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Caffeine-induced taste aversions in Lewis and Fischer rat strains: Differential sensitivity to the aversive effects of drugs

Janani M. Vishwanath *, Alexa G. Desko, Anthony L. Riley

Psychopharmacology Laboratory, Department of Psychology, American University, Washington, DC 20016, United States

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ABSTRACT

Lewis (LEW) and Fischer 344 (F344) rat strains have been reported to differ in the aversive effects induced by a number of drugs. The present studies extended this previous work and examined the ability of caffeine to induce taste aversions in the two strains. Specifically, LEW and F344 rats were given access to saccharin and injected with varying doses of caffeine (0, 0.32, 1.0 and 3.2 mg/kg—Experiment 1; 0, 10, 18 and 32 mg/kg—Experiment 2). Additionally, the effects of caffeine on locomotor activity were examined in Experiment 2. At low doses (Experiment 1), caffeine failed to induce taste aversions in either strain. At higher doses (Experiment 2), aversions were induced that were strain dependent. Specifically, caffeine induced taste aversions in both strains at 32 mg/kg, while 18 mg/kg caffeine was effective in inducing aversions only in the LEW strain. Caffeine increased activity in both strains with no strain difference. This demonstration adds to the growing list of drugs on which the LEW and F344 strains differ in relation to their affective properties. Given that drug use and abuse are a function of the balance of the rewarding and aversive effects of drugs, understanding such strain differences may provide insight into the biological (and genetic) factors impacting abuse vulnerability.

1. Introduction

The Lewis (LEW) and Fischer (F344) rat strains are genetically divergent inbred strains that have been reported to differ on a host of physiological and behavioral endpoints, including stress reactivity, inflammatory and immune response, startle reaction, locomotor activity and open-field behavior (Berton et al., 1997; Davis et al., 2007; Gomez-Serrano et al., 2009; Guitart et al., 1992, 1993; Kosten et al., 1997; Riley et al., 2009; Stöhr et al., 2000). These two strains have also been found to differ in their responses to a variety of drugs of abuse (for reviews, see Kosten and Ambrosio, 2002; Riley et al., 2009). For example, strain differences have been reported in the self-administration of morphine, cocaine, nicotine and alcohol with the LEW strain exhibiting a more rapid acquisition than the F344 strain (Brower et al., 2002; Kosten et al., 1997; Suzuki et al., 1988a,b). Further, the LEW strain has been reported to display greater cocaine and morphine conditioned place preferences than the F344 strain (Guitart et al., 1992; Kosten et al., 1994; though see Davis et al., 2007). Together, these findings suggest that the LEW strain may be more sensitive than the F344 to the rewarding effects of a variety of drugs of abuse (Guitart et al., 1992; Kosten et al., 1994, 1997; Suzuki et al., 1988a,b).

These differences in drug reactivity have been extended to other affective properties of such drugs, specifically, their aversive effects. For example, Glowa et al. (1994) reported that LEW rats displayed a greater cocaine-induced taste aversion than the F344 strain (such effects were evident at the 18 and 32 mg/kg doses of cocaine; no differences were found at 50 mg/kg). Subsequent to this report, strain differences have also been reported with morphine, nicotine and alcohol (see Lancellotti et al., 2001; Pescatore et al., 2005; Roma et al., 2006). Interestingly, for these drugs, it is the F344 rats that display a greater conditioned taste aversion, suggesting that differences in the aversive effects of such drugs are a function of the specific compound used and not a general function of differences in taste salience or learning and memory.

Although the direction of the relative sensitivity to the aversive effects of drugs (F>L; L>F) appears to be drug specific (see above), the two strains do differ for every drug of abuse examined. In this context, it is interesting to note that no consistent differences are reported when the two strains are tested on the emetic LiCl (see Foynes and Riley, 2004). Specifically, LiCl induces comparable dose-dependent aversions in both strains, suggesting that the sensitivity to the aversive effects of drugs (and not just the direction of this sensitivity) is dependent on the specific drug examined. Examination of other drugs may provide insight into the characteristics of compounds that are important for the differences in the affective properties of drugs in these two strains (see Guitart et al., 1992; Kosten et al., 1994, 1997; Riley et al., 2009; Suzuki et al., 1988a,b) and the possible role genetic factors may play in the sensitivities to such properties. Given that drug use (and abuse) vulnerability may be a function of the balance

* Corresponding author at: Psychopharmacology Laboratory, Department of Psychology, American University, 4400 Massachusetts Ave, NW, Washington, DC 200016, United States. Tel.: +1 202 885 1721; fax: +1 202 885 1023.
E-mail addresses: jans19@gmail.com (J.M. Vishwanath), alexa.desko@gmail.com (A.G. Desko), alriley@american.edu (A.L. Riley).

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between its rewarding and aversive effects (Kohut and Riley, 2010; Riley et al., 2009; Riley, 2011; Rinker et al., 2008a,b; Roma et al., 2006), this information may provide insight into possible genetic factors related to such vulnerability (Davis et al., 2007; Kosten and Ambrosio, 2002; Kosten et al., 1994, 1997; Lancellotti et al., 2001; Pescatore et al., 2005; Riley et al., 2009; Roma et al., 2006).

