ambient temperature, and 1.0 mg/kg combined with warming (up to 28°C). Mean values ± 2 S.E.M. ($n = 4$, 8, and 7, respectively) plotted for 2-h intervals. Non-REM sleep (NREMS) and REM sleep (REMS) are expressed as a percentage of recording time. SWA is expressed as a percentage of the mean 12-h value. Significant differences between saline and treatment are indicated by filled ($P < 0.01$) and open circles ($P < 0.05$; 2-tailed, paired $t$-test).

h). The samples were centrifuged, the plasma stored at −20°C and assayed with a homogenous enzyme-immunoassay (EMIT-Caffeine Test, Syva Co., Palo Alto, CA, USA; Oellerich, 1980).

### 2.5. Data analysis and statistics

The 4-s data were averaged over 2-h or 12-h intervals. The 2-h mean values of slow-wave activity in non-REM sleep, vigilance states and $T_{CRT}$ for the treatment days were compared to the corresponding baseline intervals. The cumulative slow-wave energy (i.e. the product of slow-wave activity in non-REM sleep and the duration of non-REM sleep) were determined for the 12-h light period after saline and drug administration. To analyze more closely the effect of 1.0 mg CPA and 15 mg caffeine, EEG power density was computed separately for waking, non-REM sleep and REM sleep. Overall effects of treatment were evaluated by a two-way analysis of variance (ANOVA) with the factors 'condition' (saline, drug, sleep deprivation) and 'interval' (either 1-h or 2-h intervals). Whenever significant effects were present, two-tailed paired $t$-tests were applied.

## 3. Results

### 3.1. Effects of CPA

#### 3.1.1. Behavior, vigilance states, slow-wave activity and $T_{CRT}$

The low dose of CPA had no significant effect (Table 1). After injection of CPA 1.0 mg/kg, behavior was disturbed for approximately 3 h. The rats showed severe ataxia accompanied by sporadic crawling movements.

Waking was enhanced at the expense of REM sleep which was suppressed in the first 4 h and remained reduced in the following 4 h (Fig. 1). In the last 2-h interval of the light period, REM sleep exceeded the control level. The 12-h value of slow-wave activity in non-REM sleep was significantly increased, despite its initial reduction (Table 1). The cumulative slow-wave

