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# Naloxone-precipitated conditioned taste aversions in morphine-dependent Fischer (F344) and Lewis rat strains

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ABSTRACT

The Fischer 344 (F344) and Lewis (LEW) rat strains are genetically divergent populations that are used to study the effects of and responses to drugs of abuse. In this context, LEW rats display faster acquisition of drug self-administration than F344 rats. Interestingly, these strains have also been reported to differ in their somatic responses to morphine withdrawal. To address possible strain differences in the affective response to withdrawal, the present study assessed the ability of naloxone-precipitated withdrawal from morphine to induce conditioned taste aversions in male F344 and LEW rats. Specifically, subjects from each of these strains were given chronic morphine to induce dependence and then given access to a novel saccharin solution followed by naloxone. These pairings were given every fourth day for a total of two conditioning trials after which subjects were given access to saccharin but without naloxone administration to assess extinction of the naloxone-induced aversion. Behavioral assays of withdrawal were also performed after each naloxone administration. Both F344 and LEW subjects acquired aversions to the naloxone-associated taste with no significant differences in the rate of acquisition of the aversions. Differences did appear during extinction with LEW animals extinguishing the taste aversion significantly faster than F344 animals. The data were discussed in terms of the relative strength of the affective responses during withdrawal and the role of such responses to drug use and abuse.

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## 1. Introduction

The Fischer 344 (F344) and Lewis (LEW) rat strains are genetically divergent populations that are used to study the effects of and responses to drugs of abuse (for a review, see Kosten and Ambrosio, 2002). For example, LEW rats have been shown to more rapidly acquire the self-administration of a variety of drugs of abuse than F344 rats (Kosten and Ambrosio, 2002; Kosten et al., 1997; Martin et al., 1999). The F344 and LEW rat strains also differ in a number of proteins and enzymes critical in brain areas responsible for mediating reward (Beitner-Johnson et al., 1991, 1993; Grabus et al., 2004; Guitart et al., 1992, 1993; Martin et al., 1999; Mayo-Michelson and Young, 1992; Nylander et al., 1995; Werme et al., 2000a,b), providing important insights into the biochemical bases for drug-taking behaviors. Although the focus with the F344 and LEW strains has been on the acute effects of drugs of abuse, the chronic effects of these same compounds have also been examined in the two strains. For example, following an initial examination of the effects of morphine on the levels of various neurofilament proteins in the ventral tegmental area in which LEW rats had significantly lower levels than F344 rats, Guitart et al. (1992) reported that these levels were decreased in F344 rats, but not changed in LEW rats, following chronic morphine administration.

In an extension of examining the differences between the strains in responsiveness to chronic morphine treatment, differences in withdrawal from morphine treatment have also been examined (Guitart et al., 1993). Specifically, Guitart et al. treated rats chronically with morphine and then injected them with naltrexone to assess the effects of naltrexone-precipitated withdrawal. Although the overall severity of withdrawal, as measured by the composite score of withdrawal behaviors, was not significantly different between the two strains, strain differences were evident in a number of the physical withdrawal symptoms making up the composite score. For example, F344 rats showed greater weight loss and displayed greater locomotor activity than the LEW rats. Although each of these measures assays the physical or somatic effects of opioid withdrawal (Guitart et al., 1993), little is known about any motivational or affective responses (e.g., dysphoria, malaise) that may occur during withdrawal in these two strains. Given that these affective responses have been described as important in understanding the counteradaptive mechanisms that impact addiction (Koob and Le Moal, 1997, 2001, 2005; Manning and

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Jackson, 1977), an analysis of such effects in the F344 and LEW strains may provide insight into the role of specific genetic and neurobiological systems in the abuse potential of the opiates.

One procedure that has been used to assay the affective response of drugs in general is the conditioned taste aversion procedure (Freeman and Riley, 2009; Garcia and Ervin, 1968; Hunt and Amit, 1987; Kalat and Rozin, 1970; Revusky and Garcia, 1970). In relation to drug withdrawal, it has been shown that precipitated opiate withdrawal can condition aversions (Parker and Radow, 1974). For example, morphine-dependent animals given saccharin access and then injected with the opiate antagonist naloxone rapidly acquire an aversion to the naloxone-associated saccharin solution, presumably reflective of the aversive effects of precipitated withdrawal (Pournaghash and Riley, 1991). In these instances, the effects of naloxone are dose-dependent (see Pilcher and Stolerman, 1976) and the dose of naloxone (0.1 mg/kg) needed to induce taste aversions is significantly less than that needed to induce the typical signs of opiate withdrawal in other preparations (Higgins and Sellers, 1994).

Withdrawal-induced taste aversions have been studied in outbred rats (such as the Sprague–Dawley, Long–Evans and Wistar strains), but they have not been examined in the LEW and F344 inbred rats. Accordingly, the present study extended the earlier work of Guitart et al. (1993) by assessing the ability of naloxone-precipitated withdrawal to induce taste aversions in morphine-dependent LEW and F344 rat strains. Specifically, subjects from each of these strains were given chronic morphine exposure (for 21 days) and then given access to a novel saccharin solution followed by an injection of naloxone hydrochloride (1 mg/kg). This conditioning procedure was repeated for two consecutive trials to assess acquisition of the conditioned taste aversion, at which point animals were presented daily with the saccharin solution without naloxone injections to assess extinction of the aversion. Additionally, animals were observed following each naloxone injection for behavioral signs of withdrawal.

