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Chronic caffeine exposure in rats blocks a subsequent nicotine-conditioned taste avoidance in a one-bottle, but not a two-bottle test

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Abstract

Two experiments were conducted in order to investigate nicotine-conditioned taste avoidance (CTA) following chronic preexposure to caffeine. Rats were given daily intraperitoneal injections of caffeine anhydrous (0, 10, or 30 mg/kg) for 10 or 30 days. Training of the nicotine-CTA began after the last day of caffeine preexposure. On five separate occasions access to a saccharin solution was followed immediately by an injection of 1.2 mg/kg nicotine hydrogen tartrate salt or saline. Nicotine-CTA readily developed in saline-preexposed controls. That is, paired rats drank less saccharin solution than unpaired rats after repeated saccharin–nicotine pairings. A similar pattern of nicotine-CTA was found for rats preexposed to 30 mg/kg caffeine for 10 days. Following 10 days of preexposure to 10 mg/kg caffeine, however, CTA did not develop under standard testing conditions. Thirty days of caffeine preexposure did not affect the development of a nicotine-CTA even though the anorexic effects of caffeine were evident after exposure to 30 mg/kg for this duration. Thus, caffeine exposure appears to weaken acquisition or expression of the conditioned avoidance properties of nicotine. This effect is sensitive to the dose of caffeine and duration of preexposure. Importantly, the pattern of nicotine-CTA does not appear to be due to nonspecific effects of caffeine.

Keywords: Acetylcholine; Adenosine; Conditioned avoidance; Dopamine; Pavlovian conditioning; Rats; Saccharin

1. Introduction

Organisms that experience emesis following ingestion of a novel food item will avoid that food item on subsequent encounters. This phenomenon of conditioned taste avoidance (CTA) has been utilized in the laboratory to study aversive learning processes as well as the ability of various substances, including drugs of abuse, to induce avoidance responses. In these procedures, the stimulus of interest (e.g., lithium, nicotine, X-irradiation, or rotation) is administered after the organism receives access to a novel gustatory stimulus (e.g., saccharin solution). Presumably, as a result of Pavlovian conditioning processes the pairing of these two stimuli produces avoidance of the gustatory cue on future presentations (e.g., Garcia and Koelling, 1966; Kumar et al., 1983).

Drugs of abuse such as ethanol, amphetamine, and nicotine are thought to induce CTA through their effects in the central nervous system (CNS) (Berger et al., 1973; Kumar et al., 1983; Stewart et al., 1988). This avoidance is of much theoretical and empirical interest considering that these drugs are also self-administered by humans and laboratory animals. Several authors have addressed this seeming paradox (e.g., Grigson, 1997; Hunt and Amit, 1986; Parker, 1995b; Wise et al., 1976). Hunt and Amit (1986), for example, suggested that simultaneous rewarding and aversive properties of drugs of abuse were probably not from separate processes. Indeed, Grigson and her colleagues (Gomez and Grigson, 1999; Grigson, 1997; Grigson et al., 1999) have suggested that conditioned avoidance of a hedonically rewarding saccharin taste cue may result from anticipation of the even more rewarding drug that follows access to the taste. Such anticipatory contrast is not evident when lithium chloride (LiCl), an emetic substance with no known rewarding properties, follows access to the taste cue.

Preexposure to the unconditioned stimulus (US) in a CTA preparation can attenuate the subsequent development of CTA (Cappell and LeBlanc, 1977; Cappell and Poulos, 1979; Kunin et al., 2000; Riley and Simpson, 1999). This "US preexposure effect" has been demonstrated when

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nicotine serves as the US (Iwamoto and Williamson, 1984). In that study, rats were preexposed to saline or nicotine for 2 or 4 days. These rats were then given access to a novel saccharin solution followed by a subcutaneous nicotine injection. In a two-bottle test, rats that were preexposed to saline (no drug) acquired a saccharin avoidance; rats preexposed to nicotine did not acquire this avoidance of saccharin. In the present report, we are interested in whether preexposure to caffeine will affect the later development of a nicotine-CTA. Although attenuation of CTA has been observed when the compound used during preexposure (e.g., amphetamine or ethanol) differs from that administered during conditioning trials (e.g., morphine or LiCl Cappell and Poulos, 1979; Rabin et al., 1988), this effect has not been studied for the caffeine/nicotine combination.

There are empirical, as well as practical, reasons to study the effects of caffeine preexposure on the later effects of nicotine. Practically, caffeine is the most widely used psychoactive substance in the world (Gilbert, 1976, 1984). The prevalence of caffeine in food and beverage items, as well as in prescription and over the counter medication, increases the likelihood that many individuals will be exposed to caffeine prior to experimentation with tobacco products such as cigarettes. Empirically, the psychomotor stimulant and behaviorally reinforcing effects of cigarettes and coffee have been attributed to nicotine and caffeine, respectively (Gilbert, 1976; Henningfield et al., 1995). Interestingly, previous research has indicated that the reinforcing properties of these drugs may be enhanced when they are administered together in humans (Brown and Benowitz, 1989; Istvan and Matarazzo, 1984) or in rodents (Shoaib et al., 1999; White, 1988). For instance, Shoaib et al. (1999) found that chronic exposure to caffeine enhanced the acquisition rate of nicotine self-administration in rats. Rats with continuous access to caffeine in drinking water acquired an operant response reinforced by intravenous nicotine administration more rapidly than controls that received only tap water.

Further, Gasior et al. (1999, 2000) found that caffeine preexposure altered performance in a nicotine drug discrimination paradigm. Briefly, food-deprived rats preexposed to caffeine in drinking water were subsequently trained to barpress for food. Which bar (i.e., left or right) resulted in presentation of the food reinforcement depended on whether the rat was administered nicotine before the session. At a low concentration (0.25 mg/ml), caffeine preexposure enhanced acquisition of the nicotine discrimination; preexposure to higher concentrations of caffeine (1 or 3 mg/ml) did not enhance acquisition of the discrimination (Gasior et al., 1999, 2000). Further, rats that were not preexposed to caffeine responded on the nicotine-appropriate bar when amphetamine or apomorphine was substituted for nicotine (i.e., generalization). However, the ability of amphetamine or apomorphine to substitute effectively for the nicotine cue was blocked by preexposure to the highest concentration of caffeine (3 mg/ml). The authors concluded that caffeine changed the dopaminergic quality of the nicotine cue. This conclusion is consistent with other research showing that adenosine antagonism alters dopamine receptor affinity in the CNS (see Ferre et al., 1991, 1997; Kuzmin et al., 2000; Nehlig et al., 1992 for relevant reviews).

