Acquisition of Nicotine Discrimination and Discriminative Stimulus Effects of Nicotine in Rats Chronically Exposed to Caffeine1,2

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ABSTRACT

Caffeine and nicotine are the main psychoactive ingredients of coffee and tobacco, with a high frequency of concurrent use in humans. This study examined the effects of chronic caffeine exposure on 1) rates of acquisition of a nicotine discrimination (0.1 or 0.4 mg/kg, s.c., training doses) and 2) the pharmacological characteristics of the established nicotine discrimination in male Sprague-Dawley rats. Once rats learned to lever-press reliably under a fixed ratio of 10 schedule for food pellets, they were randomly divided into two groups; 12 animals were maintained continuously on caffeine added to the drinking water (3 mg/ml) and another 12 control rats continued to drink tap water. In each group of water- and caffeine-drinking rats, there were six rats trained to discriminate 0.1 mg/kg of nicotine from saline and six rats trained to discriminate 0.4 mg/kg of nicotine from saline. Regardless of the training dose of nicotine, both water- and caffeine-drinking groups required a comparable number of training sessions to attain reliable stimulus control, although there was a trend for a slower acquisition in the caffeine-drinking group trained with 0.1 mg/kg of nicotine. Tests for generalization to different doses of nicotine revealed no significant differences in potency of nicotine between water- and caffeine-drinking groups. The nicotinic-receptor antagonist mecamylamine blocked the discriminative effects of 0.1 and 0.4 mg/kg nicotine with comparable potency and efficacy in water- and caffeine-drinking groups. There was a dose-related generalization to both the 0.1 and 0.4 mg/kg nicotine cue (maximum average of 51–83%) in water-drinking rats after i.p. treatment with d-amphetamine, cocaine, the selective dopamine uptake inhibitor GBR-12909, apomorphine, and the selective dopamine D1 receptor agonist SKF-82958, but not in caffeine-drinking rats (0–22%). There was no generalization to the nicotine cues after i.p. treatment with caffeine or the selective D2 (NPA) and D3 (PD 128,907) dopamine-receptor agonists in water- and caffeine-drinking rats. The dopamine-release inhibitor CGS 10746B reduced the discriminative effects of 0.4 mg/kg nicotine in water-drinking rats, but not in caffeine-drinking rats. There was no evidence of development of tolerance or sensitization to nicotine’s effects throughout the study. In conclusion, chronic caffeine exposure (average, 135 mg/kg/day) did not affect the rate of acquisition of the nicotine discrimination, but it did reduce the dopaminergic component of the nicotine-discriminative cue. The reduction of the dopaminergic component of the nicotine cue was permanent, as this effect was still evident after the caffeine solution was replaced with water in caffeine-drinking rats. That nicotine could reliably serve as a discriminative stimulus in the absence of the dopaminergic component of its discriminative cue may differentiate nicotine from “classical dopaminergic” drugs of abuse such as cocaine and amphetamine.

Epidemiological surveys in humans show that smokers tend to smoke more cigarettes while drinking coffee, and they also drink significantly more coffee than nonsmokers (Istvan and Matarazzo, 1984; Brown and Benowitz, 1989; Swanson et al., 1994, 1997). It is now generally accepted that this positive correlation can be ascribed to interactions between nicotine and caffeine, the main psychoactive constituents of tobacco and coffee, respectively (Rose and Behm, 1991; Swanson et al., 1994, 1997; Griffiths and Mumford, 1995). Little is known, however, of the neuropharmacological and psychopharmacological mechanisms or of their behavioral consequences that may contribute

ABBREVIATIONS: ACh, acetylcholine; FI, fixed interval; FR, fixed ratio.
to the concurrent use of nicotine and caffeine in humans. Pharmacokinetic factors such as a shorter half-life of caffeine or nicotine in coffee-drinking smokers or behavioral factors such as stress or anxiety can only partially explain their concurrent use (Brown and Benowitz, 1989; Swanson et al., 1994, 1997), suggesting that other factors are involved (Istvan and Mathur, 1984).

Both nicotine and caffeine, when administered alone, can have qualitatively comparable, often biphasic, dose-dependent effects on a variety of nonoperant and operant measures of behavior (for review, see Carney et al., 1985; Stolerman, 1990; Nehlig et al., 1992). These behavioral effects include, for example, increases in locomotor activity (Holtzman, 1983; Lee et al., 1987; Nikodijevic et al., 1993) and rates of schedule-controlled responding (White, 1988; Goldberg et al., 1989; Newland and Brown, 1997; Jaszyna et al., 1998) followed by decreases as doses of the drugs increase. Moreover, both nicotine and caffeine can each serve as discriminative stimuli (Winter, 1981; Stolerman et al., 1984; Carney et al., 1985; Rosecrans, 1989; Griffiths et al., 1990) and reinforcing stimuli under certain conditions (Goldberg and Henningfield, 1988; Heishman and Henningfield, 1992; Griffiths and Mumford, 1995; Rose and Corrigall, 1997) in laboratory animals and human subjects. Nicotine and caffeine, when coadministered acutely, have been shown to produce additive-in-nature stimulation of locomotor activity and increases in rates of operant responding maintained under a fixed-interval schedule of food reinforcement in rats (Lee et al., 1987; White, 1988) and monkeys (Howell and Landrum, 1997). Acute pretreatment with caffeine also produced significant increases in rates of responding for i.v. nicotine self-administration in squirrel monkeys (Yasar et al., 1997). Caffeine, however, is consumed chronically by humans, and behavioral responses to caffeine can be altered by repeated administration (see review by Jacobson et al., 1996), as observed with other psychomotor stimulant drugs (Goudie and Emmett-Oglesby, 1989; Stewart and Badiani, 1993), suggesting the need for more studies of the effects of chronic caffeine exposure on nicotine’s behavioral actions.

It has been well documented that daily exposure to “physiological” doses of caffeine (equivalent to the caffeine content in two to three cups of coffee) results in the development of tolerance to the diuretic, cardiovascular, and some, but not all, of the behavioral effects of caffeine in humans (Benowitz, 1990; James, 1991; Nehlig et al., 1992). Likewise, chronic caffeine exposure in rodents results in the development of tolerance to the stimulant effects of caffeine on locomotor activity and rates of food-reinforced responding, and its effects as a discriminative stimulus (Holtzman and Finn, 1988; Nikodijevic et al., 1993; Lau and Falk, 1994; Newland and Brown, 1997; Jaszyna et al., 1998). There is no clear predictive pattern of change in the behavioral effects of psychomotor-stimulant drugs that are produced by chronic caffeine exposure in experimental animals. For example, chronic caffeine exposure markedly potentiated the stimulatory effects of nicotine on locomotor activity (Shoaib et al., 1996), whereas the response to amphetamine and cocaine remained unchanged (Holtzman, 1983; Finn and Holtzman, 1987; Holtzman and Finn, 1988; Nikodijevic et al., 1993). In contrast, chronic caffeine exposure potentiated the response-rate increases produced by amphetamine and cocaine, but not by nicotine, in rats responding under a fixed interval (FI) schedule of food reinforcement (Jaszyna et al., 1998). Chronic caffeine exposure, however, increased rates of acquisition of i.v. self-administration of both nicotine (Shoaib et al., 1996) and cocaine (Horger et al., 1991) relative to control rats.

To our knowledge there are no published reports on how chronic caffeine exposure might change the discriminative stimulus properties of psychomotor stimulant drugs. There is some experimental evidence suggesting that acute presession treatment with caffeine can potentiate the discriminative stimulus effects of cocaine in rats (Harland et al., 1989; Gauvin et al., 1990), and combinations of caffeine with other over-the-counter drugs such as phenylethylamine can produce new entities distinct from their component elements and can mimic the discriminative cue of amphetamine and cocaine (Holloway et al., 1985; Gauvin et al., 1989). Thus, there are reasons to speculate that chronic caffeine exposure may change the subjective effects of psychomotor stimulants, including nicotine.