## 2. Methods

### 2.1. Subjects

Subjects were experimentally-naïve male LEW ($n=34$) and F344 ($n=34$) rats, approximately 60 days of age at the beginning of the experiment. They were maintained on a 12:12 light:dark cycle (lights on at 0800 h). They were housed in individual stainless-steel wire-mesh hanging cages and at an ambient temperature of 23 °C. Food was available ad libitum throughout the experiment. Procedures recommended by the National Institutes of Health Guide for the Care and Use of Laboratory Animals (1985), the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (2003) and the Institutional Animal Care and Use Committee at American University were followed at all times.

### 2.2. Drugs and solutions

Morphine sulfate was prepared as a 10 mg/ml solution in physiological saline (0.9% NaCl) and injected intraperitoneally (ip). Naloxone hydrochloride was prepared as a 1 mg/ml solution in physiological saline and injected ip. Both drugs were generously provided by the National Institute on Drug Abuse. Saccharin (0.1% sodium saccharin, Sigma) was prepared as a 1 g/l solution in tap water.

### 2.3. Procedure

The experiment was conducted in two replicates of 34 animals each. The same procedure was followed for each replicate, and the second replicate was performed immediately following the first. All groups were represented in each replicate, and each was represented equally.

#### 2.3.1. Phase I: habituation

Following ad-lib access to water, animals were deprived of water for $23^{2/3}$ h, during which time water bottles were removed from the cages and animals received no access to fluids. Following this, a water habituation schedule began during which animals were given 20-min access to water every day at the same time (referred to as the fluid-access period). This phase continued for 12 days until all animals' water consumption was stable and the mean water consumption was within 2 ml for 3 consecutive days. Animals were weighed daily throughout this and the following phases immediately prior to their fluid-access period. Water consumption was monitored and recorded daily throughout this and the following phases.

#### 2.3.2. Phase II: morphine dependence

Within each strain, subjects were ranked on water consumption and assigned to two groups such that water consumption was comparable among groups. At this point, subjects in each strain received an injection of either morphine or saline. Morphine was given at a dose of 10 mg/kg and increased 10 mg/kg each day for 10 consecutive days until a dose of 100 mg/kg was reached; this dose was maintained for 11 additional days (for a total of 21 days of morphine injections) (for a similar procedure, see Pournaghash and Riley, 1991). Control animals received equivolume injections of physiological saline. All injections were given daily 6 h after the fluid-access period (see Table 1 for specific group assignments during Phase II).

#### 2.3.3. Phase III: conditioned taste aversion

On Day 1 of this phase, subjects were given 20-min access to the novel saccharin solution instead of water during their normal fluid-access period. Immediately after saccharin access, subjects in each strain were assigned to four groups based on saccharin consumption such that consumption was comparable among groups in each strain. Approximately 20 min after the end of the saccharin-access period, subjects were injected with naloxone (1 mg/kg) or equivolume saline. This dose of naloxone has been reported to induce withdrawal in opiate dependent rats under a variety of induction schedules, see Chartoff et al., 2006; Easterling and Holtzman, 2004; Pournaghash and Riley, 1991) This procedure resulted in a total of eight groups: FMN ($n=10$), FMV ($n=8$), FVN ($n=8$), FVV ($n=8$), LMN ($n=10$), LMV ($n=8$), LVN ($n=8$) and LVV ($n=8$). The first letter of the group refers to the strain (F344 or LEW), the second letter refers to the maintenance injection (morphine or vehicle) and the third letter refers to the conditioning treatment (naloxone or vehicle). See Table 1 for specific group assignments during Phase III). Following conditioning, subjects were given three water-recovery days during which they received 20-min access to water during the scheduled fluid-access period. This alternating procedure of conditioning and water recovery was continued for two cycles. On the day following the second cycle, a final one-bottle aversion test was conducted in which animals were presented with the saccharin solution during their normal fluid-access period; however, no injections followed this period.

For 3 h following naloxone injections, animals were observed for behavioral symptoms of withdrawal. Animals were observed every

**Table 1**
Illustrates group assignments in Phases II (morphine dependence) and III (conditioned taste aversion) to result in the final 8 groups

| | F344 ($n=34$) | | | | LEW ($n=34$) | | | |
|---|---|---|---|---|---|---|---|---|
| Dependence morphine/vehicle | FV $n=16$ | | FM $n=18$ | | LV $n=16$ | | LM $n=18$ | |
| Conditioned taste aversion naloxone/vehicle | FVV $n=8$ | FVN $n=8$ | FMV $n=8$ | FMN $n=10$ | LVV $n=8$ | LVN $n=8$ | LMV $n=8$ | LMN $n=10$ |

F or L refers to the strain, V refers to vehicle injections, M refers to morphine injections and N refers to naloxone injections.

15 min for the 1st h and every 30 min for the following 2 h. In each observation period, animals were observed for 15 s, and the following 13 behaviors were observed as present or absent at the time of observation: jumping, wet dog shakes, torso stretching, stereotypical movements, yawning, teeth chatter, chewing (not resulting in swallowing), lacrimation, piloerection, ptosis, salivation, diarrhea and locomotor activity. At the end of the 3-h observation period, animals were weighed. Throughout this phase, subjects continued to receive their maintenance injections of either morphine (100 mg/kg) or saline 6 h following fluid access.

