Spontaneous withdrawal in opiate-dependent Fischer 344, Lewis and Sprague–Dawley rats

Jennifer L. Cobuzzi *, Anthony L. Riley

Psychopharmacology Laboratory, Department of Psychology, American University, Washington, D.C. 20016, United States

ABSTRACT

The Lewis (LEW) and Fischer 344 (F344) inbred rat strains react differentially to acute morphine administration for a variety of behavioral and neurochemical measures. Investigations into effects of chronic morphine are less common, and investigations assessing dependence have been limited to those utilizing antagonist-precipitated withdrawal. The present experiment extended these assessments by examining spontaneous withdrawal in the LEW and F344 strains. In this preparation, males of the LEW, F344 and the outbred Sprague–Dawley (SD) strain were made dependent on morphine. Following this, opiate administration was terminated and animals were examined for spontaneous withdrawal by the acquisition of a withdrawal-associated taste aversion, changes in body weight loss and the display of several behaviors characteristic of opiate withdrawal. Although all morphine-treated subjects decreased body weight and avoided consumption of the withdrawal-associated solution, indicating successful induction of dependence, no difference between the strains emerged in these indices of withdrawal severity. The only strain difference to appear in the behavioral indicators of withdrawal was with diarrhea (LEW>F344). That the strains differ in acute reactivity to opioids, but not in the overall severity of withdrawal, was discussed in relation to the need to examine the relationship between neurochemical and behavioral data in a variety of neural systems and behavioral endpoints.

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1. Introduction

The Lewis (LEW) and Fischer (F344) inbred rat strains are reported to differ on a variety of behavioral, biochemical and neuroanatomical endpoints (Guitart et al., 1993; Kosten and Ambrosio, 2002; Suzuki et al., 1992). Although these strains were initially described in relation to differences in stress (Grota et al., 1997; Riley et al., 2009; Sternberg et al., 1992; Stöhr et al., 1998) and immune (Sun et al., 1999) reactivity, more recently they have been shown to react differently in response to a variety of drugs of abuse. Interestingly, the two strains differ in their response to the rewarding and aversive effects of such drugs, displaying differential acquisition of both conditioned place preferences (Guitart et al., 1992) and conditioned taste aversions (Lancellotti et al., 2001), respectively. Given that drug abuse vulnerability has been suggested to be a function of the balance of the rewarding and aversive effects of drugs (Horan et al., 1997; Riley et al., 2009; Roma et al., 2006) and that these strains differ on these effects depending on the drug and the preparation, the LEW and F344

strains may be useful in assessing the genetic mediation of drug use and abuse (see Freeman et al., 2009; Gosnell and Krahn, 1993; Kosten and Ambrosio, 2002; Riley et al., 2009; Sánchez-Cardoso et al., 2007).

The vast majority of assessments of the differences between the two strains to drugs of abuse are reported in acute preparations in which the animals are given limited and short duration exposure to the drug (e.g., Davis et al., 2007; Gosnell and Krahn, 1993; Suzuki et al., 1988). Given the relation of chronic exposure with drug escalation, dependence and addiction (Koob and Bloom, 1988; Koob and Le Moal, 1997; Koob et al., 1998; Kreek et al., 2005), however, assessments of differences between the LEW and F344 strains in preparations in which animals are given extended exposure to the drug may provide more insight into the role of genotype in abuse vulnerability. Interestingly, it has been reported that these two strains differ in neurochemical reactivity when maintained on chronic morphine, displaying differences in several brain regions, most of which are implicated in the rewarding effects of drugs (see Guitart et al., 1992; Guitart et al., 1993; Nylander et al., 1995; Sánchez-Cardoso et al., 2007). Given these differences, it might also be expected that the two strains would differ in assays of spontaneous withdrawal as the neuroplastic changes associated with drug exposure mediate responding upon termination of opiate administration (see Koob and Le Moal, 1997, 2000). In this context, the present experiment examined strain differences in spontaneous withdrawal in animals given chronic exposure to morphine, a compound for which

* Corresponding author. Psychopharmacology Laboratory, Department of Psychology, American University 4400 Mass. Ave., NW, Washington, D.C. 20016, United States. Tel.: +1 202 885 1721; fax: +1 202 885 1081.
E-mail address: jc9378a@student.american.edu (J.L. Cobuzzi).

dependence and withdrawal have been well characterized and for which the LEW and F344 strains have been directly compared.

