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Caffeine-Induced Place and Taste Conditioning: Production of Dose-Dependent Preference and Aversion

NEIL T. BROCKWELL, ROELOF EIKELBOOM AND RICHARD J. BENINGER

Department of Psychology, Queen's University, Kingston, Canada K7L 3N6

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BROCKWELL, N. T., R. EIKELBOOM AND R. J. BENINGER. *Caffeine-induced place and taste conditioning: Production of dose-dependent preference and aversion.* PHARMACOL BIOCHEM BEHAV **38**(3) 513–517, 1991.—Although caffeine may be the most widely used behaviorally active drug, few studies have examined its rewarding properties. In the present study, the designs of place-conditioning and taste-conditioning paradigms were combined in a single experiment to provide two independent measures of drug reward. During 3 preconditioning sessions, undrugged rats received access to 2 distinctive chambers connected by a small tunnel. During the 8-session conditioning phase, groups were given home cage access to either a sweet or salty solution, administered caffeine (0.3, 1.0, 3.0, 10.0, 30.0 mg/kg IP; 30.0 mg/kg SC), and confined to one of the chambers. On alternate sessions, rats were given home cage access to the remaining solution, injected with the vehicle, and confined to the opposite chamber. On test sessions 1 and 2, undrugged animals were given home cage access to one of the flavored solutions and water, and then allowed access to both conditioning chambers. On test session 3, rats were given access to both flavored solutions, injected with the drug used during conditioning, and again allowed access to both chambers. Caffeine (3.0 mg/kg) produced a significant place preference. The highest dose (30.0 mg/kg IP and SC) produced significant place and taste aversions. A control group given (+)-amphetamine illustrated a significant place preference and taste aversion as expected. Thus caffeine appeared to produce a dose-dependent biphasic effect; a lower dose was rewarding, whereas higher doses produced aversions to environmental stimuli associated with the drug.

Caffeine Place conditioning Taste aversion

TODAY, it may be argued that caffeine is the most widely used behaviorally active drug. Current estimates of worldwide caffeine consumption range from 50 (10) to 70 (1) mg per person per day. However, despite the abundant use of caffeine, few studies have examined its rewarding properties.

Like (+)-amphetamine (AMPH) and cocaine, caffeine is classed as a psychomotor stimulant. It appears to increase alertness and enhance simple locomotor performance in humans, and dose-dependently produce hyperactivity in many animal species (2,12).

The rewarding properties of many psychomotor stimulants are well documented (18). However, to date, the rewarding effects of caffeine have generally been only equivocally demonstrated in either humans (2,12) or infra-human species (12). For example, although IV self-administration and intracranial self-stimulation (ICSS) paradigms have consistently illustrated the rewarding properties of AMPH and cocaine in several animal species [e.g., (6–9)], they have failed to reliably demonstrate the rewarding effects of caffeine (7, 8, 20, 22).

Recently, place conditioning has been utilized to demonstrate the rewarding properties of several psychomotor stimulants including AMPH [e.g., (11,16)] and cocaine [e.g., (23)]. Following several pairings of a drug injection with a distinctive environment, undrugged animals subsequently display an increase in time spent in that environment, compared to an equally distinctive alternative environment. This shift in relative preference is regarded as evidence for the rewarding effects of the drug. The main advantage of this paradigm is that, unlike IV self-administration and ICSS, subjects are tested drug free; thus the immediate motor effects of the drug do not directly influence the dependent measure.

A considerable body of evidence also suggests that psychomotor stimulants have the ability to produce aversive responses. Doses of AMPH and cocaine found to be rewarding in IV self-administration paradigms produce a paradoxical aversion to flavored solutions paired with the drug (17).

In the present study, place conditioning and taste conditioning were utilized in a single experiment to examine the rewarding and aversive properties of caffeine. The experimental procedure was designed such that a single drug injection would follow access to a specific taste, but precede placement in the conditioning environment, and thus provide two independent measures of drug reward.

METHOD

Subjects

Eighty male Wistar rats (Charles River Canada), weighing approximately 225–250 g upon arrival, were individually housed in wire mesh cages in a climatically controlled (21 ± 1°C) colony room with a 12-hour light (0600–1800 h)/dark cycle. Animals

had free access to food throughout the experiment. Water restriction is outlined below.

Drugs

Caffeine (Sigma) and (+)-amphetamine (Smith, Kline & French, Canada) were dissolved in 0.9% saline and injected in a volume of 2.0 ml/kg.