The purpose of the present series of studies was to extend the assessments of strain differences in the aversive effects of drugs to caffeine, a compound not generally regarded as a drug of abuse and for which the rewarding and aversive effects in the LEW and F344 strains have not been documented. Interestingly, in outbred rats caffeine has been reported to have both rewarding and aversive effects (Brockwell et al., 1991; also see Nehlig, 1999). For example, Brockwell et al. (1991) reported that low doses of caffeine (3.0 mg/kg) induced place preferences in Wistar rats. Conversely, higher doses have been reported to produce both place (Brockwell et al., 1991; Steigerwald et al., 1988) and taste (Vitiello and Woods, 1977) aversions. Accordingly, the present studies examined the ability of various doses of caffeine (0, 0.32, 1.0, 3.2 mg/kg, Experiment 1; 0, 10, 18, 32 mg/kg, Experiment 2) to induce taste aversions in the LEW and F344 rat strains. To assess if any strain differences with caffeine were specific to the aversion design, caffeine-induced changes in motor behavior were also examined in the two strains in Experiment 2.

2. Procedure

2.1. Experiment 1: low dose caffeine-induced conditioned taste aversion

2.1.1. Method

2.1.1.1. Subjects. Subjects in Experiment 1 were 68 experimentally naive LEW ($n=34$) and F344 ($n=34$) male rats (purchased from Harlan Sprague Dawley, Indianapolis). At the start of the experiment, animals were approximately 90 days of age and weighed between 282 and 320 g (LEW) and 182 and 240 g (F344). All animals were maintained on a 12-h light/dark cycle (lights on at 0800 h) and at an ambient temperature of 23 °C throughout the experiment. Procedures recommended by the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996), the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council, 2003) and the Institutional Animal Care and Use Committee at American University were followed at all times.

2.1.1.2. Apparatus. Subjects were housed in stainless-steel, wire-mesh cages on the front of which graduated 50 ml Nalgene centrifuge tubes could be placed for presentation of water and/or saccharin.

2.1.1.3. Drugs and solution. Anhydrous caffeine (Sigma Aldrich Co., St. Louis, MO) was dissolved in saline at a concentration of 1 mg/ml. Saccharin (sodium saccharin, Sigma) was prepared as 1 g/l solution in tap water. Caffeine weights are expressed in base form.

2.1.2. Conditioned taste aversions

2.1.2.1. Habituation. Following $23^{2/3}$ h water deprivation, all subjects were given 20-min access to water. This procedure was repeated daily until water consumption was stable (i.e., within 2 ml over 3 consecutive days with no consistent increase or decrease).

2.1.2.2. Conditioning. On Day 1 of this phase, all subjects were given 20-min access to a novel saccharin solution. Immediately after saccharin access, subjects within each strain were assigned to one of four groups such that saccharin consumption was comparable among groups. Based on these group assignments, subjects were given an intraperitoneal (IP) injection of either 0 (saline vehicle), 0.32, 1.0 or 3.2 mg/kg. This resulted in the following eight groups: L0, L0.32, L1.0, L3.2, F0, F0.32, F1.0 and F3.2 ($n=8$–9 per group). For each group, the letter denotes the strain and the number denotes the caffeine dose. Dependent upon the dose, the injection volume ranged from 0.102 to 1.02 ml for caffeine-injected LEW rats and 0.0768 to 0.768 ml for caffeine-injected F344 rats. The volume of the vehicle was matched to the volume administered at the highest caffeine dose, and all injections were given within 10 min of group assignments. On the 3 days following this conditioning trial, all subjects were given 20-min access to water (water-recovery sessions). No injections were given on recovery days. This alternating procedure of conditioning/water recovery was repeated for a total of 5 cycles.

2.1.2.3. Two-bottle aversion test. On the day following the last recovery session of the Conditioning phase, all subjects were given 20-min access to both water and saccharin in a two-bottle test of the aversion to saccharin. The location of the bottles on the front of the home cage was counterbalanced within each group. For all subjects, saccharin was initially presented for 5 s. The saccharin bottle was then removed, and water was presented for 5 s. Following this, both bottles were presented simultaneously for the remainder of the 20-min session.