Specifically, rats of the LEW and F344 strains [as well as rats from the outbred Sprague–Dawley (SD) strain] were given chronic exposure to increasing doses of morphine sulfate (to a maximum of 100 mg/kg) and then given access to a novel saccharin solution upon termination of morphine administration. Consumption of the saccharin solution was monitored for 12 days during which animals had continuous access to both water and the withdrawal-associated saccharin solution. This procedure has been reported to produce aversions to the withdrawal-associated taste in outbred rats and is used to index dependence and withdrawal (see Mucha et al., 1990; Parker et al., 1973; Parker and Radow, 1974; Zellner et al., 1984; for a general overview of taste aversion learning, see Riley and Freeman, 2004; see also www.CTAlearning.com). In addition, body weight (Guitart et al., 1993; Rasmussen et al., 1990; Schulteis et al., 1994; Stephens and Riley, 2009) and several traditional behavioral indicators of withdrawal, e.g., wet dog shakes, ptosis, and piloerection (Guitart et al., 1993; Mayo-Michelson and Young, 1992; Nylander et al., 1995; Stephens and Riley, 2009) were monitored throughout this period.

2. Material and methods

2.1. Subjects

A total of 51 experimentally naïve male rats of the LEW, F344 and SD strains ($n = 17$ per strain; Harlan Sprague–Dawley, Indianapolis, Indiana) served as the subjects in this experiment. At the start of the experiment, all animals were approximately 90 days of age and the average body weights were: LEW = 344 g; F344 = 287 g; SD = 359 g. Animals were housed in individual wire-mesh cages and maintained on a 12:12 h light/dark cycle (lights on at 0800 h) and at an ambient temperature of 23 °C. Water and food were provided *ad libitum*. Fluids were presented in 50-ml graduated Nalgene tubes affixed to the front of the cages. Animals were handled approximately 2 weeks prior to the initiation of experimental manipulations to minimize handling stress effects. All procedures were performed in the light phase of the light/dark cycle (see Gomez-Serrano et al., 2009) and were in compliance with the US National Institutes of Health and National Research Council Guidelines (1996, 2003) and approved by the Institutional Animal Care and Use Committee at American University.

2.2. Drugs and solutions

Morphine sulfate (generously supplied by the National Institute on Drug Abuse) was dissolved in isotonic saline (10 mg/ml; Sigma) and administered intraperitoneally (ip). Sodium saccharin (0.1%, Sigma) was prepared as a 1 g/l solution in tap water.

2.3. Procedure

2.3.1. Phase I: morphine-dependence acquisition
Animals in each strain were randomly divided into two groups dependent upon the injection they were to receive during this phase, i.e., morphine or vehicle. This yielded six groups: LM ($n = 9$), LV ($n = 8$), FM ($n = 9$), FV ($n = 8$), SM ($n = 9$) and SV ($n = 8$). The first letter denotes the strain of the rat (LEW, F344 or SD); the second letter refers to the treatment condition (morphine or vehicle). On Day 1, the morphine-treated animals received an injection of morphine (10 mg/ kg) at 0900 h. This dose was increased by 10 mg/kg per day until a dose of 100 mg/kg was reached on Day 10. For Days 11–15, the dose of 100 mg/kg was maintained. Vehicle-treated animals received equi-volume injections of saline. All animals were weighed prior to their injection, and all injections were given at the same time each day.

2.3.2. Phase II: assessment of withdrawal
On Day 16, at the usual injection time, two 50-ml graduated Nalgene tubes were affixed to the front of each rat's cage, one containing tap water and one containing the sodium saccharin solution. Every 12 h (at 0900 h and 2100 h) on Days 16–27, consumption of both solutions was recorded, the bottles were refilled and the position of the solutions was switched to prevent positioning effects. In addition, on Days 16–19 (see Parker and Radow, 1974) from 1200 h to 1500 h, each animal was observed for 15 s in its home cage every 15 min for the 1st hour and every 30 min for the next 2 h (resulting in a total of eight observations per animal each day). During each 15-s observation period, the presence or absence of six behavioral indicators of withdrawal was noted. Any behavior was recorded as present only once in any observation period. The behaviors scored included wet dog shakes, piloerection, ptosis, diarrhea, teeth chatter and salivation (Guitart et al., 1993; McNally and Akil, 2001; Stephens and Riley, 2009; Suzuki et al., 1992). Given this scoring scheme, the maximum value of any behavior per observation period was 1 and the maximum score for any specific behavior on each day was 8 (given eight observation periods). Total value for any specific behavior over the course of withdrawal was 32 (eight observation periods × 4 days of withdrawal). Body weights were also recorded daily after the 3-h observation period.