*2.3.4. Phase IV: extinction*

The first day of this phase was the same day as the final aversion test. Immediately following the 20-min aversion test of the preceding phase, animals were given access to the saccharin solution for an additional 100 min, for a total of 2-h access to saccharin. On each of the following four days, all animals were presented with the saccharin solution for 2 h (for a total of 5 extinction days). As above, subjects continued to receive their maintenance injections of either morphine (100 mg/kg) or saline 6 h following fluid access.

*2.4. Data analysis*

*2.4.1. Conditioned taste aversions*

Given the differences in the amount consumed between the strains and between the morphine- or vehicle-treated subjects prior to conditioning (see below), all consumption data were converted to percent of control consumption. Differences in the percent of control consumption during acquisition (see below) were analyzed using a 3×2×2 repeated measures ANOVA with a within-subjects factor of Conditioning Cycle (1–3) and between-subjects factors of Strain (F344 or LEW) and Maintenance Drug (morphine or vehicle). Differences in the percent of control consumption during extinction were analyzed using a 5×2×2 repeated measures ANOVA with a within-subjects factor of Day (1–5) and between-subjects factors of Strain (F344 or LEW) and Maintenance Drug (morphine or vehicle). In all cases, pair-wise comparisons were made using Tukey's post-hoc tests and significance was determined at a 0.05 level.

*2.4.2. Body weight*

Differences in the percent weight change from baseline (see below) were analyzed by a 2×2×2 ANOVA with between-subjects factors of Strain (F344 or LEW), Maintenance Drug (morphine or vehicle) and Conditioning Drug (naloxone or vehicle). Pair-wise comparisons were made using Tukey's post-hoc tests and significance was determined at a 0.05 level.

*2.4.3. Behavioral measures during withdrawal*

The behaviors recorded during withdrawal were analyzed in three ways. First, the total number of times each behavior was displayed was summed over the 3-h observation period, resulting in one score for each behavior for each animal; these data across all the groups were analyzed in a 13×2×2×2 repeated measures ANOVA with a within-subjects factor of Behavior and between-subjects factors of Strain (F344 or LEW), Maintenance Drug (morphine or vehicle) and Conditioning Drug (naloxone or vehicle). Second, for each animal the total number of behaviors over the 3-h withdrawal period was summed, resulting in an overall composite score for each animal; these data were analyzed in a 2×2×2 ANOVA with between-subjects factors of Strain (F344 or LEW), Maintenance Drug (morphine or vehicle) and Conditioning Drug (naloxone or vehicle). Third, the time course of withdrawal was determined by summing the total number of behaviors displayed for each observation period (eight total) over the 3-h period, resulting in eight scores for each animal; these data were analyzed in a 2×8×2×2 repeated measures ANOVA with within-subjects factors of Cycle (1 and 2) and Observation Period (1–8) and between-subjects factors of Maintenance Drug (morphine or vehicle) and Conditioning Drug (naloxone or vehicle). Pair-wise comparisons of individual observation periods between conditioning cycles were made using independent-samples *t*-tests, and significance was determined using the Holm–Bonferroni method. In all cases (unless stated otherwise), pair-wise comparisons were made using Tukey's post-hoc tests and significance was determined at a 0.05 level.

## 3. Results

*3.1. Replicate analysis*

Data from the two replicates were compared to determine if there were significant differences between any of the groups that were to be pooled for subsequent analysis (e.g., to determine if Group LMN in Replicate 1 differed from group LMN in Replicate 2; no other types of comparisons were considered in this analysis). Post-hoc analysis revealed that none of the relevant group comparisons was significant (e.g., none of the groups to be pooled was significantly different from one another; all $p$'s$>0.05$). Given these findings, the data from the two replicates were pooled for the remainder of data analysis and presentation.

*3.2. Conditioned taste aversion*

A 2×2×2 ANOVA of saccharin consumption on the first conditioning day revealed significant main effects of Strain [$F(1,60)=15.060$, $p<0.001$] and Maintenance Drug [$F(1,60)=35.365$, $p<0.001$] and a significant Strain×Maintenance Drug interaction [$F(1,60)=9.375$, $p=0.003$]. Tukey's post-hoc comparisons revealed significant differences between morphine- and saline-pretreated animals in the F344 strain (more specifically, Groups FMV and FMN drank significantly less than Groups FVV and FVN, all $p$'s$<0.01$); further, these morphine-pretreated F344 animals (Groups FMV and FMN) drank significantly

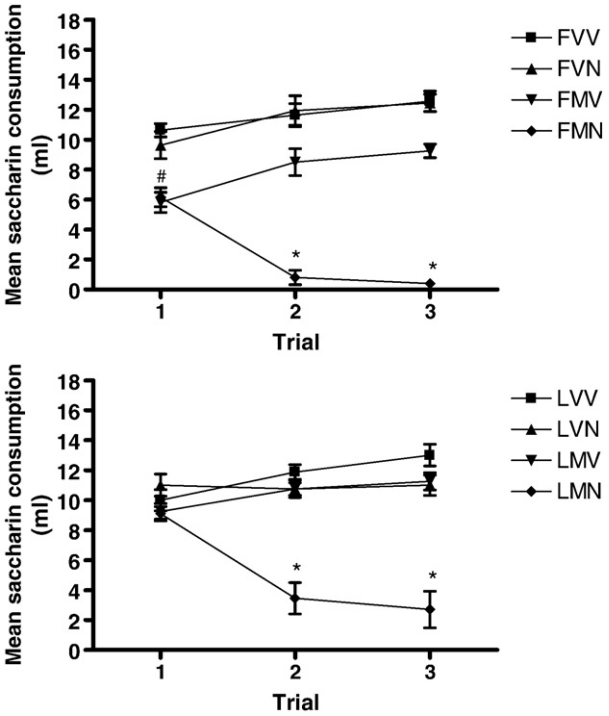


**Fig. 1.** Absolute saccharin consumption for F344 (top panel) and LEW (bottom panel) rats during conditioning. Top panel: * Indicates significant differences between Group FMN and all other groups; # Indicates significant difference between Groups FMN/FMV and FVN/FVV. Bottom panel: * Indicates significant differences between Group LMN and all other groups.

