Changes in the Ambulatory Activity and Discriminative Stimulus Effects of Psychostimulant Drugs in Rats Chronically Exposed to Caffeine: Effect of Caffeine Dose

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ABSTRACT

Caffeine is a common psychoactive constituent of coffee, carbonated beverages, and over-the-counter medications. This study examined the effects of chronic caffeine exposure on the behavioral response to acute administrations of psychostimulant drugs on ambulatory activity and on the pharmacological characteristics of nicotine discrimination in rats. Rats were maintained continuously on caffeine added to the drinking water at a concentration of 0.25 or 1.0 mg/ml that resulted in plasma caffeine concentrations ranging from 0.37 to 5.95 μg/ml. Rats maintained on tap water served as control groups. Exposure to the lower caffeine concentration (0.25 mg/ml) potentiated stimulatory effects of nicotine, amphetamine, and cocaine on ambulatory activity and failed to produce tolerance to the acute stimulatory effects of caffeine. In contrast, exposure to the higher caffeine concentration (1.0 mg/ml) did not alter the effects of the psychomotor stimulants on ambulatory behaviors but resulted in the development of complete, insurmountable tolerance to the acute stimulatory effects of caffeine. In the nicotine discrimination paradigm (0.4 mg/kg, training dose, a fixed-ratio 10 schedule of food delivery in a two-lever choice paradigm), rats exposed to the lower, but not to the higher, caffeine concentration acquired the nicotine discrimination significantly faster and were more sensitive to the effects of amphetamine and cocaine in substitution tests than water-drinking rats. Caffeine exposure did not change pharmacokinetic properties of nicotine (i.e., plasma levels, metabolism). In summary, exposure to two different caffeine solutions within a range of plasma levels observed in humans resulted in quantitatively distinct changes in psychostimulant-induced nonoperant and operant measures of behavior. These results suggest that dietary consumption of moderate doses of caffeine may be associated with enhanced reactions to some psychostimulants.

A large percentage of the human population consumes caffeine chronically as a regular part of their diet (e.g., coffee, soft drinks, over-the-counter medications) (Fredholm et al., 1999). The estimated mean consumption of caffeine in American adults is 3.0 mg/kg/day with two-thirds of it coming from coffee (Fredholm et al., 1999). The estimated daily caffeine intake in children aged 7 to 10 years ranges from 0.5 to 1.8 mg/kg with soft drinks (55%) and chocolate products (35–40%) being the two main sources of caffeine (Fredholm et al., 1999). One 150-ml cup of coffee contains 40 to 180 mg of caffeine, whereas a 12-oz (355 ml) can of a cola drink contains from 26 to 58 mg of caffeine (Fredholm et al., 1999). A dose of 0.4 to 2.5 mg/kg and a plasma concentration of 0.25 to 2.58 g/ml caffeine are typically obtained from a single cup of coffee (Fredholm et al., 1999). Habitual coffee drinking (2–3 cups/day) delivers enough caffeine to positively affect human psychomotor and cognitive performance (James, 1997). In contrast, high doses of caffeine (>300 mg/kg/day) can produce negative effects (e.g., nervousness, anxiety, sleep disturbance) (Benowitz, 1990).

Psychopharmacological studies have shown that physical dependence on caffeine may develop with its persistent use (Griffiths et al., 1986, 1990), as evidenced by development of transient withdrawal symptoms (e.g., headache, fatigue, decreased alertness, irritability) after abrupt cessation of caffeine consumption. Chronic caffeine use is also associated with development of tolerance to some of its central and peripheral effects (Holtzman, 1990; Heishman and Henningfield, 1992; Griffiths and Mumford, 1995). Although caffeine serves as a reinforcer in humans and animals under a limited...
range of experimental conditions (Heishman and Henningfield, 1992; Griffiths and Mumford, 1995), its reinforcing properties are low in comparison to prototypical illicit psychomotor stimulants (i.e., amphetamines, cocaine). Despite fulfilling certain criteria for drug dependence (i.e., tolerance and withdrawal), caffeine is not considered a drug of abuse (Fredholm et al., 1999; Nehlig, 1999). There is, however, the possibility of a link between caffeine use and abuse of illicit and illicit drugs of abuse. For example, a positive association was found between tobacco, alcohol, and caffeine use (Istvan and Matarazzo, 1984; Brown and Benowitz, 1989; Swanson et al., 1994). Caffeine use has also been reported to change patterns of cocaine and amphetamine use (Budney et al., 1993; Kozlowski et al., 1993).

There are several experimental findings in animals to support the view that caffeine use may be a positive correlate in abuse of other psychoactive drugs. With experimental methods designed to measure stimulatory (ambulatory activity, schedule-controlled responding), subjective (drug discrimination), and reinforcing (drug self-administration, conditioned place preference) properties of drugs in animals, acute parenteral administration of caffeine resulted in an enhanced response to the effects of nicotine (White, 1988) and cocaine or amphetamine (White and Keller, 1984; Misra et al., 1986; Logan et al., 1989; Gauvin et al., 1990; Horger et al., 1991; Comer and Carroll, 1996; Schenk et al., 1996). In experiments designed to model the two main characteristics of caffeine use in humans (i.e., chronic use and oral route of administration), chronic caffeine exposure had no effect on the stimulatory effects of amphetamine and cocaine on locomotor activity (Holtzman, 1983; Finn and Holtzman, 1987; Holtzman and Finn, 1988), whereas it potentiated the response rate-increasing effects of these drugs in rats responding under a fixed-interval schedule of food reinforcement (Jaszyna et al., 1998). Furthermore, chronic caffeine exposure accelerated the acquisition of self-administration of cocaine (Carroll and Lac, 1998) and nicotine (Shoaib et al., 1999) but not the rate of acquisition of nicotine discrimination (Gasior et al., 1999). These interactions, however, were assessed in rats that were exposed to relatively high daily doses of caffeine (>100 mg/kg/day).