In the present study, we adopted from Holtzman (1983) and Jaszyna et al. (1998) a method of chronically exposing rats to caffeine in their drinking water to examine possible changes in the subjective effects of nicotine using a drug discrimination procedure. Drug discrimination procedures have been successfully used to examine subjective effects of a wide range of psychoactive drugs under a variety of experimental conditions in both animals (e.g., Colpaert, 1987; Samele et al., 1992) and humans (e.g., Kamien et al., 1993). With these procedures, discriminability of a drug (percentage of subjects acquiring discrimination) and rate of acquisition of the discrimination are first established and then qualitative properties of the discriminative cue are assessed in generalization tests with other drugs permitting identification of receptor(s) mediating discriminative-stimulus properties of the drug (e.g., Colpaert, 1987; Wiley et al., 1996). All three characteristics of the discriminative effects of nicotine in rats chronically exposed to caffeine, in comparison to those of in control rats, were evaluated in the present study.

Materials and Methods

Subjects. Thirty 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 free feed for 2 weeks. Their body weights were then reduced to about 80% of free-feeding by limiting access to food. Rats continued to be kept on a restricted diet to maintain their weights at about 80 ± 5.0% of the weight of age-matched control rats until the end of the study. Rats were housed individually in stainless steel cages in a temperature- and humidity-controlled room with a 12-h light/dark cycle (7:00–19:00 h lights on).

Drugs. The drugs and their sources were as follows: (−)-nicotine hydrogen tartrate (Sigma Chemical Company, St. Louis, MO), caffeine base (Sigma), mecamylamine HCl (Research Biochemicals Internatioanl, RBI, Natick, MA); CGS 10746B (5-(4-methyl-1-piperazinyl)-imidazo[2,1-b][1,3,5]-benzothiadiazepine maleate; a gift from Novartis Pharmaceutical Corp., Summit, NJ), d-amphetamine (National Institute on Drug Abuse, Rockville, MD), cocaine HCl (National Institute on Drug Abuse), GBR-12909 2HCl (1-[2-[bis(4-fluorophenyl)methoxyl]ethyl]4-[3-phenylpropyl]piperazinyl dichloride; RBI), R(−)-apomorphine HCL (R(−)-10,11-dihydroxyapomorphine hydrochloride; RBI), (±)-SKF-82958 HBr ((±)-6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide; RBI), R(−)-NPA HCl (R(−)-10,11-dihydroxy-N-v-propynloraporphine hydrochloride; RBI), (−)-PD 128,907 HCl (S(+)-(4R,10bR)-
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3,4,4a,10b-tetrahydro-4-propyl-2H,5H-[1]benzopyrano-[4,3-b]-1,4-oxazin-9-ol hydrochloride; RBI). GBR-12909 was suspended in 40% (w/v) hydroxypropyl-γ-cyclodextrin (RBI), CGS 10746B was dissolved in sterile water, and the remaining drugs were dissolved in sterile 0.9% NaCl saline. The pH of nicotine solutions was adjusted to 7.0 with dilute NaOH. When necessary, mild heat and sonication were applied to prepare solutions of the drugs. Except for nicotine and caffeine, doses of drugs are expressed as milligrams of salt per kilogram of body weight measured before the start of the session. Doses of nicotine and caffeine refer to the free base forms. The drugs were administered either s.c. (nicotine and mecamylamine) or i.p. (the remaining drugs) in a volume of 1.0 ml/kg of body weight.

**Apparatus.** Standard operant chambers (Coulbourn Instruments Inc., Lehigh Valley, PA) located singly in sound-attenuating plastic cubicles were used. Each chamber contained two levers separated by a recessed tray into which a pellet dispenser could deliver 45-mg food pellets (Bio-Serv, Inc., Frenchtown, 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 chamber was controlled by a computer using MED-PC software (Med Associates, Inc., East Fairfield, VT).

**Training Procedure.** Twenty-four rats were trained to press the lever for food on a fixed ratio (FR) schedule of reinforcement 5 days a week (Monday through Friday), always between 9:30 AM and 12:00 PM. 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 food pellet (a fixed-ratio 10 schedule; FR 10) and initiated a 3-s timeout during which lever presses had no programmed consequences and the chamber was dark. After each timeout, 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 for each session to reduce position preferences during the training period. Once all rats responded reliably under the FR 10 schedule, they were divided into two groups: water-drinking and caffeine-drinking rats. Twelve animals received free access in their home cages to caffeine in tap water (3 mg/ml caffeine anhydride base), whereas the other 12 control rats continued to drink tap water. Daily caffeine intake was estimated once every week throughout the remainder of the study based on the rat’s body weight and 24-h fluid consumption of the established caffeine concentration (mg/kg per individual rat). Daily water intake in water-drinking rats was monitored for comparison. Training was continued for 2 weeks to eliminate temporal effects of caffeine on behavior in rats.

**Acquisition of Nicotine Discrimination.** Water- and caffeine-drinking rats were then divided into four groups of six rats each and trained, as described previously (Shoaib and Stolerman, 1986), under the FR10 schedule of food delivery to respond on one lever after s.c. injection of nicotine and on the other lever after s.c. injection of an equivalent volume of saline vehicle. Two groups of rats (water- and caffeine-drinking) were trained to discriminate 0.1 mg/kg of nicotine from saline and two groups of rats (water- and caffeine-drinking) were trained to discriminate 0.4 mg/kg of nicotine from saline. 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 remained constant throughout the study. Injections of nicotine or saline were given s.c. 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 was considered to be under stimulus control, that is, when it completed eight consecutive sessions in which at least 90% of responses during the session were on the correct lever and no more than four responses occurred on the incorrect lever during the first trial. The number of sessions required to reach this criterion for stimulus control was calculated for each rat. Test sessions with other doses of nicotine or other drugs were not started until all rats in the two groups (caffeine- and water-drinking) trained to discriminate 0.4 mg/kg nicotine from saline met the criteria for stimulus control, to assure the same duration of caffeine exposure within the group. The same criterion was applied for the rats trained to discriminate 0.1 mg/kg of nicotine from saline.

**Tests for Generalization or Antagonism.** Test sessions were identical to training sessions with the exception that 10 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 nicotine or saline injections conducted on the other days to ensure robust stimulus control. Only rats that continued to meet the above criteria for stimulus control were tested. If a rat failed to meet 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 criteria were met.

In generalization tests, rats were injected with different doses of a drug (including injection with drug vehicle) to determine the degree to which the drug generalized to nicotine. In antagonism tests, the ability of a drug to block the discriminative stimulus properties of nicotine was determined. To do so, rats were injected with a putative blocking agent in different doses (or its vehicle) first, at a time appropriate to its onset of action, and then with the training dose of nicotine (or saline) 10 min before the session. All drugs were given in doses that ranged from those without behavioral effects to doses which decreased response rates (the choice of doses was additionally confirmed by literature searches). Each dose of a respective drug was tested in a randomized order. After all doses of one drug were tested, a 1-week washout period was allowed before the next drug was tested. During this one-week period, rats continued under the training condition for maintenance of stimulus control.

**Sequence of Testing.** The following parameters, in order of their assessment, were compared in water- and caffeine-drinking rats: 1) rates of acquisition of the nicotine discrimination, 2) discrimination of different doses of nicotine (0.025–0.8 mg/kg; 10 min before test session) to establish dose-response functions, 3) ability of the nicotinic-receptor antagonist, mecamylamine (0.01–1.0 mg/kg; 20 min before test sessions), to block the discriminative stimulus actions of nicotine, and 4) abilities of a variety of drugs that act at different receptors to generalize to the nicotine-discriminative stimulus. The drugs studied (doses and pretreatment times in parentheses) were: caffeine (1.0–56 mg/kg; 15 min), d-amphetamine (0.3–3.0 mg/kg; 10 min), cocaine (0.3–17 mg/kg; 10 min), the selective dopamine-uptake inhibitor GBR-12909 (0.9–17 mg/kg; 15 min), the nonselective dopamine agonist apomorphine (0.1–0.3 mg/kg; 10 min), the selective D1, D2, and D3 dopamine-receptor agonists, SKF-82958 (0.01–0.17 mg/kg; 10 min), NPA (0.001–0.01 mg/kg; 10 min), and PD 128,907 (0.03–0.3 mg/kg; 15 min), respectively, and 5) ability of the dopamine-release inhibitor, CGS 10746B (3.0–30 mg/kg; 30 min before test sessions), to block the discriminative-stimulus actions of nicotine. Dose-response functions for nicotine were reetermined after the above tests to assess whether any tolerance or sensitivity to nicotine had developed over time or as a result of different treatments. Finally, the ability of amphetamine (0.3–3.0 mg/kg; 10 min) to generalize to the nicotine discriminative stimulus was assessed in rats subjected to a double crossover design in which caffeine was either removed or added to the drinking water of caffeine- and water-drinking rats, respectively.

