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The Influence of Chronic Caffeine Administration on Sleep Parameters in the Cat

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SINTON, C. M. AND F. PETITJEAN. *The influence of chronic caffeine administration on sleep parameters in the cat.* PHARMACOL BIOCHEM BEHAV 32(2)459–462, 1989.—Caffeine (20 mg/kg/day) was administered per os to 5 cats for 21 days and sleep parameters were measured both during drug administration and over the withdrawal phase. The initial effect of caffeine was a marked increase in waking. As the animal habituated to the stimulant action of the methylxanthine, however, total sleep time normalized, although time spent in Stage II slow wave sleep (S2) remained below, and Stage I slow wave sleep (S1) above, control levels throughout the period of drug administration. In contrast, a significant increase in the S2/S1 ratio was recorded as soon as caffeine treatment ended, and this parameter remained elevated for about 30 days. Chronic caffeine administration has been previously shown to increase the number of central adenosine receptors, and it has also been reported that adenosine agonists increase S2 at the expense of S1. The present data were thus interpreted as indicating that the action of caffeine on sleep may be mediated at a central adenosine receptor site. Results also imply that changes induced in this receptor population by chronic caffeine administration last for at least 30 days after the drug is withdrawn.

Caffeine Slow wave sleep (S2) Slow wave sleep (S1) Adenosine Cat

THE prolonged effects of acute caffeine administration on sleep in man have been well documented. Although the arousing effects of the methylxanthine on the electroencephalogram (EEG) have been reported to last for only about 3 hr (8), sleep parameters are disturbed for a much longer period. For example, Nicholson and Stone (13) showed that at a 300 mg dose, caffeine reduced slow wave sleep (SWS) in the early part of the night, and rapid eye movement (REM) sleep during the latter part of the night. These findings have also been confirmed by others (2,10) who have reported that the effects of caffeine on sleep suggest a shift of REM sleep to the early part of the sleep period, and a shift of deep SWS to the later part.

In the rat, Radulovacki and his colleagues (15,16) found that a dose of 15 mg/kg of caffeine produced a decrease in all sleep states, and at 25 mg/kg caused a delay in the appearance of REM rebound in sleep deprived rats. More recently, these workers (28) have extended this series of studies by examining the action of lower doses of caffeine on sleep in the rat. Interestingly, they found that caffeine, up to a dose of 1.25 mg/kg, did not affect total sleep time but increased shallow SWS (Stage I SWS, or S1) at the expense of deeper SWS (Stage II SWS, or S2). These changes are the converse of those induced by adenosine and the adenosine receptor agonist adenosine-5'-N-ethylcarboxamide (NECA) (29): the latter compounds increase S2 and decrease S1 (17). The data are thus consistent with the characterization of caffeine as an antagonist at the adenosine receptor (19). The implication, therefore, is that the effects of caffeine on sleep may be mediated through the action of the methylxanthine as an adenosine antagonist. This adds support to the hypothesis that the stimulating action of caffeine on motor behavior is also mediated through this receptor (20).

Binding studies in rodents have shown that chronic caffeine consumption increases the number of brain adenosine (1, 11, 27) and benzodiazepine receptors (27). In contrast, the number of adenosine uptake sites, labeled with [^{3}H] nitrobenzlthioinosine, are not changed by chronic caffeine (11). Because of the importance of these studies for the understanding of the mechanisms of tolerance (5, 7, 18, 24) to, and dependence (26) on, caffeine, the present experiments were designed to evaluate the effects of chronic caffeine consumption on sleep parameters. In view of the effects of acute caffeine administration on SWS (16,28), it was hypothesized that the relative amounts of S2 and S1, especially during the withdrawal phase, would be affected by chronic caffeine administration. Although S2 can be defined in the rat, it is apparent that this state can be more accurately quantified in the cat (9). In view of the importance of S2 in this study, therefore, the cat was chosen as the experimental subject.