The present study was undertaken to extend findings by Holtzman’s group and those from our laboratory (Gasior et al., 1999) by assessing behavioral effects of psychomotor stimulants in rats chronically exposed to moderate or low daily doses of caffeine in their drinking water that were shown to produce plasma caffeine levels comparable to those in moderate and low consumers of caffeinated beverages. Specifically, the effects of nicotine, amphetamine, cocaine, and caffeine on ambulatory activity were assessed in rats chronically exposed to either 0.25 or 1.0 mg/ml concentrations of caffeine in their drinking water. In parallel, rates of acquisition of a nicotine discrimination and the pharmacological characteristics of the established nicotine cue in generalization tests with amphetamine and cocaine were assessed in rats chronically exposed to these concentrations of caffeine in their drinking water.

Materials and Methods

Subjects. Experimentally naive, male, Sprague-Dawley rats, weighing 250 to 280 g at the beginning of the study, were used. Rats were acclimated to laboratory conditions and allowed to drink tap water and feed ad libitum for at least 2 weeks before starting experiments. All rats were housed in a temperature- and humidity-controlled room with a 12-h light/dark cycle (7:00 AM to 7:00 PM lights on). Rats were housed individually throughout the experiment in stainless steel cages with clear plastic tubes with sawdust bedding and wire mesh covers. Experiments were conducted between 10:00 AM and 6:00 PM.

Animals used in this study were maintained in facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care and all experimentation was conducted in accordance with the guidelines of the Institutional Care and Use Committee of the National Institute on Drug Abuse, National Institutes of Health, and the Guide for Care and Use of Laboratory Animals (National Research Council, 1996). Drugs. Nicotine (L-nicotinic hydrogen tartrate) and caffeine (caffeine anhydride base) were purchased from Sigma Chemical Company (St. Louis, MO). Amphetamine (L-ampetamine sulfate) and cocaine (cocaaine hydrochloride) were obtained from the National Institute on Drug Abuse (Rockville, MD). All drugs were dissolved in sterile saline and administered either subcutaneously (nicotine) or intraperitoneally (caffeine, amphetamine, cocaine) in a volume of 1.0 ml/kg of body weight. The pH of nicotine solutions was adjusted to 7.0 with dilute NaOH. Doses of the drugs were expressed as milligrams of free base (nicotine and caffeine) or salt (amphetamine and cocaine) per kilogram of body weight measured before each injection. In addition to the parenteral administration, caffeine was administered orally in the drinking water (see below).

All drugs, with the exception of cocaine, were administered in doses that produced inverted U-shaped dose-effect functions on ambulatory activity or rates of operant responding, as confirmed by literature searches and previous experiments in our laboratory (Jaszyna et al., 1998; Gasior et al., 1999). Because of the deleterious effects of cocaine injections on the peritoneum of the rats and the convulsant effects of high doses of cocaine, the range of cocaine’s doses used was reduced to the necessary minimum. All doses of a given drug were administered in a randomized order for any given subject.

Chronic Caffeine Exposure. Caffeine was administered chronically by giving the animals free access to bottles containing either 0.25 or 1.0 mg/ml caffeine anhydrate base solution in tap water. Caffeine intake was monitored throughout the experiment. Daily caffeine intake (mg/kg/day) was estimated once every week based on the subject’s fluid consumption over a 24-h period and its body weight. Daily water intake in rats not exposed to caffeine (control group) was monitored for comparison. The rats were allowed at least 1 week to acclimate to the caffeine solutions in their home cages before starting experiments.

Design of the Experiment. There were three groups of rats, each receiving a different drinking solution. The control group received tap water as a drinking solution (water-drinking group). There were two groups of rats exposed to caffeine: one group received 0.25 mg/ml caffeine solution (0.25-CAFF group) and the other one received 1.0 mg/ml caffeine solution (1.0-CAFF group) as a drinking solution throughout the experiment. Rats were assigned randomly to each of these groups.

The behavioral effects of the drugs were measured and compared in two separate experiments as described below. For each experiment, separate groups of water- and caffeine-drinking rats were used.

Ambulatory Activity. Forty-eight rats were given free access to food. The effects of nicotine and caffeine were tested in 24 rats. The effects of amphetamine and cocaine were tested in the remaining 24 rats. In each of the 24-rat groups, there were three groups: a water-drinking group, a 0.25-CAFF group, and a 1.0-CAFF group (n = 8 rats/group). Before any drug injection, rats were habituated to the locomotor activity apparatus during a 15-min test session for five consecutive days. On each of the 8 days, each rat received either an
intraperitoneal or subcutaneous injection of 0.3 ml of saline immediately before being placed in the chamber.