**Measurement of Plasma Caffeine Concentration.** A separate group of six rats was maintained on a restricted diet as above and was continuously exposed to caffeine (3.0 g/ml) added to their drinking water. Daily caffeine intake was monitored once per week.
After 3 weeks, the rats were sacrificed at the time when the nicotine-discrimination study would typically be performed and blood samples were collected into 10-ml sterile tubes containing EDTA 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 caffeine concentration were commercially performed at Labstat Incorporated (Kitchener, Ontario, Canada) according to the HPLC method described in detail by Muir et al. (1980).

Data Presentation and Statistical Analysis. 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. The response rate (expressed as responses per second) during each session was calculated by dividing the total number of responses on both levers during a session by total session length (in seconds). Average values ($\pm$ S.E.M.) for individual rats in each of the four groups were calculated. The percentage of nicotine-appropriate responses was considered as a measure of discrimination performance. The average response rate provided a second measure of the behavioral effects of treatment, a measure that was independent of the distribution of responses between the two levers. Additionally, daily fluid intake in milliliters per kilogram was calculated in water- and caffeine-drinking rats based on measured individual body weights and intakes of fluid. The daily fluid intakes of known caffeine concentration were then used to estimate intakes of caffeine in individual rats (in milligrams per kilogram per day) throughout the study. Daily caffeine intake and plasma caffeine concentration were expressed as mean $\pm$ S.E.M.

For the purpose of comparative presentation of experimental data, dose-response functions for each testing condition were plotted in paired graphs. The top graphs in Figs. 2 through 9 show absolute percentages ($\pm$ S.E.M.) of nicotine-appropriate lever selections, whereas the bottom graphs show mean percentage changes ($\pm$ S.E.M.) from baseline rates of responding after corresponding treatments in water- and caffeine-drinking rats. For each tested drug, the mean response rate during treatments with drug vehicle served as an individual baseline rate of responding (untransformed values shown in Fig. 10).

An arc-sin transformation was used to normalize distributions of percentages of nicotine-appropriate lever selections in generalization and antagonism tests (Shoaib and Stolerman, 1996). Nicotine-appropriate lever selection data were excluded from analysis if a rat emitted fewer than 10 responses during the test session; the response rate was denoted as zero in such a case and was included for analysis of changes in rates of responding. Full generalization to the nicotine cue was considered to exist if the percentage of responses on the nicotine-appropriate lever was 80% or greater. Partial generalization to the nicotine cue was defined as nicotine-appropriate lever responding ranging from 20 to 79%. No generalization to the nicotine cue was considered to exist if nicotine-appropriate responding was 19% or less. Dose-dependent effects on discrimination and changes in daily caffeine intake were analyzed using one-way repeated-measures ANOVA (within-group comparisons) or two-way repeated measures ANOVA on one repeated factor (between-group comparisons). Two-way repeated-measures ANOVA on two repeated factors was used for “before versus after” comparisons of dose-response functions. One-way ANOVA for repeated measures was used to analyze changes in fluid and caffeine intakes. Post hoc analysis was performed, when appropriate, using Dunnett’s test (multiple comparisons versus control performance within the same group). When possible, doses required to evoke 50% nicotine-appropriate responses or to decrease response rate by 50% ($ED_{50}$ values with 95% confidence limits) were calculated by linear regression analysis over the ascending or descending portion of the log dose-response curve, respectively (Internal Bioassay software, National Institutes of Health, National Institute on Drug Abuse, Intramural Research Program). Finally, when appropriate, Student’s $t$ test for unpaired groups was used. Data were considered statistically significant at $p < .05$. Two $ED_{50}$ values were considered statistically different if their 95% confidence limits did not overlap.

Results

Acquisition of Nicotine Discrimination. All 24 rats met the criteria for stimulus control (Fig. 1). There were, however, significant differences among the groups in the number of training sessions necessary before rats met the criteria for stimulus control ($H_6 = 31.055; p = .008$). Water- and caffeine-drinking rats trained to discriminate 0.4 mg/kg nicotine from saline acquired the task within a comparable number of training sessions ($p > .05$) ranging from 28 to 43 (mean $\pm$ S.E.M.: 37.2 $\pm$ 2.3) and from 29 to 56 (mean $\pm$ S.E.M.: 43.0 $\pm$ 4.3), respectively. A significantly longer period of training was required for rats that were trained with the lower 0.1 mg/kg dose of nicotine ($p < .05$ versus rats trained with the 0.4 mg/kg nicotine dose). At the 0.1 mg/kg training dose of nicotine, the number of training sessions required for water- and caffeine-drinking rats to meet the criteria of stimulus control ranged from 38 to 94 (mean $\pm$ S.E.M.: 60.3 $\pm$ 8.0) and from 39 to 115 (mean $\pm$ S.E.M.: 85.5 $\pm$ 12.0), respectively (Fig. 1). There was a trend for caffeine-drinking rats to show a slower rate of acquisition of discriminative-stimulus control with the 0.1 mg/kg training dose of nicotine (Fig. 1), but this did not reach statistical significance ($p > .05$). Caffeine exposure did not effect rates of responding. At the end of acquisition training, response rates after 0.4 mg/kg of nicotine or saline in water-drinking rats (mean $\pm$ S.E.M.: 2.01 $\pm$ 0.22 or 1.57 $\pm$ 0.10 responses/s, respectively) were not significantly different ($p > .05$) from those after 0.4 mg/kg of nicotine or saline in caffeine-drinking rats, (1.89 $\pm$ 0.28 or 1.48 $\pm$ 0.09 responses/s, respectively). Similarly, response rates after 0.1 mg/kg of nicotine or saline in water-drinking rats (mean $\pm$ S.E.M.: 2.19 $\pm$ 0.28 or 1.88 $\pm$ 0.20 responses/s, respectively) were not significantly different ($p > .05$) from those after 0.1 mg/kg of nicotine or saline in caffeine-drinking rats, (1.82 $\pm$ 0.28 or 1.67 $\pm$ 0.14 responses/s, respectively).

Dose-Response Tests with Nicotine and Blockade of the Nicotine Cue by Mecamylamine in Water- and Caffeine-Drinking Rats. The percentage of nicotine-appropriate lever-press responses increased as the dose of nicotine increased in both groups, regardless of the training dose of nicotine (Fig. 2; Table 1). In both water- and caffeine-drinking rats trained with the 0.4-mg/kg dose of nicotine, the 4-fold lower dose of nicotine (0.1 mg/kg) engendered nicotine-appropriate responding in all rats, whereas lower 0.05- and 0.025-mg/kg doses of nicotine yielded either partial or no generalization. In contrast to the rats trained with 0.4 mg/kg of nicotine, rats trained with 0.1 mg/kg of nicotine showed only partial or no generalization when nicotine dose was reduced 2- or 4-fold below the training dose. There were no statistical differences in the potency of nicotine as a discriminative stimulus between water- and caffeine-drinking rats regardless of training dose (Tables 1 and 2; Fig. 2). A nicotine dose of 0.8 mg/kg markedly and significantly ($p < .05$) decreased response rates but 0.4 mg/kg and lower doses of nicotine had little effect on response rates of water- or caffeine-drinking rats in rats trained with either 0.4 or 0.1 mg/kg of nicotine ($p > .05$). There were no significant differences in effects on
response rates of graded doses of nicotine between water- and caffeine-drinking rats trained with either 0.4 mg/kg or 0.1 mg/kg of nicotine \((p > .05; \text{Table 1})\).