This brief review indicates interesting parallels between the behavioral effects of caffeine and nicotine. Indeed, in addition to the behavioral research just described, there is pharmacological evidence for overlap in the CNS mechanisms mediating the effects of caffeine and nicotine (Shi et al., 1993, 1994; see Daly et al., 1999 for review). For example, Shi et al. (1993) found that mice exposed to caffeine (100 mg/kg/day) for 4 days showed a 40–50% increase in cortical nicotinic and muscarinic acetylcholine (ACh) receptors. Interestingly, adenosine A_1 receptors also increased by 15–20%; adenosine A_2 receptors, however, were not affected by chronic caffeine treatment.

One major goal of the present report was to investigate CTA induced by nicotine following chronic exposure to caffeine in rats. As noted earlier, the effects of chronic exposure to caffeine on conditioned avoidance properties of nicotine have not been investigated. A second goal was to examine the nonspecific effects of caffeine on weight and fluid consumption. For example, caffeine is a potent anorectic agent (Gans, 1984). Thus, the effects of caffeine preexposure on bodyweight could affect development of a nicotine-CTA. Further, the experimental protocol allowed us to examine the effects of caffeine exposure on water consumption, neophobia, and development of a saccharin preference. Indeed, these measures allow us to distinguish the unconditioned effects of caffeine from possible effects on the acquired flavor–nicotine association (see later).

2. Method

2.1. Animals

Ninety-five naive male Sprague–Dawley rats from Harlan Industries (Indianapolis, IN) were housed individually in hanging wire-mesh cages. Food was continuously available in the home cage, but water access was restricted as described later. The colony was maintained on a 12:12 light/dark cycle; all procedures were conducted in the light portion of the cycle. Each rat was handled for approximately 2 min daily for 3 days prior to the start of each experiment.

2.2. Apparatus

All fluids were presented in chambers separate from the home cage. These chambers were aluminum mailboxes converted to allow presentation of drink tubes (inside dimensions: $16 \times 47 \times 19$ cm; Solar Group, Indianapolis, IN). Two holes (1.5 cm diameter) were drilled, approximately 7.5 cm from the floor of the box, and 5 and 8.5 cm

from the door, respectively. A spring attached to the outside of each box held a 40-ml graduated drink bottle to the chamber, such that the sipper-tube extended approximately 1 cm into the chamber through one of the holes. Three rows of six holes (1.5 cm diameter) were drilled into the ceiling of each chamber to allow the passage of air.

2.3. Drugs

Caffeine anhydrous (Sigma, St. Louis, MO) was dissolved in saline (0.9% NaCl) and injected intraperitoneally (ip) at a volume of 2 ml/kg. (−)-Nicotine hydrogen tartrate salt (Sigma) was dissolved in saline and brought to a pH of 7.0 ± 0.2 with a dilute NaOH solution. Nicotine was injected subcutaneously (sc) at a volume of 1 ml/kg. Doses were based on the salt form of the drug.

2.4. Procedure

Two different experiments were conducted. In one experiment, 42 rats (278–374 g) were used to examine the effects of 10 days of chronic caffeine exposure on subsequent nicotine-CTA. In the second experiment, 53 rats (254–325 g) served to examine the effects of 30 days of chronic exposure to caffeine on later nicotine-CTA.

2.5. Ten-day preexposure

At the start of the experiment, rats were randomly assigned to one of three doses of caffeine (0, 10, or 30 mg/kg). Each rat was injected with its assigned dose of caffeine once daily for 10 days. Injections occurred in the afternoon (16:30 ± 1 h). Water bottles were removed from the home cages on the fifth day of caffeine preexposure. On Days 6–10, rats were adapted to the water restriction schedule and the experimental chambers. On each of these days, rats were transported to the experimental chambers and given access to distilled water. Following the 30-min session, fluid consumption was recorded to the nearest milliliter and the rats were transported back to the colony. Note that caffeine preexposure injections continued in the afternoons throughout the water baseline phase. Drink sessions began at 07:30 and continued at this time for the remainder of the experiment. The present experimental protocol differed from previous studies examining the behavioral effects of nicotine after caffeine preexposure (e.g., Gasior et al., 1999, 2000; Shoaib et al., 1999) in two main ways. First, caffeine was administered by injection rather than in drinking water in the present research because our protocol required restricted fluid access. Second, caffeine injections were discontinued prior to the start of nicotine conditioning in order to avoid possible pharmacological interactions between nicotine and caffeine during this phase (cf. White, 1988).

Following the last day of the water baseline phase (Day 10), rats within each caffeine dose (0, 10, or 30 mg/kg) were randomly assigned to one of two groups (paired or unpaired) with the restriction that water intake between the two groups did not differ statistically on the last day. The first conditioning trial occurred on Day 11. Procedures for this trial were identical to the water baseline phase, except that the rats were given access to a 0.1% saccharin solution (w/v) instead of distilled water. Immediately following access to saccharin, rats assigned to the paired condition ($n = 7$ per caffeine dose) received a subcutaneous injection of 1.2 mg/kg nicotine; rats in the unpaired condition ($n = 7$ per caffeine dose) were injected with saline. On the following day (Day 12), rats were given 30-min access to distilled water. Immediately after access to distilled water, unpaired rats were injected with 1.2 mg/kg nicotine and paired rats were injected with saline. This 2-day cycle was repeated such that there were five saccharin sessions and five water sessions.

Twenty-four hours after the last water session, rats were given a two-bottle test. The drink session proceeded as previously described, except two drink tubes were available in the chamber; one contained 0.1% saccharin solution and the other contained distilled water. Tube position (left or right) was counterbalanced within each experimental condition as much as allowed by the sample size.

2.5.1. Thirty-day preexposure

The protocol for this experiment was identical to that described previously, except that caffeine exposure lasted for 30 days. As such, the first water baseline session occurred on Day 26 of caffeine exposure. Conditioning began on Day 31, approximately 15 h after the last caffeine injection. There were 27 rats in the paired condition ($n = 9$ per caffeine dose), and 26 rats in the unpaired condition ($n = 9$ per caffeine dose, except $n = 8$ for 0 mg/kg caffeine).

