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BRIEF COMMUNICATION

Tolerance and Sensitization to Chronic and Subchronic Oral Caffeine: Effects on Wheelrunning in Rats[1]

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MELISKA, C. J., R. E. LANDRUM AND T. A. LANDRUM. *Tolerance and sensitization to chronic and subchronic oral caffeine: Effects on wheelrunning in rats.* PHARMACOL BIOCHEM BEHAV 35(2) 477–479, 1990.—Twenty-four male Sprague-Dawley rats were tested for wheelrunning in conjunction with chronic (continuous) or subchronic (alternate day) oral caffeine administration. As expected, chronic administration led to complete tolerance to caffeine's locomotor stimulant effect, while subchronic administration produced sensitization. Results confirm earlier reports of enhanced stimulation with spaced administration of caffeine and tolerance with chronic administration.

Caffeine Wheelrunning Chronic administration Subchronic administration

CAFFEINE has been characterized as the most widely used psychoactive drug in the world (5). While tolerance development in humans has been recognized for two decades (6), laboratory studies of tolerance to caffeine in animals have appeared only recently (1, 2, 7). The present study was designed to replicate earlier findings regarding tolerance to oral administration of caffeine using a different activity measure, wheelrunning. Furthermore, we wished to examine the effect of *spacing* of caffeine administration, since we previously observed that stimulation of wheelrunning is increased by repeated IP injections of caffeine when doses are separated by 48 or 72 hours (9,10). The present study compares the effects of repeated oral caffeine ingestion under conditions of chronic (consecutive days) vs. subchronic (alternate days) administration. We expected to observe tolerance to locomotor stimulation with chronic administration, but an *increase* in stimulation with subchronic administration.

METHOD

Subjects

Subjects were 24 male Sprague-Dawley rats (SASCO, Omaha NE), approximately 210 days old, whose weights at the start of the study ranged between 442 and 553 g (mean = 504, s.d. = 33). Light was provided from 0800 to 1630 daily.

Apparatus

Rats were housed individually in 8 standard activity wheels (Wahmann Mfg., Baltimore), 36 cm in diameter × 11 cm wide, with resting cages attached to each. Wheels were isolated in separate rooms. The sliding door separating the resting cage from each wheel was wired open. Animals occupied these cages with free access to the wheels for the duration of the study. Standard lab chow was available ad lib.

Procedure

Three groups of 8 rats each were randomly assigned to one of three drug conditions: chronic caffeine access (0.5 mg/ml hydrous caffeine alkaloid/Merck), subchronic caffeine access (24 hr of 0.5 mg/ml caffeine solution alternating with 24 hr tap water), or continuous tap water access. On the first two days of testing, all animals received tap water to establish baseline wheelrunning levels. Animals remained in the cages and wheels for 18 days—2 baseline days and 16 testing days. For the subchronic caffeine group, drinking solutions (either caffeine or tap water) were switched at 0930 daily. Drinking bottles were weighed daily,

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TABLE 1

MEAN FLUID INTAKES (±SEMs) ACROSS TEST DAYS

	Day							
Group	1	3	5	7	9	11	13	15
Caffeine	50 (5)	53 (3)	44 (3)	51 (6)	45 (4)	49 (3)	46 (4)	44 (4)
Caf(C/W)	44 (6)	39 (4)	40 (5)	42 (3)	40 (5)	42 (5)	42 (3)	42 (4)
Water	45 (5)	57 (5)	47 (5)	49 (5)	53 (4)	52 (5)	46 (3)	47 (4)

assuming 1.0 g in bottle weight to be equivalent to 1.0 ml of solution consumed.

Design

Mean wheel revolutions/day (±SEMs) for the two-day baseline period were 386 (±39), 209 (±68), and 209 (±48) for the chronic caffeine, subchronic caffeine, and water groups, respectively. A one-way ANOVA showed these differences were significant, $F(2,21)=3.71$, $p<0.042$. In order to adjust for these baseline differences, daily wheelrunning scores during drug testing were converted to percentages of baseline wheelrunning (mean of both baseline days) for each animal. Also, since the subchronic caffeine group was given caffeine only on alternate days during the 16 days of testing, this yielded 8 caffeine test days for this group. We examined wheelrunning scores on the corresponding days for all groups; i.e., days 1, 3, 5, 7, 9, 11, 13, and 15.