The nicotinic-receptor antagonist mecamylamine dose-dependently blocked the discriminative effects of nicotine in rats trained with both 0.4 mg/kg and 0.1 mg/kg of nicotine (Fig. 3; Table 1). A 1.0-mg/kg dose of mecamylamine was needed to fully block the discriminative effects of 0.4 mg/kg of nicotine in both water- and caffeine-drinking rats, whereas a lower dose of 0.3 mg/kg of mecamylamine fully blocked the discriminative effects of 0.1 mg/kg of nicotine in all of the water-drinking rats and in five of six of the caffeine-drinking rats. In both water-and caffeine-drinking rats trained with the 0.4-mg/kg dose of nicotine, mecamylamine also dose-dependently antagonized the increases in rates of responding produced by 0.4 mg/kg of nicotine \((p < .05 \text{ versus vehicle})\); at doses of 0.56 and 1.0 mg/kg of mecamylamine, rates of responding reached the baseline levels of responding \((p > .05 \text{ versus vehicle})\). This effect of mecamylamine could not be assessed in rats trained with 0.1 mg/kg of nicotine, because this dose of nicotine did not produce clear increases in rates of responding (Fig. 3). When administered alone, 1.0 mg/kg or 0.3 mg/kg of mecamylamine engendered only saline-like responses and had no effect on rates of responding in either water- or caffeine-drinking rats regardless of nicotine training dose (Fig. 3). There were no significant differences in either potency or efficacy of the blocking effects of mecamylamine in water- and caffeine-drinking rats, regardless of the training dose of nicotine (Tables 1 and 2). Likewise, the effects of mecamylamine on rates of responding in water-drinking rats did not differ from those in caffeine-drinking rats (Table 1).
**Tests for Generalization to Caffeine, Amphetamine, Cocaine, and GBR-12909.** In rats trained to discriminate 0.4 mg/kg of nicotine from saline, caffeine (1.0–56 mg/kg) failed to engender nicotine-appropriate responding in water-drinking rats or in caffeine-drinking rats (Fig. 2; Table 3). The maximum percentage of nicotine-appropriate responses was 35.1% (S.E.M., ± 20.5) after 56 mg/kg of caffeine in water-drinking rats and it did not reach the assigned level of statistical significance ($p > .05$ versus vehicle). In water-drinking rats, caffeine produced dose-dependent and biphasic changes in rates of responding (Table 3). Rates of responding increased after lower doses of caffeine (1.0 and 3.0 mg/kg) and decreased significantly after higher doses of caffeine (30 and 56 mg/kg). In contrast, in caffeine-drinking rats, there were no increases in rates of responding after lower doses of caffeine, but 30 to 56 mg/kg of caffeine did decrease response rates. There was a statistically significant interaction between two factors (water/caffeine drinking and dose of caffeine; $F_{4,40} = 2.643, p = .048$), indicative of a trend in caffeine-drinking rats to show tolerance to the rate-increasing effects of lower doses of caffeine (1.0 and 3.0 mg/kg) (Fig. 2). Higher doses of caffeine (10–56 mg/kg) produced dose-dependent decreases in rates of responding with comparable ($p > .05$) potency and efficacy in water- and caffeine-drinking rats (Fig. 2, Tables 3 and 4), indicative of the lack of tolerance to the rate-decreasing effects of caffeine in rats chronically exposed to caffeine.

Amphetamine generalized in a dose-dependent manner to both the 0.4 mg/kg and 0.1 mg/kg nicotine cue in water-drinking rats (Fig. 4; Table 3). The maximum percentage of nicotine-appropriate responses was 79.6 (S.E.M., ± 18.1) and 80.0% (S.E.M., ± 18.3) after 1.0 and 1.7 mg/kg doses of nicotine.
amphetamine in rats trained to discriminate 0.4-mg/kg and 0.1-mg/kg doses of nicotine, respectively. In contrast to water-drinking rats, caffeine-drinking rats responded only on the saline-appropriate lever after receiving the same range of doses of amphetamine, regardless of the training dose of nicotine (Fig. 4; Table 3). Amphetamine, in a dose-dependent manner, disrupted responding as indicated by statistically significant decreases in rates of responding after higher doses of amphetamine (Fig. 4; Table 3). The 3.0-mg/kg dose of amphetamine completely suppressed responding in both water- and caffeine-drinking rats. The effects of amphetamine on rates of responding were both qualitatively and quantitatively comparable in water- and caffeine-drinking rats. This was confirmed by nonsignificant (p > 0.05) differences in both potency and efficacy of amphetamine to suppress responding in water- and caffeine-drinking rats, regardless of the training dose of nicotine (Tables 3 and 4).

Like amphetamine, cocaine engendered nicotine-appropriate responses in water- but not caffeine-drinking rats (Fig. 5). There was a clear significant difference (p < 0.05) in the percentage of nicotine-appropriate responses between water- and caffeine-drinking rats trained to discriminate the lower dose of nicotine (Fig. 5). Cocaine dose-dependently increased the percentage of nicotine-appropriate responses, reaching a maximum of 83.2% (S.E.M., ± 16.6) nicotine-lever selection after a 10-mg/kg dose. In caffeine-drinking rats, however, cocaine failed to generalize to 0.1 mg/kg nicotine (p > 0.05; Table 3). In water-drinking rats trained with the higher 0.4-mg/kg dose of nicotine, there was no clear dose-response relation with cocaine. The maximum percentage of nicotine-appropriate responses was 50.7% (S.E.M., ± 22.04; p < 0.05) after a 10-mg/kg dose of cocaine, but with higher 13- and 17-mg/kg doses of cocaine the percentage of nicotine-appropriate responses was only 33.3% (S.E.M., ± 21.1). In contrast, cocaine engendered between 0.0 and 20.7% ± 19.8 nicotine-appropriate responses in caffeine-drinking rats (p > 0.05 versus vehicle). However, the percentage of nicotine-appropriate responses in water- and caffeine-drinking rats were not significantly different (p > 0.05) after treatment with cocaine (Table 3). Cocaine produced dose-dependent decreases in rates of responding, and statistical comparisons revealed that cocaine was equipotent (Table 4) and equieffective (Table 3; Fig. 5) in both water- and caffeine-drinking rats, regardless of the nicotine training dose.

The selective dopamine-uptake inhibitor, GBR-12909, produced dose-dependent nicotine-appropriate responding only in water-drinking rats, with maximal effects of 83.3% (S.E.M., ± 16.7) at 10 mg/kg and 13 mg/kg in groups trained...
to discriminate 0.4 mg/kg of nicotine and 0.1 mg/kg of nicotine from saline, respectively (Fig. 5; Table 3). In contrast, GBR-12909 produced only saline-appropriate responses in caffeine-drinking rats, regardless of the training dose of nicotine. GBR-12909 significantly and dose-dependently reduced rates of responding in both water- and caffeine-drinking rats with comparable potency and efficacy, regardless of the training dose of nicotine (Tables 3 and 4).

Tests for Generalization to Nonselective and Selective Dopaminergic Agents. The nonselective D1/D2 dopamine receptor agonist, apomorphine, significantly and dose-dependently generalized to the nicotine cue in water- and caffeine-drinking groups, with a maximal effect of 66.6% (S.E.M., ± 21.1) at 0.17 mg/kg and 63.9% (S.E.M., ± 18.0) at 0.3 mg/kg in rats trained to discriminate saline from 0.4 mg/kg of nicotine, respectively (Fig. 6; Tables 4 and 5). Apomorphine, in contrast, failed to generalize to the nicotine cue in caffeine-drinking rats, regardless of the training dose of nicotine (p > .05 versus vehicle). Apomorphine also significantly and dose-dependently decreased rates of responding (Fig. 6). The rate-decreasing potency and efficacy of apomorphine were comparable (p > .05) in water- and caffeine-drinking rats, regardless of the training dose of nicotine (Tables 4 and 5; Fig. 6).
The selective D1 receptor agonist, SKF-82958, engendered nicotine-appropriate responding in a dose-dependent manner in water-drinking rats, regardless of the training dose of nicotine. In contrast to water-drinking rats, SKF-82958 failed to generalize to either the 0.4-mg/kg or 0.1-mg/kg nicotine cues in caffeine-drinking rats. SKF-82958, however, significantly and dose-dependently decreased rates of responding with a comparable potency and efficacy in water-drinking rats in comparison with caffeine-drinking rats, regardless of the training dose of nicotine (Tables 4 and 5).

Generalization tests with selective D2 (NPA) and D3 (PD 128,907) dopamine-receptor agonists produced only saline-appropriate responding in both water- and caffeine-drinking rats (p >.05 versus vehicle), regardless of the training dose of nicotine (Figs. 6 and 7). Both compounds however, dose-dependently decreased rates of responding. Regardless of the training dose of nicotine, there were no differences in the rate-decreasing potency or efficacy of the compounds in water-drinking rats in comparison with those in caffeine-drinking rats (Tables 4 and 5; Figs. 6 and 7).

**Antagonism of the Nicotine Cue with the Dopamine-Release Inhibitor CGS 10746B.** The dopamine-release inhibitor, CGS10746B, when administered 10 min before the training dose of 0.4 mg/kg of nicotine, dose-dependently, but not completely, reduced the discriminative effects of nicotine in water-drinking rats but not in caffeine-drinking rats (Fig.

