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Morphine-induced place conditioning in Fischer and Lewis rats: Acquisition and dose-response in a fully biased procedure

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Abstract

The Fischer (F344) and Lewis (LEW) rat strains differ on a variety of behavioral assays examining the effects of morphine, with many of the differences observed during acquisition of behavioral responses. The results of these studies and others examining endogenous physiology and the biochemical effects of morphine suggest that F344 rats are more sensitive to morphine than LEW rats. However, LEW animals have shown greater conditioned place preferences (CPP) to 4 mg/kg than F344 rats. CPP is a popular assay of drug reward in which acquisition of the preference can be measured across multiple conditioning cycles, yet this aspect of CPP has not been assessed in F344 and LEW rats. As part of an ongoing effort to fully characterize the conditioned rewarding effects of abused drugs in these strains, the present study assessed the effects of 0, 1, 4 and 10 mg/kg subcutaneous (SC) morphine in adult male F344 and LEW rats (n=12/strain/dose). A fully biased place conditioning procedure was employed where morphine's effects were paired with the initially non-preferred chamber on Day 1, saline was paired with the preferred chamber on Day 2 and drug-free access to the entire apparatus was allowed on Day 3. This conditioning and testing regimen was repeated for four consecutive cycles. The F344 animals acquired CPP at 1 mg/kg only; this effect emerged after only two conditioning cycles. LEW rats never acquired a CPP at any dose tested. Peak blood morphine levels following SC injections of 1, 4 or 10 mg/kg revealed no significant strain or dose effects. These behavioral data are consistent with the hypothesis that F344 rats are more sensitive to the rewarding effects of morphine than LEW rats. Additional implications for the Fischer–Lewis model of drug abuse and the utility of CPP acquisition procedures are discussed.
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1. Introduction

The Fischer (F344) and Lewis (LEW) inbred rat strains differ in their behavioral responses to a variety of drugs of abuse (Kosten and Ambrosio, 2002; Riley et al., in press), and as such, have been used to explore the underlying mechanisms mediating differential sensitivities to various drug effects (Flores et al., 1998; Grabus et al., 2004; Guitart et al., 1992, 1993; Herradón et al., 2003a,b; Selley et al., 2003). Although much of the work on drug sensitivity has focused on the rewarding effects of drugs of abuse, F344 and LEW rats also reportedly differ in their responses to the aversive effects of such drugs, as assessed by the conditioned taste aversion (CTA) procedure (Glowa et al., 1994; Grigson and Freet, 2000; Kosten et al., 1994; Lancellotti et al., 2001; Pescatore et al., 2005; Roma et al., 2006, 2007). Strain-dependent differences in sensitivity to the discriminative stimulus effects of morphine and nicotine, as assessed by drug discrimination procedures, have also been reported (Morgan et al., 1999; Philibin et al., 2005).

Of particular interest to those investigating vulnerability to drug abuse are differences observed during drug self-administration procedures. Interestingly, many of the differences between F344 and LEW rats observed during self-administration studies are seen during the acquisition phase. For example, LEW animals acquire self-administration of cocaine, morphine and other opioids, and ethanol faster than F344 rats, even though comparable intake is often seen during maintenance or at the

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conclusion of self-administration studies with these drugs (Ambrosio et al., 1995; Kosten et al., 1997; Martín et al., 1999, 2003; Suzuki et al., 1988a,b). Although this pattern is not always seen with cocaine (Haile and Kosten, 2001; Haile et al., 2005; Kruzich and Xi, 2006), LEW rats are usually described as being generally more sensitive to the reinforcing effects of drugs of abuse than F344 rats (Camp et al., 1994; Flores et al., 1998; Kearns et al., 2006; Martín et al., 1999).