Apparatus

The place conditioning study utilized 4 similar, dual-chambered, rectangular boxes (84 × 27 × 36 cm height) constructed of wooden sides and removable Plexiglas covers. The two chambers (38 × 27 × 36 cm) were joined by a wooden tunnel (8 × 8 × 6 cm height) which could be blocked by the insertion of two Plexiglas guillotine doors. The chambers differed in wall pattern and floor design. In two of the boxes, one chamber had brown walls and a wire mesh floor (1 × 1 cm), while the other chamber had black and white vertically striped (1 cm) walls and a floor consisting of wire rods spaced 1 cm apart. In the other two boxes the floor and wall pairings were reversed. The floor of each box was positioned on a fulcrum such that the weight of a rat in either compartment would close a microswitch and activate a timer in an adjoining room. Each box was housed in an outer plywood chamber which was insulated with sound-attenuating styrofoam, illuminated by a 7.5-W light, and ventilated with a small fan.

The taste-conditioning study utilized standard water bottles, inserted into the home cages, which were weighed to record the amount of fluid consumed. Tests conducted prior to the experiment indicated that less than 0.5 ml per bottle per day was lost due to spillage.

Procedure

Following random assignment to one of eight groups (n = 10) all animals were handled approximately 10 min per day for a period of 10 days. Water was initially freely available during handling but was restricted 72 h prior to the experiment to 30 min access per day.

The experimental design consisted of three phases: preconditioning, conditioning, and test, conducted over a total of 27 days. Previous evidence has suggested that acute administration of caffeine may produce hypoactivity 24 h following IP administration (15). Therefore, in the present study, experimental sessions were conducted at 48-h intervals to limit the influence of possible withdrawal effects on conditioning. Animals were confined to their home cages during the intersession interval. Following each session, and on days in which animals were confined to the home cage, a water bottle was mounted on the home cage for 30 min to ensure adequate fluid intake.

Preconditioning. During 3 15-min sessions, undrugged animals were placed in one of the two chambers (designated the start side) and given access to the entire conditioning box with the guillotine doors removed. The choice of start side was counterbalanced across rats, but remained the same for each rat throughout the experiment.

Conditioning. The design of the conditioning phase took advantage of the fact that in taste-conditioning paradigms drug administration follows fluid consumption, whereas in place-conditioning paradigms drug administration precedes placement in the conditioning apparatus. This feature allowed a single drug injection to be paired with both a distinctive taste and a distinctive environment to provide two independent measures of reward.

During the conditioning phase 6 groups received caffeine: five received IP injections (0.3, 1.0, 3.0, 10.0, 30.0 mg/kg); the sixth group received 30.0 mg/kg SC to determine whether possible aversions were the product of the direct action of caffeine at the IP injection site. Two control groups were also included: one received the vehicle (0.9% saline), while the other received 2.0 mg/kg IP AMPH known to produce both a conditioned place preference and a conditioned taste aversion (16).

During each of the 8 conditioning sessions, a bottle containing either a salty (0.9% saline) or sweet (0.1% saccharin) solution was mounted on the home cage. Following 15-min access to this solution, rats were removed from their home cages and injected. On odd-numbered sessions rats were administered the drug; the vehicle was administered on even-numbered sessions. In each group, half of the animals received access to the sweet solution prior to drug injection and the salty solution prior to vehicle injection. In the remaining animals this order was reversed.

Immediately following injection, animals were placed in the conditioning box with the guillotine doors in place, and confined to one chamber for a period of 30 min. During odd-numbered sessions rats were confined to the nonstart side, while on even-numbered sessions rats were confined to the start side.

Test. During each of 3 sessions, two bottles were mounted on the home cage for a period of 15 min. Rats received access to one of the flavored solutions and water on the first session, and the remaining solution and water on the second session. The flavored solution presented during each session was counterbalanced such that half of the animals in each group received the drug-paired solution on the first test session. Thus the first two test sessions were components of one overall test rather than discrete measures of taste conditioning. Immediately following fluid access, subjects were removed from their home cages, placed in the start side of the conditioning box with guillotine doors removed, and given access to the entire box for 15 min.

On the third test session, animals were presented with both the sweet and salty solutions in a standard two-bottle test. Following 15-min fluid access, rats were removed from their home cages and injected with the drug used during conditioning. This procedure was employed to examine the effect of drug state on prior conditioning. The rats were then placed in the conditioning box for 15 min following the procedure outlined above.

The position of bottles on the home cage was counterbalanced across rats within each group on each of the test sessions. The amount (g) of all fluids consumed was recorded throughout all phases of the experiment. The amount of time (s) spent in each chamber was recorded during the preconditioning and test phases.