RESULTS

Separate, two-factor mixed (1 between, 1 within) analyses of variance (ANOVAs) were performed on percentage wheelrunning scores and fluid intake data.

Fluid Intake

Mean daily fluid intakes for rats consuming caffeine solutions ranged between 35 and 53 ml across the 16 experimental days, yielding mean daily caffeine consumptions of approximately 40–60 mg/kg. ANOVA revealed a significant test day main effect, $F(7,147)=2.30$, $p<0.05$. Post hoc comparisons indicated that total fluid intake varied across test days, but in an unsystematic fashion. More importantly, the absence of a significant Drug effect or Drug × Day interaction confirmed that all three drug groups consumed equivalent volumes of fluid each day (see Table 1). Thus, the caffeine solutions administered were well tolerated by rats in this study.

Percent Baseline Wheelrunning

ANOVA indicated that the Drug main effect and the Drug × Day interaction both approached significance, $F(2,21)=2.79$, $p=0.08$ and $F(14,147)=1.64$, $p=0.07$, respectively. Planned comparisons showed significant differences between groups (see Fig. 1). Rats that drank caffeine on alternate days were significantly more active than those receiving continuous caffeine on sessions 9, 11, 13, and 15, $Fs(1,21)=5.00$, 6.25, 6.59, and 7.83, respectively (all p's<0.05). Furthermore, subchronic (alternate day) caffeine administration stimulated wheelrunning significantly above tap water on the latter three test sessions, $Fs(1,21)=5.02$, 5.86, and 7.80, respectively (all p's<0.05). Rats given continuous caffeine access did not differ significantly at any time from those given tap water.

A separate, within-subjects (Drug × Day) ANOVA on percent of baseline wheelrunning in the subchronic (Caffeine/Water) group showed, as expected, that rats ran significantly more on days when they drank caffeine than on days when they drank water, $F(1,7)=7.84$, $p<0.03$. Although mean wheelrunning scores were nearly four times as great after caffeine administration on Day 8 than after water administration, compared to only 1.75 times as great after caffeine on Day 1, the Drug × Day interaction did not attain statistical significance, $F(7,49)=1.63$, $p<0.16$ (see Table 2). Additionally, a separate ANOVA performed on the chronic caffeine, subchronic water, and chronic water groups showed no significant effect for Drug, $F(1,7)=0.46$, $p<0.05$, or for the Drug × Day interaction, $F(7,147)=0.69$, $p>0.05$, indicating that chronic and subchronic water groups were comparable in wheelrunning across days, and neither differed from the chronic caffeine group.

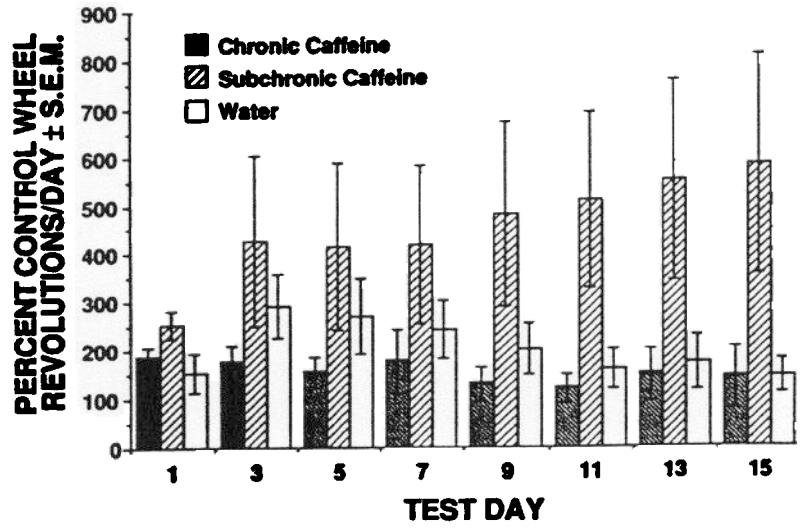

FIG. 1. Effect of caffeine administration on wheelrunning in rats. Wheelrunning is expressed as percent control wheel revolutions. Vertical bars represent ±1 S.E.M.