2.4. Statistical analysis

2.4.1. Morphine-dependence acquisition
Throughout this phase, differences in body weight were compared using a $3 \times 2 \times 15$ repeated measures Analysis of Variance (ANOVA) with between-subjects factors of Strain (LEW, F344 or SD) and Drug (Morphine or Vehicle) and a within-subjects factor of Day (Days 1–15 of morphine-dependence acquisition). Differences in mean water consumption were compared using a $3 \times 2 \times 15$ repeated measures ANOVA with between-subjects factors of Strain (LEW, F344 or SD) and Drug (Morphine or Vehicle) and a within-subjects factor of Day (Days 1–15).

2.4.2. Assessment of withdrawal
Throughout this phase, body weight was analyzed by a $3 \times 2 \times 12$ repeated measures ANOVA with between-subjects factors of Strain (LEW, F344 or SD) and Drug (Morphine or Vehicle) and a within-subjects factor of Day (Days 16–27). Total fluid consumption (water plus saccharin) was analyzed by a $3 \times 2 \times 12$ repeated measures ANOVA with between-subjects factors of Strain (LEW, F344 or SD) and Drug (Morphine or Vehicle) and a within-subjects factor of Day (Days 16–27). Percent saccharin consumption (saccharin/water plus saccharin) was analyzed by a $3 \times 2 \times 12$ repeated measures ANOVA with between-subjects factors of Strain (LEW, F344 or SD) and Drug (Morphine or Vehicle) and a within-subjects factor of Day (Days 16–27). Each behavioral indicator of withdrawal was analyzed by a 3×2 ANOVA with between-subjects factors of Strain (LEW, F344 or SD) and Drug (Morphine or Vehicle).

In the event of a significant overall effect, a one-way ANOVA was utilized to examine specific factors. Where appropriate, pair-wise comparisons were made using Tukey's HSD post-hoc tests and significance was assessed at $\alpha \leq 0.05$.

3. Results

3.1. Morphine-dependence acquisition

3.1.1. Body weight
The $3 \times 2 \times 15$ repeated measures ANOVA on body weight during chronic morphine exposure (Days 1–15) revealed significant main effects of Strain [$F(2,45) = 100.428$, $p < 0.001$] and Drug [$F(1,45) = 7.081$, $p = 0.011$], but no Strain × Drug interaction. With respect to the

effect of Strain, Tukey's post-hoc analysis revealed that the F344 strain weighed significantly less than the LEW and SD strains (all ps<0.001) and the LEW strain weighed significantly less than the SD strain ($p=0.034$; see Fig. 1A). With respect to the effect of Drug, morphine-treated animals weighed significantly less than vehicle-treated animals ($p=0.011$; see Fig. 1B).

3.1.2. Water consumption

The $3\times2\times15$ repeated measures ANOVA on water consumption during chronic morphine exposure (Days 1–15) revealed a significant main effect of Strain [$F(2,45)=56.509$, $p<0.001$] as well as a significant Drug×Strain interaction [$F(2,45)=3.699$, $p=0.033$]. In relation to the Drug×Strain interaction, Tukey's post-hoc analysis revealed that Groups SM and SV drank significantly more water than Groups LM, LV, FM and FV (all ps<0.001; see Fig. 2). No other comparisons were significant.

3.2. Assessment of withdrawal

3.2.1. Body weight

An independent-samples *t*-test on body weight on the last day of morphine-dependence acquisition (Day 15) revealed that morphine-treated animals weighed significantly less than vehicle-treated animals [$t(49)=3.7$, $p=0.001$] (see Fig. 3). A $3\times2\times12$ repeated measures ANOVA of body weight over withdrawal revealed significant main effects of Drug [$F(1,45)=74.979$, $p<0.001$] and Strain [$F(2,45)=80.958$, $p<0.001$] as well as significant Day×Drug [$F(11,495)=8.27$, $p<0.001$] and Day×Strain [$F(22,495)=1.74$, $p=0.02$] interactions. With respect to the Day×Drug interaction, a one-way ANOVA revealed that on all days (16–27) morphine-treated animals weighed significantly less than vehicle-treated animals (all ps≤0.022; see Fig. 3). With respect to the Day×Strain interaction,

Fig. 2. Absolute water consumption during morphine-dependence acquisition. *Groups SM and SV drank significantly more water than all other groups. There were no other significant differences in absolute water consumption between morphine- and vehicle-treated animals of each strain.