2.6. Data analyses

Analyses of variance (ANOVAs) were used for overall analyses. For example, saccharin intake across conditioning trials was analyzed using a $3 \times 2 \times 5$ mixed factorial ANOVA in which dose (0, 10, or 30 mg/kg caffeine) and group (paired or unpaired) were the between-subjects factors and trial (1–5) was the within-subject repeated measure. Tukey's Honestly Significant Differences tests or pairwise t tests were used for planned contrasts. Before analysis of the two-bottle test data, intake was converted to a preference ratio using the following formula: saccharin intake/(saccharin intake + water intake). Bonferroni's multiple comparison test was used to contrast each paired group from its respective unpaired control on the test. Statistical significance for all analyses was set at $P \leq .05$, two-tailed.

The present design allowed us to assess the effects of caffeine preexposure on bodyweight, water intake, and initial saccharin intake (neophobia). Because these measures were taken before nicotine treatment and/or do not involve a learned association with nicotine, we will refer to them as "unconditioned effects."

3. Results

3.1. Ten-day preexposure

3.1.1. Unconditioned effects

Body weight, water intake, and initial saccharin consumption were assessed before rats were differentially treated with nicotine. Hence, data from paired and unpaired groups were pooled to increase statistical power. Exposure to caffeine for 10 days did not alter rats' weight as assessed on the last day of caffeine preexposure [$F(2,39) = 1.54$, $P = .36$] (Fig. 1A). Across the water baseline sessions, there was a main effect of trial [$F(4,156) = 19.751$, $P < .05$], indicating that water consumption increased across trials. There was no main effect of dose [$F(2,39) = 2.09$, $P = .13$], and no interaction ($F < 1$), indicating that caffeine preexposure did not differentially alter water consumption (Fig. 1B). Caffeine preexposure did not affect saccharin intake on the first exposure [$F(2,39) = 1.60$, $P = .22$] (Fig. 1C).

3.1.2. Conditioned effects

Fig. 2A–C show saccharin intake in each paired group compared to its respective unpaired control group across conditioning trials. The overall $3 \times 2 \times 5$ ANOVA revealed a significant main effect of dose [$F(2,36) = 4.11$, $P < .05$], a main effect of group [$F(1,36) = 30.37$, $P < .001$], and a main effect of trial [$F(4,144) = 7.94$, $P < .001$]. Further, there was a significant Group × Trial interaction [$F(4,144) = 17.68$, $P < .01$], and a significant Dose × Group interaction [$F(2,36) = 4.59$, $P < .05$]; the Dose × Trial interaction was not significant [$F(8,144) = 1.62$, $P = .12$]. Importantly, there was a significant Dose × Group × Trial interaction [$F(8,144) = 3.44$, $P < .01$], suggesting an effect of caffeine preexposure on the shift in saccharin consumption that was isolated to a particular group (i.e., paired rats preexposed to 10 mg/kg caffeine; see Fig. 2B).

To determine the source of the interactions, we first conducted contrasts comparing the unpaired or paired saline (0 mg/kg)-preexposed rats to their respective caffeine-preexposed groups. Paired rats preexposed to 10 mg/kg caffeine consumed more saccharin on Trials 3 to 5 than the paired rats preexposed to saline [t's$(12) \geq 2.47$, P's $< .03$]. Paired rats preexposed to 30 mg/kg caffeine did not differ from saline-preexposed controls on any trial (t's < 1). On Trial 2, unpaired rats preexposed to saline consumed more saccharin than unpaired rats preexposed to 10 or 30 mg/kg caffeine [t's$(12) \geq 2.27$, P's $< .05$]. No other significant differences among the unpaired rats were found [t's$(12) \leq 1.92$, P's $\geq .08$]. This pattern suggests that saccharin avoidance conditioned by nicotine was weaker only in rats preexposed to 10 mg/kg of caffeine.

This specificity of caffeine's effects was confirmed by contrasts comparing each paired group to its respective unpaired control. Conditioned avoidance in the rats preexposed to saline emerged by the second conditioning trial and was maintained through the rest of conditioning [t's$(12) > 3.15$, P's $< .01$] (Fig. 2A). Similarly, CTA emerged on the third conditioning trial for rats preexposed to 30 mg/kg caffeine and was present through the rest of

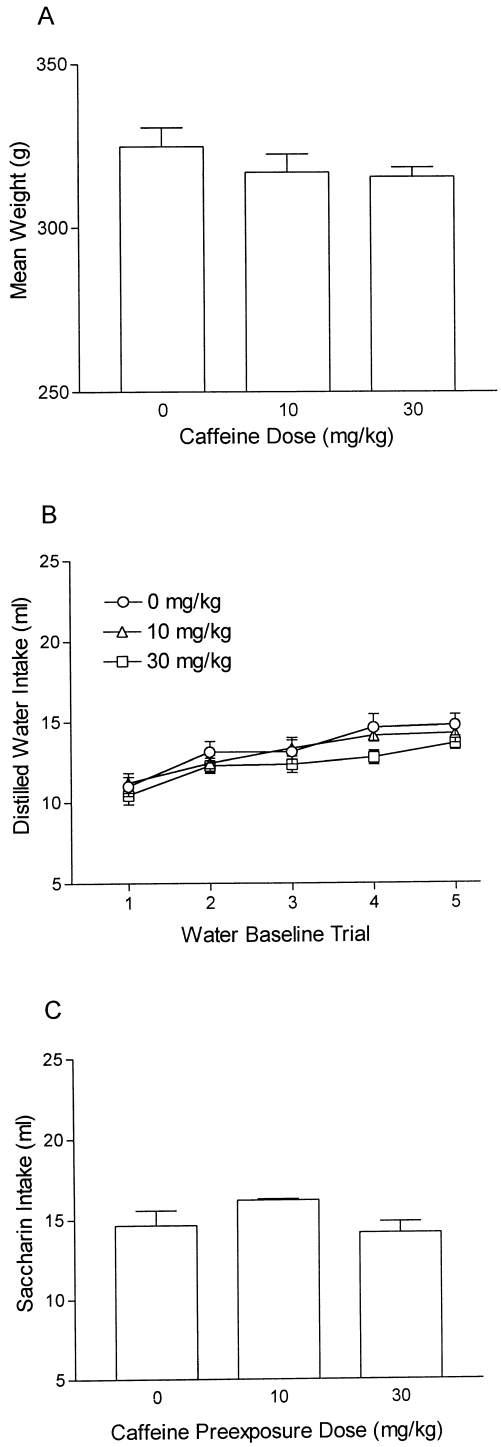

Fig. 1. This figure illustrates unconditioned effects of chronic caffeine in the 10-day preexposure experiment. Panel A shows the mean (+1 S.E.M.) weight in grams for rats on the last day of caffeine preexposure. Panel B shows distilled water intake in milliliters during the water baseline phase. Panel C illustrates saccharin intake on the first conditioning day for rats preexposed to 0, 10, or 30 mg/kg caffeine.