RESULTS

Due to a mechanical difficulty with one of the conditioning boxes, one subject in the AMPH group was eliminated from the study. All other groups retained the initial number of subjects (n = 10).

Place Conditioning

Figure 1 presents the amount of time spent in the drug-paired chamber during the preconditioning and test phases for all groups. The data have been averaged across the three preconditioning sessions and the first two test sessions. Figure 1 also presents the data for test session 3.

As discussed above, test session 3 occurred following an injection of the drug used during conditioning. Therefore, the primary statistical analyses utilized data from test sessions 1 and 2 only, hereafter referred to as the test phase. A two-way analysis

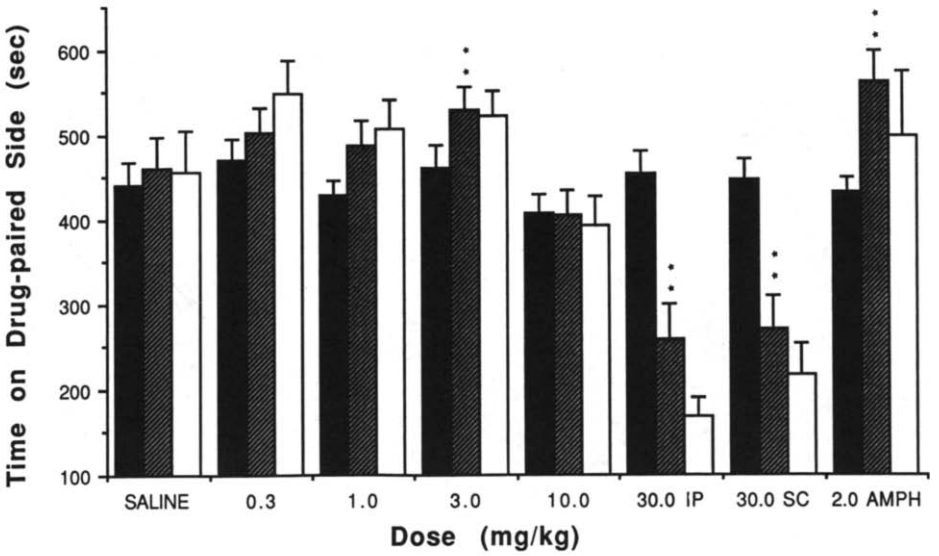

FIG. 1. Average (± SEM) amount of time spent in the drug-paired chamber during the preconditioning phase (black bar), test sessions 1 and 2 (striped bar), and test session 3 (white bar). $^{**}p<0.01$. Differs significantly from preconditioning phase.

of variance (ANOVA), with phase as a repeated measure, revealed a significant group effect, $F(7,71)=5.63$, $p<0.001$, and a significant phase × group interaction, $F(7,71)=12.25$, $p<0.001$. To isolate the source of the two-way interaction, one-way ANOVAs were conducted for each of the eight groups. For the sake of clarity, the terms preference and aversion will be used to denote the direction of each phase effect. Results revealed that a significant place preference occurred with 3.0 mg/kg caffeine, $F(1,9)=10.36$, $p<0.01$. In contrast, 30.0 mg/kg caffeine produced significant place aversions with IP, $F(1,9)=22.39$, $p<0.01$, and SC, $F(1,9)=22.64$, $p<0.01$, administration. AMPH also produced a significant place preference, $F(1,8)=14.29$, $p<0.01$.

Orthogonal contrasts were conducted to compare the amount of time spent in the drug-paired chamber during test session 3 to the average time in the drug-paired side during the previous two test sessions. Results indicated that no significant differences were found for any of the 8 groups.

Taste Conditioning

Analyses were conducted to assess relative preference for the two flavored solutions, based on drug pairing, during test sessions 1 and 2. A two-way ANOVA, with solution (drug-paired vs. vehicle-paired) as a repeated measure, revealed a significant group × solution interaction, $F(7,71)=2.83$, $p<0.05$. The results of one-way ANOVA's, conducted for each group to isolate the source of this interaction, indicated that 30.0 mg/kg caffeine IP and AMPH produced significant taste aversions, $F(1,9)=11.32$, $p<0.01$; $F(1,8)=10.68$, $p<0.05$; respectively. These results are presented in Fig. 2.

The two-bottle test, used on the third test day, provided a more direct comparison of solution preference, based upon drug pairing, since water was not available as an alternate solution. A two-way ANOVA revealed a significant main effect for solution, $F(1,71)=14.42$, $p<0.001$, and a significant group × solution interaction, $F(7,71)=4.68$, $p<0.001$. To isolate the source of the interaction, one-way ANOVA's were again conducted for each group. Significant taste aversions were produced by 30.0 mg/kg caffeine IP, $F(1,9)=10.91$, $p<0.01$, 30.0 mg/kg caffeine SC, $F(1,9)=40.11$, $p<0.001$, and AMPH, $F(1,8)=36.55$, $p<0.001$. These results are presented in Fig. 3.