Tukey's post-hoc analysis revealed that on all days the F344 strain weighed significantly less than the LEW and SD strains (all ps<0.001) and on Days 26 and 27 the LEW strain weighed significantly less than the SD strain (all ps≤0.028) (data not shown).

Paired-samples t-tests comparing body weight of morphine-treated animals on the day immediately prior to cessation of morphine administration (Day 15) to each day of withdrawal revealed that body weight decreased significantly on Days 16–19 (all ps<0.01). There was no difference in body weight on Day 20, and body weight significantly increased on Days 21–27 (all ps<0.05). Similar comparisons for vehicle-treated animals revealed that body weight decreased significantly on Day 17 ($p=0.011$) and significantly increased on Days 20–27 (all ps<0.01).

3.2.2. Consumption

The $3\times2\times12$ repeated measures ANOVA on total fluid consumption during spontaneous withdrawal (Days 16–27) revealed significant main effects of Strain [$F(2,45)=67.318$, $p<0.001$] and Drug [$F(1,45)=15.775$, $p<0.001$]. With respect to the effect of Strain, Tukey's post-hoc analysis revealed that the LEW and F344 strains drank significantly less than the SD strain ($p<0.001$; see Fig. 4A). With respect to the effect of Drug, a one-way ANOVA revealed that morphine-treated animals drank significantly less than vehicle-treated animals ($p<0.05$; see Fig. 4B).

Given the significant differences observed in overall fluid consumption over this phase, saccharin consumption was analyzed as a percent of overall fluid consumption. A $3\times2\times12$ repeated measures
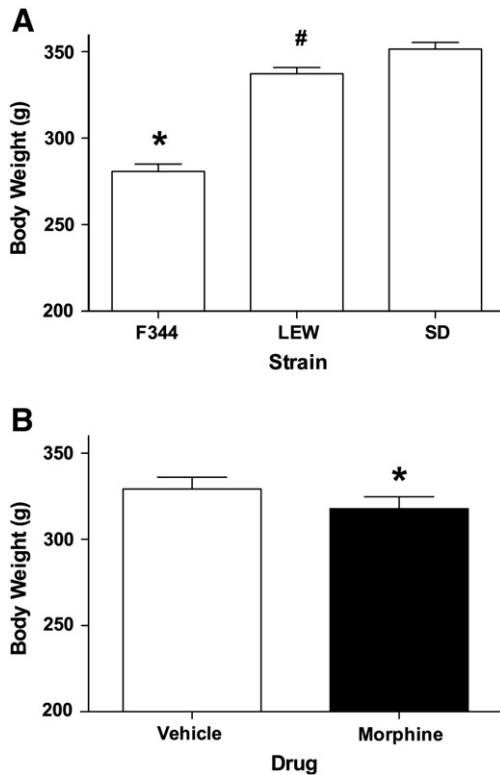
Fig. 1. Panel A: Body weight of the rat strains collapsed across all days of morphine-dependence acquisition. * F344 strain weighed significantly less than the LEW and SD strains. # LEW strain weighed significantly less than the SD strain. Panel B: Body weight of the drug groups collapsed across all days of morphine-dependence acquisition. *morphine-treated animals weighed significantly less than vehicle-treated animals.
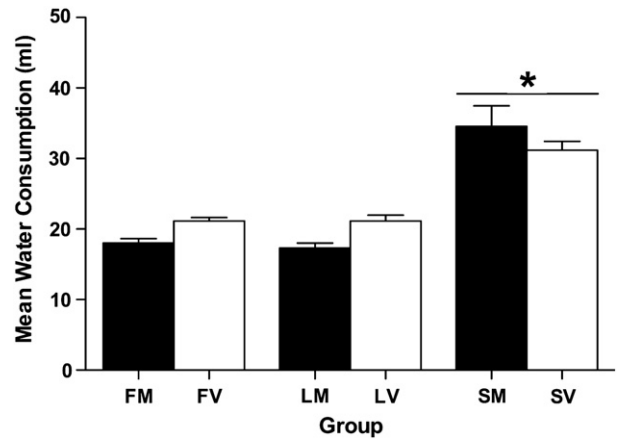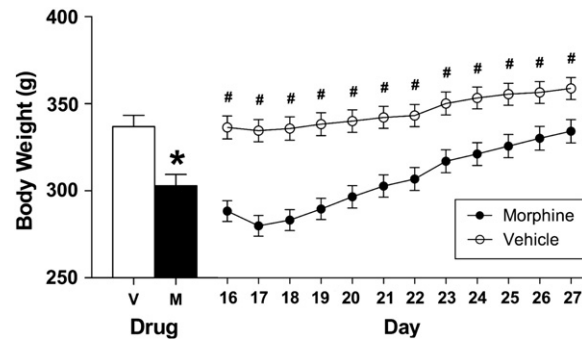
Fig. 3. Body weight of morphine- and vehicle-treated animals on Day 15 and throughout the assessment of withdrawal phase indicating the significant Day×Drug interaction. *morphine-treated animals weighed significantly less than vehicle-treated animals on Day 15. #morphine-treated animals weighed significantly less than vehicle-treated animals throughout the assessment of withdrawal (Days 16–27).
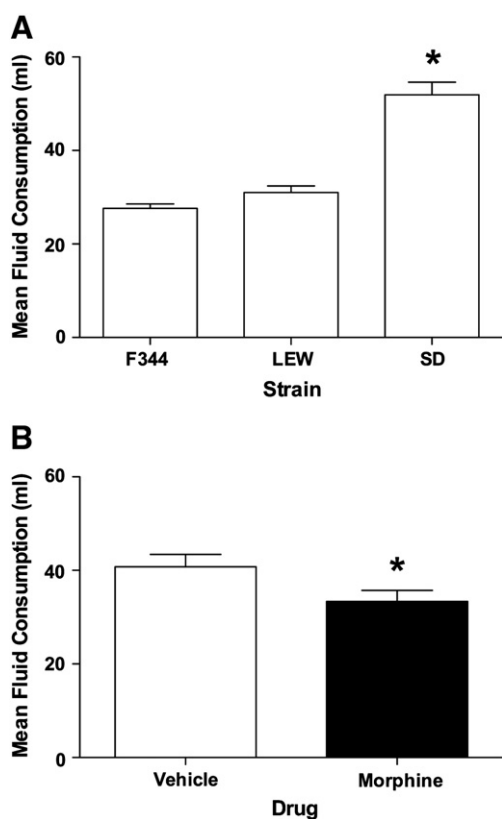
Fig. 4. Panel A: Mean fluid consumption of the strains collapsed across all days of withdrawal assessment. *LEW and F344 strains drank significantly less than the SD strain. Panel B: Mean fluid consumption of the drug groups collapsed across all days of withdrawal assessment. *Morphine-treated animals drank significantly less than vehicle-treated animals.