It is evident that monitoring the acquisition of drug-induced behaviors is a useful tool when investigating genetic factors in behavioral responses to drugs of abuse. Another valuable and increasingly popular behavioral preparation in which acquisition can be assessed is the conditioned place preference procedure (CPP; Bardo and Bevins, 2000; Tzschentke, 1998; Cunningham et al., 2003). When studying acquisition in the CPP design, animals are tested for approach behavior to the cues associated with the drug after each conditioning cycle, thereby allowing investigators to determine how many drug-environment pairings are required to elicit a significant preference. It is believed that faster acquisition of CPP represents greater sensitivity to the drug's rewarding effects, as opposed to its reinforcing effects *per se* (Bardo and Bevins, 2000; Gaiardi et al., 1991; Shippenberg et al., 1996; also see Meisch and Carroll, 1987).

Place conditioning to several drugs has been examined in F344 and LEW rats (see Kosten and Ambrosio, 2002; Roma et al., 2006), but despite the important information to be gained from studying acquisition, monitoring the acquisition of drug-induced CPP over conditioning has yet to be done in these strains. Therefore, as part of a larger effort to more fully characterize the behavioral responses to the conditioned rewarding effects of drugs of abuse in F344 and LEW rats, the present study investigated the acquisition of CPPs induced by several doses of morphine. Previously, Guitart and colleagues (1992) found that both strains exhibited preferences induced by 4 mg/kg morphine, but the LEW strain had a preference double that of the F344 rats. Another recent report by Grakalic et al. (2006) assessed the effects of stress on morphine-induced CPP at doses of 1, 4 and 10 mg/kg in F344 and LEW animals; although no direct strain comparisons were reported, both strains acquired CPP at all training doses. Most relevant to the present study was the fact that acquisition was not assessed in either of the above experiments.

The current study examined the acquisition of morphine CPPs at 1, 4 and 10 mg/kg using a fully biased procedure, meaning that all animals experienced morphine's effects in the initially non-preferred conditioning chamber. Although many advocate use of an unbiased procedure where drug-paired chamber assignments are counterbalanced across the equally-preferred conditioning chambers (Carr et al., 1989; Cunningham et al., 2003; van der Kooy, 1987), the biased design has some potential advantages. Biased procedures may be more sensitive to increases from pre-conditioning to post-conditioning due to the lower amount of time spent in the initially non-preferred chamber (Schenk et al., 1985; Scoles and Siegel, 1986). Although empirical support is limited (Blander et al., 1984), it has also been argued on theoretical grounds that biased procedures may provide more room for the emergence of dose-response functions, which are somewhat rare in unbiased assessments, possibly due to "ceiling effects" (Cunningham et al., 2003; Roma and Riley, 2005). Nonetheless, monitoring acquisition of CPP allows for the detection of differences in how rapidly asymptotic preference levels are achieved, a variable absent in designs featuring a single preference test at the conclusion of multiple conditioning cycles (Simpson and Riley, 2005). If the LEW animals are indeed more sensitive to morphine's rewarding effects than F344 rats, then the assessment of place conditioning over multiple trials provides an opportunity for such differences to emerge in the form(s) of differential CPP acquisition at any of the three doses tested.

2. Method

2.1. Subjects

A total of 96 adult male rats served as subjects; 48 rats were of the Fischer strain (F344/SsNHsd), and 48 were of the Lewis strain (LEW/NH). The respective mean (±SD) weights for the two strains at the beginning of the experiment were 251±54 g and 286±56 g. All animals were housed in individual hanging wire cages (24×19×18 cm) with *ad libitum* access to food and water. Animal housing rooms operated on a 12-h light/dark schedule (lights on at 0800 h) and were maintained at an ambient temperature of 23 °C; all procedures were conducted between 0900 h and 1400 h. All procedures described in this report were in compliance with National Research Council guidelines (NRC, 1996, 2003) and were 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 prepared in a 5 mg/ml solution in saline and administered via subcutaneous (SC) injection at doses of 1, 4 or 10 mg/kg; non-drug saline injections within the drug-treated groups were also administered SC and were equivolume to morphine. Exclusively vehicle-treated control animals (0 dose) were injected with either SC saline equivolume to 4 mg/kg morphine ($n=6$) or 3 ml/kg intraperitoneal (IP) saline ($n=6$).