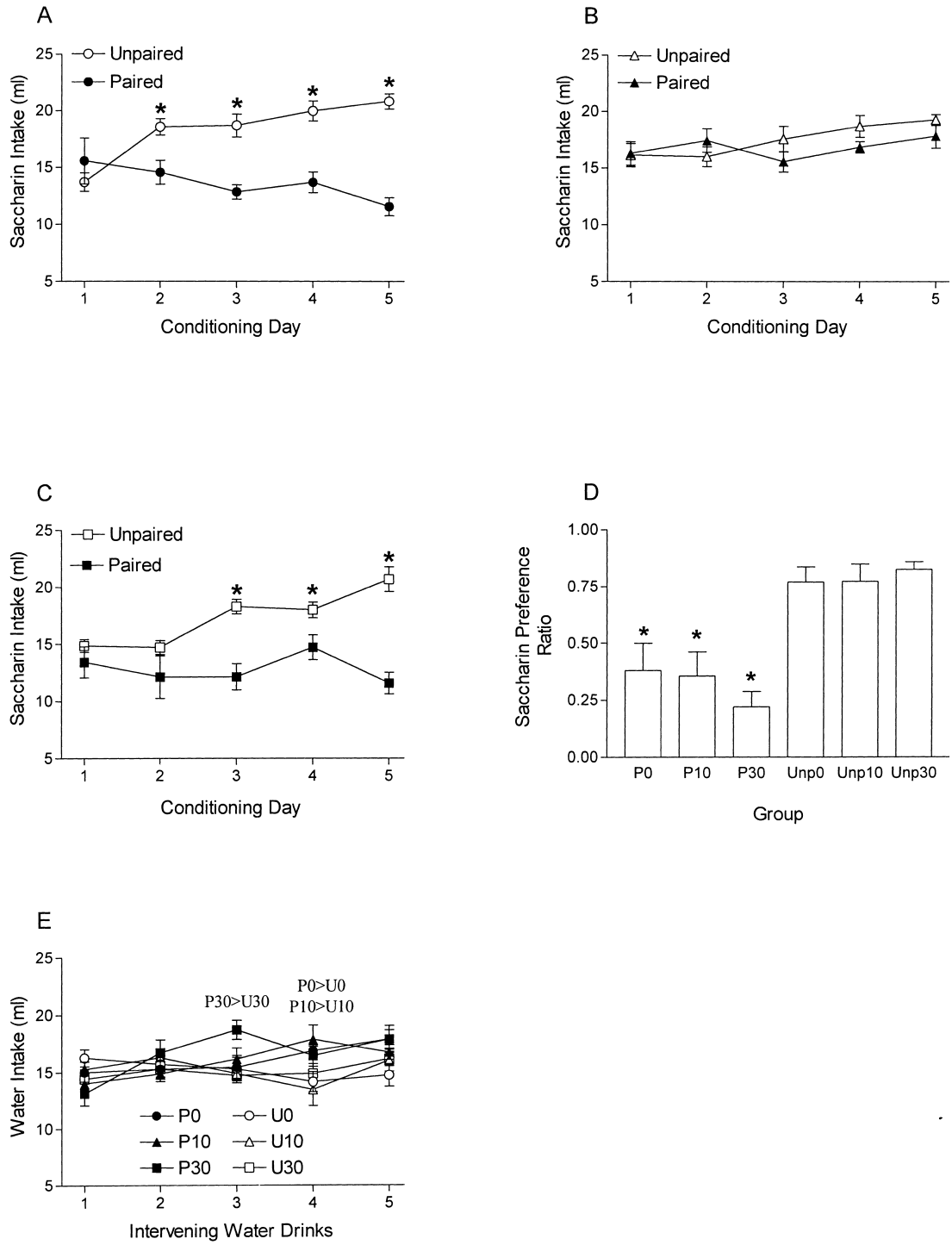

Fig. 2. This figure illustrates the conditioned effects of saccharin paired with nicotine after exposure to caffeine for 10 days. Panels A–C show mean (±1 S.E.M.) saccharin intake for paired vs. unpaired rats preexposed to 0, 10, and 30 mg/kg, respectively, over the five conditioning trials. Panel D shows the preference ratio for each group on the two-bottle test. Panel E illustrates water consumption on intervening water days during the conditioning phase. Asterisk denotes significant difference ($P<.05$) from comparable control condition.

conditioning [t's(12)>2.55, P's $<.05$] (Fig. 2C). In contrast, saccharin intake did not differ between paired and unpaired rats on any trial when the preexposure dose of caffeine was 10 mg/kg (t's <1) (Fig. 2B). Thus, the significant Dose × Group × Trial interaction detected in the overall analyses indicates that the effect of caffeine preexposure on the shift in saccharin consumption was isolated to the paired rats preexposed to 10 mg/kg caffeine.

Fig. 2D illustrates saccharin preference ratios for the two-bottle test. Analysis revealed a main effect of group

[$F(1,36) = 49.45$, $P < .01$], denoting that paired groups had significantly lower preference for saccharin than the unpaired controls. The main effect of dose and the Group × Dose interaction were not significant (F's < 1). Planned contrasts confirmed that each paired group had a significantly smaller preference ratio for saccharin than its respective unpaired control (P's < .01). This data pattern suggests that caffeine preexposure did not influence conditioned saccharin avoidance as indexed with this testing procedure.