less than all LEW groups (all $p$'s<0.02). Saccharin consumption on this day (as well as throughout conditioning) is illustrated in Fig. 1 for F344 (top panel) and LEW (bottom panel) subjects. Given the differing baselines of saccharin consumption for each strain on the initial conditioning trial, intake was transformed to a percent shift of control subjects to allow for direct comparisons among groups. Specifically, consumption for each group injected with naloxone during conditioning was presented as a percentage of the amount consumed by its vehicle-injected control. These data are illustrated in Fig. 2.

A 3×2×2 repeated measures ANOVA on the transformed data revealed significant main effects of Conditioning Cycle [$F(2.64)$= 37.272, $p$<0.001] and Maintenance Drug [$F(1,32)$=106.980, $p$<0.001] and significant Conditioning Cycle×Maintenance Drug [$F(2,64)$= 35.798, $p$<0.001] and Conditioning Cycle×Strain×Maintenance Drug [$F(2,64)$=3.873, $p$=0.026] interactions. There were no significant group differences on the initial exposure to saccharin (relative to their own controls). Tukey's post-hoc tests showed significant differences between groups maintained on morphine, i.e., Groups FMN and LMN, and groups maintained on saline, i.e., Groups FVN and LVN, on Trials 2 and 3 (all $p$'s<0.001). Specifically, subjects maintained on morphine drank significantly less on these trials than subjects maintained on saline. There were no strain differences, however, in that the relative decreases in saccharin consumption were not different between the F344 and LEW strains (within either morphine/naloxone or vehicle/naloxone comparison; all $p$'s>0.1). Over conditioning, the saccharin consumption of all control groups (animals injected with saline during conditioning) remained high and stable and did not differ from one another (all $p$'s>0.5).

Water consumption on water-recovery sessions throughout conditioning remained at pre-conditioning baseline levels. Further, water consumption during recovery sessions did not differ among groups (all $p$'s>0.05), indicating that changes in saccharin consumption were not a general function of changes in fluid consumption but were instead a function of the association of saccharin with the aversive state of withdrawal.

### 3.3. Extinction

Fig. 3 illustrates mean saccharin consumption for F344 (top panel) and LEW (bottom panel) subjects during extinction. Given the differing levels of saccharin consumption between control subjects in the two strains, consumption was transformed to percent of saline-treated controls (within each strain) and presented in Fig. 4. A 5×2×2 repeated measures ANOVA on the transformed data showed significant main effects of Day [$F(4,128)$=18.925, $p$<0.001], Strain [$F(1,32)$=5.690, $p$=0.023] and Maintenance Drug [$F(1,32)$= 32.558, $p$<0.001] and significant Day×Strain [$F(4.128)$=6.962, $p$<0.001], Day×Maintenance Drug [$F(4,128)$=13.554, $p$<0.001], Day×Strain×Maintenance Drug [$F(4,128)$=5.361, $p$=0.001] and Strain×Maintenance Drug [$F(1,32)$=33.195, $p$<0.001] interactions.

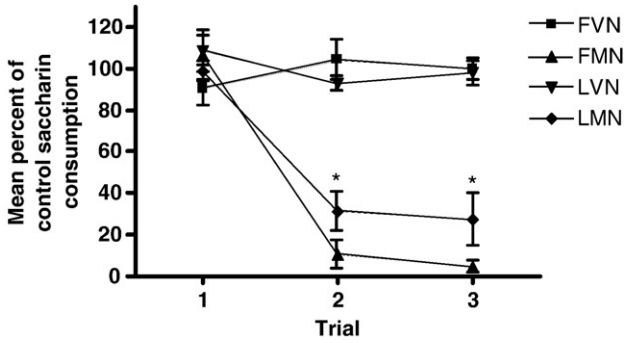


**Fig. 2.** Percent of saccharin consumption of all naloxone-treated groups relative to their saline-treated controls over conditioning. * Indicates significant differences between Groups FMN and FMV and between Groups LMN and LMV.