2.3. Place conditioning apparatus

The CPP apparatus was constructed of wood and consisted of two main conditioning chambers (30×30×39 cm each) joined by a smaller middle chamber (10×30×39 cm). One of the conditioning chambers had a smooth Plexiglas floor, the other conditioning chamber had a textured plastic floor and the smaller middle chamber had heavy steel mesh attached directly to the floor. Vertically sliding wood panels separated the chambers. Six identical apparatuses were utilized for running multiple animals simultaneously. The procedure room was illuminated only by an 85-watt red light mounted to the ceiling in the center of the room; a white noise generator was also used in the room throughout all procedures. The CPP tests were

digitally recorded by a light-sensitive ceiling-mounted camera (Sony DVR201) and coded by trained observers. An animal was operationally defined as "in a chamber" once both forepaws crossed the threshold into the same chamber.

2.4. Place conditioning regimen

2.4.1. Pre-test

Baseline chamber preferences of the animals were determined by placing each animal in the center compartment of the CPP apparatus, then removing the barriers and allowing it free access to the entire apparatus for 15 min (PRE). Consistent with previous work from our laboratory with these strains (Roma et al., 2006), a paired-samples *t*-test revealed that all animals as a group spent less time in the smooth chamber than the textured chamber (190 versus 412 s, $t(95)=12.14$, $p<.001$), indicating a significant apparatus bias (Cunningham et al., 2003; Roma and Riley, 2005); time spent in the non-preferred smooth chamber did not differ between strains (independent samples $t(94)=1.92$, $p>.05$). All animals ($n=12$ per combination of strain and dose) were assigned to experience morphine's effects in the initially non-preferred smooth chamber with intervening saline injections paired with the initially preferred textured chamber. The place conditioning strategy employed may thus be considered "fully biased," with biased drug-paired stimulus assignment in a biased apparatus.

2.4.2. Acquisition

The CPP Acquisition phase (CPP) began 2 days after the Pre-Test session. On Day 1 of the conditioning cycle, half of the animals were administered their respective doses of morphine and confined to the smooth chamber for 30 min. The remaining animals received their respective saline injections and were confined to the textured chamber for 30 min. On Day 2, animals experienced injections and chamber confinement opposite those of Day 1. On Day 3, each animal was given access to the entire apparatus in a 15-min CPP test session. This 3-day sequence constitutes one conditioning cycle, and the Acquisition phase consisted of four such cycles culminating in a final CPP test on Day 12 (CPP 4). Exclusively vehicle-treated animals received vehicle injections on both days of each cycle, but were otherwise tested identically to their morphine-treated counterparts.

2.5. Blood morphine assessment

In order to determine if any strain differences seen in the acquisition of morphine-induced CPPs were attributable to differences in morphine absorption, blood morphine levels were determined in a separate experiment at 15- and 60-min post-injection of 1, 4 or 10 mg/kg morphine. Blood morphine levels after injections of a single dose have been assessed in these animals with no differences being reported (Gosnell and Krahn, 1993; Guitart et al., 1992). The current study adds to the literature by 1) assessing the effects of three doses of morphine under identical parameters instead of a single dose, and 2) using a within-groups design across multiple time points rather than between-groups.

After a one month wash-out period following the conclusion of the place conditioning procedures, blood morphine concentrations in response to SC morphine injections were assessed in 24 animals from each strain. Animals were assigned to the 1, 4 or 10 mg/kg dose groups ($n=8$ per strain) such that equal numbers of animals of each experimental history (i.e., CPP dose) were represented in each group. For the assessment itself, each rat was briefly removed from its homecage for receipt of its respective morphine injection and returned to the homecage immediately after injection. At 15- and 60-min post-injection, animals were moved to an unfamiliar room for blood sampling and then immediately returned to their homecage.

Immediately prior to the 15-min sampling, each rat's tail was soaked in warm water for 45–75 s and wiped dry with a paper towel. The rat was then placed in an oversized restraint tube (Plas-Labs, Lansing, MI) while approximately 1 mm of the tip of the tail was cut with surgical scissors. For the subsequent sampling, the tail was re-soaked and dried, but no further incisions were made, and the restraint tube was employed on an as-needed basis. For all samplings, approximately 40–90 μl of whole blood were collected via heparinized capillary tubes (Drummond Scientific, Broomall, PA) and the contents immediately transferred to microcentrifuge vials.