A Columbus Instruments Auto-Track system (Coulbourn Instruments, Lehigh Valley, PA) with a resolution of 0.1 s was used to record locomotor activity. Each of three sound-attenuation chambers enclosed two clear, Plexiglas cages (26.4 × 26.4 cm, 44 cm in height) bedded with sawdust. The cages were positioned vertically within the chamber, one cage resting on the floor of the chamber, the other on a shelf midway up the chamber. These cages were equipped with a 15 × 15 array of photocells spaced every 2.4 cm near the base of the cage. Vertical-activity monitoring bars were lined with a strip of photocells located approximately 15 cm above the floor. Any movement that interrupted a photobeam was recorded as an activity count, and this event provided no feedback to the rat. A dim red light (power indicator light) was positioned above each cage. Two measures of locomotor activity were assessed: horizontal activity and vertical activity. Horizontal activity was defined as the number of horizontal beams that was interrupted by the subject during a particular interval. Vertical activity was defined as the number of vertical beams that was interrupted by the subject during a particular interval.

After an injection of a test drug (or its vehicle), rats were immediately placed into the locomotor activity chambers. Each session lasted 60 min and data were collected in 15-min blocks. Because each drug was tested after a pretreatment time of 10 min in the nicotine discrimination study (see below), data collected during the first 15 min were not included in statistical analysis to ensure drug effects were measured at comparable pretreatment times in both studies.

Nicotine Discrimination Study. Twenty-four rats were kept on a restricted diet to maintain their weights at about 80 ± 5.0% of the weight of age-matched control rats. The rats of all three groups (water-drinking, 0.25-CAFF, and 1.0-CAFF) were simultaneously tested for their ability to acquire the discrimination (rate of discrimination) of a fixed dose of nicotine (0.4 mg/kg) versus saline. After all rats acquired the nicotine discrimination, a generalization gradient to different doses of nicotine administered 10 min before the test session was determined. Finally, a generalization gradient to different doses of the nontraining drugs amphetamine and cocaine was studied.

The technical parameters of operant training and subsequent testing of drugs in the present study were identical to those described in Gasior et al. (1999) to make data from both studies comparable. Specifically, standard operant chambers (Coulbourn Instruments) were located singly in sound-attenuating plastic cubicles. Each chamber contained two levers separated by a recessed tray into which a pellet dispenser could deliver food pellets (BioServe, Free- town, NJ), a house light that was centrally mounted on the front wall below the ceiling, and a device producing white noise to mask extraneous sounds. Each press of a lever with a force of 0.4 N through 1 mm was recorded as a response and was accompanied by an audible click. The operant chambers were controlled by a computer using MED-PC software (Med Associates, Inc., East Fairfield, VT).

The rats were trained to press the lever for food on a fixed-ratio (FR) schedule of reinforcement 5 days a week (Monday through Friday). At the start of each session, the white house light was turned on and in its presence 10 consecutive responses on the active lever delivered a 45-mg food pellet (a fixed-ratio 10 schedule; FR10) and initiated a 3-s time-out during which lever presses had no programmed consequences and the chamber was dark. After each time-out, the house light was turned on and food was again available. Each session lasted 15 min. The location of the active lever (left versus right) was randomly changed each session to reduce position preferences during the training period. Once all 24 rats responded reliably under the FR10 schedule, they were randomly divided into three equally sized groups: a water-drinking group, a 0.25-CAFF group, and a 1.0-CAFF group (n = 8/group). After a 2-week habituation period, nicotine discrimination training (acquisition phase) was started. Water- and caffeine-drinking rats were trained under the FR10 schedule of food delivery to respond on one lever after an injection of nicotine (0.4 mg/kg) and on the other lever after injection of an equivalent volume of saline vehicle. For half the rats in each group, the right lever was the drug lever and for the other half the left lever was the drug lever. This assignment remained constant throughout the study. Injections of nicotine or saline were given 10 min before the session.

During discrimination training, 10 consecutive responses on the stimulus-appropriate (correct) lever resulted in delivery of a food pellet. Responses on the stimulus-inappropriate (incorrect) lever were recorded but had no programmed consequences other than to reset the FR requirement on the active lever. There were an equal number of nicotine and saline sessions during each 2-week period of training and neither nicotine nor saline sessions prevailed for more than three consecutive sessions. Discrimination training continued until an animal concurrently met two criteria of stimulus control during eight consecutive training sessions: 1) at least 90% of responses during the session on the correct lever, and 2) no more than four responses on the incorrect lever during the first trial. The number of sessions required to reach both criteria for stimulus control was calculated for each rat. Test sessions with other doses of nicotine or other drugs were not started until all 24 rats in the three groups met the criteria for stimulus control, to ensure the same duration of handling, caffeine exposure, and nicotine history.

Test sessions were identical to training sessions with the exception that both levers were active and 10 consecutive responses on either lever resulted in delivery of a food pellet. There were no more than two test sessions conducted per week (usually on Tuesdays and Fridays) and there were regular training sessions with either the training dose of nicotine or saline injections conducted on the other days to ensure robust stimulus control. If a rat failed to meet the criteria for stimulus control during one of the training sessions, it remained in the training condition until at least five consecutive sessions were completed in which the criteria for stimulus control were met.

In generalization tests, rats were injected with different doses of a drug (including injection with drug vehicle) 10 min before the session to determine the degree to which the drug generalized to the training dose of nicotine. After all doses of one drug were tested, a 1-week washout period was allowed before the next drug was tested. During this 1-week period, rats continued under the training condition.