DISCUSSION

The results of the present experiment suggest that caffeine produced a biphasic effect; a lower dose was rewarding, whereas higher doses produced aversions to both place and taste cues associated with the drug. As expected, saline failed to induce conditioning and AMPH produced both a place preference and a taste aversion, indicating that the experiment provided a valid assessment of drug-induced reward.

Results of place conditioning illustrate that IP and SC injections of 30.0 mg/kg caffeine produced comparable effects. This similarity suggests that route of systemic administration was not a significant factor in the production of caffeine-induced place aversion; the aversion was not the product of peritoneal irritation at higher caffeine doses.

As previously discussed, the drug injection employed during test session 3 was used to examine the effect of the drug on prior conditioning. The results of the orthogonal contrasts revealed that for all groups, the drug did not significantly alter the time spent in the drug-paired chamber. Although this finding is complicated by the presence of two previous test sessions in the nondrugged state, it does suggest that the place preference and aversions were not simply a product of drug state. Similar findings have been reported by two recent studies which tested animals in the drugged state and reported place preferences with AMPH (16) and cocaine (21).

Comparison of Figs. 2 and 3 reveals that AMPH produced significant taste aversions as expected, and that the highest dose of caffeine generally produced significant taste aversions across both tests. Although 30.0 mg/kg SC caffeine failed to produce a significant taste aversion in test sessions 1 and 2, it should be noted that only one subject in this group did not demonstrate a preference for the vehicle-paired solution. However, it is possible that the magnitude of this one discrepant preference (25 vs. 1

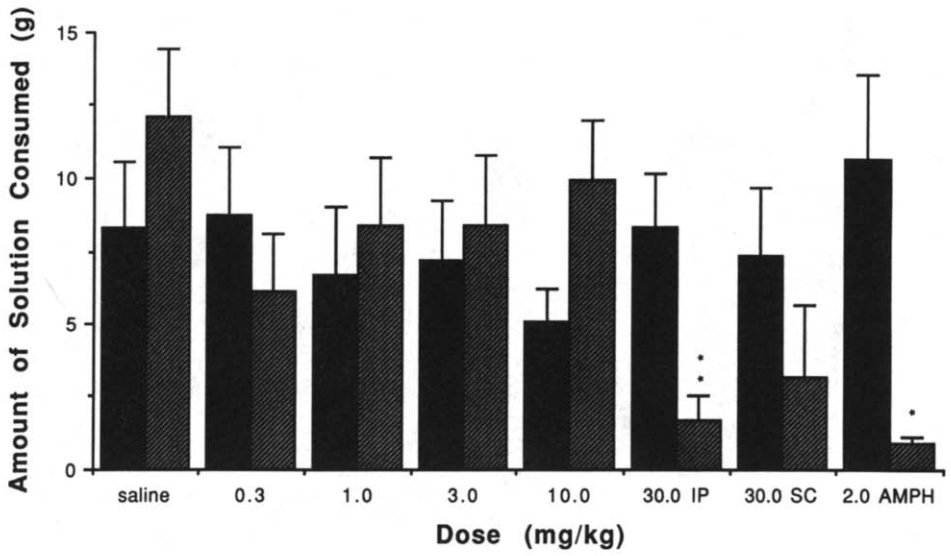

FIG. 2. Average (±SEM) amount of vehicle-paired solution (black bar) and drug-paired solution (striped bar) consumed during test sessions 1 and 2. *$p<0.05$, **$p<0.01$. Differs significantly from vehicle-paired solution.

g) may have obscured potential aversive effects. The similarity of the taste aversions produced with 30.0 mg/kg caffeine IP and SC suggests that, as in place conditioning, the effects of the drug were produced regardless of route of systemic administration.

The taste aversions produced in the present study are congruent with previous research conducted on laboratory animals. White and Mason (26) reported that 30.0 mg/kg IP caffeine appeared to be very close to the threshold dose for producing taste aversions in rats following a single injection. Although these authors also concluded that 10.0 and 20.0 mg/kg caffeine produced enhanced intake of flavoured solutions paired with the drug (26), a similar relationship was not found in the present study. The reason for this discrepancy is not clear. An accumulating body of evidence suggests that doses of psychomotor stimulants which readily produce IV self-administration also produce paradoxical taste aversions (17). Based upon this evidence it might be expected that caffeine doses which produced a significant place preference would also produce a taste aversion. However, again this relationship was not found. Future research is warranted to resolve these discrepancies.