2.2. Experiment 2: high dose caffeine-induced conditioned taste aversion

2.2.1. Method

Subjects in Experiment 2 were 67 experimentally naïve LEW ($n=33$) and F344 ($n=34$) subjects of the same sex, strain and age and maintained under the same conditions as those used in Experiment 1. The subjects were run in two replicates ($n=33$ and 34 animals for Replicates 1 and 2, respectively). In Replicate 1, there were 16 and 17 LEW and F344 rats, respectively; in Replicate 2, there were 17 rats of each strain. At no point did subjects in the two replicates differ [$(F(12, 204)=0.739, p=0.712)$], and data from the two replicates were pooled for analysis and presentation. The taste aversion conditioning procedure in this phase was identical to that described in Experiment 1 with the following exceptions. During conditioning, subjects in each strain were assigned to four groups and injected with either 0 (vehicle), 10, 18 or 32 mg/kg, resulting in the following eight groups: L0, L10, L18, L32, F0, F10, F18 and F32 ($n=7$–9 per group). Dependent upon the dose, the injection volume ranged from 0.914 to 2.926 ml for caffeine-injected LEW rats and 0.688 to 2.194 ml for caffeine-injected F344 rats. The volume of the vehicle was matched to the volume administered at the highest caffeine dose, and all injections were given within 10 min of group assignments. To accommodate the larger doses in Experiment 2, the concentration of caffeine was increased to 3.5 mg/ml (from 1 mg/ml in Experiment 1). As in Experiment 1, a two-bottle test was given following conditioning. Given that consumption at the highest dose of caffeine (32 mg/kg) was dramatically suppressed for both strains on this test (see below), all animals were given 3 water-recovery days and then a second two-bottle test (in anticipation that some recovery of saccharin consumption would be evident allowing group differences to be detected). No injections were given after either test.

2.2.2. Locomotor assessment

Following the second two-bottle test, the effects of caffeine on fine locomotor activity and ambulation were assessed in animals from the second replicate (17 LEW and 17 F344 rats). On the 2 days during which locomotor activity was assessed, all animals were restricted to 20-min access of water each day. This was given 1 h prior to the locomotor tests. These assessments were made in modified place preference chambers (San Diego Instruments Place Preference System, San Diego, CA). The chambers (68.5 cm wide × 34.5 cm high × 21 cm deep) were equipped with white LED lights and a 16 × 4 photobeam array used for recording the animals' movement. An 85-watt red light illuminated the otherwise unlit room, and white noise masked background sound. Each individual chamber was made of clear Plexiglas walls, and a single 68.5 cm × 21 cm

sheet of haircell textured gray Kydex plastic covered the floor of each chamber.

On the first day of locomotor assessment, rats from both strains were injected with saline (vehicle) and placed in the chambers for 60 min. On the following day, subjects in each strain were injected with caffeine (or vehicle) based on their prior group assignments, i.e., 0, 10, 18 and 32 mg/kg (n = 4–5 per group; similar injection volumes as noted above), and placed in the same locomotor chambers for 60 min. Changes in motor activity (both fine and ambulation) were determined by breaks in the photobeam array. Fine motor activity was defined as repeated breaks of the same beam (indicative of repetitive activities such as head weaving and grooming). Ambulation was defined as successive breaks of different beams (indicative of gross activities such as walking).

Between each session, all chambers were cleaned with soap and water.

2.3. Statistical analysis

For each experiment, consumption on the first exposure to saccharin was analyzed using an Independent Sample's *t*-test to determine differences between strains. Since this test revealed that F344 and LEW rats differed in consumption on this exposure (see below), each strain in each experiment was evaluated independently. Thus, for each strain, saccharin consumption was analyzed using a 4 × 5 mixed analysis of variance (ANOVA) with the between-subjects variables of Dose (0, 0.32, 1.0, 3.2 mg/kg, Experiment 1; 0, 10, 18, 32 mg/kg, Experiment 2) and the within-subjects variable of Trial (1–5). Where appropriate, one-way ANOVAs followed by Tukey's post-hoc tests were performed on each trial to analyze differences in saccharin consumption between groups. Percent saccharin consumption on the two-bottle tests in each experiment was analyzed using a 2 (Strain) × 4 (Dose) ANOVA. Locomotor activity in Experiment 2 was calculated as number of beam breaks in a 60-min period broken down into 5-min intervals. Since there were no baseline differences between LEW and F344 strains in motor activity (see below), ambulation and fine activity scores were independently analyzed using a 2 × 4 × 12 mixed ANOVA with between subjects variables of Strain (LEW and F344) and Dose (0, 10, 18, 32 mg/kg) and the within-subjects factor of Interval (1–12). As above, one-way ANOVAs followed by Tukey's post-hoc tests were performed on any main effects or interactions revealed by the repeated measures ANOVA.

3. Results

3.1. Experiment 1: low dose caffeine-induced conditioned taste aversion

3.1.1. One bottle mean saccharin consumption

An Independent Sample's *t*-test revealed that the LEW and F344 rats differed in consumption on the first exposure to saccharin prior to conditioning ($t = 6.68$, $p < 0.001$). Consequently, each strain was independently evaluated using a 4 × 5 (Dose by Trial) mixed ANOVA.

3.1.1.1. LEW. The 4 × 5 mixed ANOVA did not reveal any significant main effects or interaction. LEW animals in all groups drank high levels of saccharin across the five trials (see Fig. 1; top left panel).

3.1.1.2. F344. The 4 × 5 mixed ANOVA revealed a significant effect of Trial [$F(4,120) = 28.309$, $p < 0.001$]. However, subsequent post-hoc tests on Trial did not reveal any significant effects. F344 animals in all groups drank high levels of saccharin across the five trials (see Fig. 1; top right panel).