Measurement of Plasma Levels of Nicotine and Cotinine. Groups of rats were maintained on a restricted diet and were continuously exposed to either tap water or caffeine solutions (0.25 and 1.0 mg/ml). Daily caffeine intake was monitored once per week. After 3 weeks, the rats received a single subcutaneous injection of 0.4 mg/kg nicotine 10 or 60 min before decapitation. Immediately after decapitation, blood samples (mixture of arterial and venous blood) were collected into 10-ml sterile tubes containing ethylenediamine- neteracetic acid as an anticoagulant. Tubes were centrifuged at 3500 rpm/min for 15 min to separate plasma from blood cells. Plasma samples were then transferred to transport tubes. Measurements of plasma concentrations of nicotine and cotinine were commercially performed at Labstat Incorporated (Kitchener, Ontario, Canada) according to the HPLC method described in detail by Jacob et al. (1981).

Measurement of Plasma Levels of Caffeine, Theophylline, Paraxanthine, and Theobromine. Separately maintained on a nonrestricted and restricted diet and were continuously exposed to either 0.25 or 1.0 mg/ml caffeine solution. After 3 weeks of caffeine exposure, all rats received a single subcutaneous injection of saline. After 10 min, the rats were sacrificed. Blood samples were collected and handled as described above. Plasma levels of caffeine and its metabolites were commercially performed at Labstat Incorporated according to the HPLC method described in detail by Muir et al. (1983).

Statistical Analysis of Experimental Data. The two measures of ambulatory activity (horizontal and vertical) after drug treatment
were expressed as a percentage of change from baseline activity. Baseline activity reflected the average of baseline activity recorded after saline injection during three 45-min control sessions, which were conducted before, during, and after the testing of a given drug. A baseline level for each individual rat was calculated separately and compared with data only for that specific rat when determining the percentage of baseline activity for a given drug. Changes from baseline activity in individual rats were then averaged for each of the experimental groups and expressed as mean ± S.E.M.

In the nicotine-discrimination study, there were two independent measures of behavior: a measure of discrimination performance (percentage of nicotine-appropriate responses) and a measure of motor performance (response rate). The percentage of nicotine-appropriate responses during each training or test session was obtained by dividing the number of responses on the nicotine-appropriate lever by the total number of responses on both levers during a session and was expressed as a mean ± S.E.M. of absolute percentages of nicotine-appropriate lever selections. Response rate (responses/s) during each session was calculated by dividing total number of responses on both levers during a session by total session length (900 s) and was expressed as a mean ± S.E.M. percentage change from baseline rates of responding. For each tested drug, a baseline rate of responding was measured in individual rats treated with saline instead of drug. Nicotine-appropriate lever selection data were excluded from analysis if a rat emitted less than 10 responses during the test session; response rate was denoted as zero in such a case and was included for analysis of changes in rates of responding. Estimated daily caffeine intake as well as plasma levels of nicotine, cotinine, and caffeine were expressed as mean ± S.E.M. A ratio of nicotine/cotinine levels was calculated by dividing a plasma level of nicotine by a plasma level of cotinine in an individual rat. Then, ratios were averaged for each experimental group and expressed as mean ± S.E.M.

Statistical analysis of data within a group was performed using one-way repeated measures ANOVA. Two-way repeated measures ANOVA on one repeated factor was used to determine differences in the effects of drugs among different experimental groups. When appropriate, post hoc analysis was performed using either Tukey’s or Dunnett’s test following one-way ANOVA on one repeated factor. See Table 1 for the outcome of between-group comparisons of these dose-response functions. There were no qualitative or quantitative differences in the patterns of locomotor activity in water-drinking, 0.25-CAFF, and 1.0-CAFF groups after saline injection during control sessions.

![Ambulatory Activity: Changes in Activity Levels after Increasing Doses of Caffeine and Nicotine](image)

**Results**

**Ambulatory Activity: Activity Levels over a 5-Day Habitation Period.** A steady decrease in horizontal activity from approximately 3750 to 2500 counts was observed in water-drinking, 0.25-CAFF, and 1.0-CAFF groups during 15-min habitation sessions conducted daily for 5 days. The levels of ambulatory activity stabilized by the third day. There were no significant differences between the water-drinking and caffeine-drinking rats over the habituation period ($F_{(2,150)} = 0.133, P = .875$) (data not shown).

**Ambulatory Activity: Effects of Acute Caffeine.** Parenteral caffeine produced biphasic (i.e., inverted U-shaped) and dose-dependent changes in all three measures of ambulatory activity with maximal increases in activity after 10 and 30 mg/kg caffeine in water-drinking and 0.25-CAFF groups. In contrast, chronic exposure to the 1.0 mg/ml caffeine solution attenuated the locomotor stimulating effects of acute caffeine, because there were no statistically significant increases in ambulatory activity after acute caffeine (Fig. 1). The latter is further supported by statistically significant “drinking × caffeine-dose” interaction (Table 1), indicative of qualitative changes in the dose-response functions for acute caffeine.

There were also significant quantitative differences in the response to graded doses of caffeine among the water- and caffeine-drinking rats in the case of horizontal and vertical activity (Table 1). Specifically, acute caffeine produced a greater response (upward shift) in the water-drinking and 0.25-CAFF groups relative to the 1.0-CAFF group ($P < .05$, Dunnett’s test).

**Ambulatory Activity: Effects of Acute Nicotine.** Graded doses of nicotine significantly and dose dependently affected horizontal and vertical activity in all three experi-
TABLE 1
Ambulatory activity: ANOVA table for the effects of drugs on ambulatory activity in water- and caffeine-drinking rats
Shown are the F values, degrees of freedom (df), and significance levels of difference (P values) revealed by ANOVA. Two-way repeated measures ANOVA on one repeated factor was used to depict quantitative (main effect) and qualitative (interaction) differences in the effects of drugs on the two measures of ambulatory activity in water, 0.25-CAFF, and 1.0-CAFF groups. The dose-response functions are plotted in Figs. 1 and 2.

