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Caffeine and Nicotine: Differential Effects on Ambulation, Rearing and Wheelrunning[1]

CHARLES J. MELISKA AND WING HONG LOKE[2]

Psychology Department, Monmouth College, Monmouth, IL 61462

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MELISKA, C. J. AND W. H. LOKE. *Caffeine and nicotine: Differential effects on ambulation, rearing, and wheelrunning.* PHARMACOL BIOCHEM BEHAV **21**(6) 871–875, 1984.—Male Sprague Dawley rats were tested for open field ambulation and rearing, and for wheelrunning, following repeated injections of either caffeine or nicotine, given according to a Latin Square design. Caffeine enhanced ambulation and rearing at 5 and 15 mg/kg, IP, and increased wheelrunning with 15 and 45 mg/kg. Nicotine (0.63 mg/kg) also enhanced ambulation, but not rearing, and depressed wheelrunning during the first 20 min of testing. Caffeine's enhancement of wheelrunning was not significant during the first two drug administrations. Results suggest that caffeine and nicotine affect activity via different neuropharmacological mechanisms. Previous experience with these drugs may modulate animals' reactivity to them.

Caffeine Nicotine Open Field Exploration Wheelrunning Tolerance Drug experience

WHILE numerous studies have investigated the effects of caffeine and nicotine on rodent activity, results have been variable. Caffeine has been observed to increase ambulation in the open field and in various types of mazes [8, 10, 15, 24, 31], but nonsignificant effects are also reported [12,23]. Similarly, increased rearing has been found with caffeine [8], but not always [10]. Nicotine has been found to reduce ambulation [6, 15, 25], but increases are also reported, depending upon genetic strain [1], for tobacco smoke; [21], dosage [17], age and sex [3], and previous experience with the drug [4, 5, 16].

Another popular measure of rodent activity is wheelrunning, which is increased by caffeine [12,23]. Nicotine has been found to depress wheelrunning in rats [14], but some reports indicate enhancement, depending upon basal activity level [2] and time since administration [3].

An animal's response to a drug may also be affected by prior experience with it. Sometimes mere exposure to the drug—i.e., without behavioral testing—changes reactivity to the drug in test situations (e.g., [26]). In other cases, changed responsiveness results only when repeated drug administrations occur in conjunction with a specific behavioral test. Such "behavioral tolerance" involves learned adaptations which occur irrespective of drug-dispositional or pharmacodynamic adaptations to the drug [9, 13, 19]. Various kinds and degrees of adaptation to the behavioral effects of caffeine [18,28] and nicotine [4, 5, 15, 16, 25, 27] are reported.

Few studies compare the effects of caffeine and nicotine on measures of activity within a single experiment. The present study tests the effects of various doses of these drugs on ambulation, rearing, and wheelrunning in drug-experienced subjects. The experimental design also permitted assessment of the effects of repeated drug administrations. A second aim was to sample the time course of the actions of the drugs.

METHOD

Subjects

For open field tests, 16 male, Harlan/Sprague Dawley rats, approximately 200 days of age were used. A comparable group of 16 animals was used for tests of wheelrunning. All rats were naive with respect to the open field and wheels; but all had been previously trained to barpress on a multiple FR/FI schedule of food reinforcement, and had been tested on two occasions, two weeks earlier, once following an injection of caffeine and once following an injection of nicotine. Body weights were maintained at about 80% of ad lib feeding weights with Purina Lab Chow, given 1–2 hours after testing. Water was available ad lib, and the lights of the animal room were maintained on for 24-hr day.

Apparatus

Open field tests were conducted in a 90 cm^2 field constructed of masonite, with 25 cm high walls. The floor was divided into 25 squares, 18 cm on a side. Manual closure of a switch advanced a counter in an adjacent room which printed the number of squares traversed (ambulations) made during each of 10 consecutive minutes of testing. A second

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[2]Presently at the University of Iowa, Iowa City, IA 52242.