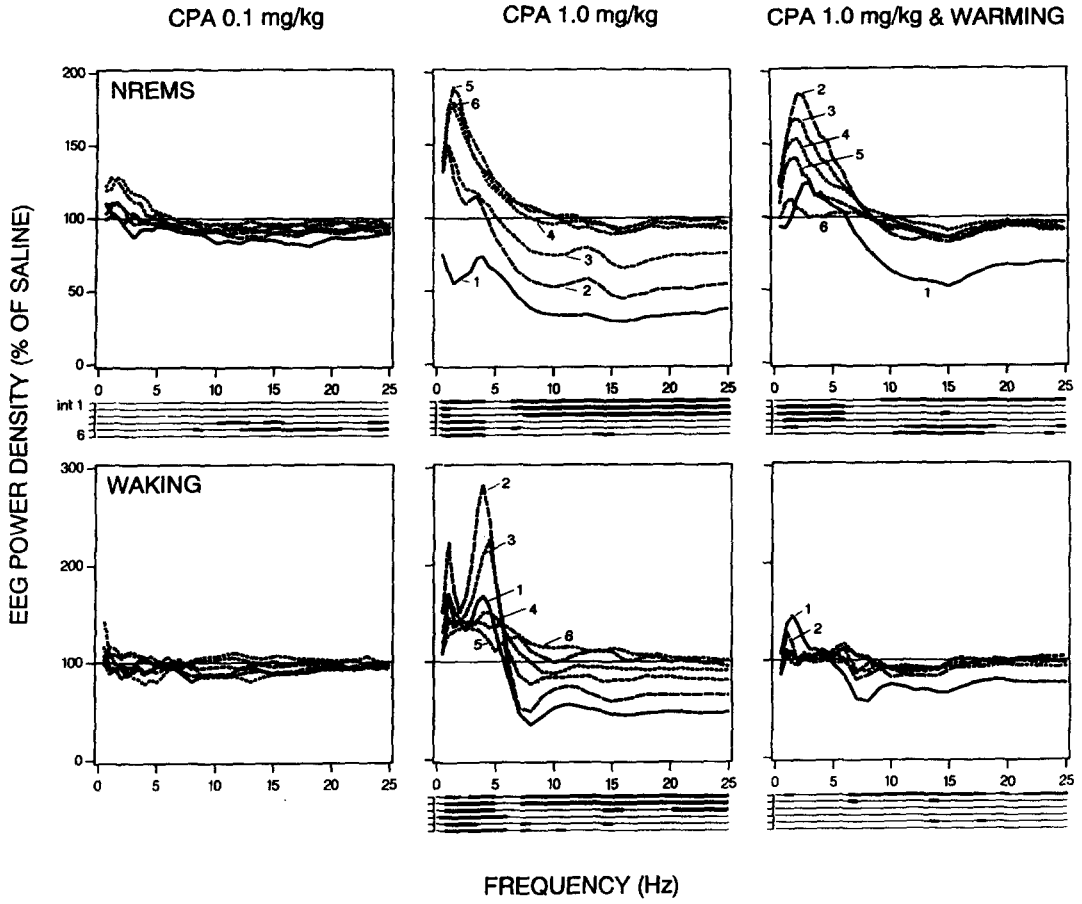


Fig. 2. Effects of CPA on the EEG spectrum in non-REM sleep and waking. The spectral distribution of relative EEG power density in non-REM sleep (top panels) or waking (bottom panels) was computed for consecutive 2-h intervals after injection of saline and CPA (0.1 mg/kg and 1 mg/kg at 23°C and during warming to 28°C). Curves connect mean values ($n = 4$, 8 and 7, respectively) plotted for 0.5-Hz or 1.0-Hz bins. The values are plotted as percentage of corresponding 2-h intervals of the saline treatment. The numbers near the curves designate the consecutive 2-h intervals. Lines below the panels indicate bins which differ significantly from corresponding bins of the saline injection ($P < 0.05$; paired $t$-test).

Table 2
Mean 12-h values of vigilance states, slow-wave activity (SWA; mean EEG power density 0.75–4.0 Hz) in NREM sleep, sleep onset latency (SOL) and cortical temperature ($T_{CRT}$) for saline and the treatments

| | Caffeine 10 mg/kg vs. NaCl ($n = 6$) 0–12 h | | | Caffeine 15 mg/kg or SD vs. NaCl ($n = 8$) 0–12 h | | | | Caffeine 15 mg/kg and 6 h SD vs. NaCl and 6 h SD ($n = 6$) 6–18 h | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | NaCl | CAF | | NaCl | CAF | | SD | | NaCl | CAF | |
| Waking | 38.4 (2.1) | 49.8 [a] | (2.6) | 35.3 (1.9) | 49.7 [a] | (2.0) | 46.9 [a] | (0.9) | 32.8 (1.4) | 34.3 | (1.0) |
| NREMS | 48.2 (2.1) | 37.2 [a] | (1.8) | 51.2 (1.8) | 38.8 [a] | (1.3) | 40.9 [a] | (0.7) | 52.2 (1.0) | 51.0 | (1.0) |
| REMS | 13.5 (0.8) | 13.0 | (1.3) | 13.5 (0.8) | 11.5 [b] | (0.9) | 12.0 | (0.6) | 14.9 (0.5) | 14.7 | (0.4) |
| SWA in NREMS | 100 | 118.2 | (7.3) | 100 | 121.9 [a] | (3.9) | 140.9 [a] | (8.0) | 100 | 85.0 [a] | (4.2) |
| SOL | 49.5 (8.8) | 171.7 [a] | (32.8) | 39.9 (5.8) | 218.0 [a] | (15.7) | 237.7 [a] | (12.9) | – | – | |
| $T_{CRT}$ (°C) | 36.9 (0.1) | 37.0 | (0.1) | 36.9 (0.1) | 37.1 | (0.1) | 37.1 | (0.1) | 36.5 (0.0) | 36.6 | (0.0) |