Each whole blood sample was centrifuged at 3000 rpm for 20 min; immediately afterwards, the plasma was transferred via micropipette to new vials. The plasma samples were then stored in a freezer at −80 °C until ready for analysis. Undiluted plasma was later thawed and assayed for morphine using high-performance liquid chromatography with electrochemical detection (HPLC-EC), similar to procedures established in Dominguez et al. (2001, 2006). Generally, the chromatography system consisted of a Valco (Houston, TX) injector with a 2-μl sample loop, and an Antec microelectrochemical detector, equipped with a microflow cell (11-nl cell volume), with a glassy carbon working electrode and an Ag/AgCl reference electrode. The analytical column was an LC Packings Fusica reversed-phase capillary column (300 μm inner diameter, 5 cm long, packed with 3 μm C-18 particles). The working electrode was maintained at an applied potential of 0.8 V relative to the reference electrode. The mobile phase was prepared in HPLC-grade water and included (in mM concentration): 32 citric acid, 54.3 sodium acetate, 0.074 EDTA, 0.215 octyl sulfonic acid (Sigma, St. Louis), and 4% methanol (v/v; pH 3.45).

2.6. Data analysis

The place conditioning data were expressed as the time (s) spent in the initially non-preferred, morphine-paired chamber during each of the 15-min test sessions. Preliminary analyses showed that the vehicle-treated control animals did not differ as a function of route of saline administration (SC versus IP), so their data were collapsed within each strain for formal analyses. Plasma morphine concentrations were expressed in picograms per microliter (pg/μl), and each animal's peak level (regardless of time post-injection) was used for analysis. Preliminary results confirmed that blood morphine levels did not vary as a function of experimental history, so this factor

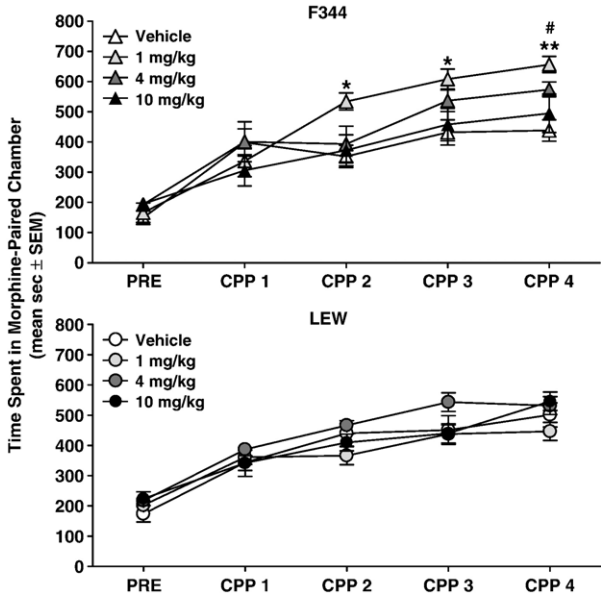

Fig. 1. Dose-response relationships throughout acquisition of morphine-induced place conditioning in F344 and LEW rats. The *y*-axes present time spent in the initially non-preferred, morphine paired chamber across the multiple 900-s test sessions by animals conditioned with saline vehicle (white) or 1 (light gray), 4 (dark gray) or 10 (black) mg/kg SC morphine. The top panel represents the F344 animals ($n=12$ per dose) while the bottom panel represents LEW ($n=12$ per dose). Within each strain, a significant difference between 1 mg/kg and vehicle is indicated by * and a significant difference between 1 and 10 mg/kg is indicated by #. A single icon denotes $p<.05$ and two icons denote $p<.01$ as determined by Tukey-corrected comparisons at each trial.

was excluded from further consideration. All data were analyzed by Analyses of Variance (ANOVA) with warranted post-hoc analyses accomplished via Tukey-corrected comparisons and independent-samples *t*-tests. These and all other procedures are described in detail below. Statistical significance was set at $\alpha=.05$ for all analyses.