<table>
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<tr>
<th>Drug</th>
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<th>Vertical Activity</th>
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<td></td>
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Ambulatory Activity: Effects of Acute Amphetamine. Graded doses of amphetamine produced dose-dependent and biphasic changes in horizontal and vertical activity in water- and caffeine-drinking rats (Fig. 2) with maximal increases after 1.0- and 3.0-mg/kg doses. Only with horizontal activity were qualitative changes in the effects of amphetamine on ambulatory activity between groups revealed by ANOVA (P > .05 for “drinking × nicotine-dose” interaction, Table 1).

There were, however, quantitative differences in the effects of nicotine among the three experimental groups, i.e., the 0.25-CAFF group was more sensitive to the stimulatory effects of nicotine relative to other experimental groups (Table 1). Specifically, a statistically significant upward shift of the dose-effect function was observed in the 0.25-CAFF group relative to the water group in the case of horizontal and vertical activity.

Ambulatory Activity: Effects of Acute Cocaine. The pattern of cocaine-induced changes was characterized by proportional increases in horizontal and vertical activity after graded doses of cocaine (Fig. 2). Despite the apparent trend for the 0.25-CAFF group to show higher levels of activity relative to the water-drinking and 1.0-CAFF groups (Fig. 2), no significant differences were found by ANOVA among the experimental groups (Table 1), probably due to large standard errors. Only with horizontal activity was a statistically significant “drinking × cocaine-dose” interaction revealed by ANOVA (Table 1), indicative of qualitative differences in the dose-effect functions produced by cocaine in the groups studied.

Nicotine Discrimination: Rate of Acquisition. All 24 rats met the criteria for stimulus control (Fig. 3). There were, however, significant differences among the groups in the number of training sessions necessary to meet the criteria for stimulus control (F<sub>2,21</sub> = 10.645, P < .001). The 0.25-CAFF group showed the fastest rate acquisition of discriminative-stimulus control. The mean ± S.E.M. number of sessions required in this group was 25.8 ± 1.76 and was significantly smaller (P < .05) from that in the water group (40.4 ± 3.03) (Fig. 3, bottom). Relative to the water-drinking group, the 1.0-CAFF group needed a comparable number of training sessions to meet the criteria of stimulus control (mean ± S.E.M., 34.8 ± 1.75) (P > .05 versus water group).

Chronic caffeine exposure did not affect rates of responding (Fig. 4). At the end of acquisition training, response rates in water- and caffeine-drinking groups did not differ after nic-
otine ($F_{2,21} = 0.467, P = .634$) or saline injection ($F_{2,21} = 0.399, P = .676$).

**Nicotine Discrimination: Dose-Response Tests with the Training Drug Nicotine.** The percentage of nicotine-appropriate lever-press responses increased in a dose-dependent manner in water- and caffeine-drinking groups (Fig. 5, top). Comparison of the dose-response functions for nicotine revealed no quantitative (main effect, $P > .05$) nor qualitative changes (drinking $\times$ nicotine-dose interaction, $P > .05$) in the effects of graded doses of nicotine (0.025–0.8 mg/kg) in water-drinking, 0.25-CAFF, and 1.0-CAFF groups (Table 2). Furthermore, there were no statistical differences in the potency of nicotine as a discriminative stimulus among the three experimental groups (Table 3).

Nicotine in a dose range of 0.025 to 0.4 mg/kg had little effect ($P > .05$) on rates of responding, whereas the 0.8-mg/kg dose of nicotine markedly ($P < .05$) depressed responding (Fig. 5, bottom). Nonetheless, there were no significant differences in the effects of graded doses of nicotine on response rates between water- and caffeine-drinking rats (Table 2). Likewise, the potency of nicotine to reduce response rates was comparable ($P > .05$) in all three groups (Table 3). There was however a separation between the potency of nicotine as a discriminative stimulus and its potency in reducing rate of responding (Table 3), indicating that these two behavioral measures are independent.

**Nicotine Discrimination: Generalization of Amphetamine and Cocaine to the Nicotine Discriminative Cue.** Both amphetamine and cocaine (Fig. 6) generalized in a dose-dependent manner to the 0.4 mg/kg nicotine cue in water- and caffeine-drinking rats. The maximum percentage of nicotine-appropriate responses was engendered by 1.7 mg/kg amphetamine and 10 and 13 mg/kg cocaine. Rats exposed to the 0.25 mg/ml caffeine solution appeared more sensitive to the effects of intermediate doses of amphetamine and cocaine than water-drinking control rats, resulting in statistically significant differences among the groups (Table 2). Specifically, there was a statistically significant upward shift of the dose-response function of amphetamine and cocaine in the 0.25-CAFF group (but not the 1.0-CAFF group) relative to the water-drinking group without any qualitative changes in the dose-response function (Table 2). This was further confirmed by a statistically significant difference ($P < .05$) in the potency of both amphetamine and cocaine in the 0.25-CAFF group (but not the 1.0-CAFF group) relative to the water-drinking group (Table 3).