METHOD

Five adult male cats weighing between 2.4 and 3.6 kg were used in these experiments. Recording electrodes were chronically implanted under sodium pentobarbital anesthesia (Nembutal, 25 mg/kg IV) with the animal restrained in a stereotaxic instrument. The cranium was exposed under

aseptic conditions and stainless steel screw electrodes were inserted into the skull bilaterally over the occipital and frontal cortices for EEG recording. In addition, screws were inserted into the bone of the eye socket for eye movement potential recordings. Electromyogram (EMG) activity was recorded from multistranded stainless steel wires, insulated except for 5 mm at the tip, inserted bilaterally into the dorsal neck muscles. A bipolar electrode was also stereotaxically placed (AP 6.5, ML 9.5, DV 13.5) in the lateral geniculate nucleus for recording of PGO wave activity. The electrodes were soldered to a miniature socket which was fixed to the skull with dental acrylic.

The cat was left to recover from surgery for at least 14 days before experiments began. For sleep recording the electrodes were connected to a counterweighted, low noise cable which allowed freedom of movement and, via a rotating slip ring commutator, to an 8 channel polygraph recorder (Alvar Minihuit; Alvar Electronics, Montreuil, France). Food, water and a litter basket were freely available at all times, and the cat was housed in a Plexiglas recording chamber (75×50×60 cm) throughout the experiment, with the temperature maintained at 22±2°C. The recording cable was connected to the cat during the whole experiment, except for brief periods necessitated by daily cleaning. Polygraphic records were visually scored in 60-sec periods, according to previously published criteria (9), into waking (W), the two stages of slow wave sleep (S1 and S2), and rapid eye movement (REM) sleep. S2 was scored when the EEG was dominated by the characteristic low frequency, high amplitude waves.

Baseline sleep data were recorded initially for 5 or 6 consecutive 24-hr periods, the control sleep state quantities being taken as the mean of these periods. Cats subsequently received caffeine while sleep recording continued. Caffeine (20 mg/kg/day) was administered per os as two capsules, one at 0900 hr and one at 1800 hr, for a period of 21 days. The dose and period of administration of caffeine chosen for these experiments were based on data from the receptor binding studies in the rodent (11,27). At the completion of caffeine treatment, sleep state recording continued until sleep times had returned to baseline, and thereafter for a few days to ensure that there were no further changes in sleep patterns. Recordings thus continued for approximately 30 days after the cessation of caffeine treatment. During this drug washout period, cats received placebo capsules.

RESULTS

Baseline sleep state data for the 5 cats are displayed in Table 1. During and after caffeine treatment, no qualitative changes were noted in the EEG recordings, so that sleep states maintained the same characteristics throughout the experiment. Quantitatively, however, caffeine administration had a marked effect on sleep. As soon as the drug treatment began there was a notable increase in waking, followed by a gradual return of total sleep time to control levels during the course of the 21-day treatment period. The time course for this normalization differed slightly between cats: Fig. 1 displays these changes for cat H73 as a representative example. But in all animals at the end of the treatment period S2 remained reduced and S1 increased with respect to control levels. This effect is shown in Fig. 2 which displays the variation in the mean S2/S1 ratio for the 5 cats. Towards the end of the drug treatment period this S2/S1 ratio appeared to be reaching an asymptote at a level of about 60% of the control value.

TABLE 1

SLEEP STATE PARAMETERS UNDER CONTROL CONDITIONS

	Waking	S1	S2	REM Sleep
Z68 (N=5)	554 ± 22	485 ± 24	248 ± 22	153 ± 11
R70 (N=5)	641 ± 41	428 ± 24	212 ± 14	159 ± 11
E72 (N=6)	551 ± 22	463 ± 27	244 ± 19	182 ± 9
S72 (N=6)	420 ± 48	484 ± 43	353 ± 9	183 ± 13
H73 (N=5)	396 ± 42	623 ± 31	259 ± 13	162 ± 10

Mean times (± SEM) in min/24 hr spent by each cat in waking and the different sleep states during the control recording period, prior to caffeine administration. N=number of consecutive 24 hr recording periods; S1=Slow wave sleep, Stage I; S2=Slow wave sleep, Stage II.