Recall that each saccharin trial was followed by an intervening water day in which the unpaired rats received an injection of nicotine. Although the original intent of this protocol was to insure that the rats remained hydrated (cf. Bevins et al., 1996), this protocol may have allowed the unpaired rats to associate distilled water with nicotine administration. In order to assess this interesting possibility, distilled water intake from intervening water days was analyzed using a 3 × 2 × 5 mixed factorial ANOVA (see Fig. 2E for the distilled water intake data). Overall, there were significant main effects of group [$F(1,36) = 4.74$, $P < .05$], and trial [$F(4,144) = 4.62$, $P < .01$], and a Group × Trial interaction [$F(4,144) = 7.53$, $P < .001$], indicating that water intake in unpaired rats tended to diverge from the intake of the paired rats over the 5 days. Planned contrasts revealed that each paired group drank significantly more distilled water than its comparable unpaired control on only one of the five intervening water days [t's(12) ≥ 2.35, P's ≤ .04] (Day 3: 30 mg/kg; Day 4: 0 and 10 mg/kg; Fig. 2E). The main effect of dose and the Group × Dose interaction were not significant (F's < 1). The Dose × Trial and Dose × Group × Trial interactions were also not significant (F's ≤ 1.91, P's ≥ .08).

3.2. Thirty-day preexposure

3.2.1. Unconditioned effects

Body weight, water intake, and initial saccharin consumption were assessed before rats were differentially treated with nicotine. Data from the paired and unpaired groups were pooled as described earlier. A one-way ANOVA revealed a significant effect of dose on bodyweight [$F(2,53) = 3.82$, $P < .05$]. Post hoc analysis revealed that rats preexposed to 30 mg/kg caffeine weighed significantly less than those exposed to saline [$P < .05$] (Fig. 3A). This difference in weight was the result of caffeine exposure because weights were statistically similar between groups at the start of the experiment ($F < 1$).

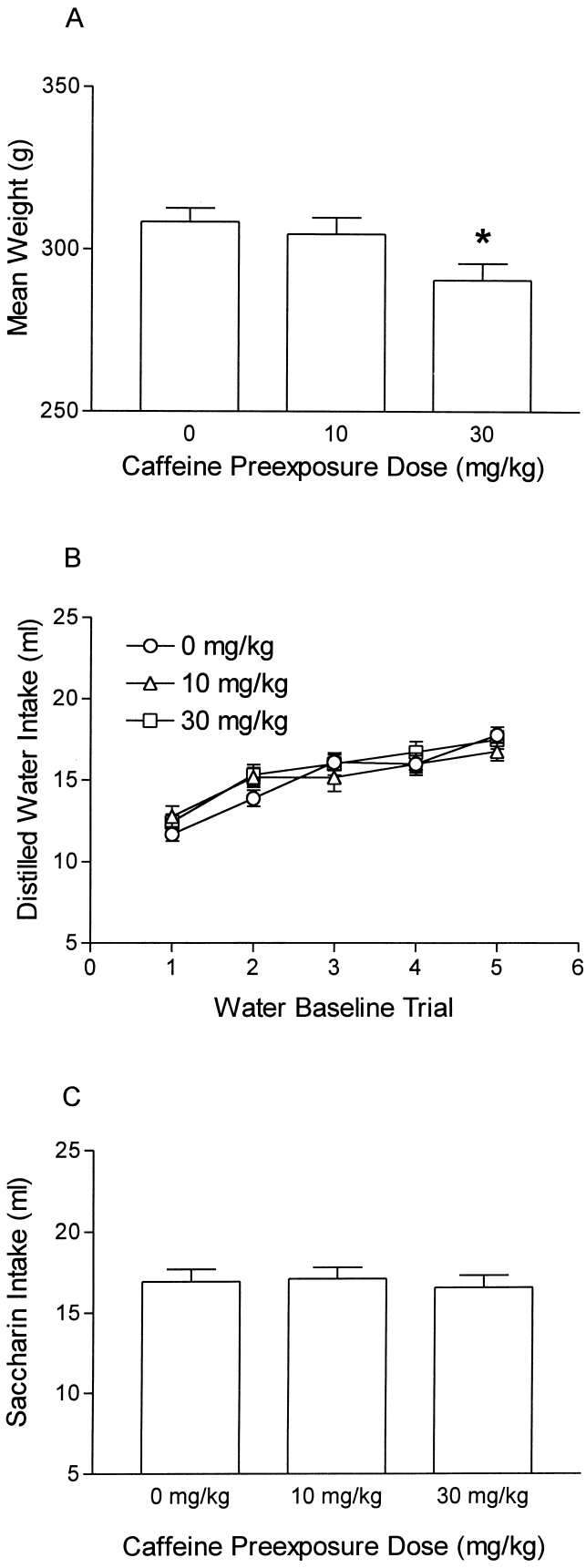

Fig. 3. This figure illustrates unconditioned effects of chronic caffeine in the 30-day preexposure experiment. Panel A shows the mean (+1 S.E.M.) weight in grams for rats on the last day of the caffeine preexposure. Panel B shows distilled water intake in milliliters over the course of water baseline. Panel C illustrates saccharin intake on the first conditioning day for rats preexposed to 0, 10, or 30 mg/kg caffeine. Asterisk denotes significant difference ($P < .05$) in weight from saline-preexposed controls.