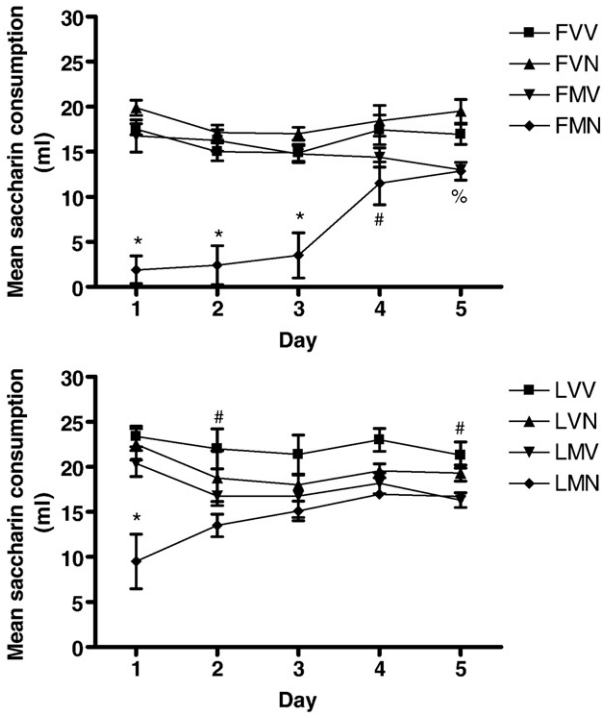


**Fig. 3.** Absolute saccharin consumption for F344 (top panel) and LEW (bottom panel) rats during extinction. Top panel: * Indicates significant differences between group FMN and all other groups; # Indicates significant differences between Groups FMN and FVN; % Indicates significant differences between Group FVN and Groups FMN/FMV. Bottom panel: * Indicates significant differences between Groups LMN and all other groups; # Indicates significant differences between Group LVV and Groups LMN/LMV.

On Day 1, both Groups FMN and LMN, subjects maintained on morphine, drank significantly less than the LEW and F344 groups maintained on saline (Groups FVN and LVN) (all $p$'s<0.01). On Days 2 and 3, only the F344 group maintained on morphine continued to avoid saccharin, drinking significantly less than the saline-maintained subjects (all $p$'s<0.01). On these trials, Group FMN also drank significantly less than its LEW counterpart (Group LMN). By Trial 4, there were no significant difference among any groups, i.e., Groups FMN and LMN had extinguished their aversion to saccharin. Over extinction, saccharin consumption by the control groups remained high and stable and did not differ consistently from one another (all $p$'s>0.1).

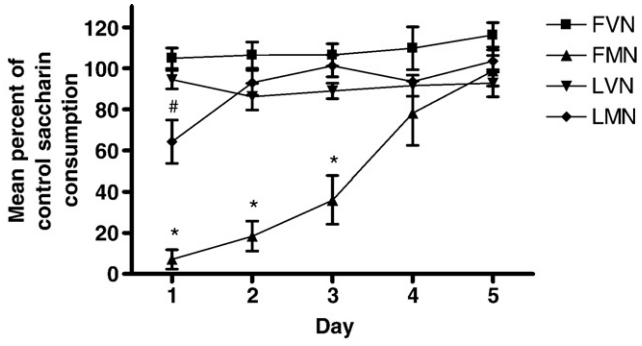


**Fig. 4.** Percent of saccharin consumption of all naloxone-treated groups relative to their saline-treated controls over extinction. * Indicates significant differences between Groups FMN and all other groups; # Indicates significant differences between Groups LMN and all other groups.

**Table 2**
Presents scores for various measures following injections of naloxone or vehicle during conditioning

| Measure | FMN | FVN | FMV | FVV | LMN | LVN | LMV | LVV |
|---|---|---|---|---|---|---|---|---|
| Weight change (%) | −4.22±0.38 | 4.30±0.31 | 3.10±0.42 | 4.87±0.30 | −3.73±0.56 | 3.51±0.16 | 3.54±0.28 | 3.78±0.21 |
| Jumping | 0.05±0.05 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 |
| Wet dog shakes | 0.15±0.11* | 0±0 | 0±0 | 0±0 | 0.80±0.24* | 0±0 | 0±0 | 0±0 |
| Abdominal stretching | 0.85±0.24 | 0±0 | 0±0 | 0±0 | 0.95±0.30 | 0±0 | 0±0 | 0±0 |
| Chewing | 0.10±0.07 | 0±0 | 0±0 | 0±0 | 0.05±0.05 | 0±0 | 0±0 | 0±0 |
| Piloerection | 4.25±0.46* | 0±0 | 0±0 | 0±0 | 1.60±0.44* | 0±0 | 0±0 | 0±0 |
| Ptosis | 3.35±0.66 | 0±0 | 0±0 | 0±0 | 2.10±0.33 | 0±0 | 0±0 | 0±0 |
| Diarrhea | 0.90±0.13 | 0±0 | 0±0 | 0±0 | 1.00±0.15 | 0±0 | 0±0 | 0±0 |
| Composite score | 9.65±1.09 | 0±0 | 0±0 | 0±0 | 6.50±0.80 | 0±0 | 0±0 | 0±0 |

For body weight, the numbers refer to the change from baseline weight (pre-naloxone injection). For individual behaviors, the numbers refer to the frequency with which the specific behavior was noted over the entire withdrawal period. For the composite score, the number refers to the sum of the frequencies of the individual behaviors. All scores are the averages collapsed across the two naloxone cycles. *Indicates significant differences between Groups FMN and LMN.

### 3.4. Withdrawal assays

Table 2 presents changes in body weight and behavior for subjects in both strains during the 3-h observation period following injections of naloxone or vehicle during the conditioning phase.