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**TABLE 3**

ANOVA table for the effects of caffeine, amphetamine, cocaine, GBR-12909, and CGS 10746B in water- and caffeine-drinking rats trained to discriminate either 0.4 mg/kg of nicotine from saline or 0.1 mg/kg of nicotine from saline.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Water-dr</th>
<th>Caff-dr</th>
<th>Water-dr versus Caff-dr</th>
<th>Water-dr</th>
<th>Caff-dr</th>
<th>Water-dr versus Caff-dr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeine</td>
<td>F_{25,00} = 1.924 F_{14,00} = 0.999 F_{14,00} = 3.032</td>
<td>F_{0.00} = 12.67 F_{0.00} = 26.05 F_{0.00} = 0.106</td>
<td>F_{p &lt; .001} &lt; F_{p &lt; .001} = 0.752</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amphetamine</td>
<td>F_{3,15} = 6.458 F_{3,15} = 0.994 F_{1.36} = 6.010</td>
<td>F_{p &lt; .001} = 11.98 F_{0.25} = 9.792 F_{0.40} = 0.261</td>
<td>F_{p &lt; .001} &lt; F_{p &lt; .001} = 0.621</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cocaine</td>
<td>F_{5,25} = 2.824 F_{5,20} = 0.977 F_{5,20} = 1.017</td>
<td>F_{p &lt; .001} = 0.010 F_{p &lt; .001} = 0.040 F_{p &lt; .001} = 0.450</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GBR-12909</td>
<td>F_{4,15} = 4.113 F_{1.24} = 13.18 F_{1.24} = 7.527</td>
<td>F_{p &lt; .001} = 0.014 F_{p &lt; .001} = 0.005 F_{p &lt; .001} = 0.693</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CGS 10746B</td>
<td>F_{4,13} = 4.656 F_{4,12} = 1.001 F_{4,12} = 5.801</td>
<td>F_{p &lt; .001} = 0.261 F_{p &lt; .001} = 28.99 F_{p &lt; .001} = 3.427</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Shown are the F values, degrees of freedom, and significance levels of differences (p values) revealed by ANOVA of the dose-response functions (percentage of generalization to the training dose of nicotine and changes in rates of responding) for each treatment as listed in the first column. See Figs. 2 to 5 for dose-response functions of the drugs and the footnote to Table 1 for other details.

**TABLE 4**

ED_{50} values (with 95% CL) of drugs tested.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Training dose: 0.4 mg/kg nicotine</th>
<th>Training dose: 0.1 mg/kg nicotine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ED_{50} (% generalization)</td>
<td>ED_{50} (% response rate)</td>
</tr>
<tr>
<td>Caffeine</td>
<td>n.c. (36.3–56.2)</td>
<td>n.e. (42.9–57.3)</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>0.51 (0.33–0.79)</td>
<td>n.c. (0.61–0.92)</td>
</tr>
<tr>
<td>Cocaine</td>
<td>n.c. (17.8–35.2)</td>
<td>n.e. (12.6–21.9)</td>
</tr>
<tr>
<td>GBR-12909</td>
<td>5.58 (3.66–9.28)</td>
<td>n.e. (15.2–23.5)</td>
</tr>
<tr>
<td>CGS 10746B</td>
<td>10.1 (2.36–42.6)</td>
<td>n.e. (9.43–12.9)</td>
</tr>
<tr>
<td>Apomorphine</td>
<td>0.21 (0.09–0.49)</td>
<td>n.c. (0.21–0.7)</td>
</tr>
<tr>
<td>PD 128,907</td>
<td>0.05 (0.01–0.20)</td>
<td>n.c. (0.07–0.09)</td>
</tr>
<tr>
<td>SKF-82958</td>
<td>n.c. (0.003–0.0039)</td>
<td>n.c. (1.8–5.5–10)</td>
</tr>
<tr>
<td>NPA</td>
<td>n.c. (2.3–4.0–10)</td>
<td>n.e. (3.4–10–3)</td>
</tr>
</tbody>
</table>

n.c., not calculated; n.e., not evaluated. Data represent ED_{50} values of drugs in mg/kg (with 95% CI in parentheses) calculated, where appropriate, from dose-response functions shown in Figs. 2 to 8. Data from dose-response functions were treated quantitatively. Each ED_{50} value reflects a dose of a drug in mg/kg predicted to produce 50% nicotine-appropriate responses (% generalization) or reduce rates of responding to 50% of the individual baseline level of responding. In the case of CGS 10746B, the ED_{50} value reflects a dose of CGS 10746B predicted to block nicotine-appropriate responses by 50% in rats treated with a training dose of nicotine that produced 100% nicotine-appropriate responses. See the footnote to Table 1 for details.
The most effective doses of CGS10746B appeared to be 10 and 17 mg/kg (reduction of the percentage of nicotine-appropriate responses from 100% to 38.8% (S.E.M., ± 21.2) and 47.7% (± 18.3), respectively, p < .05 versus 0.4 mg/kg of nicotine alone. These doses of CGS 10746B also significantly decreased the rate of responding. A higher dose of CGS 10746B (30 mg/kg) almost completely suppressed responding. In contrast to the different effects of CGS1076B upon the discriminative effects of nicotine in water- and caffeine-drinking rats, CGS 10746B dose-dependently and with a comparable (p < .05) efficacy and potency reduced rates of responding in water- and caffeine-drinking rats (Table 4).

Re-evaluation of the Dose-Response Curves of the Discriminative-Stimulus Effects of Nicotine. Dose-response functions for the discriminative-stimulus effects of nicotine were re-evaluated in each group after completion of the above-mentioned tests. In each group, nicotine again produced dose-dependent increases in the percentage of nicotine-appropriate responding with a comparable (p > .05) potency in water- and caffeine-drinking rats, regardless of the training dose of nicotine (Fig. 8; Tables 1 and 2). Likewise, the discriminative-stimulus effects of nicotine did not change significantly over time (Fig. 8). This was further confirmed by comparable ED50 values of nicotine (Table 2).

Similarly, the effects of nicotine on rates of responding did not differ between water- and caffeine-drinking rats, and the effects of nicotine on rates of responding did not change over the time (p > .05) (Fig. 8; Table 1 and 2).

Generalization of Amphetamine to the Nicotine Cue in Rats When the Water- and Caffeine-Drinking Conditions Were Reversed. In rats trained to discriminate 0.4 mg/kg of nicotine from saline, caffeine solution (3 mg/ml) was substituted for water solution in water-drinking rats and caffeine solution was replaced with water solution in caffeine-drinking rats. Rats were then allowed 3 weeks to habituate to the new drinking solutions, during which time they were kept in their home cages with no training. After 3 weeks, the ability of amphetamine (10 min before testing, i.p.) to generalize to the 0.4-mg/kg nicotine cue was re-evaluated (Fig. 9; Table 6). The water-drinking group of rats, in
which amphetamine had previously fully generalized to the nicotine cue, no longer showed generalization of amphetamine to the nicotine cue after 3 or more weeks of caffeine exposure. Surprisingly, the caffeine-drinking group of rats, in which amphetamine had previously failed to generalize to the nicotine cue, continued to show no generalization to the nicotine cue with amphetamine after 3 or more weeks maintenance on water.

There continued to be no differences between water- and caffeine-drinking rats in the effects of amphetamine on rates of responding after the exchange of solutions. Amphetamine decreased rates of responding with a comparable (\( p < .05 \)) efficacy and potency in both groups (Fig. 9; Table 6). Adding caffeine to the drinking water did result in a significant decrease (1.99-fold; \( p < .05 \)) in sensitivity to the rate-decreasing effects of amphetamine (Table 6), which was most evident with doses of 1.0 and 1.7 mg/kg of amphetamine (Fig. 9). In contrast, sensitivity to the rate-decreasing effect of amphetamine did not change as a result of removal of caffeine from the drinking water in caffeine-drinking rats (Fig. 9; Table 6).