3.1.2. Two-bottle aversion test

The 2 × 4 ANOVA performed on the two-bottle aversion test did not reveal any significant main effects or interaction. Animals in both strains displayed a high preference for saccharin (see Fig. 1; bottom panel).

3.2. Experiment 2: high dose caffeine-induced conditioned taste aversion

3.2.1. One bottle mean saccharin consumption

An Independent Sample's *t*-test revealed that the LEW and F344 subjects differed in consumption on the first exposure to saccharin prior to conditioning ($t = 5.443$, $p < 0.001$). Consequently, each strain was independently evaluated using a 4 × 5 (Dose by Trial) mixed ANOVA.

3.2.1.1. LEW. The 4 × 5 mixed ANOVA revealed a significant effect of Trial [$F(4, 116) = 12.650$, $p < .001$] and a significant Trial × Dose interaction [$F(12, 116) = 6.053$, $p < 0.001$]. In relation to the Trial × Dose interaction, subsequent one-way ANOVAs followed by Tukey's post-hocs revealed that although there were no significant differences between groups on the initial saccharin exposure (Trial 1) [$F(3, 32) = 0.400$, $p = 0.754$], significant differences were evident on Trials 2–5 [all ($F(3, 32) \geq 7.477$, $p \leq 0.001$)]. Specifically, on Trials 2–4 LEW rats injected with 18 and 32 mg/kg drank significantly less saccharin than those injected with either vehicle or 10 mg/kg [all $p < 0.05$]. On Trial 5, subjects in Group L32 drank significantly less than those in all remaining groups [all $p < 0.05$] (see Fig. 2; top left panel). There were no other significant comparisons.

3.2.1.2. F344. The 4 × 5 mixed ANOVA revealed a significant effect of Trial [$F(4, 120) = 5.110$, $p < 0.001$] and a significant Trial × Dose interaction [$F(12, 120) = 6.052$, $p < 0.001$]. In relation to the Trial × Dose interaction, subsequent one-way ANOVAs followed by Tukey's post-hocs revealed that although there were no significant differences between groups on the initial saccharin exposure (Trial 1), [$F(3, 33) = 0.196$, $p = 0.899$], significant differences were evident on Trials 2–5 [all ($F(3, 33) \geq 14.712$, $p < 0.001$)]. Specifically, F344 rats injected with 32 mg/kg caffeine drank significantly less than all other groups [all $p < 0.05$] (see Fig. 2; top right panel). There were no other significant comparisons.

3.2.2. Two-bottle aversion tests

The 2 × 4 ANOVA performed on saccharin preference on the first two-bottle test revealed a significant effect of Strain [$F(1, 59) = 12.661$, $p = 0.001$] and Dose [$F(3, 59) = 28.056$, $p < 0.001$], as well as a significant Strain × Dose interaction [$F(3, 59) = 4.832$, $p = 0.004$]. In relation to the Strain × Dose interaction, Groups L18 and L32 had a significantly lower saccharin preference than Groups L0 and L10 [all $p \leq 0.001$]. Group F32 had a lower saccharin preference than Groups F0, F10 and F18 [all $p < 0.05$]. Finally, Group L18 had a lower saccharin preference than Group F18 [$p < 0.001$]. No other strain comparisons were significant on the first two-bottle assessment (see Fig. 2; bottom left panel).

The 2 × 4 ANOVA performed on the second two-bottle test revealed a significant effect of Strain [$F(1, 59) = 18.452$, $p < 0.001$] and Dose [$F(3, 59) = 30.368$, $p < 0.001$] as well as a significant Strain × Dose interaction [$F(3, 59) = 4.299$, $p = 0.008$]. In relation to the Strain × Dose interaction, Groups L18 and L32 had a lower saccharin preference than Groups L0 and L10 [all $p \leq 0.001$]. Group F32 showed a significantly lower saccharin preference than the other three F344 groups [all $p \leq 0.001$]. Again, Group L18 had a lower saccharin preference than Group F18 [$p < 0.001$] (see Fig. 2; bottom right panel). No other strain comparisons were significant.

3.2.3. Locomotor activity

3.2.3.1. Fine motor activity. There were no baseline differences between LEW and F344 strains in fine motor activity [$F(11, 286) = 1.671$, $p = 0.079$]. Consequently, the strains were analyzed together and