#### 3.4.1. Body weight

Given that the two strains had different baseline weights [$t(66)=9.256$, $p=0.002$], weight change from pre- to post-naloxone injection was transformed as a percent shift from baseline weight. Also, because there was no significant difference in weight changes on the two conditioning cycles [$t(67)=0.610$, $p=0.544$] these data were averaged for representation and analysis. A 2×2×2 ANOVA on the averaged, transformed weight data showed main effects of Maintenance Drug [$F(1,60)=286.883$, $p<0.001$] and Conditioning Drug [$F(1,60)=215.937$, $p<0.001$] and significant Strain×Maintenance Drug [$F(1,60)=7.164$, $p=.010$] and Maintenance Drug×Conditioning Drug [$F(1,60)=171.628$, $p<0.001$] interactions. Tukey's post-hoc tests showed that subjects maintained on morphine and injected with naloxone, i.e., Groups FMN and LMN, displayed significant reductions in body weight from their baselines relative to all other groups (all $p$'s<0.001). There was no difference in this reduction between the two strains ($p=0.968$). No other comparisons were significant.

#### 3.4.2. Behavior

During the 3-h observation period, a variety of behaviors were scored for their presence or absence. The 13×2×2×2 ANOVA showed significant main effects of Behavior [$F(12,720)=33.798$, $p<0.001$] and significant Maintenance Drug×Conditioning Drug [$F(1,60)=100.737$, $p<0.001$] and Behavior×Strain×Maintenance Drug×Conditioning Drug [$F(12,720)=5.412$, $p<0.001$] interactions (other significant interactions not indicated). Given the significant four-way interaction, Tukey's post-hocs on each behavior revealed significant differences between Groups FMN and LMN and their respective controls on all behaviors (only 7 of the 13 behaviors were observed to occur during the observation period). Further, significant strain differences were observed in Wet Dog Shakes, with LEW animals displaying higher levels than F344 animals ($p=0.001$) and Piloerection, with F344 animals displaying higher levels than LEW animals ($p<0.001$); a strain difference in Ptosis neared significance, with F344 animals displaying higher levels than LEW animals ($p=0.058$).

The total number of behaviors displayed over the 3-h observation period was summed for each animal, resulting in an overall composite score. There was no significant difference between these scores on the two conditioning cycles [$t(67)=0.718$, $p=0.475$], so they were averaged for representation and analysis. A 2×2×2 ANOVA on the averaged overall composite data revealed main effects of Maintenance Drug [$F(1,60)=100.737$, $p<0.001$] and Conditioning Drug [$F(1,60)=100.737$, $p<0.001$] and a significant Maintenance Drug×Conditioning Drug [$F(1,60)=274.550$, $p<0.001$] interaction (other significant interactions not indicated). The main effect of Strain approached significance [$F(1,60)=3.832$, $p=0.055$].

#### 3.4.3. Time course of withdrawal

Fig. 5 illustrates the number of withdrawal-associated behaviors observed during each observation period (hour) collapsed across Groups FMN and LMN (subjects maintained on morphine and injected with naloxone; there were no significant strain differences). A 2×8×2×2×2 repeated measures ANOVA showed a main effect of Observation Period [$F(7,420)=11.358$, $p<0.001$] and a significant Cycle×Observation Period [$F(7,420)=3.829$, $p<0.001$] interaction (other significant interactions not indicated; as with the prior behavioral data, a significant Maintenance Drug×Conditioning Drug interaction was seen [$F(1,60)=100.737$, $p<0.001$). In relation to this Cycle×Observation Period interaction, the time course of withdrawal was shifted 30 min earlier from the first to the second cycle. This difference was seen between the two cycles at Observation Periods 0.25 and 0.5 ($p$'s=0.001 and 0.005, respectively), with the mean number of behaviors displayed at these observation periods significantly higher in the second cycle than in the first. Specifically, the time to peak number of symptoms displayed shifted from Observation Periods 0.75, 1.0 and 1.5 (all having the same mean) in Cycle 1 to Observation Period 0.5 in Cycle 2. Further, the initial presentation of symptoms at Observation Period 0.25 in Cycle 2 neared the peak of symptoms not evident until Observation Period 0.75 in Cycle 1, while the initial presentation of symptoms at Observation Period 0.25 in Cycle 1 neared 0. There were no strain differences.

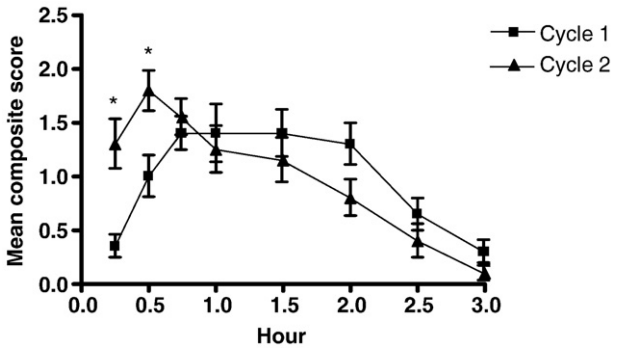


**Fig. 5.** Mean composite scores of all behaviors over the multiple observation periods following the first (Cycle 1) and second (Cycle 2) injections of naloxone. The onset and termination of behavioral withdrawal symptoms shifted 30 min earlier from the first to the second cycle. Data are collapsed across strains. * Indicates significant differences between Cycles 1 and 2.

## 4. Discussion

Although differences between the F344 and LEW rat strains in response to both acute and chronic drug exposure are well documented (see above), little work has been done studying such differences in response to opiate withdrawal. Differing responses to withdrawal from nicotine, ethanol, pentobarbital and diazepam have been reported, with mixed findings regarding which strain displays a more severe withdrawal (Suzuki et al., 1999, 1987, 1992a,b). In a behavioral assay of opiate withdrawal, F344 and LEW strains have been reported to have similar severity of withdrawal, with differences reported only in select individual withdrawal symptoms (Guitart et al., 1993). The aim of the present study was to determine what, if any, strain differences exist in the affective response to withdrawal (as measured by the conditioned taste aversion procedure). Specifically, animals from both strains were given chronic morphine exposure and then underwent naloxone-precipitated withdrawal within a conditioned taste aversion design.