Neither amphetamine nor cocaine had any effect on rates of responding after low and intermediate doses (Fig. 6). Higher doses of both drugs, however, disrupted responding. The 3.0-mg/kg dose of amphetamine and the 17-mg/kg dose of cocaine almost completely suppressed responding in all three experimental groups. A comparison of the effects of amphetamine and cocaine on rates of responding revealed no significant qualitative or quantitative differences ($P > .05$) among
the three experimental groups when the dose-response functions (Table 2) and potencies (Table 3) of these drugs were analyzed. There was, however, a separation between the potencies of amphetamine and cocaine in the generalization test and their potencies in depressing rates of responding (Table 3), indicative of the independence of these two behavioral measures.

**Nicotine Discrimination: Plasma Levels of Nicotine and Cotinine.** Plasma levels of nicotine and cotinine in water-drinking, 0.25-CAFF, and 1.0-CAFF groups were comparable regardless of whether nicotine was administered 10 or 60 min before blood sampling (Table 4). In all three groups, plasma levels of nicotine 60 min after administration were about 38.5% lower relative to those 10 min after administration, whereas the levels of cotinine increased by about 80%. This resulted in different nicotine/cotinine ratios 10 min (ratio: 3) and 60 min (ratio: 1) after nicotine administration in the experimental groups (Table 4). However, nicotine/cotinine ratios were comparable across the three experimental groups both 10 and 60 min after nicotine administration (Table 4).

**Ambulatory Activity and Nicotine Discrimination: Caffeine Intake and Plasma Levels.** Daily caffeine intakes in caffeine-drinking rats are plotted in Fig. 7. In the free-feeding groups (ambulatory activity study), caffeine intakes ranged from 18.3 ± 0.86 mg/kg/day to 27.0 ± 2.76 mg/kg/day in the 0.25-CAFF group and from 66.0 ± 3.69 mg/kg/day to 97.5 ± 7.71 mg/kg/day in the 1.0-CAFF group. Due to lower fluid intakes in the food-deprived groups (nicotine discrimination study), daily caffeine intakes were lower relative to the free-feeding groups exposed to the corresponding caffeine concentrations. Specifically, they ranged from 8.72 ± 1.28 mg/kg/day to 15.7 ± 1.42 mg/kg/day in the 0.25-CAFF group and from 33.0 ± 1.36 mg/kg/day to 57.6 ± 4.72 mg/kg/day in the 1.0-CAFF groups. Plasma levels of caffeine corresponded to daily caffeine intake and they are listed in Table 5.

**Discussion**

Chronic exposure to a low (0.25 mg/ml), but not a higher (1.0 mg/ml), concentration of caffeine in the drinking water of rats in the present study changed the behavioral responses to psychomotor stimulant drugs in ambulatory activity and in the nicotine discrimination paradigm. In agreement with previous reports (Holtzman, 1983; Finn and Holtzman, 1987; Antoniou et al., 1998), nicotine, caffeine, and amphetamine increased ambulatory activity at intermediate doses and decreased it at higher doses, whereas cocaine produced only increases in ambulatory activity over the range of doses tested. Rats chronically exposed to a low 0.25 mg/ml caffeine solution responded to nicotine, amphetamine, or cocaine with increases in activity higher than water-drinking control rats, whereas the response to caffeine injections was unchanged. In contrast, chronic exposure to a higher 1.0 mg/ml caffeine solution did not alter the stimulatory effects of nicotine, amphetamine, or cocaine on ambulatory activity but did result in the development of insurmountable tolerance to the stimulatory effects of parenterally administered caffeine.

In the nicotine discrimination paradigm, the rate of acquisition of the nicotine discrimination and the findings of partial generalization of amphetamine and cocaine to the nicotine cue in the water-drinking rats were similar to previous reports (Chance et al., 1977; Stolerman et al., 1984; Rosecrans, 1989; Desai et al., 1999; Gasior et al., 1999). Rats chronically exposed to the low, but not to the high, caffeine concentration in the present study acquired the nicotine discrimination at a significantly faster rate than water-drinking rats and appeared more sensitive to the effects of amphetamine and cocaine in substitution tests. Thus, exposure to two different caffeine solutions resulted in quantitatively distinct changes in drug-induced nonoperant and operant measures of behavior: exposure to the low, but not to the high, concentration of caffeine potentiated the behavioral effects of psychomotor stimulant drugs. Further studies with even lower levels of caffeine exposure are needed to determine whether the effects observed with the 0.25 mg/ml concentration of caffeine represent the maximum effect of chronic exposure to low doses of caffeine.
The present findings both confirm and extend previous reports. Finn and Holtzman (1987) found that rats continuously exposed to 1.0 mg/ml caffeine added to the drinking water developed complete tolerance to the stimulatory effects of caffeine but not to amphetamine and cocaine. In the present study, there was no cross-tolerance to the effects of nicotine (1.0 mg/kg) in NIH Swiss mice chronically exposed to a 1.0 mg/ml caffeine solution. This finding extends a previous observation on the lack of cross-tolerance to the effects of nicotine (225–6 mg/kg) on ambulatory activity in rats exposed chronically to a 1.0 mg/ml caffeine solution. This finding extends a previous observation on the lack of cross-tolerance to the effects of nicotine (1.0 mg/kg) in NIH Swiss mice chronically exposed to a 1.0 mg/ml caffeine solution (Nikodijevic et al., 1993).