TABLE 2

PERCENT WHEELRUNNING SCORES FOR SUBCHRONIC (ALTERNATING CAFFEINE/WATER) GROUP: CAFFEINE VS. WATER DAYS

	Administration							
Drug	1st	2nd	3rd	4th	5th	6th	7th	8th
Caffeine	254	428	414	418	480	510	549	579
Water	144	179	172	118	90	152	126	145
CAF/WAT Ratio	1.75	2.39	2.41	3.54	5.33	3.36	4.35	3.98

DISCUSSION

We previously reported finding greater locomotor stimulation following a series of subchronic, IP administrations of 15 mg/kg caffeine than after acute injections (9,10). In that case, subchronic injections given at 72-hour intervals sensitized rats to the locomotor stimulating effects of caffeine, but only when injections were combined with wheelrunning; sensitization did not develop when caffeine-injected animals were confined to home cages without the opportunity to run. The present results confirm that sensitization also occurs with a 24-hour-on, 24-hour-off regimen of oral caffeine availability, when rats are allowed continuous access to wheelrunning.

The absence of sensitization in rats drinking caffeine daily confirms an earlier report (4) showing complete tolerance to the locomotor stimulating effects of caffeine in an activity monitor, within a day or two of the beginning of oral administration, using doses comparable to ours. Taken together, these results suggest that chronic intake diminishes caffeine's locomotor stimulant actions in the dose ranges tested, while subchronic administration produces a sensitization effect.

A variety of explanations could account for the observed difference between chronic and subchronic administration in the present study. The most obvious one is that daily exposure to caffeine leads to metabolic, drug-dispositional, or pharmacodynamic alterations not produced by alternate-day administration. At the very least, under the conditions of the present experiment rats given continuous exposure ingested roughly twice as much caffeine in a given two-day period as those given alternate day exposure. Nevertheless, behavioral tolerance to locomotor stimulation with daily oral caffeine doses of only 5–10 mg/kg/day—well below the 40–60 mg/kg ingested on alternate days by rats in the present experiment—has been reported (2). Thus, while daily ingestion of 5–10 mg/kg was sufficient to produce tolerance in the earlier (2) study, ingestions averaging 20–30 mg/kg/day in the present study produced sensitization, rather than tolerance, when caffeine was given on alternate days. Therefore, temporal spacing of doses, rather than dose magnitude would appear to determine whether sensitization or tolerance to oral caffeine administration occurs.

Many of the behavioral effects of caffeine and the methylxanthines appear to be related to their ability to block inhibitory actions of adenosine (3,8). Alterations in adenosine receptor function following chronic caffeine exposure may also provide a mechanism for the development of tolerance to caffeine's behavioral effects. For example, an increase in adenosine receptor binding sites in mesencephalic reticular formation in rats that ingested caffeine orally for 14 days has been reported (2). Changes in adenosine receptor affinity were absent. Up-regulation of adenosine receptors in mouse cerebellum following chronic exposure to subcutaneously implanted theophylline has also been found (12).

Siegel and colleagues (11) proposed that some behavioral adaptations to drugs are environment-specific; i.e., tolerance to a particular agent is greater when that agent is administered repeatedly in the same environment than when it is given in different environments. In the present study, rats in the chronic caffeine group were housed continuously in the same wheels while ingesting caffeine in their water. This protocol would have maximized behavioral tolerance according to Siegel's hypothesis. By the same argument, rats given alternate-day exposure would have become less tolerant because they ran in the same wheel (environment) under nondrug conditions on alternate days, thereby producing a kind of extinction of tolerance development. Future studies are needed to determine whether alternate-day oral caffeine administration produces tolerance, or possibly diminished sensitization, in rats given wheel access only on caffeine test days and not on tap water days.

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