As described, naloxone rapidly induced taste aversions in all morphine-pretreated animals, an effect consistent with previous work assessing aversions induced by antagonist-precipitated opiate withdrawal in outbred rats (Higgins and Sellers, 1994; Pilcher and Stolerman, 1976; Pournaghash and Riley, 1991). Interestingly, there were no strain differences in the acquisition of the aversions. That is, both F344 and LEW animals markedly suppressed saccharin consumption following a single conditioning trial (on the second saccharin exposure). On the first day of extinction (during which animals were given 2-h access to the saccharin solution), significant differences emerged between the two strains. Although both strains drank less than their respective controls, LEW animals drank significantly more than F344 animals. By the second extinction session, LEW subjects no longer differed from their controls, indicative of extinction of the aversion, whereas F344 subjects continued to drink significantly less than all other groups. F344 subjects gradually increased saccharin consumption over extinction, eventually drinking at control levels by the fourth extinction session. At this point, the F344 and LEW subjects no longer differed.

Although there was a clear strain difference in the extinction of the naloxone-induced aversions, the basis for this difference is not known. It is certainly possible that this difference reflects differential learning during extinction between the two strains, i.e., the ability to learn that saccharin was no longer associated with the naloxone-precipitated withdrawal. Although possible, there are no clear learning differences between the two strains when tested in standard learning preparations (Tang et al., 2005; Wieland et al., 1986) and the two strains do not show consistent differences even within the taste aversion design (i.e., it is drug dependent) (Davis and Riley, 2007; Foynes and Riley, 2004; Kosten et al., 1994; Lancellotti et al., 2001). In one of the few preparations in which the LEW and F344 animals have been tested for their ability to display reversal learning (stop responding to a cue previously associated with food), there are no strain differences (Kearns et al., 2006). A more parsimonious way of accounting for the strain differences in extinction reported here might be to reconsider the absence of strain differences in acquisition. That is, although there were no significant differences in acquisition of the naloxone-induced aversions in the morphine-exposed rats, this may reflect a floor effect. As noted, subjects of both strains displayed rapid acquisition of the aversion (within a single trial). It is possible that suppression was sufficiently complete by the second exposure to saccharin that differences between the strains simply could not be detected because subjects could not decrease consumption any further. If there were true differences between the strains that could not be measured in acquisition, it might be expected that differences would be detected as consumption increased for both groups during extinction. The data from the extinction phase are consistent with this argument and would support the position that the affective response to morphine withdrawal is different between the two strains with the F344 subjects showing a greater response than the LEW rats.

Stating that the affective response differs between the two strains is quite different from identifying the basis for this difference. Two possibilities include often-reported neurobiological and hormonal differences between the two strains. For example, there are well-documented neurobiological differences between the F344 and LEW strains in response to morphine (Beitner-Johnson et al., 1991, 1993; Guitart et al., 1993; Werme et al., 1999, 2000a); however, the majority of this work has focused on acute drug administration and changes in systems typically mediating the rewarding effects of drugs. As such, the relevance of such findings to the current work on the aversive effects of chronic opioid administration remains unknown. Interestingly, Nylander et al. (1995) have reported that F344 and Lew rats differ significantly in basal dynorphin peptide levels in a variety of brain areas, e.g., substantia nigra, striatum, ventral tegmental area, pituitary and nucleus accumbens. Further, the F344 rats display increases in dynorphin A levels in response to chronic morphine, while the LEW rats either show no change or deceased dynorphin A levels with this drug regime. Although these brain areas are generally associated with motor activation or reward, changes in kappa opioid activity, e.g., dynorphin levels, in the accumbens have been implicated in the aversive effects of dependence and withdrawal and the subsequent vulnerability to continued drug taking (see Koob and Le Moal, 2006). The fact that the F344 animals in the present study displayed the stronger taste aversion is certainly consistent with these differences in kappa brain activity. Further, F344 and LEW animals differ significantly in stress reactivity with the F344 strain generally showing greater reactivity to stress (Kosten and Ambrosio, 2002). It is possible that the effects of withdrawal may have been potentiated in the F344 strain as a result of their well-documented HPA hyperreactivity (see Grakalic et al., 2006; Sternberg et al., 1992) and that this potentiation impacted withdrawal. However, given that neither corticosterone nor ACTH levels were measured in the present experiment, it remains unknown to what extent (if any) there were changes in these indices of stress during withdrawal and whether there were differences between the strains. Such information would be important to determining their role in the reported differences in aversions (or other behavioral indices of withdrawal; see below) between the two strains.