Fig. 5. Panel A: Percent saccharin of total consumption during assessment of withdrawal indicating the significant Day×Drug interaction. *morphine-treated animals drank a significantly smaller percentage of saccharin than vehicle-control animals throughout the assessment of withdrawal. Panel B: Percent saccharin of total consumption during assessment of withdrawal indicating the significant Strain×Drug interaction collapsed over days. *Groups LM and FM drank a significantly smaller percentage of saccharin than Group SM. ^morphine-treated animals consumed a significantly smaller percentage of saccharin than vehicle-treated animals.

ANOVA on percent saccharin consumption during spontaneous withdrawal revealed significant main effects of Drug [$F_{(1,45)} = 100.068$, $p < 0.001$] and Strain [$F_{(2,45)} = 4.174$, $p = 0.022$] and significant Day×Drug [$F_{(11,495)} = 11.344$, $p < 0.001$] and Strain×Drug [$F_{(2,45)} = 3.270$, $p = 0.047$] interactions. With respect to the Day×Drug interaction, a one-way ANOVA revealed that morphine-treated animals drank a significantly smaller percentage of saccharin than vehicle-control animals on all days (all $ps < 0.001$; see Fig. 5A). With respect to the significant Strain×Drug interaction, Tukey's post-hoc analysis revealed that Groups LM and FM drank a significantly smaller percentage of saccharin than Group SM ($p = 0.014$ and 0.023, respectively; see Fig. 5B). Further, each morphine-treated group drank a significantly smaller percentage of saccharin than its vehicle-control. Groups LM and FM did not differ in their percent saccharin consumption. There were no differences in consumption among the vehicle-treated controls.

3.2.3. Behavioral assays

Two of the six behavioral indicators of withdrawal were never observed to occur (ptosis and salivation), and thus the analyses focused on the four remaining behaviors, i.e., teeth chatter, wet dog shakes, diarrhea and piloerection. For each of these behavioral indicators, data were collapsed across the eight observation periods and 3 days of observations, allowing for assessments of the effects of Strain, Drug and their interaction.