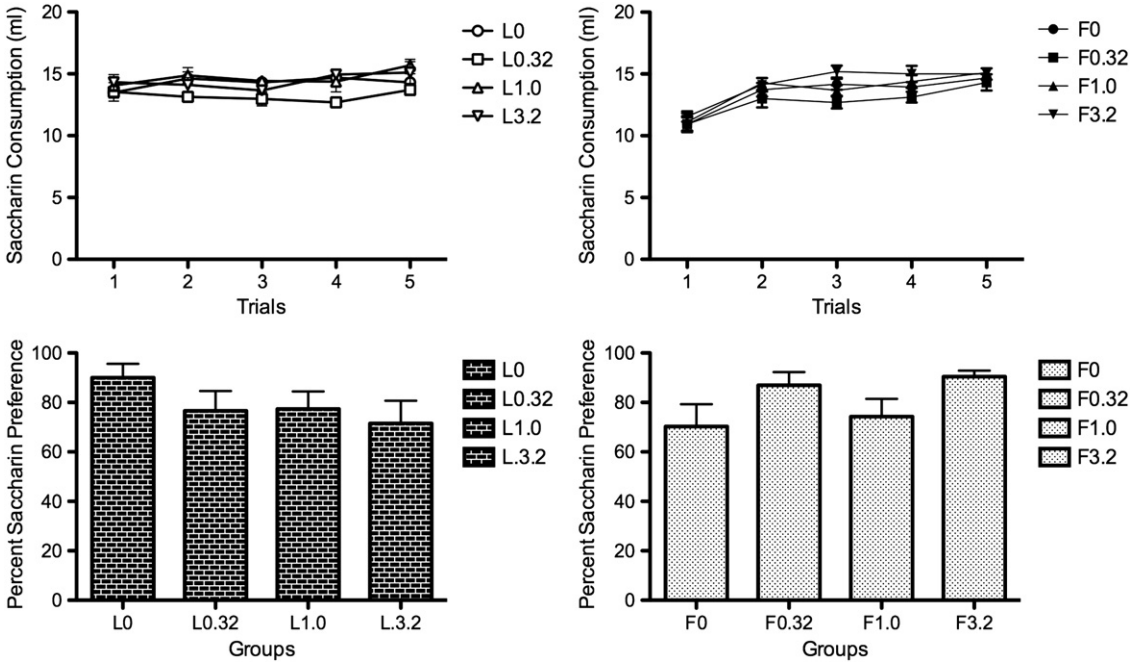

Fig. 1. The top panel presents mean (+/−SEM) saccharin consumption (ml) over repeated conditioning trials in which LEW (left) and F344 (right) animals were given saccharin paired with varying doses of caffeine (0, 0.32, 1.0, 3.2 mg/kg). For neither strain was there a significant effect of Dose or a significant Dose×Trial interaction. The bottom panel presents mean saccharin preference for the LEW (left) and F344 (right) strains on the two-bottle aversion test. There were no significant main effects of Strain or Dose (or any significant interaction).

directly compared. The 12×2×4 mixed ANOVA on fine motor activity revealed that there was a significant effect of Interval [$F(11, 286) = 3.804$, $p<0.001$], Strain [$F(1, 26) = 9.836$, $p<0.05$] and Dose [$F(3, 26) = 17.430$, $p<0.001$] as well as a significant Interval×Dose interaction [$F(33, 286) = 1.948$, $p<0.05$]. In relation to the main effect of Interval (collapsed across Dose and Strain), fine motor activity in Interval 2 was significantly greater than that in Interval 11 ($p = 0.043$). In relation to the main effect of Strain (collapsed across Interval and Dose), Independent Sample *t*-tests revealed that F344 subjects displayed greater fine motor activity than LEW animals [$t = 5.198$, $p<0.001$]. In