With respect to the behavioral data, of the seven somatic symptoms of opiate withdrawal that were displayed with any frequency, F344 animals showed a greater frequency of piloerection while LEW animals showed a greater frequency of wet dog shakes. Interestingly, no strain differences were seen in the other behavioral indices. There was also no significant difference between the strains in the overall composite score representing the total number of behaviors seen over the 3-h observation period. Additionally, both strains displayed significant weight loss from baseline and in comparison to their controls following naloxone administration. The fact that these physical symptoms of morphine withdrawal did not parallel the changes seen with aversion learning is consistent with other reports of dissociations between somatic and motivational symptoms of withdrawal (Chartoff et al., 2006; Frenois et al., 2002; Koob and Le Moal, 2006; Papaleo et al., 2008; Schulteis and Koob, 1996; Valverde et al., 2004). As noted for a variety of drugs of abuse, including the opiates, somatic and motivational (or affective) symptoms have different time courses, dose-dependencies and neurobiological substrates, all of which may impact their display in any given preparation (see Koob and Le Moal, 2006). Thus, the lack of a parallel between differences seen in the two measures may be due to these established dissociations between the somatic and affective symptoms of opiate withdrawal.

It should be noted that the differences in the somatic symptoms reported here are not consistent with those previously reported by Guitart et al. (1993) who also assessed opiate antagonist-induced

withdrawal in morphine-exposed F344 and LEW rats. For example, in the present study significant differences were seen between the strains in piloerection (F344>LEW) and wet dog shakes (LEW>F344). There were no differences between the strains for the remaining five symptoms that occurred with sufficient frequency to be recorded. Further, there were no strain differences in weight loss following the naloxone injection (although both groups of subjects displayed significant loss relative to their own baselines and to non-dependent controls). On the other hand, Guitart et al. (1993) found significant strain differences in jumping (LEW>F344), wet dog shakes (LEW>F344), salivation (LEW>F344), locomotor activity (LEW>F344), ptosis (F344>LEW), irritability (F344>LEW) and chewing (F344>LEW). Also, the F344 strain displayed a significantly greater decrease in body weight relative to the LEW subjects. It is difficult to know what specific factor(s) was responsible for the differences between the two studies, although a variety of parametric differences exist that could impact the degree of severity of these somatic responses. For example, the two studies differed in the manner by which dependence was produced (chronic intraperitoneal injection of morphine vs. pellet implantation); dose of morphine on which subjects were maintained (100 mg/kg vs. 75 mg/kg), duration of morphine maintenance (21 days vs. 5 days), specific antagonist used to induce withdrawal (naloxone vs. naltrexone) and the dose of the antagonist used (1 mg/kg vs. 100 mg/kg). Any (or all) of these factors may have contributed to the reported differences between the present study and that of Guitart et al. (1993). The fact that differences were reported, however, does indicate that the display of somatic withdrawal symptoms (and likely affective ones as well) are parameter-dependent and any conclusions drawn regarding strain differences must be made in the context of the specific parameters under which they were tested.

An interesting finding with respect to the somatic withdrawal symptoms reported here was the fact that the time course of withdrawal appeared to change over repeated naloxone challenges (for both strains). As described, the onset of withdrawal (as indexed by the appearance of a variety of withdrawal behaviors) occurred 30 min earlier during the 3-h observation period from the first to the second conditioning cycles. Although the basis for this shift is not known, it likely reflects a conditioned effect of saccharin, i.e., the presentation of saccharin that had been paired with naloxone elicited withdrawal. Such a conditioning effect is consistent with prior research showing that presentation of environmental cues present during withdrawal produce opioid withdrawal symptoms in the absence of antagonist administration (Koob and Le Moal, 2006).

In most assessments of drug effects in the LEW and F344 strains, LEW rats are described as more vulnerable to drug use and abuse (Kosten and Ambrosio, 2002). Specifically, the LEW strain generally displays more pronounced morphine- and cocaine-induced place preferences and displays a more rapid acquisition of cocaine and morphine self administration. Based on these differences, the LEW strain is considered addiction prone, whereas the F344 strain is considered addiction resistant and the two strains are presented as animal models for these different characteristics of drug intake. It is important to note that the majority of these assessments focus on the acute rewarding effects of drugs. Interestingly, in more recent assessments that have modeled more chronic exposure, it is the F344 strain that displays greater drug taking behavior. For example, Kosten and her colleagues (2007) have recently reported that following prolonged cocaine exposure, F344 subjects maintain a higher overall level of cocaine intake than LEW rats (see also Haile et al., 2001; Kosten et al., 1994, 1997). Freeman and his colleagues (in press) have noted that when F344 and LEW rats are given extended access to cocaine (in a preparation reported to produce escalation of drug intake in outbred subjects), it is again the F344 rats that self administer greater amounts of cocaine. Christensen et al. (in press) have similarly noted that when demand functions are established for food and cocaine in the two strains (within a procedure giving extended access to either reinforcer), cocaine is more essential as a reinforcer (with less elasticity) in the F344 strain compared to the LEW strain (the reverse is true for food). Thus, in procedures with more extended access to cocaine, the F344 rats seem a better model for drug intake than the LEW strain. Such comparisons suggest that the two strains model different aspects about drug taking behavior, i.e., initial rewarding effects vs. chronic compulsive intake. It is interesting in this context that Belin and his colleagues (Belin et al., 2008) have recently noted a similar dichotomy in the use of specific animal models for drug initiation and abuse. Specifically, they reported that animals highly reactivity to novelty (the HR strain) model a vulnerability to the acute reinforcing effects of cocaine. The HI strain, one displaying high levels of impulsivity, models behaviors related to the compulsive (and more protracted) use of drugs. Clearly, more work assessing the F344 and LEW strains in acute and chronic preparations with a variety of compounds at a range of doses is necessary to assess the generality of the present findings and the implications for the use of these strains (as well as others) as models of drug use and abuse.

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