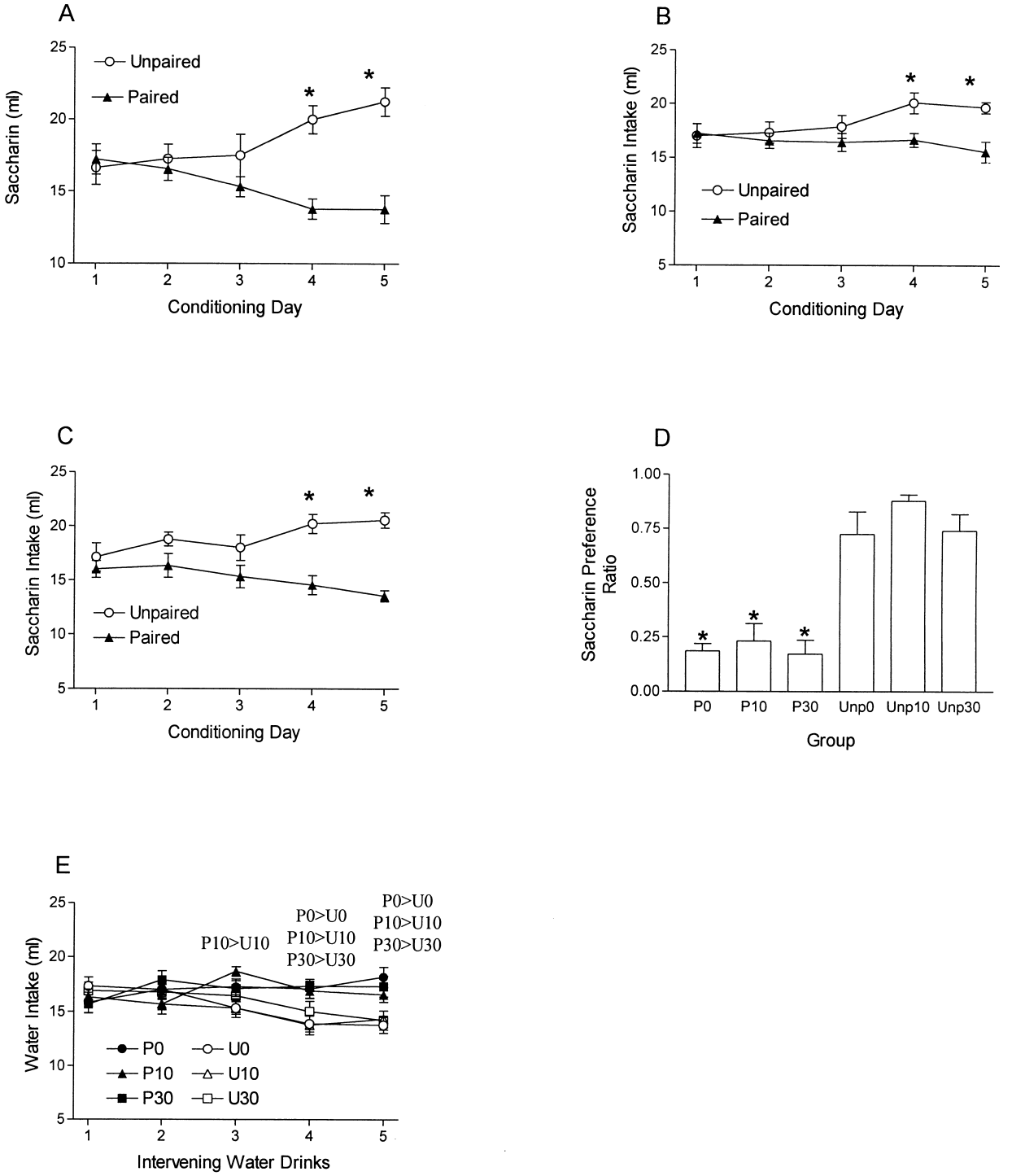

Fig. 4. This figure illustrates the conditioned effects of saccharin paired with nicotine after exposure to caffeine for 30 days. Panels A–C show mean (±1 S.E.M.) saccharin intake for paired vs. unpaired rats preexposed to 0, 10, and 30 mg/kg, respectively, over the five conditioning trials. Panel D shows the preference ratio for each group on the two-bottle test. Panel E illustrates water consumption on intervening water days during the conditioning phase. Asterisk denotes significant intake difference ($P<.05$) between paired and respective unpaired control.

During the water baseline phase, there was a main effect of trial [$F(4,212)=44.49$, $P<.05$], denoting that rats increased water intake during this phase (Fig. 3B). The lack of a main effect of dose ($F<1$), or an interaction [$F(4,212)=1.32$, $P>.23$], indicates that caffeine preexposure did not affect water intake. Exposure to caffeine for 30 days did not yield any unconditioned effects on initial intake of a novel saccharin solution ($F<1$) (Fig. 3C).

3.2.2. Conditioned effects

Fig. 4A–C show saccharin intake in each paired group compared to its respective unpaired control across conditioning trials. The overall $3 \times 2 \times 5$ mixed factorial ANOVA revealed no main effect of trial ($F < 1$), but there was a main effect of group [$F(1,47) = 37.85$, $P < .001$], and a Group × Trial interaction [$F(4,188) = 15.03$, $P < .001$], indicating that across trials, paired groups decreased saccharin intake relative to unpaired groups. None of the analyses including dose as a factor was significant (F's < 1). This pattern indicates that 30 days of preexposure to caffeine did not differentially affect the development of a nicotine-conditioned saccharin avoidance. Planned comparisons confirmed this impression; conditioned avoidance was present on the fourth and fifth conditioning trials for all paired rats [t's(16)>2.93, P's < .01] (Fig. 4A–C).

Fig. 4D illustrates mean saccharin preference ratios for the two-bottle test. Analysis revealed a main effect of group [$F(1,47) = 109.82$, $P < .01$], denoting that paired rats had a significantly lower preference for saccharin than unpaired rats. The main effect of dose was not significant [$F(2,47) = 1.44$, $P > .05$], and there was no interaction ($F < 1$). Planned contrasts revealed that each paired group demonstrated significantly less preference for saccharin than its respective unpaired control (P's < .001). This data pattern suggests that caffeine preexposure did not influence conditioned saccharin avoidance as indexed with this procedure.

As noted previously, the design of this experiment may have promoted an association between distilled water and the effects of nicotine in unpaired groups. We again examined this possibility with a $3 \times 2 \times 5$ repeated measures ANOVA on distilled water intake across the five intervening water days (see Fig. 4E). Overall analysis revealed main effects of group [$F(1,47) = 11.32$, $P < .01$], and of trial [$F(4,188) = 2.99$, $P < .05$]. There was also a Group × Trial interaction [$F(4,188) = 10.05$, $P < .001$], indicating that across days, paired rats water intake diverged from the unpaired rats. The lack of any effect including dose as a factor (F's ≤ 1.31, P's $\geq .34$), indicates that this effect did not vary as a function of caffeine preexposure dose. Planned contrasts revealed that paired rats preexposed to 10 mg/kg caffeine consumed more distilled water than their respective unpaired controls on Days 3, 4, and 5 [t's(16) ≥ 2.19, P's < .05]. Paired rats preexposed to 0 and 30 mg/kg caffeine consumed more distilled water than respective unpaired controls on Days 4 and 5 [t's(16) ≥ 2.19, P's < .05].