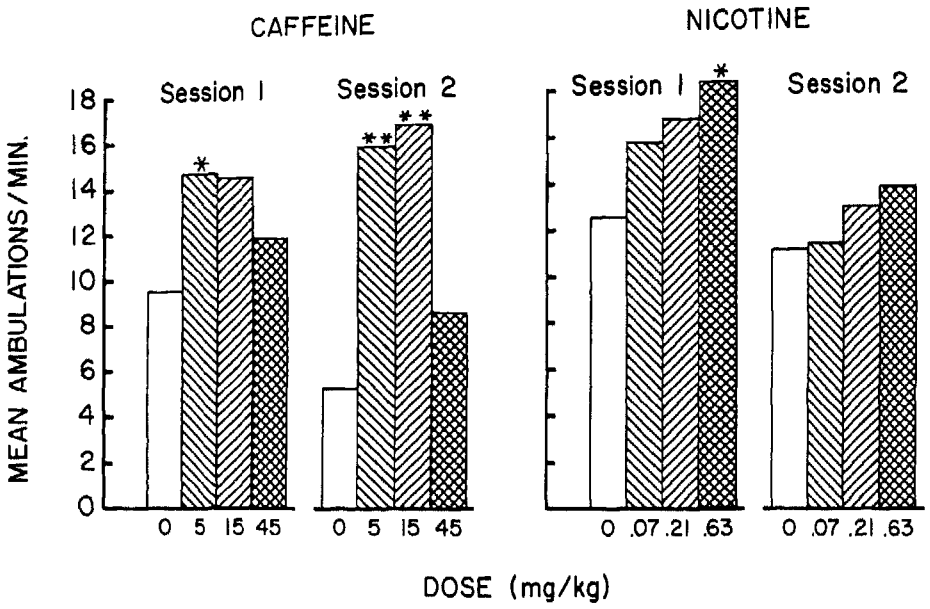

FIG. 1. Effects of caffeine and nicotine on open field ambulation. Session 1: 20–30 min post-injection; Session 2: 80–90 min post-injection. Data are collapsed over 10-min sessions, for N=8 rats per data point. Asterisks indicate significance of differences from Distilled Water (0 mg/kg) control: * $p<0.05$; ** $p<0.01$.

counter recorded rearings. The field was illuminated by standard overhead fluorescent lighting.

Wheelrunning was measured in six activity wheels (Wahman Mfg., Baltimore, MD), 36 cm in diameter×11 cm wide. A mechanical counter attached to each wheel recorded the number of revolutions made in either direction of motion. A sliding door at the entrance of each wheel was closed to prevent the rat from exiting during testing. Each wheel was housed in a separate room.

Procedure

For open field tests rats were randomly assigned to either caffeine (CAF) or nicotine (NIC) groups, 8 rats/group. To reduce possible behavior-disrupting side-effects of the drugs (e.g., nausea, diarrhea), pre-test injections of the highest doses of caffeine and nicotine were given 72 hours before behavioral tests. CAF rats received 45 mg/kg caffeine (Merck), IP; NIC rats received 0.63 mg/kg nicotine hydrogen tartrate (J. T. Baker), IP. Behavioral tests were run on alternate days with a "rest" day between to minimize carryover effects. CAF rats received 0 (distilled water; DW), 5, 15, or 45 mg/kg caffeine, IP and NIC rats received 0 (DW), 0.07, 0.21, or 0.63 mg/kg nicotine hydrogen tartrate, IP, both drugs given according to a balanced Latin Square design. Drugs were dissolved in DW and given in volumes of 2.0 ml/kg. To minimize deterioration, fresh nicotine solutions were prepared for the pretreatment, test day 1, and test day 3 injections.

On each test day rats were tested in the open field twice. Twenty min after injection, each rat was placed into the center of the field and the timer was started. Ambulations (number of floor units traversed by all four limbs) and rearings (number of times the animal stood on hind legs) were recorded at one minute intervals, for 10 min. Subjects were then returned to home cages. About 60 min after the start of the first test, they were retested. Each rat was tested in this manner on four separate occasions, with a different dose of drug or DW.

For wheelrunning, the same assignment, pre-test drug injections, and Latin Square order of doses was used. Rats were placed into the wheels twenty min after injection, and revolutions made during the next hour were recorded at 20 min intervals.