The present study also provides the first characterization of significant changes in effects of psychomotor stimulant drugs on ambulatory activity of rats chronically exposed to very low levels of caffeine. Exposure to a low 0.25-mg/ml concentration of caffeine in the drinking water resulted in an average daily dose of caffeine of 23 mg/kg and an average plasma level of 1.45 μg/ml. Unlike with the 1.0 mg/kg caffeine solution, tolerance to the stimulatory effects of caffeine failed to develop. Similarly, Holtzman and Finn (1988) reported incomplete tolerance to the stimulatory effects of caffeine on ambulatory behavior in rats chronically exposed to a 0.25 mg/ml caffeine solution. In the present study, rats exposed to the low concentration of caffeine solution also showed enhanced stimulatory effects of nicotine and amphetamine on ambulatory behavior compared with water-drinking rats; a similar trend was observed with cocaine. These findings were somewhat unexpected given that chronic exposure to higher doses of caffeine has not been found to modify the ambulatory response to amphetamine, cocaine, or nicotine (Holtzman, 1983; Finn and Holtzman, 1987; present study). Taken together, the present study together with previous reports shows that the effects of psychomotor stimulant drugs on ambulatory activity qualitatively differ (potentially versus no effect) in rats exposed to low (0.25 mg/ml) compared with higher (0.5, 1.0, or 3.0 mg/ml) caffeine concentrations. Furthermore, exposure to the low compared with the higher caffeine concentrations also produced qualitative differences in the stimulatory effects of acute parenteral caffeine (no tolerance versus complete insurmountable tolerance).

Qualitative differences in behavioral responses to psychomotor stimulant drugs resulting from chronic exposure to different caffeine concentrations were also demonstrated with a two-lever choice nicotine-discrimination paradigm (Gasior et al., 1999; present study). Chronic exposure to the low caffeine concentration (0.25 mg/ml) in the present study significantly facilitated the acquisition of a nicotine discrimination, whereas higher caffeine concentrations (1.0 and 3.0 mg/ml) had no significant effect on the rate of acquisition. Furthermore, rats chronically exposed to the 0.25 mg/ml (but not 1.0 mg/ml) caffeine solution were more sensitive to the
Effects of amphetamine and cocaine when they were substituted for the nicotine training cue relative to water-drinking group. In marked contrast, amphetamine and cocaine failed to generalize to the nicotine cue in rats chronically exposed to a 3.0 mg/ml caffeine solution (Gasior et al., 1999). Thus, depending on the concentration of caffeine, the ability of amphetamine and cocaine to generalize to the nicotine cue changed in a biphasic manner from potentiation through no effect to attenuation in rats chronically exposed to 0.25, 1.0, and 3.0 mg/ml caffeine concentrations, respectively. In contrast, none of the caffeine solutions appeared to affect sensitivity to the training drug nicotine, when nicotine dose was varied after acquisition of the discrimination. This, however, would not be expected with the constant retraining after acquisition of the nicotine discrimination that is part of the experimental design. It should be noted that the enhanced responses to nicotine, amphetamine, and cocaine with the food-deprived rats exposed to the low 0.25 mg/ml caffeine solution in the nicotine-discrimination study occurred at average caffeine plasma levels of 0.37 mg/ml that were lower than the already low caffeine levels (1.45 mg/ml) in the non-food-deprived rats exposed to the same caffeine solution in the ambulatory-activity study. Plasma levels of caffeine in both studies, however, were in agreement with plasma levels of 0.25 to 2.0 mg/ml that would be produced by a single cup of coffee in humans (Fredholm et al., 1999).

**Fig. 6.** Nicotine discrimination: dose-response functions for the discriminative-stimulus generalization of amphetamine and cocaine to the nicotine cue in water- and caffeine-drinking rats. Amphetamine, cocaine, and saline were administered intraperitoneally 10 min before the test session. See Fig. 5 for other details. See Table 2 for the outcome of between-group comparisons and Table 3 for ED50 values calculated, where appropriate, from these dose-response functions. *, performance significantly (P < .05) different from vehicle (Dunnett's test following one-way repeated measures ANOVA.