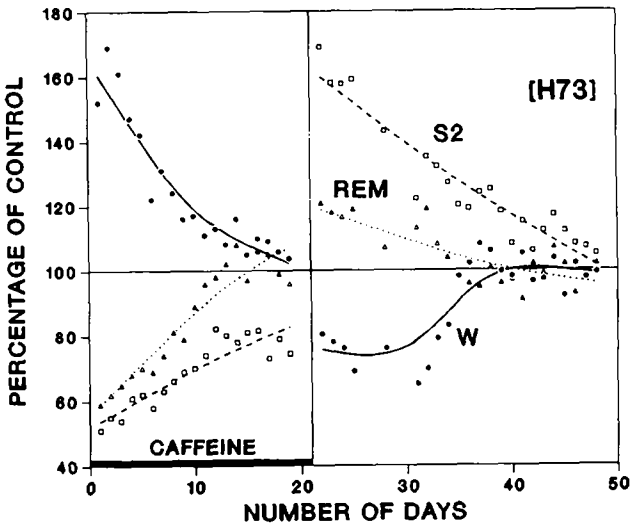

FIG. 1. Variation in sleep states, expressed as a percentage of control values, during and after caffeine administration for cat H73 as a representative example. W=Waking, S2=Stage II slow wave sleep, and REM=rapid eye movement sleep. For clarity of display, Stage I slow wave sleep is not included. Note the increase in waking at the beginning of caffeine treatment, and the opposite effect at the beginning of the withdrawal phase. The time spent in S2 also did not return to control levels by the end of the 21-day drug treatment period.

Within 24 hr of the end of caffeine treatment, sleep increased markedly with the principal effect apparent in S2. Figure 1 displays these sleep changes for cat H73, and Table 2 itemizes the effects on the S2/S1 ratio for each animal at the beginning of the washout period. The mean S2/S1 ratio for the 5 animals (Fig. 2) increased to over 200% of the baseline value during the first 24-hr period after drug treatment ended. Considering each cat individually, the S2/S1 ratio for the first 4 days after the end of caffeine administration was also significantly different from control values (Table 2). Thereafter, normalization of SWS occurred gradually and the mean S2/S1 ratio returned to control levels about 30 days later. REM sleep

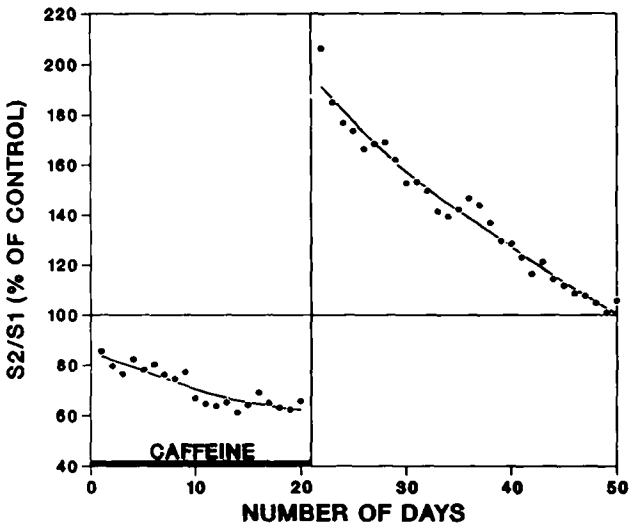

FIG. 2. Variation in the mean S2/S1 ratio, expressed as a percentage of control values, during and after caffeine administration for the 5 cats. Note that this ratio remained below baseline throughout the 21-day drug treatment period.

TABLE 2

SLEEP PARAMETER CHANGES AFTER THE END OF CAFFEINE ADMINISTRATION

	S2/S1	*t*-Statistic*	N
Z68	165.2	$t(7)=2.20, p<0.05$	32
R70	185.8	$t(7)=5.19, p<0.001$	27
E72	251.1	$t(8)=5.67, p<0.001$	23
S72	154.1	$t(3)=3.77, p<0.02$	29
H73	170.8	$t(7)=3.97, p<0.01$	21

Change to the ratio S2/S1 during the first 4 days of the drug withdrawal period for each animal, expressed as a percentage of the control value; and the number of days (N) before this ratio returned to baseline.