Table 1
Peak blood-morphine levels in F344 and LEW rats

Strain	Dose		
	1 mg/kg	4 mg/kg	10 mg/kg
F344	343±107	313±50	415±114
LEW	379±112	489±130	311±56

Tail blood was sampled from adult male F344 and LEW rats 15 and 60 min after morphine injection ($n=8$ per combination of strain and dose). The peak blood morphine level from each animal was determined, the means±SEM of which are presented in the table above. No effects of dose or strain were observed.

3. Results

3.1. Morphine-induced place conditioning

A 5×2×4 mixed ANOVA with a repeated-measures factor of Trial (PRE, CPP 1, CPP 2, CPP 3 and CPP 4) and between-groups factors of Strain (F344 or LEW) and Dose (0, 1, 4 or 10 mg/kg) was performed with seconds spent in the morphine-paired chamber as the dependent variable. This analysis yielded a significant main effect of Trial ($F(4352)=173.22$, $p<.001$) and a significant Trial×Strain×Dose interaction ($F(12,352)=3.35$, $p<.001$); no other terms in the ANOVA achieved statistical significance (Fs<2.52, ps>.06). Given the significant interaction of all three factors, further post-hoc analyses were conducted to identify specific dose-response relationships within each strain as well as strain differences at each dose.

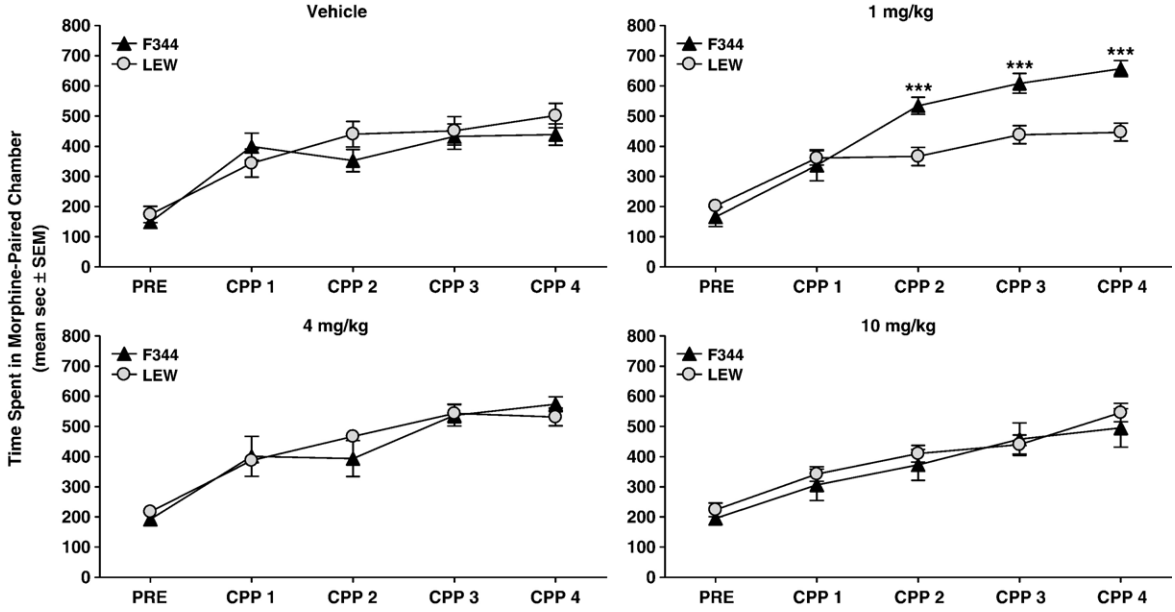

Fig. 2. Strain differences between F344 and LEW rats throughout acquisition of morphine-induced place conditioning. The *y*-axes present time spent in the initially non-preferred, morphine paired chamber across the multiple 900-s test sessions. Each panel displays responses by the F344 (▲) and LEW (●) animals conditioned at each dose (vehicle, 1, 4 or 10 mg/kg SC morphine, $n=12$ per strain and dose). Within each dose, a significant difference between F344 and LEW rats at a given trial is indicated by *** ($p<.001$, independent-samples *t*-tests).