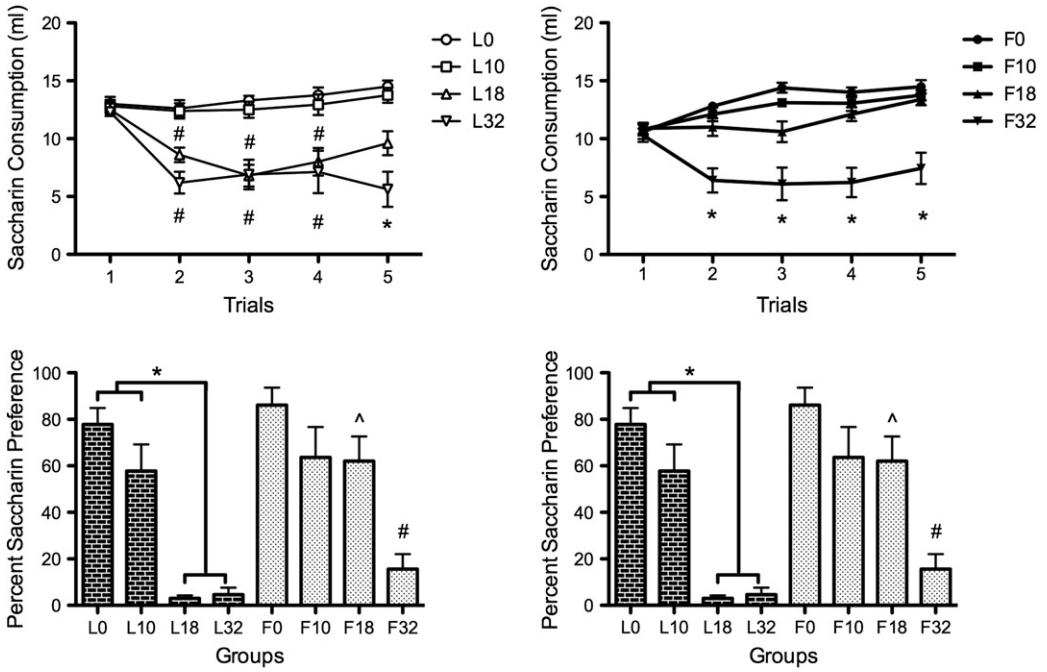

Fig. 2. The top panel presents mean (+/−SEM) saccharin consumption (ml) over repeated conditioning trials in which LEW (left) and F344 (right) animals were given saccharin paired with varying doses of caffeine (0, 10, 18, or 32 mg/kg). For LEW animals, on Trials 2–4, LEW rats in Groups L18 and L32 drank significantly less than animals in the Vehicle Group (L0) or Group L10. On Trial 5, LEW rats in Group L32 drank significantly less than those in the remaining groups (L0, L10, L18). For F344 animals, on Trials 2–5 Group F32 drank significantly less than all other groups (F0, F10, F18). *Indicates a significant difference from any other group (for each strain). #Indicates a significant difference from Vehicle (0 mg/kg) and the 10 mg/kg group. The bottom panel presents mean saccharin preference for the LEW (left) and F344 (right) strains on the two-bottle aversion test. *Indicates a significant difference between Groups L18 and L32 and Groups L0 and L10. ^Indicates a significant difference between Groups L18 and F18. #Indicates a significant difference between Group F32 and all other F344 groups.

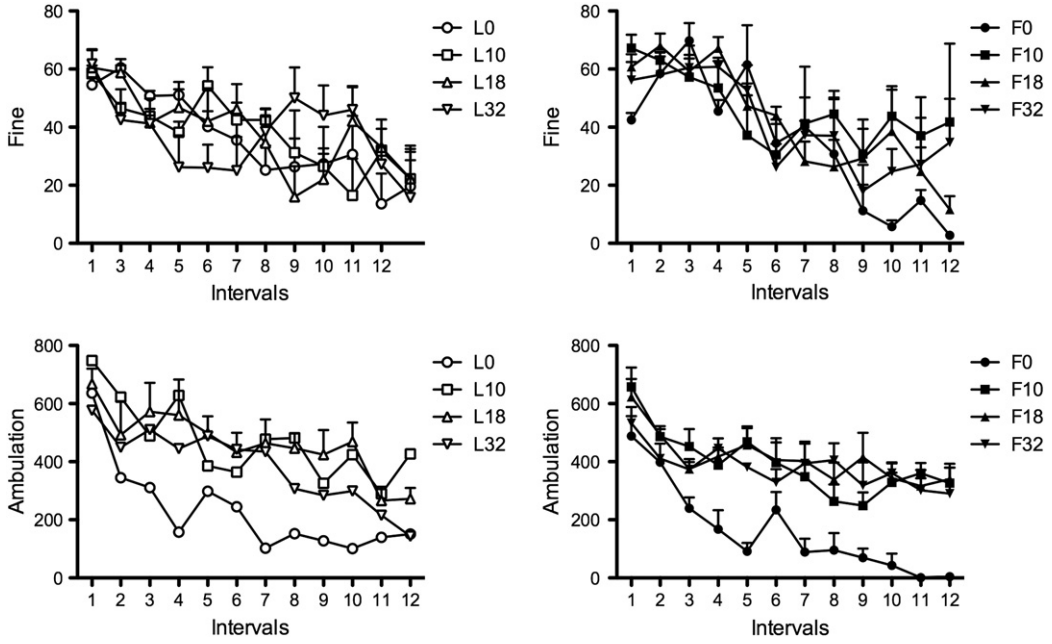

Fig. 3. The top panel presents mean (+/−SEM) fine motor activity scores at each dose for the LEW (left) and F344 (right) strains in 5-min intervals over the duration of the locomotor assessment (60 min). The bottom panel presents mean (+/−SEM) ambulation scores for the LEW (left) and F344 (right) strains in 5-min intervals over the duration of the locomotor assessment (60 min).

relation to the main effects of Dose (collapsed across Interval and Strain), animals injected with caffeine had a significantly greater fine motor score than those injected with vehicle [all $p<0.001$]. Fig. 3 illustrates the fine motor activity scores across all the sessions (in 5-min intervals) at all doses for animals in the LEW (top left panel) and F344 (top right panel) strains.

3.2.3.2. Ambulation. There were no baseline differences between LEW and F344 strains in ambulation [$F(11, 286)=1.696$, $p=0.074$]. Consequently, the strains were analyzed together and directly compared. The $12\times2\times4$ mixed ANOVA revealed a significant effect of Interval [$F(11, 286)=24.019$, $p<001$] and Dose [$F(3, 26)=26.254$, $p<0.001$] but no significant interaction [$F(33, 286)=1.117$, $p=0.309$]. In relation to the main effect of Interval (collapsed across Dose and Strain), subjects had higher ambulation during Interval 1 relative to the remainder of the session [all $p<0.001$]. Further, subjects had higher ambulation over the first five intervals relative to the last two [all $p<0.05$]. In relation to the main effect of Dose (collapsed across Interval and Strain), animals injected with caffeine had significantly higher ambulation than those injected with vehicle [all $p<0.001$]. No other comparisons were significant. Fig. 3 illustrates the ambulation scores across all the sessions (in 5-min intervals) at all doses for animals in the LEW (bottom left panel) and F344 (bottom right panel) strains.