**t*-Statistic, and associated probability values, are quoted as a measure of the difference between S2/S1 values during control and drug withdrawal periods. Calculations were based on a comparison between the S2/S1 ratio during the 5/6 control recording periods and the initial 4 periods after caffeine administration ended. All tests were for equal variance samples, with the exception of the test for S72, which was tested with unequal variance samples.

was also increased above baseline during this drug washout phase, and in 3 of the cats an increase in the number of REM sleep episodes was also noted.

DISCUSSION

These results have demonstrated that chronic caffeine administration to the cat produces marked and prolonged changes in sleep patterns. The initial effect of an increase in waking was expected from the known stimulating properties of the drug. Subsequently, two important changes to sleep were observed during the course of this study. First, although total sleep time returned to baseline by the end of the 21-day drug treatment period, the ratio of deep SWS to shallow SWS (S2/S1) remained depressed while the methylxanthine was being administered. Second, the S2/S1 ratio increased significantly as soon as caffeine treatment ended, and remained above control values for the next 30 days. This effect on the S2/S1 ratio is similar to that obtained in the rat after acute administration of the drug (28).

The dose of caffeine used in this study is comparable to that which is known to induce changes in the number of central adenosine receptors in other species (1, 11, 27). Furthermore, the present data can be interpreted as supporting a receptor-mediated action of caffeine. For example, during administration of the ligand, receptor adaptation would be expected to occur, resulting in gradual habituation to the effect of the drug. When treatment ends, the half-life of caffeine is short, of the order of a few hours (21), and the methylxanthine is rapidly removed from receptor occupancy. An endogenous ligand, acting at the receptor in a way that is opposite to that of caffeine, would then be expected to produce a rebound effect. This follows from the altered receptor population caused by chronic drug exposure. In view of the effects of adenosine on sleep parameters (17), this explanation is most consistent with the known antagonistic action of caffeine at the adenosine receptor (19). But the hypothesis is also supported by the prolonged up-regulation of adenosine receptors that is seen during washout following chronic administration of the methylxanthine (27). Nonspecific rebound in sleep parameters typically observed after pharmacological sleep deprivation lasts only a few days (14,15). This makes it unlikely that the present results were due to this type of sleep rebound, but other explanations are feasible. Caffeine is known to act at a number of central sites (4). One possibility, for example, is the action of caffeine at another receptor such as the benzodiazepine site (12,25). In this case, however, prolonged changes to the receptor population are not seen during withdrawal after chronic drug exposure in the rat (27).

Further studies with adenosine analogs are now required to evaluate the hypothesis of an action at an adenosine receptor as the cause of the present effects. In addition, caffeine does not show high selectivity for either of the two principal types of the adenosine receptor, the A_2 and A_1 sites (3,6); the doses of caffeine used in the present study were high enough to antagonize both sites. Previously, the A_1 receptor has been implicated in the action of adenosine on sleep in the rat (17). Additional similar experiments in the cat could therefore also examine more specific antagonists to evaluate if chronic caffeine is also acting in this species at the A_1 site.

An action of caffeine at the adenosine receptor enables a further hypothesis to be derived from these results. This follows from the fact that in the mouse the most significant increases in adenosine receptor population following chronic caffeine administration were observed in the cerebellum, brain stem, cortex and thalamus (11). Although similar studies have not been performed in the cat, extrapolation of these data from the mouse suggest that brain stem or thalamic mechanisms may be involved in the changes to sleep parameters seen in the present study. In this regard, the concomitant effect on the number of REM sleep episodes during the withdrawal phase in 3 of the cats may be significant. This is because the ventromedial and intralaminar thalamic nuclei have been critically implicated in both the maintenance of SWS (23) and the gating of cortical activation

(22)—a necessary condition for the development of an episode of REM sleep. A further possible conclusion from this study is thus that the site of action of caffeine on sleep may be related to medial thalamic nuclei or the associated reticulo-thalamic and thalamo-cortical circuits.

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