The vigilance states are expressed as percentages of the 12-h recording time. In the first two experiments data analyses started with light onset; in the experiment of caffeine and 6 h sleep deprivation (SD) data analyses started after SD. SWA in NREM sleep is expressed relative to the 12-h mean value in saline (= 100%). The values are means and S.E.M. in brackets, $n$ = number of animals. Significant differences between saline and treatments [a] $P < 0.01$, [b] $P < 0.05$; 2-tailed paired $t$-test. CAF = caffeine.

energy did not differ significantly from saline (12-h mean values as percentage of saline: CPA 0.1 mg/kg, 117.4 ± 11.0; 1.0 mg/kg, 123.7 ± 11.9). $T_{CRT}$ exhibited a marked, prolonged decline for 8 h (Fig. 1).

Warming the animal after administration of CPA 1.0 mg/kg successfully prevented the drug-induced hypothermia. In fact, $T_{CRT}$ exceeded the control level by 0.5°C (Table 1). Under these conditions, the CPA-induced increase in waking was no longer present, and the suppression of REM sleep was shortened (Table 1, Fig. 1). Also the initial suppression of slow-wave activity was prevented by the warming procedure.

### 3.1.2. EEG power spectrum

While CPA 0.1 mg/kg exerted only minor effects, the 1.0 mg/kg dose induced an initial reduction of EEG power density in non-REM sleep, which in the first 2 h encompassed almost the entire frequency range (Fig. 2, middle upper panel). Over the subsequent three 2-h intervals, spectral activity showed a progressive rise. Whereas