The aversions found in the present study are also congruent with the results of research conducted with human subjects. In order to make direct comparisons between the results of experiments conducted on rats and humans, it is essential that doses in-

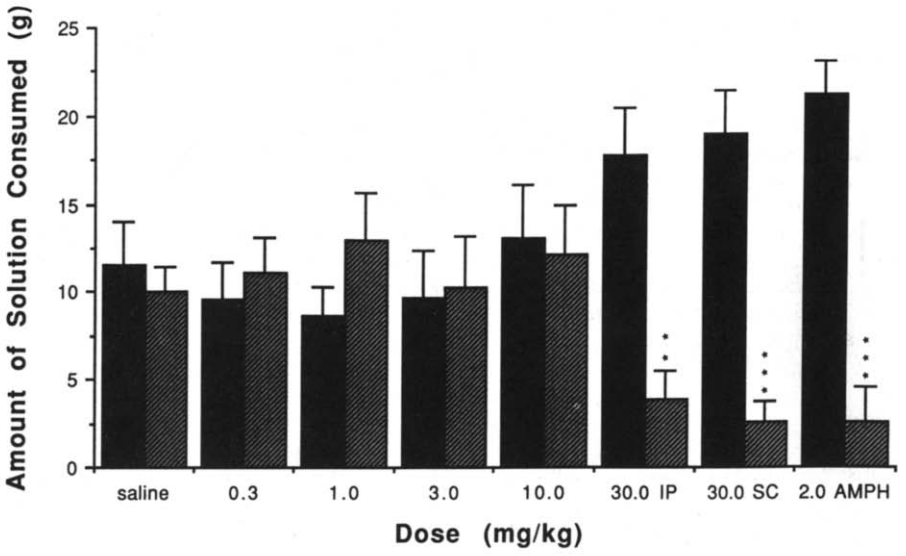

FIG. 3. Average (±SEM) amount of vehicle-paired solution (black bar) and drug-paired solution (striped bar) consumed during test session 3. **$p<0.01$, ***$p<0.001$. Differs significantly from vehicle-paired solution.

dicated as mg/kg body weight be corrected for differences in interspecies metabolic rates. Based upon the work of Bonati, Latini, Tognoni, Young and Garattini (3) which examined in vivo caffeine pharmacokinetics and bioavailability, a dose of 30.0 mg/kg in rats is roughly equivalent to 7.5 mg/kg in humans. Therefore, assuming that the average human weighs approximately 50.0 to 80.0 kilograms, a comparable single dose of caffeine would be 375–600 mg. Recent evidence has suggested that this dose range is associated with caffeine-induced aversions in humans (14,24).

To date, previous studies have generally provided equivocal evidence regarding the rewarding properties of caffeine. Thus the present study may provide the first clear experimental documentation that caffeine is rewarding. Direct comparison of the present study to previous research utilizing infra-human species is difficult due to the utilization of different dose levels. Nevertheless, a number of IV self-administration studies and one ICSS study failed to find reward utilizing caffeine doses in the 1.0–5.0 mg/kg range (7, 8, 20, 22). The reason for this discrepancy is unclear. However, it may be argued that the place conditioning paradigm is simply more sensitive to drug reward than either IV self-administration or ICSS paradigms. Indeed, it has recently been asserted that place conditioning has replaced self-administration as the most popular method for assessing the rewarding properties of drugs (5).

A considerable body of evidence suggests that the rewarding effects of many substances may involve enhanced dopaminergic neurotransmission (4). The results of two recent studies suggest that caffeine also influences dopamine (DA) systems. Using in vivo voltammetry, Morgan, Dunn and Vestal (19) concluded that acute administration of 15.0 mg/kg caffeine enhanced, whereas 50.0 and 100.0 mg/kg decreased, caudate DA release. In contrast, Taylor et al. (25) found enhanced striatal DA release with 25.0 and 50.0 mg/kg, but not 10.0 mg/kg caffeine following 30 days of administration. Together, these findings suggest that the effect of caffeine on DA systems is influenced by factors related to both dose level and drug tolerance. In caffeine-naive animals, enhanced central DA release may be associated with lower caffeine doses. However, following chronic administration, doses which originally inhibited DA release may begin to enhance DA activity.

In conclusion, the results of the present study suggest that caffeine produces a dose-dependent biphasic effect; a lower dose is rewarding, whereas higher doses produce aversions to environmental stimuli associated with the drug.

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