**TABLE 4**
Plasma levels of nicotine and cotinine (ng/ml)
Shown are the mean ± S.E.M. plasma levels of nicotine and cotinine 10 and 60 min after a single subcutaneous injection of nicotine (0.4 mg/kg) in the three experimental groups (number of rats per group as indicated by the n value). The ratio of plasma levels of nicotine and cotinine was calculated in individual animals and then expressed as a group mean ± S.E.M. value.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pretreatment Time</th>
<th>n</th>
<th>Nicotine (ng/ml)</th>
<th>Cotinine (ng/ml)</th>
<th>Ratio (Nicotine/Cotinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water group</td>
<td>10 min</td>
<td>11</td>
<td>117 ± 4.57</td>
<td>41.9 ± 3.87</td>
<td>3.06 ± 1.24</td>
</tr>
<tr>
<td>0.25-CAFF group</td>
<td>10 min</td>
<td>8</td>
<td>115 ± 2.73</td>
<td>37.8 ± 1.82</td>
<td>3.09 ± 0.46</td>
</tr>
<tr>
<td>1.0-CAFF group</td>
<td>10 min</td>
<td>8</td>
<td>114 ± 8.96</td>
<td>44.4 ± 5.57</td>
<td>2.80 ± 0.50</td>
</tr>
<tr>
<td>Water group</td>
<td>60 min</td>
<td>10</td>
<td>72.3 ± 3.33</td>
<td>72.4 ± 3.50</td>
<td>1.02 ± 0.06</td>
</tr>
<tr>
<td>0.25-CAFF group</td>
<td>60 min</td>
<td>10</td>
<td>69.2 ± 4.04</td>
<td>72.2 ± 2.82</td>
<td>0.96 ± 0.05</td>
</tr>
<tr>
<td>1.0-CAFF group</td>
<td>60 min</td>
<td>10</td>
<td>71.7 ± 4.62</td>
<td>79.3 ± 2.28</td>
<td>0.91 ± 0.05</td>
</tr>
</tbody>
</table>
The complex effects produced by caffeine are not likely to be explained by a single mechanism (Fredholm et al., 1999). Like behavioral effects, the molecular actions of caffeine depend on dose. In doses that result from dietary consumption of coffee, caffeine acts as a competitive, nonselective A1/A2 adenosine-receptor antagonist. In higher doses, caffeine additionally inhibits phosphodiesterase activity and thus increases levels of cAMP. In very high doses, caffeine can also inhibit calcium uptake and stimulate release of calcium from the sarcoplasmic reticulum and, thus, stimulate the release of different neurotransmitters. Recent research implicates the involvement of dopaminergic systems in caffeine-induced behavioral effects (for review, see Garrett and Griffiths, 1997) based on the anatomical colocalization and the existence of an antagonistic interaction between adenosine and dopamine receptors in the brain (Ferre et al., 1992). Specifically, caffeine, by blocking A1 and A2 adenosine receptors, can remove the inhibitory tone of endogenous adenosine from D1 and D2 dopamine receptors (Ferre et al., 1992; Fredholm et al., 1999). These effects can also depend on caffeine dose. For example, involvement of dopaminergic activation in the discriminative stimulus effects of the low (10 mg/kg), but not the high (56 mg/kg), dose of caffeine has been postulated (Powell et al., 1999). Of note, stimulation of dopaminergic neurotransmission appears to be the final common mechanism of action (Pich et al., 1997) linked to the behavioral effects of amphetamine, cocaine, and nicotine (also see discussion in Jaszyma et al., 1998; Gasior et al., 1999). One can speculate that chronic exposure to low caffeine doses resulted in an enhanced behavioral response to nicotine, amphetamine, and cocaine in the present study as a result of selective potentiation of a dopaminergic component mediating the behavioral effects of these drugs. However, chronic caffeine exposure can also affect functioning of other neurotransmitter systems (Shi et al., 1993; Jacobson et al. 1996; Fredholm et al., 1999) and the involvement of these other neurotransmitter systems cannot be ruled out. Further studies are needed to find molecular and anatomical substrates differentially affected by chronic exposure to different doses of caffeine.

In summary, the present study provides the first evidence that very low levels of chronic caffeine exposure can potentiate the behavioral effects of common drugs of abuse. This seems to be opposite to a general belief that low, unlike high, doses of caffeine are generally ineffectual and harmless. It is important to note that plasma levels of caffeine in the

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**TABLE 5**

Average (±S.E.M.) water intake, caffeine intake, and plasma levels of caffeine in rats maintained on ad libitum or restricted diet

<table>
<thead>
<tr>
<th>Group</th>
<th>Food Availability</th>
<th>n</th>
<th>Water Intake (ml/kg/day)</th>
<th>Caffeine Intake (mg/kg/day)</th>
<th>Caffeine Plasma Levels (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water group</td>
<td>Ad libitum</td>
<td>10</td>
<td>102 ± 8.2</td>
<td>23.1 ± 1.0</td>
<td>1.45 ± 0.36</td>
</tr>
<tr>
<td>0.25-CAFF group</td>
<td>Ad libitum</td>
<td>10</td>
<td>107 ± 9.8</td>
<td>90.5 ± 6.6</td>
<td>81.9 ± 3.5</td>
</tr>
<tr>
<td>1.0-CAFF group</td>
<td>Ad libitum</td>
<td>10</td>
<td>90.5 ± 6.6</td>
<td>52.2 ± 1.4</td>
<td>42.5 ± 1.1</td>
</tr>
<tr>
<td>Water group</td>
<td>Restricted</td>
<td>6</td>
<td>61.8 ± 1.3</td>
<td>43.3 ± 1.1</td>
<td>43.3 ± 1.1</td>
</tr>
<tr>
<td>0.25-CAFF group</td>
<td>Restricted</td>
<td>6</td>
<td>52.2 ± 1.4</td>
<td>43.3 ± 1.1</td>
<td>42.5 ± 1.1</td>
</tr>
<tr>
<td>1.0-CAFF group</td>
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<td>6</td>
<td>43.3 ± 1.1</td>
<td>43.3 ± 1.1</td>
<td>42.5 ± 1.1</td>
</tr>
</tbody>
</table>

n, number of animals per group.
present study are well within the levels found in humans exposed to relatively low doses of caffeine as a result of habitual consumption of caffeine-containing products (see Introduction). The present finding that low levels of chronic caffeine exposure can facilitate acquisition of the nicotine discrimination may have important clinical implications for those individuals who begin to smoke cigarettes. Further clinical studies are needed to determine whether dietary consumption of caffeine-containing beverages is associated with increased use of tobacco and enhanced reactions to psychomotor stimulants. This is an important question given the wide spread availability of caffeine-containing beverages. The effects of chronic caffeine exposure on behavioral responses to other psychomotor stimulants uncovered in the present study and in previous studies (Jaszyra et al., 1998; Gasior et al., 1999; Shoab et al., 1999) add to the complexity of caffeine-induced effects, suggesting the involvement of specific neuroanatomical and molecular substrates for different behavioral measurements and different levels of chronic caffeine exposure.

References