**Fig. 5.** Dose-response functions for the stimulus generalization of cocaine and GBR-12909 in water- and caffeine-drinking rats. Circles represent water (-)- and caffeine (●)-drinking rats trained to discriminate 0.4 mg/kg of nicotine from saline. Triangles represent water (▲)- and caffeine (▼)-drinking rats trained to discriminate 0.1 mg/kg of nicotine from saline. Top, mean percentage of nicotine-appropriate responses (±S.E.M.; \( n = 5–6 \) rats) after injections with increasing doses of cocaine, GBR-12909, or control vehicle (i.p.; 10 min and 15 min before test). Bottom, mean percentage of change (±S.E.M.) from the individual baseline rates of responding (responses per second) after different doses of the drugs. The individual baseline level of responding was recorded during a test session with appropriate vehicles administered instead of either cocaine or GBR-12909. The dashed line at 0% denotes no change from the individual baseline rate of responding. Doses shown on the abscissa are in mg/kg, log scale. Asterisks represent performance significantly (\( p < .05 \)) different from vehicle (Dunnnett’s test after one-way repeated measures ANOVA). See Fig. 2 for other details and Tables 3 and 4 for the outcome of between-group comparisons and for ED\(_{50}\) values calculated, where appropriate, from these dose-response functions.

**Absolute Values of Rates of Responding during the Study.** Absolute values of rates of responding after administration of the appropriate vehicle under the testing condition were grouped (Fig. 10) and analyzed for changes in the baseline levels that might result from repeated exposure to different compounds or chronic caffeine exposure. One-way repeated measures ANOVA revealed a stable baseline level of responding throughout the study in water- (\( F_{12.58} = 1.649, p = .103 \)) and caffeine- (\( F_{12.58} = 0.525, p = .888 \)) drinking rats trained to discriminate 0.4 mg/kg of nicotine from saline and in water- (\( F_{9.45} = 1.008, p = .449 \)) and caffeine- (\( F_{9.45} = 1.750, p = .105 \)) drinking rats trained to discriminate 0.1 mg/kg of nicotine from saline. There were also no significant differences between water- and caffeine-drinking groups trained with 0.4 mg/kg (\( F_{1.101} = 0.259, p = .622 \)) or 0.1 mg/kg of nicotine (\( F_{1.87} = 4.677, p = .056 \)) according to two-way
repeated measures ANOVA. In the groups trained with 0.1 mg/kg of nicotine, however, there was a tendency for water-drinking rats to show higher levels of baseline responding than caffeine-drinking rats.

**Daily Intake and Plasma Concentration of Caffeine in Caffeine-Drinking Rats.** The bottom plot in Fig. 10 shows the daily intake of caffeine. The daily caffeine intake ranged from 93.9 ± 15.2 to 199.2 ± 17.1 mg/kg in the group of rats trained to discriminate 0.4 mg/kg of nicotine from saline, and from 86.1 ± 9.1 to 187.9 ± 8.0 mg/kg in the group of rats trained to discriminate 0.1 mg/kg of nicotine from saline, with average values from 76 measurements being 138.9 ± 24.5 mg/kg/day and 131.0 ± 23.3 mg/kg/day of caffeine in these groups. Overall, throughout the study (over 1½ years), daily caffeine intakes in these two groups were indistinguishable during acquisition training and drug testing.

Consumption of caffeinated water, however, remained from 10 to 15% below that of tap water throughout the study (data not shown). The latter had no effect on food consumption, body weights, or baseline performance (Fig. 10) of rats throughout the study.

Relative to the above mentioned groups of caffeine-drinking rats, the rats used for determination of plasma levels of caffeine showed a comparable daily caffeine intake (ranged from 131.6 ± 8.1 to 167.3 ± 18.2 mg/kg/day) that resulted in a mean plasma caffeine concentration of 29.11 ± 6.61 μg/ml.

**Discussion**

In the present study, chronic caffeine exposure during acquisition of a nicotine discrimination by rats changed the qualitative nature of the nicotine discrimination without af-
fecting the rate at which the discrimination was acquired. The effect of chronic exposure to caffeine on the acquired nicotine cue in the present study appeared to be selective to its dopaminergic component, since a number of dopaminergic compounds, including amphetamine, cocaine, GBR-12909, apomorphine, and SKF-82958, failed to generalize to the nicotine cue in caffeine-consuming rats, whereas there was a complete or partial generalization with these compounds in water-consuming rats. However, chronic caffeine exposure did not appear to change the nicotinic component of the nicotine cue, as there were no differences between caffeine- and water-consuming rats in the nicotine dose-response functions nor in the ability of the nicotinic-receptor blocker mecamylamine to block the nicotinic discriminative cue.

Rates of acquisition of nicotine discrimination for both lower and higher doses of nicotine in the present study with rats were similar to those reported in the literature (Chance et al., 1977; Stolerman et al., 1984; Rosecrans, 1989). As with Shoaib et al. (1997), there was no evidence for the development of either tolerance or sensitization to the discriminative-stimulus effects of nicotine over time as a result of repeated treatment with nicotine and with various other compounds in the generalization and antagonism tests (Fig. 8). For example, the effects of graded doses of nicotine on rates of responding at the end of the study were comparable to those at the beginning of the study. Furthermore, the present findings from the generalization and antagonism tests generally resembled previous findings. Specifically, nicotine discrimination has been reported to be dose-related, with ED50 values typically ranging from 0.04 to 0.1 mg/kg depending on the training dose and conditions (Stolerman, 1988; Rosecrans, 1989; Schechter and Meehan, 1992; Shoaib et al., 1997). In the present study, the ED50 values of nicotine ranged from 0.044 to 0.088 mg/kg. The noncompetitive nicotinic-receptor antagonist mecamylamine dose-dependently blocked the discriminative-stimulus effects of nicotine in the present study (Fig. 3) with comparable potency and efficacy to those found in the earlier studies (Romano et al., 1981; Stolerman et al., 1983, 1984). The present findings with partial generalization of amphetamine, cocaine, and the nonselective dopamine receptor agonist apomorphine to the nicotine cue, as well as the failure of cocaine to generalize to the nicotine cue (Figs. 2, 4, 5, and 6), have also been previously reported (Chance et al., 1977; Stolerman et al., 1984; Rosecrans, 1989). Finally, the dopamine-release inhibitor CGS 10746B has previously been shown to attenuate a nicotine discrimination in rats (Schechter and Meehan, 1992). In the present study, the blocking effect of CGS 10746B was also incomplete and its behavioral effects were characterized by a small separation between doses attenuating the discriminative-stimulus effect of nicotine and those producing a marked reduction in rates of responding.

In a previous study with Long-Evans rats trained to discriminate nicotine from saline, 5.0- and 30-mg/kg doses of GBR-12909 failed to generalize to the nicotine cue (Corrigall and Coen, 1994); the higher dose produced complete suppression of responding in four of six rats. In the present study, however, GBR-12909 produced full generalization in water-drinking Sprague-Dawley rats to both the 0.1- and 0.4-mg/kg nicotine cues at doses of 10 and 13 mg/kg (intermediate doses 5, 6), have also been previously reported (Chance et al., 1977; Stolerman et al., 1984; Rosecrans, 1989). Finally, the dopamine-release inhibitor CGS 10746B has previously been shown to attenuate a nicotine discrimination in rats (Schechter and Meehan, 1992). In the present study, the blocking effect of CGS 10746B was also incomplete and its behavioral effects were characterized by a small separation between doses attenuating the discriminative-stimulus effect of nicotine and those producing a marked reduction in rates of responding.

The role of D1 and D2 dopamine receptors in the mediation of nicotine’s discriminative stimulus effects has been studied by Stolerman and coworkers. The selective D1 dopamine-
Dopamine receptor agonist SKF 38393 partially generalized to the nicotine cue (Stolerman and Reavil, 1989). Moreover, the selective D1 dopamine antagonist SCH 23390 significantly attenuated nicotine discrimination, whereas two neuroleptics with selectivity for D2 dopamine receptors had no effect (Reavil and Stolerman, 1987). In the present study, the selective D1 dopamine-receptor agonist SKF-82958 generalized to the nicotine cue in water-drinking rats, whereas the selective D2 dopamine-receptor agonist NPA produced only saline-appropriate responses (Fig. 7), supporting these earlier findings indicating involvement of D1 but not D2 dopamine receptors in mediation of the discriminative-stimulus effects of nicotine in rodents.