4. Discussion

As described, the LEW and F344 rat strains differ in their acquisition of taste aversions induced by a variety of drugs of abuse (Gomez-Serrano et al., 2009; Lancellotti et al., 2001; Pescatore et al., 2005; Roma et al., 2006; see Riley et al., 2009 for a review) with the specific direction of this relative sensitivity (i.e., F>L; L>F) dependent upon the drug examined. Further, the two strains do not differ in aversions induced by the classical emetic LiCl (Foynes and Riley, 2004), suggesting that the demonstration of differential sensitivity is drug dependent as well. The present series of experiments examined caffeine-induced aversions in the two strains to assess the generality of strain differences to a drug that is widely used but not considered abused and for which the affective properties have not been examined.

In the present experiments, rats from the LEW and F344 inbred rat strains were given saccharin to drink followed by varying doses of caffeine. At low doses (0, 0.32, 1.0 and 3.2 mg/kg; see Experiment 1), caffeine failed to induce taste aversions in either the LEW or F344 strain (in either one or two-bottle tests). This failure to induce taste aversions at these doses is consistent with earlier work by Vitiello and Woods (1977) who reported similar results in outbred rats, suggesting that such doses of caffeine are generally not aversive (at least as indexed by the taste aversion design). At higher doses (0, 10, 18 and 32 mg/kg; see Experiment 2), caffeine induced taste aversions in both strains. Specifically, 32 mg/kg caffeine induced taste aversions in the LEW and F344 strains after only a single conditioning trial. Interestingly, although 18 mg/kg caffeine was ineffective in the F344 strain, this dose conditioned aversions in the LEW strain, again after only a single pairing. This relative sensitivity of the two strains to caffeine was also evident in the two-bottle test in which the LEW rats displayed strong aversions to saccharin at both of the two higher doses of caffeine, while only the higher dose suppressed consumption in the F344 strain. The ability of caffeine to induce taste aversions at these higher doses is consistent with earlier work by Steigerwald et al. (1988) who reported conditioned taste aversions at 20 mg/kg, but not at 5.0 mg/kg, in outbred rats. Additionally, Brockwell et al. (1991) reported that their highest dose (30 mg/kg; but not 10 mg/kg) produced taste aversions, suggesting that higher doses of caffeine do induce aversions not only in outbred rats but in inbred strains as well. The fact that caffeine induced strain dependent taste aversions extends the compounds for which such differences have been reported and to a compound not generally classified as a drug of abuse.

Following the last two bottle test in Experiment 2, locomotor (fine and ambulation) activity was recorded as another general test of possible strain differences in the sensitivity to caffeine. As reported, all animals (regardless of strain) injected with 10, 18 and 32 mg/kg caffeine were more active (both fine and ambulation) than those injected with vehicle. These results are consistent with earlier work by Bedingfield et al. (1998) who reported that their highest dose of caffeine (10 mg/kg) increased locomotor activity in outbred rats. There was no

effect of Strain on ambulation. The only significant strain difference was in fine motor activity for which the F344 rats displayed greater fine motor activity than the LEW strain (indicative of greater repetitive motor movements). There was no significant Strain × Dose interaction, however, precluding any conclusions that such differences were related to caffeine. The fact that the LEW strain displayed greater taste aversions, while there was no clear strain effect with caffeine on motor activity, suggests that the differences in taste aversion learning are not a function of a general differential sensitivity to caffeine's effects in the two strains (at least within the parameters of the current study). Given the limited number of subjects in this assessment, however, examination with larger groups is important before concluding that the two strains do not differ in caffeine-induced motor effects or that such effects are not related to the reported differences in caffeine-induced taste aversions. Further, given that all doses tested in Experiment 2 induced significant increases in activity with no apparent dose–response differences, lower doses may have differentially affected motor activity that was dependent upon strain.

The present data add to a growing list of compounds for which the LEW and F344 strains differ in relation to aversion learning. For many compounds, it is the LEW strain that displays greater aversions than F344 subjects (Glowa et al., 1994); for others F344 subjects display greater aversions than LEW rats (see Lancellotti et al., 2001; Pescatore et al., 2005; Roma et al., 2006); yet for others, e.g., LiCl, the strains do not differ (Foynes and Riley, 2004). Such variation argues against a simple explanation for the strain differences, e.g., differential ability in associative learning, differential processing of taste stimuli, differential memory of acquired associations (see Cunningham et al., 2009; Reilly, 2009; Riley, 2011), and suggests instead that the two strains may be differentially sensitive to the aversive effects of drugs and that such differential sensitivity is drug dependent.