3.1.1. Dose-response

Tukey-corrected comparisons at each trial within the F344 strain revealed a greater preference for the initially non-preferred chamber in the 1 mg/kg morphine group compared to their vehicle-treated counterparts at CPP 2, CPP 3 and CPP 4 ($ps < .05$). In addition, the mean asymptotic preference level of the 1 mg/kg F344 strain at CPP 4 was significantly greater than the 10 mg/kg group (657 versus 495 s, $p < .05$); however, no other groups differed from each other at any trial ($ps > .06$). Among the LEW animals, there were no significant between-groups differences at any trial ($ps > .10$). These data suggest an inverse dose-response function in the morphine-treated F344 animals, a conclusion confirmed by the significant negative linear correlation between dose and shift in time spent in the morphine-paired chamber from PRE to CPP 4 (Pearson $r(36) = -.448$, $p < .01$); no significant effects were observed in the morphine-treated LEW rats ($r(36) = .248$, $p > .10$). The dose-response relationships within each strain are presented in Fig. 1.

3.1.2. Strain differences

Independent-samples *t*-tests at each dose revealed a significantly stronger place preference at 1 mg/kg in the F344 animals versus LEW at CPP 2, CPP 3 and CPP 4 ($t(22)s > 3.84$, $ps < .001$). The strains did not differ from each other at any other trial or dose ($t(22)s < 1.58$, $ps > .10$; see Fig. 2).

3.2. Blood morphine assessment

A 2×3 univariate ANOVA with between-groups factors of Strain and Dose (1, 4 or 10 mg/kg) was performed on peak plasma morphine levels. This analysis revealed no significant main or interaction effects ($Fs < 0.98$, $ps > .30$). As seen in Table 1, blood morphine levels averaged 375 pg/μl with considerable variability, but no systematic effects of strain and a general insensitivity to the 1, 4 and 10 mg/kg doses tested.

4. Discussion

The F344 and LEW rat strains have been reported to differ in morphine-induced place preference, with LEW animals showing a more robust CPP to 4 mg/kg morphine (Guitart et al., 1992). However, in the current study, strain differences emerged only at the 1 mg/kg dose, with the F344 animals exhibiting a sustained preference for the morphine-paired chamber after only two conditioning cycles. Surprisingly, the LEW animals never displayed significant preferences at any of the doses tested, findings in opposition to the previous investigations of morphine-induced CPP in these strains. Interestingly, place preferences to 4 and 10 mg/kg also never developed in the F344 animals.

Exactly why morphine CPP appeared different when compared to previous reports is not readily apparent. However, methodological differences between the past and present experiments may underlie some of these differences. For example, both previous reports employed essentially unbiased designs where all animals as a group showed no significant preference for a particular conditioning chamber, whereas we employed a biased design where morphine was paired with the initially (and strongly) non-preferred chamber. It has been argued that unbiased designs provide a "purer" assay of reward than do biased designs, possibly due to the lack of anxiety presumably elicited by the non-preferred chamber (Carr et al., 1989; van der Kooy, 1987). With this idea comes the assumption that increases in time spent in the drug-paired chamber within a biased design are a function of the anxiolytic effects of the drug rather than it's positively reinforcing effects. Relevant to this issue is the fact that F344 rats are generally more reactive to physical and psychosocial stressors than are LEW rats (Dhabhar et al., 1993; Rex et al., 1996; Sternberg et al., 1992; Stöhr et al., 2000); however, the strains did not differ in amount of time spent in the non-preferred chamber during the pre-test, and the vehicle-treated control animals of both strains showed identical habituation responses over the multiple CPP test trials. It is still possible that both strains were equally anxious (cf. Chaouloff et al., 1995), with the F344 animals still being more sensitive to morphine's anxiolytic effects, but this argument is countered by the inverse dose-response function: that is, greater anxiety reduction provided by higher doses of morphine did not produce greater CPP. An additional criticism of the biased design is that this preparation favors significant increases in time spent in the drug-paired chamber, possibly due to habituation, but not absolute preferences for that chamber. However, the highest preference levels found in the current study approached 70% of total test session time and nearly 85% of the time spent in just the main conditioning chambers—clearly an absolute preference for the drug-paired chamber over the vehicle-paired chamber, and not simply a statistically significant increase. Moreover, the 1 mg/kg F344 animals spent significantly more time in the initially non-preferred, drug-paired chamber compared to their exclusively vehicle-treated counterparts, an effect inconsistent with a purely habituation account of changes in preference. Despite the reasonable concerns inherent to the biased procedure, the most parsimonious explanation of CPP in the F344 animals at 1 mg/kg still seems to be one of differential sensitivity to morphine's rewarding effects.