It has recently been suggested that D3 dopamine autoreceptors may be involved in the pathogenesis of neuropsychiatric disorders such as schizophrenia and drug addiction, and the potential clinical use of selective D3 dopamine receptor agonists is now being explored (Caine and Koob, 1993; Acri et al., 1995; Lamas et al., 1996; Levant, 1997; Sanger et al., 1997; Witkin et al., 1998). Stimulation of D3 dopamine autoreceptors by selective compounds results in a dose-dependent inhibition of dopamine release in vivo and in vitro, and also has been implicated in blocking the reinforcing effects of amphetamine and cocaine (for review see Levant, 1997). Of importance for the present study, the discriminative stimulus effects of D3 dopamine-receptor agonists (e.g., 7-hydroxydipropylaminotetralin hydrobromide, PD 128,907) are similar to those of cocaine and the nonselective dopamine receptor agonist apomorphine in both rats and monkeys (Acri et al., 1995; Lamas et al., 1996; Sanger et al., 1997). Thus, the selective D3 dopamine receptor agonist PD 128,907 was tested in the present study to verify the extent to which it was similar to the other dopaminergic compounds that partially generalized to the nicotine cue. PD 128,907 engendered

Fig. 7. Dose-response functions for the stimulus generalization of SKF-82958 and NPA in water- and caffeine-drinking rats. Circles represent water (○)- and caffeine (●)-drinking rats trained to discriminate 0.4 mg/kg of nicotine from saline. Triangles represent water (△)- and caffeine (▽)-drinking rats trained to discriminate 0.1 mg/kg of nicotine from saline. Top, mean percentage of nicotine-appropriate responses (± S.E.M.; n = 5–6 rats) after injections with increasing doses of SKF-82958, NPA, or control vehicle (i.p.; 10 min before test). Bottom, mean percentage of change (± S.E.M.) from the individual baseline rate of responding (responses per second) after different doses of SKF-82958 or NPA. The individual baseline rate of responding was recorded during a test session with appropriate vehicles administered instead of SKF-82958 or NPA. The dashed line at 0% denotes no change from the individual baseline rate of responding. Doses shown on the abscissa are in mg/kg, log scale. Asterisks represent performance significantly (p < 0.05) different from vehicle (Dunnett’s test after one-way repeated measures ANOVA). See Fig. 2 for other details and Tables 4 and 5 for the outcome of between-group comparisons and for ED50 values calculated, where appropriate, from these dose-response functions.
only saline-appropriate responses in water-drinking rats up to doses that markedly suppressed responding. Moreover, chronic caffeine exposure in caffeine-drinking rats did not change the discriminative or response rate-decreasing effects of PD 128,907. Although more studies will be needed to assess the role of D3 dopamine autoreceptors in mediating the discriminative stimulus effects of nicotine, the present study provides the first evidence suggesting their minimal role and adds to accumulating evidence differentiating the nicotine cue from those of other psychomotor stimulants such as amphetamine and cocaine.

It has been observed for a number of pharmacologically diverse drugs, including nicotine, that the results of generalization tests may vary depending on the training dose of drug (Stolerman et al., 1984; Mumford and Holtzman, 1991; Schechter, 1997). Typically, pharmacological specificity in drug discrimination studies decreases with lower and increases with higher doses of a training drug. In the present study, a 4-fold difference in the training dose of nicotine was insufficient to produce marked differences in the pharmacological specificity of the nicotine cue. Patterns of generalization to dopaminergic agents were qualitatively identical for the lower and higher training doses of nicotine in water-drinking rats. Likewise, ED50 values calculated from dose-response functions were statistically comparable (Table 4). Only in the case of cocaine did different nicotine-training doses quantitatively affect the outcome. Cocaine appeared more efficacious in producing nicotine-appropriate responses in water-drinking rats trained with 0.1 mg/kg of nicotine relative to water-drinking rats trained with 0.4 mg/kg of nicotine (83.2% versus 50.7%).

Different training doses of nicotine also had little effect on its final potency as a discriminative stimulus after acquisition. When dose-response functions of nicotine’s discriminative

**Fig. 8.** Re-evaluation of the dose-response functions for the discriminative-stimulus effects of nicotine in water- and caffeine-drinking rats. Open and solid symbols represent water- and caffeine-drinking rats, respectively. Circles and triangles represent dose-response curves evaluated at the beginning of the study (re-plotted from Fig. 2 for comparison); squares represent dose-response curves re-evaluated in the same groups. Top, mean percentage of nicotine-appropriate responses (±S.E.M.; n = 5–6 rats) after injections with increasing doses of nicotine or saline (s.c.; 10 min before test). Bottom, mean percentage of change (±S.E.M.) from the individual baseline rate of responding (responses per second) after different doses of nicotine. The individual baseline rate of responding was recorded during a test session with saline administered instead of nicotine. The dashed line at 0% denotes no change from the individual baseline rate of responding. Doses shown on the abscissa are in mg/kg, log scale. Asterisks represent performance significantly (p < .05) different from vehicle (Dunnett’s test after one-way repeated measures ANOVA). See Fig. 2 for other details and Tables 1 and 2 for the outcome of between-group comparisons and for ED50 values calculated, where appropriate, from these dose-response functions.
tive stimulus effects were evaluated, immediately after all rats met the criteria of stimulus control, the ED_{50} values for nicotine were similar in water- and caffeine-drinking rats trained with 0.1 mg/kg relative to 0.4 mg/kg of nicotine (Table 2). However, when dose-response functions were re-evaluated after generalization and antagonism tests were completed, the ED_{50} values for nicotine were 1.9-fold lower in water-drinking rats and 2.4-fold lower in caffeine-drinking rats trained with 0.1 mg/kg of nicotine relative to rats trained with 0.4 mg/kg of nicotine. Furthermore, the ED_{50} values for mecamylamine needed to block the discriminative effects of the 0.1-mg/kg training dose of nicotine were 5.1-fold lower in water-drinking rats and 3.8-fold lower in caffeine-drinking rats relative to the doses needed to block the discriminative effects of the 0.4 mg/kg training dose of nicotine. As expected (Stolerman et al., 1984), a 4-fold decrease in the training dose of nicotine appeared sufficient to significantly (1.6- and 2.0-fold; \( p < .05 \)) decrease the rate of acquisition of the nicotine discrimination in both water- and caffeine-drinking rats.

A large body of evidence suggests that nicotine exerts stim-
The failure of dopaminergic compounds to generalize to the nicotine cue in caffeine-drinking rats in the present study is unlikely due to an inability of rats chronically exposed to caffeine to respond to dopaminergic drugs. Similar regimens of caffeine exposure in rats failed to change amphetamine- and cocaine-induced stimulation of ambulatory activity but potentiated that of nicotine (Holtzman, 1983; Finn and Holtzman, 1987; Shoaib et al., 1996). More recently, we have shown that a chronic regimen of caffeine exposure, identical with that in the present study, markedly potentiated the response-rate increasing effects of amphetamine and cocaine, but not those of nicotine, in rats responding under a FI schedule of food reinforcement (Jaszyna et al., 1998). In contrast, caffeine-drinking rats appeared to be less sensitive to the response-rate decreasing effect of the selective D1 dopamine receptor agonist SKF-82958 than water-drinking rats, but sensitivity to the behavioral effects of the selective D2 dopamine receptor agonist NPA remained unaffected by chronic caffeine exposure. Chronic caffeine exposure also appears to have no effect on the overall behavior of rats, as there were no differences in FR or FI rates of responding between water- and caffeine-drinking rats throughout the previous study by Jaszyna et al. (1998) and throughout the present study (Fig. 10). Furthermore, the baseline level of ambulatory activity in caffeine-drinking rats did not differ from that of water-drinking rats (Shoaib et al., 1996). Thus, it can be concluded that chronic caffeine exposure changed the discriminative stimulus properties of nicotine and these changes could be due to specific pharmacological effects of caffeine on the dopaminergic component of nicotine’s discriminative cue.