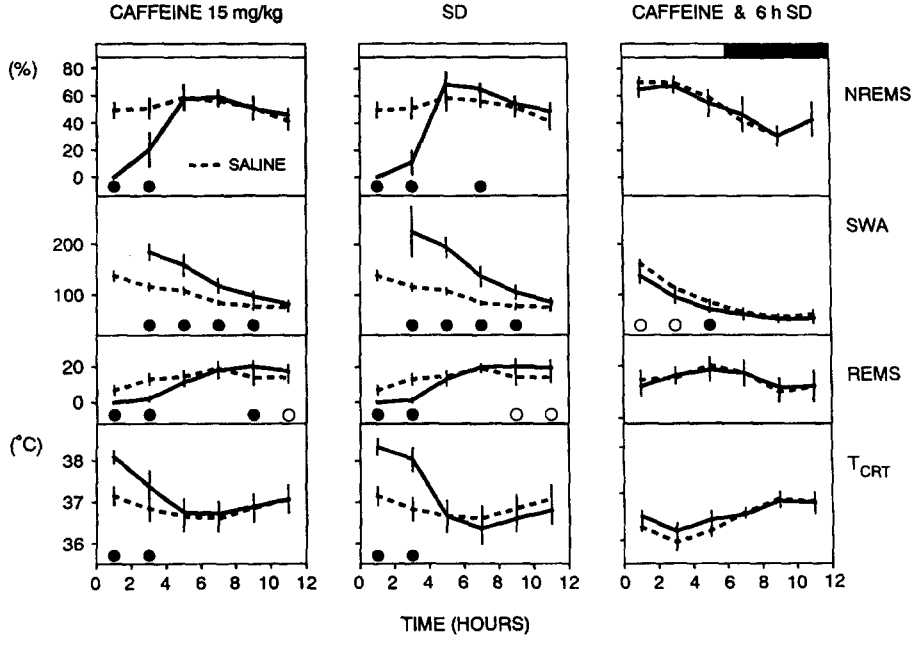


Fig. 3. Vigilance states, slow-wave activity (SWA; EEG power density 0.75–4.0 Hz) and cortical temperature ($T_{CRT}$) for saline and caffeine (15 mg/kg), saline and sleep deprivation (SD; mean duration (± 2 S.E.M.) 237.7 ± 12.9 min) and after either saline or caffeine combined with 6-h sleep deprivation. Mean values ± 2 S.E.M. ($n = 8$) for 2-h intervals. All treatments began at the onset of light and the recordings were obtained for 12 h. Note that in the right panel the recordings continued 6 h into the dark period. Non-REM sleep (NREMS) and REM sleep (REMS) are expressed as a percentage of recording time. SWA is expressed as a percentage of the mean 12-h value. Significant differences between saline and treatment are indicated by filled ($P < 0.01$) and open circles ($P < 0.05$; 2-tailed, paired $t$-test).

slow-wave activity was enhanced (maximum increase in the 1.25–1.5 Hz bin), activity in frequencies higher than 6–7 Hz were reduced for 6 h and then reverted towards the baseline level.

In waking, power density in the 0.5–5.0 Hz range was markedly elevated in the first three 2-h periods, the changes exhibiting a biphasic pattern (Fig. 2, middle lower panel). In the initial hours, slow waves appeared in the behaviorally awake animal whose eyes were open and whose EMG level was typical for quiet waking. Slow-wave activity remained increased throughout the 12-h recording period. In contrast, activity in frequencies above 7 Hz was markedly reduced after drug administration and then reverted gradually towards baseline.

The combination of CPA and warming caused a more rapid and shorter lasting slow-wave activity response in non-REM sleep than CPA alone (Fig. 2, upper right panel). In waking, warming prevented almost completely the drug-induced enhancement of slow-wave activity (Fig. 2, lower right panel). Moreover, in both vigilance states, the reduction of high-frequency activity was attenuated and largely limited to the first 2-h interval.

### 3.2. Effects of caffeine and sleep deprivation

#### 3.2.1. Vigilance states, slow-wave activity and $T_{CRT}$

After caffeine administration sleep was markedly disturbed. Several short episodes of sleep were followed by longer episodes of waking. Sleep onset latency was prolonged by both doses of caffeine. These doses increased waking and reduced non-REM sleep (Table 2, Fig. 3). The higher dose inhibited also REM sleep. Slow-wave activity in non-REM sleep was enhanced in the 2–10 h interval following the 15 mg/kg dose (Fig. 3). This effect was most prominent in the first 2-h interval and then gradually subsided. Neither dose affected significantly the cumulative 12-h value of slow-wave energy (saline, 100%; caffeine 10 mg/kg, 91.0 ± 6.1%; 15 mg/kg, 93.0 ± 5.2%; $P > 0.3$, paired $t$-test). $T_{CRT}$ was elevated in the first 4 h after caffeine 15 mg/kg when the animals were predominantly awake.

Non-REM sleep and REM sleep were not significantly affected by sleep deprivation (Fig. 3; Table 2). In contrast, slow-wave activity in non-REM sleep was increased during four 2-h intervals and its 12-h value was above the saline level. The cumulative 12-h value of slow-wave energy did not differ significantly from the corresponding value after saline (saline, 100%; sleep deprivation 111.5 ± 7.4%; $P > 0.2$). $T_{CRT}$ was increased during sleep deprivation but did not deviate from the saline level during recovery.

The 12-h value of slow-wave activity was lower after caffeine than after sleep deprivation (Table 1), and a similar trend was present for cumulative slow-wave energy (sleep deprivation, 100%; caffeine, 85.4 ± 6.4%; $P < 0.06$; two-tailed paired $t$-test).