A final consideration that may resolve the discrepancy between our data and the existing morphine CPP assessments in F344 and LEW rats is the actual use of the acquisition procedure. Specifically, exposure to the drug-paired chamber in the absence of drug during the CPP tests may be considered a quasi-extinction trial. As described above, LEW animals appear less sensitive to morphine's effects in several biobehavioral assays. As such, the absence of CPP could have been the product of diminished unconditioned stimulus salience due to this lack of sensitivity coupled with the repeated drug-free exposures to the morphine-paired chamber during the testing component across CPP acquisition trials. These putative extinction days may have limited the LEW animals from strongly associating the positive effects of morphine with the drug-paired chamber, allowing no increases in time beyond the simple habituation pattern exhibited by the vehicle-treated control animals. This suggests that in order to produce morphine CPPs like those shown in previous reports, the LEW animals might need uninterrupted conditioning cycles, if not daily morphine exposure as was the case with Guitart et al. (1992; see also Grakalic et al., 2006). The empirical effects of

CPP acquisition procedures have not been systematically studied, but the reasoning outlined above suggests that regardless of biased methodology, CPP acquisition in general may actually be a more *conservative* estimate of drug reward, with motivation to seek drug-paired stimuli evident only in the animals most sensitive to the drug's rewarding effects. Once again, we are led to conclude that, insofar as CPP to fixed doses of drug models abuse liability, the F344 animals appear more susceptible than LEW. Although the effects reported in the present study were clear, a fully unbiased assessment of morphine CPP acquisition would provide an additional procedural link to the existing literature, and either strengthen the conclusions drawn here or reveal an interaction between genotype and unconditioned motivational states in morphine-induced place conditioning in these strains. Regardless of outcome, these data would be valuable to those interested in place conditioning and the effects of genotype in animal models of drug abuse, and such an assessment is currently being undertaken by our laboratory.

The assessment of blood morphine levels in the present study showed no strain differences and revealed no significant dose-response function. These findings agree with earlier reports of blood morphine levels in F344 and LEW rats at doses comparable to those presented here (4 mg/kg, Guitart et al., 1992; 3 mg/kg, Gosnell and Krahn, 1993). The absence of differences in these measures suggests that the mechanism(s) mediating the differences in CPP between the strains may lie in the brain. Several investigations have suggested that the F344 strain is more sensitive than the LEW strain to morphine administration. For example, compared to LEW rats, F344 animals acquire discrimination of morphine at a lower dose and show greater antinociception with this and other opioid drugs (Morgan et al., 1999; Terner et al., 2003a,b; although see Herradón et al., 2003b). Moreover, μ opioid receptors in the F344 rat are more responsive than those of LEW rats to stimulation by μ-receptor agonists (Herradón et al., 2003a; Selley et al., 2003), and F344 animals show greater behavioral responses to the administration of low-and intermediate-efficacy μ agonists (Morgan et al., 1999). In addition, F344 rats have higher μ opioid receptor binding than LEW animals in the central amygdala (Oliva et al., 1999), an area specifically implicated in appetitive conditioning (Knapska et al., 2006). The greater sensitivity of the F344 strain to lower doses of morphine and low-efficacy μ agonists predicts that these animals would show greater responding to low-dose morphine compared to the LEW strain, so our findings of CPP in F344, but not LEW, rats at 1 mg/kg are not surprising, if not more concordant with the molecular literature than the other two published reports of morphine CPP in these strains.