The 3×2 ANOVA on the presence or absence of teeth chatter revealed a significant effect of Drug [$F_{(1,45)} = 13.612$, $p = 0.001$]. A one-way ANOVA revealed that morphine-treated animals displayed significantly more teeth chatter than vehicle-treated animals ($p = 0.001$). The 3×2 ANOVA on the presence or absence of wet dog shakes revealed significant effects of Strain [$F_{(2,45)} = 7.794$,
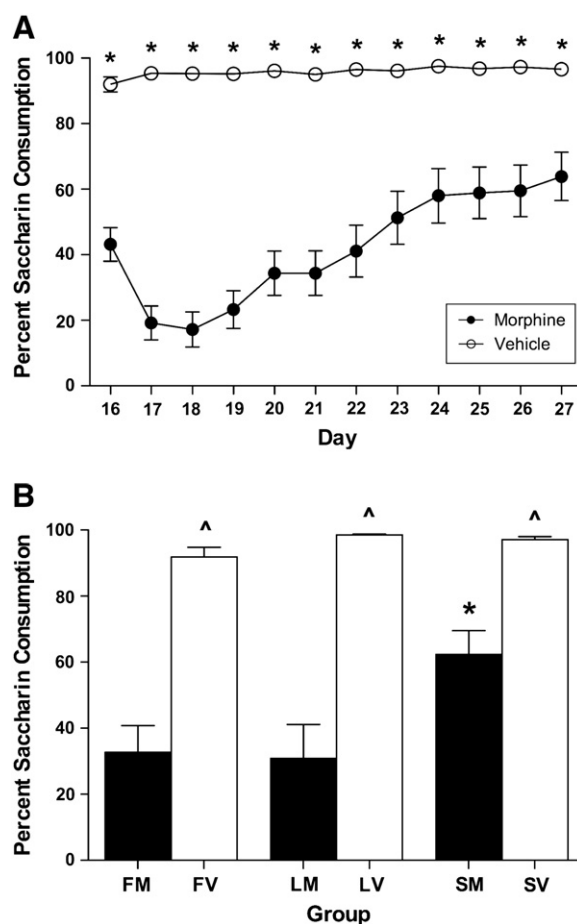
$p = 0.001$] and Drug [$F_{(1,45)} = 57.161$, $p < 0.001$]. With respect to the effect of Strain, Tukey's post-hoc analysis revealed the LEW strain displayed significantly more wet dog shakes than the SD strain ($p < 0.05$). With respect to the effect of Drug, a one-way ANOVA revealed that morphine-treated animals displayed significantly more wet dog shakes than the vehicle-treated animals ($p < 0.001$). There was no Strain×Drug interaction. The 3×2 ANOVA on the presence or absence of piloerection revealed significant effects of Strain [$F_{(2,45)} = 54.688$, $p < 0.001$] and Drug [$F_{(1,45)} = 77.325$, $p < 0.001$] as well as a significant Strain×Drug interaction [$F_{(2,45)} = 9.303$, $p < 0.001$]. With respect to the Strain×Drug interaction, Tukey's post-hoc analysis revealed that Group FM displayed significantly more piloerection than both Groups LM and SM (both $ps < 0.001$) and Group FV displayed significantly more piloerection than both Groups LV and SV (both $ps \leq 0.01$). Groups FM and LM displayed significantly more piloerection than the vehicle-treated animals of their own strain (both $ps < 0.01$). The 3×2 ANOVA on the presence or absence of diarrhea revealed significant effects of Strain [$F_{(2,45)} = 5.53$, $p < 0.01$] and Drug [$F_{(1,45)} = 27.417$, $p < 0.001$] as well as a significant Strain×Drug interaction [$F_{(2,45)} = 6.359$, $p < 0.01$]. With respect to the Strain×Drug interaction, Tukey's post-hoc analysis revealed that Groups LM and SM displayed significantly more diarrhea than Group FM (both $ps < 0.01$). Further, Group LM displayed significantly more diarrhea than the vehicle-control of its own strain ($p < 0.001$); see Table 1 for a summary of the behavioral indicators of withdrawal.

Table 1
Mean presence of behavioral indicators of withdrawal collapsed across the eight observation periods and 4 observation days (maximum value 32). *Group FM displayed significantly more piloerection than both Groups LM and SM. #Group FV displayed significantly more piloerection than both Groups LV and SV. ^Morphine-treated LEW and F344 animals displayed significantly more piloerection than the vehicle-treated animals of their own strain. ᵛGroups LM and SM displayed significantly more diarrhea than Group FM. +Group LM displayed significantly more diarrhea than its own vehicle-control.