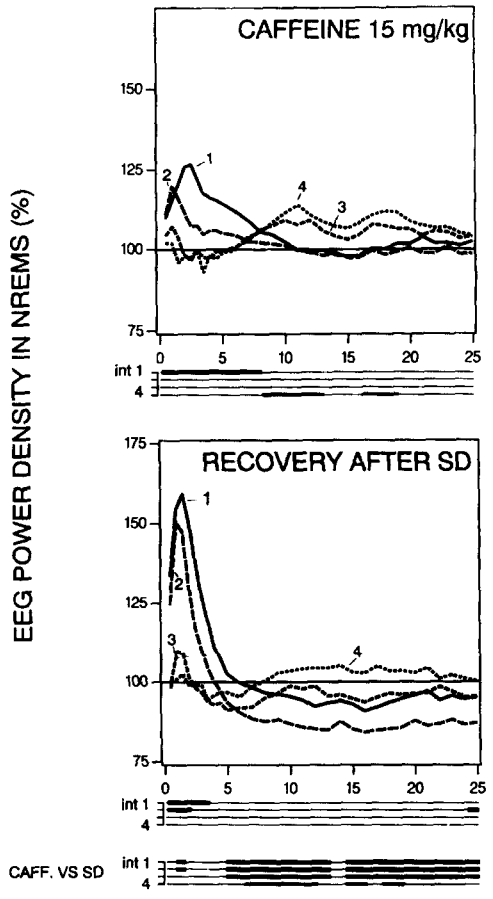


Fig. 4. Effects of caffeine and sleep deprivation (SD) on the EEG spectrum in non-REM sleep (NREMS). The spectral distribution of relative EEG power density in NREMS was computed for consecutive 2-h intervals (int) after injection of saline and caffeine (15 mg/kg). Curves connect mean values ($n = 8$) plotted for 0.5-Hz or 1.0-Hz bins. The values are plotted as percentage of corresponding 2-h intervals of the saline treatment. Due to the varying duration of waking after saline and caffeine and immediately after the sleep deprivation, the first 2-h interval starting with sleep onset was individually determined. The numbers near the curves designate consecutive 2-h intervals. Lines below the panels indicate bins which differ significantly from corresponding bins after the saline injection, or the bins which differ between caffeine and sleep deprivation (paired $t$-test, $P < 0.05$).

#### 3.2.2. EEG power spectrum

Whereas the low dose of caffeine had no significant effect (data not shown), the high dose enhanced power density in non-REM sleep in the $\delta$ and $\theta$ band in the first 2-h interval (Fig. 4, upper panel). An increase in higher frequency bands was observed in the fourth interval. The maximum increase in the first 2 h (126.7%) occurred in the 2.25–2.5 Hz bin.

In the waking spectrum, caffeine induced a prominent increase in a single bin of the $\theta$ band (7.25–8.0 Hz) which was accompanied by a reduction in the adjacent low-frequency and high-frequency range, changes that subsided after 4 h (Fig. 5). The same spectral pattern was seen when waking epochs with high $\theta$ ($> 400\ \mu V^2$; 6.25–9.0 Hz) were plotted against waking epochs with low $\theta$ (Fig. 5, inset).

Sleep deprivation enhanced EEG power density in non-REM sleep in the range of 0.25–3.5 Hz (Fig. 4, bottom panel). This effect was largest in the first 2-h interval (maximum value 159.2% in the 1.25–1.5 Hz bin) and subsided after 4 h. The higher frequencies were not affected.

### 3.3. Comparison of caffeine and sleep deprivation

The duration of sleep deprivation was individually matched to the caffeine records to obtain a corresponding suppression of sleep. The resulting sleep latencies (sleep deprivation, 237 min; caffeine, 218 min; Table 2) did not differ significantly (ANOVA factor 'treatment', $P < 0.349$). There was also no significant difference between the vigilance states and $T_{CRT}$. However, slow-wave activity in non-REM sleep was lower after caffeine than after sleep deprivation (sleep deprivation, 100%; caffeine, 87.8 ± 4.0%; $P < 0.05$). Significant differences were present in the 0.75–1.5-Hz band in the first 4 h as well as in frequencies beyond 4.5 Hz throughout the recovery period (Fig. 4, bottom lines).