The suggestion that F344 rats are more sensitive to morphine reward than LEW rats may be consistent with the molecular pharmacological data, but may seem counter-intuitive in relation to the existing morphine self-administration literature: How could the strain that more rapidly acquires morphine self-administration behavior be *less* sensitive to that drug's rewarding effects? One advantage of classical conditioning preparations like CPP is that the amount of drug exposure can remain constant while the animals' responses to the fixed dose(s) are free to vary. This differs from self-administration where the individual infusion dose is fixed, but the actual amount of infusions ultimately consumed in any given session (i.e., dose administered) varies by individual. Given this, the fact that LEW rats initially self-administer more morphine is still consistent with the hypothesis that they are insensitive to morphine, in that they may require more drug to achieve the same level of reward that F344 rats experience at lower levels of intake. Although the semantics of "reinforcement" versus "reward" in interpreting self-administration studies are beyond the scope of this report, the fact that LEW rats did not develop preferences at any of the three doses tested in the present study remains in agreement with the observed patterns of operant self-administration and molecular work with these strains. Moreover, another possible view of the strain difference in self-administration acquisition is one of impulsivity. LEW rats are generally more exploratory than F344, and recent efforts from our laboratory (Kearns et al., 2006) revealed more rapid acquisition and higher levels of autoshaping behavior in LEW rats versus F344, while others have shown LEW rats to be more susceptible than F344 to delay discounting for food rewards (Anderson and Woolverton, 2005). Endogenous insensitivity to opiates combined with greater impulsivity provides a possible explanation for LEW rats' more rapid acquisition of morphine self-administration. Unlike self-administration, the use of fixed doses for CPP precludes any putative "self-correction" responses by the LEW rats, leaving only the lack of CPP consistent with a neurobiological insensitivity hypothesis, once again leading to the conclusion that F344 rats may be more sensitive than LEW rats to the rewarding effects of morphine.

If F344 rats are generally more sensitive to morphine than LEW rats, then the lack of CPP in the LEW animals may be explained, but the inverse dose-response function in the F344 animals is somewhat puzzling. However, this relative sensitivity to morphine's rewarding effects may also reveal sensitivity to its aversive effects. If so, then it follows that the aversive effects of the drug would hinder appetitive responding at increasing doses in the F344 animals. Indeed, reports show that F344 rats display conditioned taste aversions to morphine at doses as low as 5 mg/kg, whereas LEW rats do not acquire aversions at any dose reported (Davis and Riley, submitted for publication; Lancellotti et al., 2001). Differential sensitivity to morphine's aversive properties could have contributed to the lack of strong preferences in the F344 rats at 4 and 10 mg/kg versus their vehicle-treated controls, and we also speculate that these aversive effects may have contributed to the previously reported differences between F344 and LEW rats in morphine CPP at 4 mg/kg (Guitart et al., 1992). This view of dose-dependent shifts in the balance between competing motivational states is supported by the fact that, in addition to the μ receptor differences described above, F344 rats also have higher basal dynorphin peptide levels in various brain regions relevant to behavioral responses to morphine (Nylander et al., 1995). Discovery of strain differences specifically in terms of density and sensitivity of κ opioid receptors or behavioral responses to κ-specific compounds would be valuable in this regard, but such assessments remain to be made (although see Barrett et al., 2002).

In summary, responses to 1, 4 and 10 mg/kg morphine were assessed in male F344 and LEW rats. Using a biased design, only the F344 animals exhibited a significant conditioned place preference, and did so after only two drug-environment pairings. Despite the behavioral differences, there were no significant dose or strain effects on peak blood morphine concentrations. Although a number of important issues await resolution, these results indicate increased sensitivity to morphine reward in F344 over LEW animals and add to the literature dedicated to the comparison between F344 and LEW rats for modeling genetic factors in the etiology of drug abuse. The present results, along with a growing body of research, suggest that the "addiction-resistant" Fischer versus "addiction-prone" Lewis dichotomy sometimes ascribed to this model may benefit from further refinement.

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