In the present study, rats were exposed to caffeine for 2 weeks before acquisition training was initiated. Chronic caffeine exposure had little effect on rates at which rats learned to discriminate nicotine from saline, as the number of sessions required for robust stimulus control was comparable in water- and caffeine-drinking rats. There was, however, a trend for acquisition of the 0.1-mg/kg nicotine discrimination to be retarded in caffeine-drinking rats. It is possible that chronic caffeine exposure weakened the discriminative stimulus cue of nicotine, and in turn, more training sessions were needed to establish stimulus control. Given that there were no changes in the nicotinic component but marked changes in

### Table 6

ANOVA table and ED$_{50}$ values for the effects of amphetamine evaluated in water- and caffeine-drinking rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>% Generalization</th>
<th>ED$_{50}$</th>
<th>Response Rate</th>
<th>ED$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamine (training dose: 0.4 mg/kg nicotine)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water-dr (W)</td>
<td>F$_{1,15} = 6.458$; p = 0.005</td>
<td>0.51 (0.32–0.79)</td>
<td>F$_{1,25} = 11.98$; p &lt; 0.001</td>
<td>0.82 (0.61–0.92)</td>
</tr>
<tr>
<td>Water-dr now Caff-dr (W→C)</td>
<td>F$_{1,10} = 1.062$; p = 0.407</td>
<td>n.c.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caff-dr (C)</td>
<td>F$_{1,16} = 0.994$; p = 0.423</td>
<td>n.c.</td>
<td>F$_{1,25} = 9.792$; p &lt; 0.001</td>
<td>1.00 (0.61–1.45)</td>
</tr>
<tr>
<td>Caff-dr now Water-dr (C→W)</td>
<td>F$_{1,16} = 0.995$; p = 0.439</td>
<td>n.c.</td>
<td>F$_{1,20} = 7.557$; p &lt; 0.001</td>
<td>1.37 (1.04–1.88)</td>
</tr>
</tbody>
</table>

W versus C*              | F$_{1,16} = 6.019$; p = 0.034 | F$_{1,40} = 0.361$; p = 0.621 |
W→C versus C→W           | F$_{1,16} = 0.448$; p = 0.522 | F$_{1,32} = 0.091$; p = 0.770 |
W versus C               | F$_{1,16} = 12.96$; p = 0.023 | F$_{1,16} = 22.33$; p = 0.009 |
C versus C→W             | F$_{1,16} = 1.006$; p = 0.373 | F$_{1,16} = 4.457$; p = 0.102 |

* Data from Table 3.
Fig. 10. Top and middle plots show untransformed mean values (±S.E.M.) of the baseline rates of responding in water- (open symbols) and caffeine- (solid symbols) drinking rats trained to discriminate 0.4 mg/kg nicotine (top) or 0.1 mg/kg of nicotine (middle) from saline (n = 5–6 rats per group) during all test sessions with the different drugs as indicated in the legend. The average response rate during a session (expressed as responses per second) in an individual rat was calculated by dividing the total number of responses emitted by a rat on both levers by the total session length in seconds (900 s). These rates of responding were used as baseline levels of responding to calculate mean percentage of changes from individual level of responding produced by the respective drugs (Figs. 2–9). Bottom, calculated average intake (±S.E.M., n = 5–6 per data point) of caffeine (in mg/kg/day) during successive weeks in rats trained to discriminate 0.4 mg/kg of nicotine (○) or 0.1 mg/kg of nicotine (▼) from saline. The shaded horizontal bars represent the number of weeks necessary for all rats from groups trained with either 0.4 mg/kg or 0.1 mg/kg of nicotine to meet the criteria of reliable stimulus control (see Fig. 1), whereas generalization and antagonism tests were performed during the remaining weeks (see Figs. 2–9).
the dopaminergic component of the discriminative stimulus cue of nicotine, as revealed by later studies, it seems reasonable to speculate that chronic caffeine exposure reduced this dopaminergic component and thus retarded the acquisition of the 0.1 mg/kg of nicotine discrimination. Because 0.4 mg/kg of nicotine would produce stronger stimulation of nicotinic receptors than 0.1 mg/kg, this could overshadow the effect of caffeine on the dopaminergic component of the nicotine cue during the acquisition phase. There was also a trend for caffeine-drinking rats trained with 0.1 mg/kg of nicotine to show lower response rates than water-drinking rats trained with the same dose of nicotine (Fig. 10). This could additionally affect the rate of acquisition of the nicotine discrimination in this group. Nevertheless, the general lack of effect of chronic caffeine exposure on rates of acquisition of the nicotine discrimination were somewhat surprising based on our previous findings that Sprague-Dawley rats chronically exposed to the same concentrations of caffeine in their drinking water acquired self-administration of i.v. nicotine significantly faster and reached higher rates of responding than did water-drinking control animals (Shoaib et al., 1996). This apparent difference may be attributed to different brain regions mediating the discriminative and reinforcing effects of nicotine (Stolerman and Shoaib, 1991; Nisell et al., 1995; Shoaib and Stolerman, 1996) and/or to different dose thresholds for caffeine to produce facilitation and retardation of these effects.

Caffeine appeared to have both acute and long-lasting effects on the discriminative stimulus properties of nicotine. In contrast to water-drinking rats, amphetamine engendered only saline-appropriate responses in rats chronically exposed to caffeine (Fig. 4). When caffeine solution was replaced by tap water and dose-response functions for amphetamine were re-evaluated in the same subjects 3 weeks later, amphetamine still failed to generalize to the nicotine cue (Fig. 9). This suggests that chronic caffeine exposure during the acquisition phase and subsequent testing and training phases produced long-lasting changes in the discriminative stimulus properties of nicotine, as no caffeine or its metabolites would be expected 21 days after termination of chronic caffeine exposure in rodents (Bonati et al., 1984–1985; Gasior et al., 1996). On the other hand, although amphetamine was shown to generalize to the nicotine cue in water-drinking rats (Fig. 4), when tap water was replaced by caffeine solution and amphetamine was re-evaluated 3 weeks later, amphetamine failed to generalize to the nicotine cue. It is important to note that there was no training during this 3-week period to avoid retraining animals under new conditions in terms of water and caffeine exposure.

Chronic oral exposure to caffeine in the drinking water, as in the present study, has previously been shown to produce rapid, complete, and insurmountable tolerance to the stimulatory effects of caffeine on behavior in rats (Holtzman, 1983; Finn and Holtzman, 1987; Newland and Brown, 1997; Jaszyma et al., 1998), and similar tolerance is seen after repeated daily i.m. injections of caffeine in monkeys (Katz and Goldberg, 1987; Howell and Landrum, 1997). Although caffeine intake in rats remained stable throughout the present study, week-to-week variations were considerable (Fig. 10). Similar degrees of variation in oral caffeine intake have been reported (Holtzman, 1983; Jaszyma et al., 1998). To minimize these variations, each dose of a respective drug was tested in a randomized order. Nevertheless, the present regimen of chronic caffeine exposure had no effect on body weights, baseline levels of FR responding (Fig. 10), FI responding (Jaszyma et al., 1998), or on ambulatory activity (Shoaib et al., 1996).

In the present study, the plasma level of caffeine in rats drinking water containing 3 mg/ml caffeine and showing an average 135 mg/kg/day caffeine intake was, on average, 29.11 μg/ml. Such a plasma concentration of caffeine is comparable to those measured in rats after a single bolus injection of behaviorally active 20- to 40-mg/kg doses of caffeine (e.g., Modrow et al., 1981; Hirsh, 1984; Nehlig et al., 1992; Lau and Falk, 1994). In humans, an oral dose of 1 mg/kg of caffeine (equivalent to the caffeine content in one cup of coffee) produces plasma concentrations of 1 to 2 μg/ml, whereas doses of 5 to 8 mg/kg (equivalent to the caffeine intake of a heavy coffee drinker) would produce plasma concentrations of about 8 to 10 μg/ml (e.g., Benowitz, 1990; James, 1991; Sawnynok, 1995). Any direct comparisons of doses and plasma levels of caffeine in experimental animals relative to humans ought to be interpreted with extreme caution, because there are large between-species differences in metabolism and sensitivity to the stimulatory effects of caffeine (James, 1991). In general, any direct comparisons of doses and plasma levels of caffeine in experimental animals relative to humans ought to be interpreted with extreme caution, because there are large between-species differences in metabolism and sensitivity to the stimulatory effects of caffeine (James, 1991).
In conclusion, the present study provides evidence that the discriminative properties of nicotine can be markedly changed by chronic caffeine exposure. Changes produced by chronic caffeine exposure appear to be selective to the dopaminergic component of nicotine’s discriminative cue. Nicotine, however, could reliably serve as a discriminative stimulus even in the absence of the dopaminergic component of its discriminative cue. This reinforces the notion that the dopamine neurotransmitter system plays a secondary role in the discriminative stimulus properties of nicotine. Furthermore, the present findings add to accumulating evidence differentiating nicotine from “classical dopaminergic” drugs of abuse such as cocaine and amphetamine. Finally, involvement of other neurotransmitter systems in the effects observed in the present study cannot be ruled out and warrants further study given that caffeine can alter the density and function of a number of different receptors in the brain (Jacobson et al., 1996).

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