### 3.4. Caffeine and sleep deprivation vs. saline and sleep deprivation

To investigate whether the effect of sleep deprivation is affected by the presence of caffeine, either a 15 mg/kg dose or saline was administered at the beginning of the 6-h enforced waking period. The two treatments had similar effects on the vigilance states and $T_{CRT}$ (Table 2). However, in comparison to the saline condition, caffeine lowered slow-wave activity in non-REM sleep as well as the power density in bins below 5 Hz (Table 2, Fig. 6).

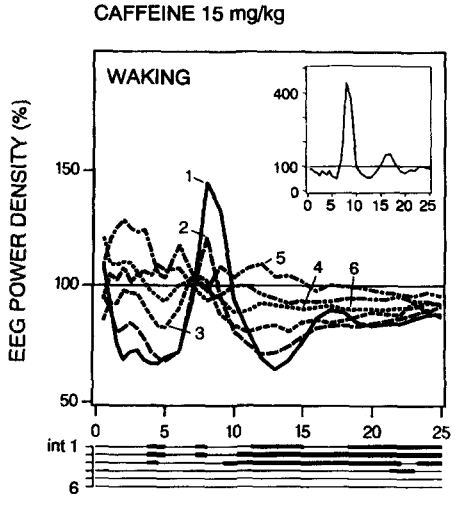


Fig. 5. Effects of caffeine on the EEG spectrum in waking. The spectral distribution of relative EEG power density in waking was computed for consecutive 2-h intervals after injection of saline or caffeine (15 mg/kg). Curves connect mean values ($n = 8$) plotted for 0.5-Hz or 1.0-Hz bins. The values are plotted as a percentage of corresponding 2-h intervals of the saline treatment. The numbers near the curves designate consecutive 2-h intervals. Lines below the panels indicate bins which differ significantly from corresponding bins after the saline injection (paired $t$-test, $P < 0.04$). The inset represents the EEG power spectrum in waking for episodes with high $\theta$ ($n = 310$ epochs) in an individual animal within the first 2 h after lights on. The curve is plotted as a percentage of EEG power density in low-$\theta$ waking in hour 0–2.

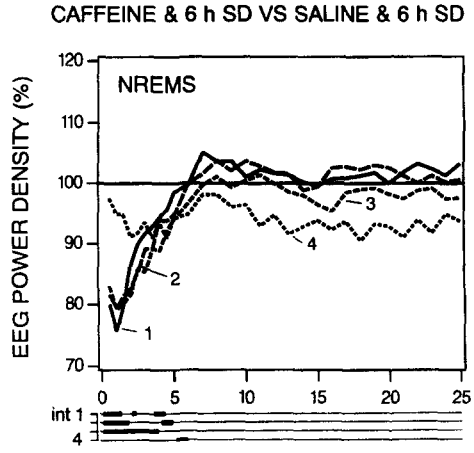


Fig. 6. Comparison of the effects of caffeine combined with 6-h sleep deprivation (SD) and saline combined with 6-h SD. The spectral distribution of relative EEG power density in non-REM sleep (NREMS) was computed for consecutive 2-h intervals (int) after injection of saline or caffeine (15 mg/kg). Immediately after either treatment the animals were subjected to 6-h SD. Curves connect mean values ($n = 6$) plotted for 0.5-Hz or 1.0-Hz bins. The values are plotted as a percentage of corresponding 2-h intervals of the saline/SD treatment. The numbers near the curves designate consecutive 2-h intervals. Lines below the panels indicate bins which differ significantly from corresponding bins of the saline/sleep deprivation treatment (paired $t$-test, $P < 0.05$).