4. Discussion

Rats preexposed to 30 mg/kg caffeine for 30 days weighed less than those preexposed to saline, confirming previous observations that caffeine can have anorexic effects (e.g., Gans, 1984). Given that the weight difference was specific to the high dose of caffeine for the longest preexposure period, weight differences cannot account for the effects of caffeine on conditioned saccharin avoidance. Further, the effects of caffeine on conditioned saccharin avoidance are likely not due to changes in the pharmacokinetic properties of nicotine. For example, Gasior et al. (2000) preexposed rats to caffeine (0.25 or 1.0 mg/ml in drinking water) for 3 weeks. Rats were then treated acutely with nicotine (0.4 mg/kg, base) and blood samples were collected 10 or 60 min later. Rats preexposed to caffeine had plasma levels of nicotine and cotinine (major metabolite) similar to control rats that had access to tap water regardless of when blood was collected. Chronic exposure to caffeine for 10 or 30 days did not alter water intake for rats preexposed to 10 or 30 mg/kg caffeine. Therefore, preexposure to caffeine does not have nonspecific effects on fluid intake within our experimental protocol. Further, caffeine does not appear to affect neophobia as evidenced by comparable initial water intake (novel drink situation) and initial saccharin intake (novel flavor).

In general, caffeine preexposure did not alter the development of a saccharin preference. Except for a transient difference between the saline and 30 mg/kg caffeine conditions in the 10-day preexposure experiment, unpaired controls in both experiments displayed a similar increase in saccharin intake over repeated exposures (trials). The comparable development of a preference suggests that caffeine neither enhances nor attenuates the palatability of saccharin. The inability of caffeine to affect flavor intake distinguishes it from other compounds. For example, chlordiazepoxide is a benzodiazepine that nonspecifically enhances saccharin intake (Parker, 1991, 1995a). Also, morphine can enhance or attenuate saccharin intake depending on the dose of morphine and the concentration of the saccharin solution (Touzani et al., 1990). This apparent dissociation in the effects of caffeine from opiates or benzodiazepines on taste palatability likely reflects differential involvement of adenosine versus GABA or opioid brain systems in the consumption of sweet flavors.

Rats not exposed to caffeine for 10 days prior to saccharin–nicotine pairings developed a conditioned avoidance of saccharin (i.e., paired rats drank less than unpaired rats). Ten days of preexposure to 30 mg/kg caffeine appeared to slow the development of nicotine-CTA, as it did not develop until the third conditioning trial. However, this retardation is probably due to the transient difference in the unpaired groups mentioned previously (i.e., less intake than saline controls on Trial 2). Interestingly, rats preexposed to 10 mg/kg caffeine for 10 days did not appear to acquire a nicotine-CTA as measured in repeated one-bottle tests. In contrast, chronic caffeine preexposure for 30 days yielded a different data pattern. That is, caffeine exposure for 30 days, regardless of dose, did not affect the acquisition of nicotine-CTA.

Chronic preexposure to caffeine attenuated expression of nicotine-CTA only after 10 days of preexposure, and only for rats preexposed to 10 mg/kg. Interestingly, when given a

two-bottle choice test, paired rats preexposed to 10 mg/kg caffeine for 10 days displayed an avoidance tendency comparable to those preexposed to saline or 30 mg/kg caffeine. The discrepancy between one- and two-bottle tests in the 10 mg/kg condition of the 10 day preexposure experiment is intriguing. When saccharin was the only available fluid, paired rats performed similar to unpaired controls with a similar history of caffeine preexposure. Indeed, like the controls, intake of saccharin increased even though saccharin was paired with nicotine after each exposure (trial). However, when these rats were presented with a choice between saccharin and water, the rats avoided saccharin similar to other paired rats. This outcome indicates that rats that were preexposed to 10 mg/kg caffeine for 10 days prior to taste conditioning acquired the saccharin–nicotine association even though it was not expressed during training (i.e., one-bottle tests).

The discrepancy between one- and two-bottle tests in this experiment requires further discussion. Note that we do not have one-bottle intake data for the sixth trial (i.e., the day that the two-bottle test took place). Although unlikely, it is conceivable that paired rats preexposed to 10 mg/kg caffeine for 10 days may have demonstrated nicotine-CTA in a one-bottle test on this trial. A more likely explanation, however, rests on the research indicating that a two-bottle test for CTA, under some conditions, is more sensitive than a one-bottle test (Batsell and Best, 1993; Bevins et al., 1997; Dragoin et al., 1971; Grote and Brown, 1971). We take this lack of nicotine-CTA in the one-bottle tests to indicate that 10 days of preexposure to 10 mg/kg caffeine attenuates the avoidance tendencies conditioned by nicotine. A learning or performance deficit may account for this attenuation; thus a more sensitive test was required to reveal the nicotine-CTA. Data from the present experiments do not permit us to distinguish between these accounts. Regardless, our main conclusion stands; expression of nicotine-CTA was attenuated. One methodological implication of this result is that studies assessing the effects of preexperimental manipulations (e.g., drug preexposure) on subsequent development of a CTA should employ both one- and two-bottle tests. To date, research on nicotine-CTA is mixed (e.g., one-bottle test: Cappell and Poulos, 1979; Cappell and LeBlanc, 1977; two-bottle test: Iwamoto and Williamson, 1984; or both: Kunin et al., 2000). This methodological implication may extend to CTA research with other abused drugs.

If the data pattern for the one- and two-bottle tests reflects a weakened association, then one question that arises is how caffeine attenuates the avoidance properties conditioned by nicotine. Specifically, we propose that caffeine could attenuate the conditioned avoidance effect by altering processing of the flavor cue, altering processing of the nicotine-US, or disrupting the processes that enable the flavor cue to be associated with nicotine administration via Pavlovian conditioning. Our enthusiasm for the first account, altered processing of the flavor cue, is severely diminished by our data. If processing of the flavor cue were affected by caffeine preexposure, then it would be altered in all rats (paired and unpaired) exposed to 10 mg/kg caffeine for 10 days. However, initial saccharin intake was not affected by caffeine preexposure. Further, development of a saccharin preference in unpaired rats was not affected by preexposure to 10 mg/kg caffeine for 10 days. Presumably, preference development provides an additional index of cue processing.