Data Analyses

Individual ambulation and rearing scores were collapsed over two-minute time blocks. A multifactor, Latin Square ANOVA was used to test for effects of Drug, Dose, Session, Time, and Order of Dose Administration. Where Order and Drug factors proved nonsignificant, data were collapsed across orders, and individual Dose × Session × Time ANOVAs were run for CAF and NIC groups, separately. Similar analyses were done for wheel activity. Significant main effects and interactions ($p<0.05$) were subjected to analyses of simple effects and Dunnett's tests for comparisons of treatment means with a control.

RESULTS

No significant effect of Order of Dose Administration was detected with either drug for open field ambulation or rearing. Separate ANOVAs showed that overall, both drugs increased ambulation in an approximately dose-dependent manner: $F(3,21)=7.75$, $p<0.001$ for caffeine; $F(3,21)=3,54$, $p<0.05$ for nicotine. However, effects were time dependent (Fig. 1). Nicotine (0.63 mg/kg) enhanced ambulation significantly above control ($p<0.05$) only during Session1, 20–30 min post-injection; caffeine (5 mg/kg) enhanced ambulation during Session 1 ($p<0.05$), but produced even greater effects during Session 2, 80–90 min post-injection ($p<0.01$ for 5 and 15 mg/kg).

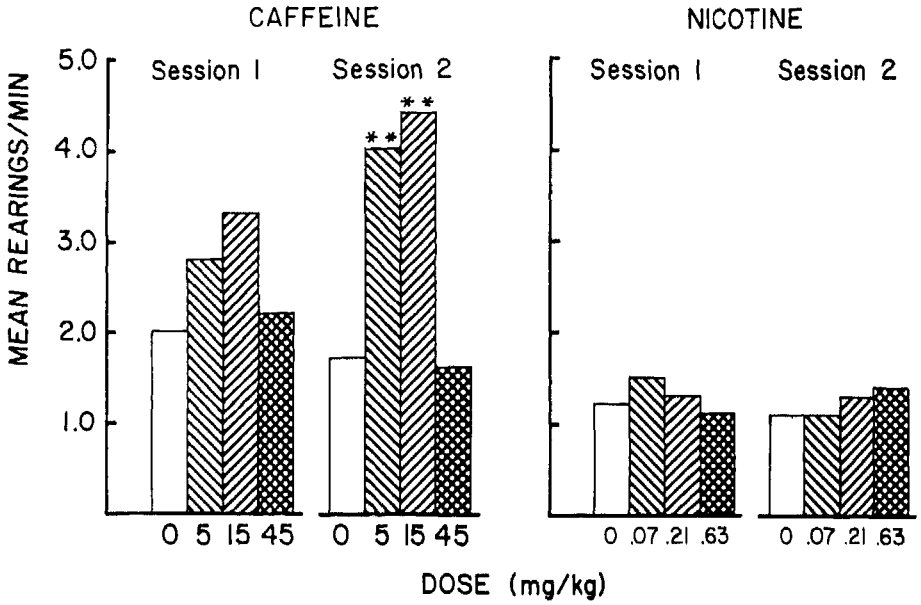

FIG. 2. Effects of caffeine and nicotine on open field rearing. Session 1: 20–30 min post-injection; Session 2, 80–90 min post-injection. Data are collapsed over 10-min sessions, for N=8 rats per data point. Asterisks indicate significance of difference from Distilled Water (0 mg/kg) control: $^{**}p<0.01$.

The pattern of caffeine's effects on rearing closely paralleled its effects on ambulation (Fig. 2). Rearing was elevated ($p<0.01$) during Session 2 with 5 and 15 mg/kg, but increments in Session 1 were not significant. In contrast, nicotine did not increase rearing significantly in either session.

Caffeine also increased wheelrunning in a dose-related manner, $F(3,12)=6.2$, $p<0.01$. The 15 and 45 mg/kg doses exceeded control ($p<0.05$, at least); 5 mg/kg did not. A negligible interaction with Time, $F(6,8)=0.65$, $p>0.05$, showed that the effect was quite constant across the hour-long test, (Fig. 3). In contrast, nicotine depressed wheelrunning 20–40 min post-injection ($p<0.05$ for 0.21 mg/kg; $p<0.01$ for 0.63 mg/kg), but not thereafter.