### 3.5. Pharmacokinetics of caffeine

The 15 mg/kg dose induced a peak plasma concentration 30 min after administration. Thereafter, the caffeine level declined but was still elevated after 8 h. The mean values (±S.E.M.; $n = 6$, last time point: $n = 5$) for the time points 0.5, 1, 2, 3, 4, 6 and 8 h after the injection were: 92.5 ± 8.3, 73.5 ± 2.5, 66.2 ± 2.8, 70.3 ± 3.4, 56.0 ± 2.6, 51.7 ± 3.0 and 20.4 ± 2.8 μmol/l.

## 4. Discussion

CPA, a potent and selective adenosine $A_1$ receptor agonist (Bruns et al., 1986), enhanced EEG slow-wave activity and thereby mimicked the effect of sleep deprivation. This result is in accordance with previous findings (Benington et al., 1995) which were the corner stones of the hypothesis linking brain energy metabolism to sleep regulation (Benington and Heller, 1995). Although the increase of slow-wave activity reported by Benington et al. (1995) was larger than the one observed in the present study, this apparent discrepancy appears to be simply due

to differences in the reference level of slow-wave activity to which the effects of CPA were compared. Thus Benington and associates injected CPA 4 h after light onset, at a time when slow-wave activity had spontaneously declined to a relatively low level, whereas we injected the drug at light onset when slow-wave activity was at its maximum.

Apart from enhancing slow-wave activity, CPA had also other effects. Initially, the rats exhibited abnormal behavior (e.g. ataxia, crawling movements) coupled with a marked and prolonged hypothermia. A decrease of body temperature by CPA and other adenosine receptor agonists has been reported previously (Snyder et al., 1981; Dunwiddie and Worth, 1982; Ticho and Radulovacki, 1991; Benington and Heller, 1995). When the decrease in brain temperature was prevented by warming the animal, the rise in slow-wave activity appeared much earlier. The warming experiment showed also clearly that the dramatic suppression of high-frequency activity in the EEG spectrum of non-REM sleep and waking as well as the enhancement of slow-wave activity in waking were mainly due to hypothermia. A temperature-dependent suppression in the sleep EEG has been recently documented during natural hypothermia in the Djungarian hamster (Deboer and Tobler, 1995). Thus the present results clearly show that major drug-induced alterations in the EEG may be due to non-specific effects (e.g. hypothermia) and do not necessarily reflect a pharmacological influence on sleep regulating mechanisms. Other effects of CPA (e.g. the hypotensive effect: Barrett et al., 1994; Bonizzoni et al., 1995) may have contributed to the initial changes such as the suppression of REM sleep which persisted when CPA was combined with warming.

Caffeine caused a dose-dependent increase in sleep onset latency, a finding that is in accordance with previous data (e.g. Snyder et al., 1981; Radulovacki et al., 1982; Yanik et al., 1987; Virus et al., 1990). The effect of the drug-induced waking period was compared to a closely matched enforced drug-free waking period. Both manipulations caused a delayed REM sleep rebound, and the drug-free sleep deprivation protocol induced an increase of non-REM sleep. Slow-wave activity in non-REM sleep was enhanced after both caffeine-induced and drug-free sleep deprivation. However, the latter caused a more prominent and longer lasting rise of power density in the $\delta$ frequency range as well as a marked depression of activity between 5 and 25 Hz. This result indicated that waking induced by an adenosine receptor antagonist may not be equivalent to a usual, drug-free waking period in terms of the consequence on subsequent sleep. To further investigate these two conditions, a 6-h sleep deprivation was performed either in the presence or in the absence of caffeine. Also in this protocol, the enhancement of slow-wave activity was attenuated by caffeine administered at the beginning of the enforced waking period.