Measure	FM	FV	LM	LV	SM	SV
Teeth chatter	1.22 ± 0.32	0.25 ± 0.16	1.22 ± 0.55	0.75 ± 0.31	2.11 ± 0.56	0.0 ± 0.0
Wet dog shakes	2.11 ± 0.39	0.38 ± 0.18	4.11 ± 0.68	0.75 ± 0.25	2.0 ± 0.24	0.13 ± 0.13
Piloerection	28.11 ± 1.02*^	9.5 ± 2.57#	9.67 ± 1.67^	1.5 ± 0.76	7.44 ± 1.69	1.13 ± 0.55
Diarrhea	0.56 ± 0.24	0.38 ± 0.18	1.78 ± 0.22ᵛ+	0.0 ± 0.0	1.67 ± 0.29ᵛ	0.75 ± 0.25

4. Discussion

The present study examined spontaneous withdrawal from morphine in the LEW and F344 rat strains. Specifically, LEW and F344 rats were chronically exposed to morphine and given access to a novel saccharin solution upon the termination of opiate administration. As described, morphine-treated animals from both strains acquired aversions to saccharin, indicative of the association of the taste with the aversive effects of spontaneous withdrawal (see Mucha et al., 1990; Parker et al., 1973; Parker and Radow, 1974; Zellner et al., 1984). Further, animals from both strains significantly decreased body weight in a manner characteristic of morphine withdrawal (Guitart et al., 1993; Rasmussen et al., 1990; Schulteis et al., 1994; Stephens and Riley, 2009). Finally, morphine-exposed subjects displayed a variety of behaviors characteristic of opiate withdrawal, e.g., teeth chatters, wet dog shakes, piloerection and diarrhea. With the single exception of diarrhea (LEW>F344), there were no systematic strain differences in withdrawal (as assessed by the acquisition of the withdrawal-induced aversions, body weight loss or behavioral indicators).

The fact that spontaneous withdrawal from morphine induced a taste aversion is consistent with work in outbred rats that has demonstrated that animals exposed to a novel taste while undergoing spontaneous withdrawal from morphine display significant aversions to the withdrawal-associated solution (see Mucha et al., 1990; Parker et al., 1973; Parker and Radow, 1974; Zellner et al., 1984), suggesting that spontaneous withdrawal can be assessed by the aversion design in both outbred and inbred strains. Further, the present results parallel those by Stephens and Riley (2009) who reported that morphine-dependent F344 and LEW rats acquired comparable aversions to a novel saccharin solution paired with naloxone-precipitated withdrawal. Withdrawal from morphine (either spontaneous or precipitated) does not appear to differ between the two strains as indexed by the acquisition of withdrawal induced aversions.

The significant decreases in body weight with the termination of morphine administration also parallel other work assessing opiate withdrawal in outbred rats (see Mucha et al., 1990; Parker and Radow, 1974). Such decreases appear to be relatively small (approximately 1–2%) and short-lived (in the present case, approximately 4 days). These effects contrast from those seen with precipitated withdrawal for which body weight decreases are more robust but shorter lived (see Guitart et al., 1993; Pournaghash and Riley, 1991; Rasmussen et al., 1990; Stephens and Riley, 2009). Although the data reported here are the first examining body weight changes in LEW and F344 rats undergoing spontaneous withdrawal from morphine, the data are similar to those reported by Stephens and Riley (2009) wherein opiate-dependent LEW and F344 rats decreased body weight following the administration of naloxone, but again there were no strain differences observed (Rasmussen et al., 1990; Stephens and Riley, 2009; though see Guitart et al., 1993). Withdrawal from morphine (either spontaneous or precipitated) does not appear to differ between the two strains as indexed by decreases in body weight.

The analysis of changes in body weight during spontaneous withdrawal in the present experiment are somewhat complicated by the fact that there were strain differences in body weight prior to the onset of withdrawal (LEW>F344). As reported by Gomez-Serrano et al. (2001), LEW and F344 rats differ in body weight at birth and maintain this difference through adulthood. Although such differences do exist, it is important to note that in the present experiment there was no Strain×Drug interaction when morphine treatment was terminated. Both LEW and F344 strains significantly decreased weight upon morphine termination, a decrease reflective of withdrawal.