A Dose × Order of Administration interaction for caffeine was also found, $F(6,12)=2.98$, $p<0.05$. Caffeine did not elevate wheelrunning significantly until the third and fourth administrations (Fig. 4). No Order of Administration effect for nicotine was detected, $F(6,12)=0.71$, $p>0.05$.

DISCUSSION

Caffeine increased all three measures of activity, confirming earlier reports of enhancement of ambulation [8, 10, 24, 31], rearing [8] and wheelrunning [12,23] in rodents. Greatest effects occurred 80–90 min after injection. It is unclear whether this delay in maximal enhancement is due to slow onset of action, or whether caffeine affects later activity more than earlier activity, e.g., by attenuating response-produced decrements which normally occur with unreinforced responding. Delaying testing an hour or more after injection might clarify this point. It is noteworthy that two studies reporting no effects of caffeine on ambulation used short duration tests—e.g., 5–10 min [12] and 15 min [23]. However, two other studies using tests of only two min [8] and 10 min [10] reported enhancement. The effect of interval between injection and testing also needs clarification.

Nicotine increased ambulation as reported previously with some doses and some genetic strains [3, 4, 5, 16, 17, 21]. However, reduced ambulation with nicotine has also been found [6, 15, 25]. Methodological differences among the present and earlier studies might account for these discrepancies. For example, the present study used repeated drug administration over several days, rather than a single test. Some studies report that nicotine-naive rats react to the drug with behavioral depression, while nicotine-experienced rats show stimulation [3, 4, 5, 16, 25]. Since we pretreated with nicotine three days before the open field tests, this may have produced behavioral stimulation rather than depression.

While caffeine's effects in the present study could all be attributed to "general locomotor stimulant" actions, nicotine's cannot. A nicotine dose (0.63 mg/kg) which increased open field ambulation (Fig. 1) decreased wheelrunning (Fig. 3), and had not effect on rearing (Fig. 2). The dissociation of its effects on these three measures implies that nicotine enhances ambulation via different means than mere locomotor stimulation, and presumably by a more selective neuropharmacologic mechanism than caffeine's. Nicotine's effects are usually attributed to stimulation of central cholinergic receptor sites [4, 5, 27]. Caffeine has been thought to stimulate by augmenting cellular metabolism through inhibition of breakdown of cyclic AMP [19]. However, recent evidence shows that the concentration of caffeine required to produce activity changes via cyclic AMP enhancement greatly exceed doses which increase activity. An alternative suggestion is that caffeine and other methylxanthines enhance neural firing and behavior by blocking inhibitory actions of adenosine [11,22].

Whether either drug stimulates "exploration" or "novelty seeking" is a matter of theoretical interest. While often regarded as indices of exploration, open field ambulation and rearing may actually be motivated by fear (see [20]