According to the hypothesis of Benington and Heller (1995), adenosine release in the brain acts as a feedback signal which ensures that the depletion of cerebral glycogen stores results in an increased sleep drive and a potentiation of slow-wave activity in the non-REM sleep EEG. A reduced availability of glycogen to astrocytes is supposed to increase the release of adenosine during both waking and non-REM sleep. In the framework of the hypothesis one would predict that the presence of an adenosine receptor antagonist during sleep deprivation would counteract the *manifestation* of an increasing sleep drive but not the underlying cause (i.e. the glycogen depletion). After the elimination of the drug, the normal response to sleep deprivation would occur. This means that the reduction in slow-wave activity which was observed during recovery in the caffeine protocol must be due to the residual level of caffeine 6–10 h after its administration, and not to the action of caffeine during the waking period. In fact, although the plasma level of caffeine steadily declined, a concentration of 20.4 $\mu$mol/l was still present 8 h after the injection. A similar situation had been observed in a human sleep study in which the intake of 200 mg caffeine at 7:00 in the morning reduced low-frequency activity and enhanced spindle frequency activity in the non-REM sleep EEG during the subsequent night (Landolt et al., 1995). At the time of sleep onset, the caffeine concentration of saliva had declined to a very low level (2.6 $\mu$mol/l). In both studies the question arises whether the effects on sleep can be attributed (1) to the residual concentration of caffeine; (2) to a secondary, delayed process triggered by caffeine; or (3) to a drug-induced attenuation of the buildup of sleep pressure in the course of the waking period. Several arguments favor the latter interpretation. First, in the present study, the same difference in slow-wave activity was present throughout the first 6 h of recovery (i.e. the last 6 h of the light period (Figs. 3 and 6)); if the effect were due to the residual concentration of caffeine, a progressively diminishing difference in slow-wave activity between sleep deprivation alone and sleep deprivation combined with caffeine would be expected. Second, the prominent, drug-induced changes in the spectra of the waking EEG reverted progressively towards baseline in the course of the sleep deprivation period and were no longer significant in the recovery period (Fig. 5). Thus a putative residual drug level did not affect the waking EEG. Third, in the human caffeine study, the enhancement of spindle frequency activity was significant throughout the first three non-REM sleep episodes and there was no indication of a gradual decline (Landolt et al., 1995; Fig. 4). Taken together, the two studies are consistent with the assumption that caffeine attenuates the buildup of the sleep drive during waking, although the biochemical mechanism remains to be elucidated. Thus, in addition to their counteracting the manifestation of sleep as proposed by Benington and Heller (1995), adenosine receptor antagonists may interfere with the gradual rise of sleep propensity in the waking episode. The question then arises in what respect, the caffeine-induced waking differs

from drug-free waking. The analysis of the waking EEG showed that caffeine promoted the θ-dominated waking pattern, an effect that gradually subsided. As the same spectral pattern occurs also spontaneously (Fig. 5, inset), caffeine does not appear to alter the waking EEG but rather to shift the balance between θ-dominated and non-θ-dominated waking. Muscarinic antagonists are known to inhibit θ activity (Vanderwolf, 1992). It is an intriguing possibility that the putative attenuation of the buildup of sleep propensity by adenosine receptor antagonists could be associated with a specific mode of waking which can be characterized by electrophysiological criteria.

In conclusion, the data of this study are consistent with the hypothesis that the increase in slow-wave activity during recovery from sleep deprivation is mediated by the activation of adenosine $A_1$ receptors. Administration of the adenosine $A_1$ receptor agonist CPA enhances slow-wave activity for several hours. However, the effects of sleep deprivation were not entirely mimicked by this drug since the increase in power density occurred over a broader frequency range (cf. Figs. 2 and 4). Adenosine receptors may be also involved in the buildup of sleep propensity during waking. This could explain why caffeine-containing beverages are so popular in the morning when, according to the two-process model, the rise rate of homeostatic sleep propensity is highest.

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