Although caffeine preexposure does not appear to alter the quality of the flavor cue in our situation, it may alter processing of the nicotine-US. Nicotine-CTA is produced by the central cholinergic action of nicotine (Kumar et al., 1983; Shoaib and Stolerman, 1995). Notably, Shi et al. (1993) found that chronic caffeine exposure increased cortical nicotinic ACh receptors by as much as 50%. Daly et al. (1999) suggested that the upregulation of nicotinic ACh receptors could indicate conversion to a desensitized state. Further support for desensitization of nicotinic receptors is provided by the observation that chronic caffeine administration reduces the sensitivity of cortical neurons to the excitatory action of microiontophoretically applied ACh (Lin and Phillis, 1990). If there are more desensitized nicotinic ACh receptors following caffeine preexposure, this would likely decrease the impact of nicotine in the CTA situation.

Another possible way that caffeine preexposure attenuated nicotine-CTA could be that caffeine altered processes allowing formation of the associative link between the flavor cue and nicotine. For example, extracellular ACh levels increase in the nucleus accumbens when a saccharin cue previously paired with LiCl is presented alone (Mark et al., 1995). In addition, intracranial nicotine administration only produced reliable CTA when infused directly into the nucleus accumbens; infusion into the interpeduncular nucleus, ventral tegmental area, and dorsal hippocampus yielded inconsistent effects at best (Shoaib and Stolerman, 1995). Thus, ACh transmission in the nucleus accumbens appears important for processing both the flavor cue (Mark et al., 1995) and the nicotine-US (Shoaib and Stolerman, 1995) in CTA preparations. Caffeine alteration of cholinergic processes in the nucleus accumbens could disrupt the formation of the flavor–US association in this preparation. One possible mechanism described earlier was an increase in desensitized nicotinic ACh receptors (Daly et al., 1999; Kumar et al., 1983; Shi et al., 1993).

Another possible neurobiological mechanism in the nucleus accumbens may involve dopaminergic processes. For example, there is a functional interaction between adenosinergic and dopaminergic receptors in the basal ganglia (Ferre et al., 1991, 1997). Indeed, chronic preexposure to caffeine enhances nicotine-induced locomotor activity, acquisition of a nicotine discrimination, and dopamine release in the nucleus accumbens (Gasior et al., 2000; Tanda and Goldberg, 2000). Dopaminergic activity in the nucleus accumbens appears to be involved in acquisition of CTA

(Mark et al., 1991); presentation of a saccharin flavor that has been previously paired with LiCl results in decreases in extracellular dopamine in the nucleus accumbens (Mark et al., 1991). Thus, chronic preexposure to caffeine may attenuate CTA in this preparation by altering important dopaminergic processes in the nucleus accumbens.

One question that requires some attention is why caffeine preexposure only affects nicotine-CTA after brief exposure (10 days) to the lowest dose (10 mg/kg). Interestingly, this outcome is consistent with other behavioral effects of chronic caffeine exposure. Indeed, in humans, one researcher (Nehlig, 1999) indicated that "The most notable behavioral effects of caffeine occur after low to moderate doses..." (p. 563). For example, Richardson et al. (1995) tested the mood and performance effects of caffeine in human caffeine consumers and nonconsumers. Following acute administration of 70 or 250 mg of caffeine, jitteriness increased whereas headache, tiredness, and hand-steadiness decreased as a function of dose. However, performance on a reaction-time task was enhanced by the 70 mg, but not the 250 mg dose of caffeine. In a different study, a low dose of caffeine (2 mg) administered to human participants delayed habituation of eye-blink responses to an acoustic stimulus, a higher dose (6 mg) did not (Schicatano and Blumenthal, 1995). Further, in juvenile rats, chronic exposure to moderate doses of caffeine (approximately 19.6 or 37.5 mg/kg/day) in drinking water for 11 days increased play fighting (Holloway and Thor, 1984). However, chronic exposure to a high dose (approximately 150 mg/kg/day) had no effect on play fighting. As described previously, chronic preexposure to a low concentration of caffeine in drinking water (0.25 mg/ml) potentiated locomotor hyperactivity induced by nicotine and enhanced acquisition of a nicotine discrimination task in rats (Gasior et al., 2000). Preexposure to a higher concentration of caffeinated water (1.0 mg/ml) did not affect nicotine-induced motor activity or acquisition of the discrimination. Our results add to the list of caffeine effects that are specific to low to moderate doses. Systematic research examining caffeine dose, duration of exposure, and administration protocol will be required to elucidate the mechanisms mediating the differential behavioral effects of caffeine.

In contrast to saccharin CTA, caffeine preexposure did not differentially alter consumption of distilled water on the days that intervened between each saccharin conditioning trial. However, we did find that water intake between the paired and unpaired rats diverged across trials. That is, unpaired rats drank less than the paired rats in the later trials; this result was more robust in the 30-day preexposure experiment. Recall that access to water on the intervening water day was followed by nicotine in the unpaired rats; saline was given to the paired rats. This protocol may have allowed the unpaired rats to associate distilled water with nicotine administration. This association is one potential account for the difference in water intake during the intervening water days. Alternatively, perhaps the paired rats were consuming more water on these days to compensate for the decrease in saccharin intake seen in the later saccharin conditioning trials. Analysis comparing intake from intervening water day 1 to that of day 5 does not provide unequivocal support for either account. For example, two of the six unpaired groups displayed a significant decrease in intake (10 day 30 mg/kg unpaired and 30 day unpaired 0 mg/kg); this decrease is predicted by the conditioning and the compensatory intake account. Only three of the six paired groups displayed a statistically significant increase in water intake suggesting a compensatory mechanism.

In summary, the potential avoidance properties of nicotine can be altered by chronic caffeine preexposure. In our preparation, a 10-day treatment with 10 mg/kg caffeine masked a CTA produced by nicotine on one-bottle tests for conditioning. Although conditioned avoidance was not expressed during one-bottle tests, conditioning was revealed in a two-bottle choice test. The present research can eliminate an account based on caffeine alteration of processing of the saccharin cue. Further research will be required to determine whether the mechanisms mediating US processing or the saccharin–nicotine association were altered. To our knowledge, these are the first experiments to systematically investigate the Pavlovian conditioned avoidance properties of nicotine following chronic exposure to caffeine. It is our view that the investigation of the effects of caffeine preexposure on the Pavlovian conditioned avoidance properties of nicotine will help to elucidate the behavioral interactions of these drugs.

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