There were significant behavioral changes upon termination of morphine treatment, all of which are consistent with other assessments of withdrawal. As noted, all morphine-treated animals, irrespective of strain, displayed significantly greater teeth chatter, piloerection, wet dog shakes and diarrhea than those injected with vehicle. Such effects parallel those seen in other assessments of opiate withdrawal (both spontaneous and precipitated; see Guitart et al., 1993; Mayo-Michelson and Young, 1992; Nylander et al., 1995; Rasmussen et al., 1990; Stephens and Riley, 2009). The only effect of strain in the present assessment was with respect to diarrhea (LEW>F344). Interestingly, preparations assessing precipitated withdrawal with the opiate antagonist naloxone report no strain difference between LEW and F344 animals on the measure of diarrhea (Mayo-Michelson and Young, 1992; Stephens and Riley, 2009). There also appeared to be an effect of strain on piloerection in the present experiment with the morphine-treated F344 strain displaying significantly more piloerection than the LEW. It is important to note, however, that the vehicle-treated F344 strain also displayed significantly more piloerection than the vehicle-treated LEW strain suggesting that the greater piloerection in the F344 strain may be due to its hyperactive stress response and not necessarily an effect of withdrawal (see Sternberg et al., 1992). Thus, with the single exception of diarrhea, withdrawal from morphine (either spontaneous or precipitated) does not appear to differ between the two strains as indexed by a variety of behavioral indicators.

It is surprising that the LEW and F344 strains that are consistently characterized as differentially responsive to acute administration of morphine (Ambrosio et al., 1995; Davis et al., 2007; Gomez-Serrano et al., 2009; Gosnell and Krahn, 1993; Lancellotti et al., 2001) and with respect to basal levels of opioid peptides (Martín et al., 1999; Nylander et al., 1995; Sánchez-Cardoso et al., 2007) do not differ in their response to spontaneous morphine withdrawal. This is also surprising given the limited work assessing neuroplastic changes seen with chronic morphine in these two strains. For example, Guitart et al. (1993) has reported greater firing rates in the locus coeruleus (LC) as well as increased levels of adenylate cyclase (AC) and cyclic adenosine monophosphate (cAMP)-dependent protein kinase activity in the LC of the LEW strain relative to the F344 strain during chronic treatment with morphine. It is difficult to know the extent to which these data address the work reported here in that the changes reported by Guitart et al. were during chronic morphine administration and not upon its termination. Clearly, assessments of strain differences in these brain systems and others must be made and correlated with the behavioral changes seen in dependence and withdrawal. Such assessments may allow conclusions regarding the possible biochemical mediation of any reported behavioral differences between the strains.

Although the present investigation focused on the possible differences between the LEW and F344 strains, outbred SD rats were run as a baseline condition with which to compare the two inbred strains. As described, SD rats displayed a number of differences relative to the LEW and F344 strains. Specifically, the morphine-exposed SD rats had a significantly greater saccharin preference during withdrawal than their counterparts in the LEW and F344 strains, suggesting that withdrawal was not as aversive in these animals. Interestingly, most assessments investigating differences between F344 and LEW strains do not include outbred animals for comparison, and when they are included, they do not always display behaviors in the same direction relative to the LEW and F344 strains. For example, stress reactivity has been a topic of much investigation with these strains and it has been shown that the F344 strain displays greater responsivity to stress than the LEW strain, whereas the SD strain lies between the two (Sternberg et al., 1992). Further, while investigating strain differences in male rat sexual behavior, Hurwitz and Riley (in press) reported that LEW males were slower to initiate copulation and displayed fewer behaviors overall than F344 males with SD males being intermediate between the two. In an assessment on the effects of light cycle phase on morphine induced conditioned taste aversions, Gomez-Serrano et al. (2009) reported greater aversions in the F344 strain relative to the SD strain with the LEW strain falling in the intermediate position during the light portion of the cycle and no differences between any of the strains during the dark portion. Thus, differences between the LEW and F344 strains and the outbred SD strain are dependent on the specific endpoint being assayed.

Taken together, these data demonstrate comparable conditioned taste aversions, body weight loss and behavioral changes induced by withdrawal (with the exception of diarrhea) between the LEW and F344 inbred rat strains. That the two strains displayed an overall behavioral profile reflective of opiate withdrawal, but with no differences between them, is somewhat surprising given the consistently reported differential responsivity of these strains with respect to opiate administration. Although the spontaneous withdrawal preparation utilized in the current assessment is more similar to the human condition relative to withdrawal from chronic opiate administration, this preparation is not commonly utilized in animal models of dependence and withdrawal due to the effects being less pronounced and harder to detect. A complementary analysis to the current preparation would be to examine changes in overall opioid tone in discrete brain areas after chronic opiate exposure in these strains. Such assessments might provide more insight into the differences in drug taking and drug escalation in these strains and the possible mechanisms for reported genotypic differences in drug self-administration.

Acknowledgements

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