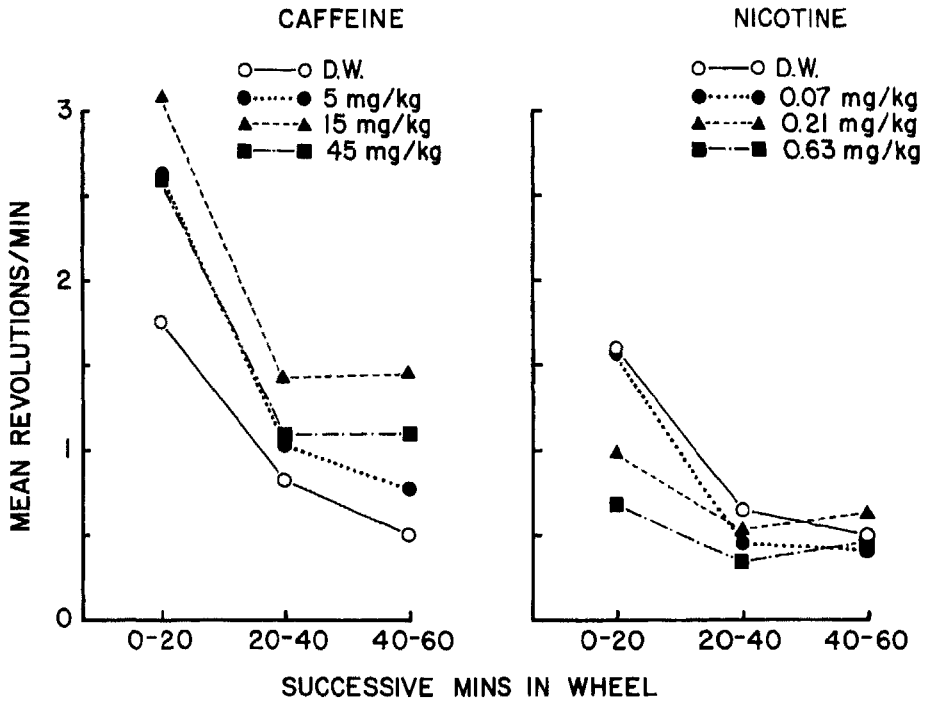

FIG. 3. Effects of caffeine and nicotine on wheelrunning. Rats were injected 20 min before being placed in the wheels. Data points represent means for N=8 rats at successive 20 min intervals.

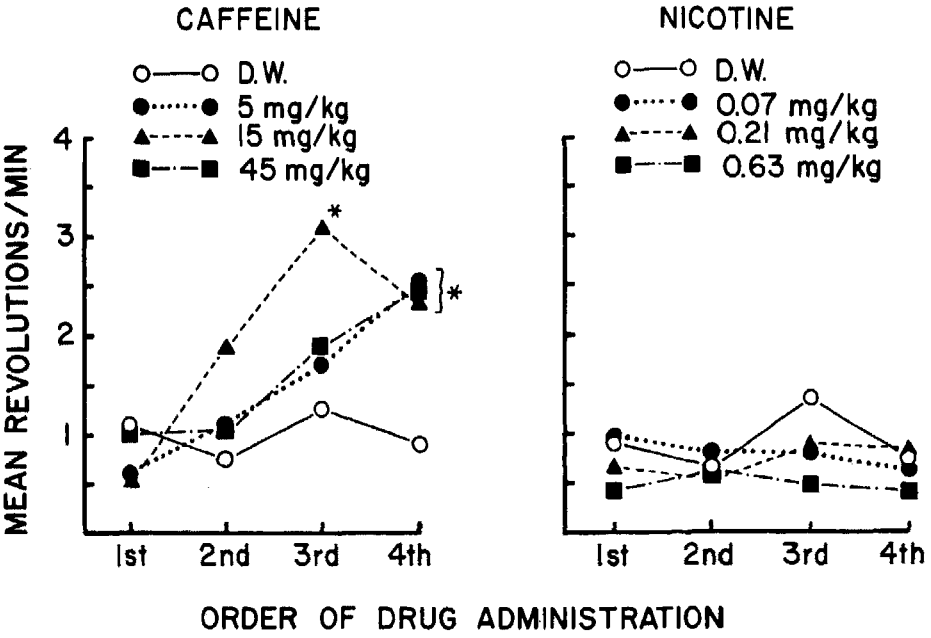

FIG. 4. Effects of order of drug administration on wheelrunning. Data points represent means for N=2 rats, averaged across the 60 min tests. Asterisks indicate significance of difference from Distilled Water control: * $p<0.05$.

for a review), or by the drive to escape from the field [29,30]. File [7] notes that measures of an animal's tendency to nose into holes in the floor, or approach an object, or emerge from a darker into a lighter part of the field, might be better indices of exploration, *per se*, than sheer locomotion.

The unanticipated effect of order of caffeine administration on wheelrunning (Fig. 4) may be important. Our data suggest that in some tests, prior drugging with caffeine enhances its stimulant actions. Future studies could evaluate the relative contributions of drugging alone, test experience alone, and their combination, in determining the nature and magnitude of this effect.

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