Although the LEW and F344 strains appear differentially sensitive to the aversive effects of a variety of compounds (abused and non-abused), the basis for these differences remains unknown. Part of the difficulty in attempting to determine the basis for these strain differences stems from the fact that although a wide variety of mechanisms have been proposed to account for taste aversion learning, e.g., drug novelty, reward, general malaise, there is no consensus for specific drugs, such as caffeine, or for drugs in general (for a discussion, see Freeman and Riley, 2009; also see Hunt and Amit, 1987; Parker, 2003; Riley et al., 2009; Riley, 2011). It is interesting in this context that one of the few other drugs for which LEW rats display greater aversions than the F344 strain is cocaine (see Glowa et al., 1994; though see also Kosten et al., 1994). Work from our laboratory and others have suggested that cocaine's aversive effects may be mediated via its activity as a NET (norepinephrine transporter) inhibitor (Freeman et al., 2005, 2008; Serafine and Riley, 2009) by which it increases the overall levels of NE. Although caffeine has no effects on NET, acting instead as an adenosine antagonist (for review see Nehlig, 1999; also see Bedingfield et al., 1998), it is a CNS stimulant whose stimulus effects partially generalize to those of cocaine (see Gauvin et al., 1990).

Interestingly, Geist and Ettenberg (1997) have reported that rats given cocaine in a goal box display an approach–avoidance response after conditioning. Geist and Ettenberg have argued that the approach to the goal box reflected the rewarding effects of cocaine, whereas its avoidance reflected an underlying negative effect of cocaine. This negative effect was likely a function of cocaine's anxiogenic properties since the avoidance component was blocked by pretreatment with the anxiolytic diazepam. In this context, it is important to note that caffeine can also induce anxiety not only in humans, but also in rats, by activating the HPA axis via its interaction with adenosine receptors in the hypothalamus (Bhattacharya et al., 1997; Nicholson, 1989; Patz et al., 2006).

Although cocaine and caffeine may each produce anxiogenic effects, it remains unknown if such effects mediate aversions induced by these compounds. Further, it is not known if and to what extent the LEW and F344 strains differ in such effects (if stress is involved). In relation to the role of stress in cocaine and caffeine-induced aversions, there are little data to suggest that stress itself consistently impacts aversion learning (see Revusky and Reilly, 1989). Further, drugs known to reduce stress (e.g., chlordiazepoxide, diazepam, alcohol) can induce taste aversions (Brockwell et al., 1991; Cappell and LeBlanc, 1973; Roma et al., 2006; Lancellotti et al., 2001; Steigerwald et al., 1988; Vitiello and Woods, 1977). In relation to the role stress might play in the strain difference reported here (and with cocaine), although the LEW strain displays stronger aversions than the F344 strain (Glowa et al., 1994), it is the F344 strain that displays greater stress reactivity (Baumann et al., 2000; Dhabhar et al., 1993, 1997; Kosten and Ambrosio, 2002; Stöhr et al., 2000; Ortiz et al., 1995). Although this suggests that stress may not be mediating the aversive effects of caffeine in these two strains, it would be necessary to assess directly the effects of caffeine on stress reactivity in the LEW and F344 strains to determine its potential role in caffeine-induced aversions.

Independent of the basis for the differences seen between the LEW and F344 strains in aversion learning, it is important to note that this differential sensitivity to the aversive effects of drugs does not indicate absolute sensitivities. That is, although the LEW strain displays greater caffeine-induced aversions than the F344 strain, the F344 strain does display aversions with caffeine at some dose (in the present analysis at 32 mg/kg caffeine). This is similar to work with other drugs in this design, e.g., cocaine, alcohol, amphetamine, and nicotine, where strain differences are evident, but only at specific doses (for an exception with morphine, see Lancellotti et al., 2001). As dose increases, a dose is reached at which both strains show aversions that do not differ. Such dose-dependent effects have been reported for other behavioral preparations, e.g., self administration (Brower et al., 2002; Kosten et al., 1997) and conditioned place preferences (Kosten et al., 1994), arguing that the differential sensitivity in these strains to the aversive (or reinforcing) effects of drugs is one of degree.

As noted above, given that drug use (and abuse) vulnerability may be a function of the balance between its rewarding and aversive effects (Kohut and Riley, 2010; Riley et al., 2009; Riley, 2011; Rinker et al., 2008a,b; Roma et al., 2006), work with strain differences in assessments of the affective properties of drugs (both rewarding and aversive) may be important in the understanding of possible genetic factors related to this vulnerability (Davis et al., 2007; Kosten and Ambrosio, 2002; Kosten et al., 1994, 1997; Lancellotti et al., 2001; Pescatore et al., 2005; Riley et al., 2009; Roma et al., 2006). Both inbred and selected strains provide a useful animal model in such investigations (Koob and Le Moal, 2006) and may provide additional insight into the differential neurochemical responses to these drugs (and/or the different neuroanatomical mediation of their specific effects; see Grabus et al., 2004; Nestler, 2002).

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