

Increased Vulnerability to Stress Following Opiate Exposures: Behavioral and Autonomic Correlates

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The authors used rats to study the impact of a history of opiate exposures on behavioral and autonomic responses to restraint stress. Brief restraint (30 min) provoked tachycardia and a pressor response, anxiety (as indexed by social interaction), grooming, and reduced exploration. The pressor response was reduced at 1 day, but not 7 days, after last opiate exposure; tachycardia was unaffected (Experiment 1). Stress-induced anxiety was potentiated 1 and 7 days after last opiate exposure (Experiment 2), and this potentiation was a function of dose (Experiment 3) and duration (Experiment 4) of opiate exposure. The results show that a history of opiate exposures alters vulnerability to stress and has implications for understanding coping, anxiety, and emotionality in former opiate users.

Keywords: stress, opiate, blood pressure, heart rate, anxiety

Stress has a profound influence on drug dependence. For example, animal models have shown that stressors provoke relapse to drug-seeking (e.g., Shaham & Stewart, 1995) and potentiate behavioral sensitization to opiates and psychostimulants (e.g., Deroche et al., 1992). Stressors also increase drug self-administration (e.g., Goeters & Guerin, 1994), drug-induced conditioned place preference (Will, Watkins, & Maier, 1998), and drug withdrawal (Williams, Drugan, & Maier, 1984). Likewise, studies of human drug users reveal that stress and negative affect are potent antecedents to drug craving, drug-use, and relapse to drug-seeking (e.g., Brewer, Catalano, Haggerty, Gaine, & Fleming, 1998; Kosten, Rounsaville, & Kleber, 1986; Shiffman et al., 1996; Sinha, Fuse, Aubin, & O'Malley, 2000).

An increasing body of evidence suggests that this interaction between stress and drug dependence is bidirectional. Just as a history of exposure to stressors can increase vulnerability to drug dependence, so too can a history of drug dependence increase vulnerability to stress. For example, repeated exposures to cocaine potentiate subsequent behavioral responses (e.g., locomotor activity) to intracerebroventricular infusions of the stress-related peptide corticotropin-releasing hormone (CRH; Erb, Funk, & Le, 2003) and the expression of c-Fos mRNA to footshock stress (Erb, Lopak, & Smith, 2004). Repeated exposures to opiates alter hypothalamic–pituitary–adrenal (HPA) axis function (e.g., Houshyar, Cooper, & Woods, 2001; Houshyar, Galigniana, Pratt, & Woods, 2001) and levels of key genes in the HPA axis, such as

CRH mRNA in the paraventricular nucleus of the hypothalamus (Houshyar, Gomez, Manalo, Bhargava, & Dallman, 2003; Houshyar, Manalo, & Dallman, 2004; McNally & Akil, 2002) and glucocorticoid receptor mRNA in the hippocampus (McNally & Akil, 2003). These alterations also extend beyond the HPA axis to include neural circuits for anxiety and autonomic function, such as the extended amygdala (e.g., McNally & Akil, 2002) and locus coeruleus (e.g., Xu, Van Bockstaele, Reyes, Bethea, & Valentino, 2004).

These findings show that an alteration of central stress circuits is an important consequence of drug dependence and may have implications for understanding coping, emotionality, and relapse to drug seeking in former opiate users (Kreek & Koob, 1998). However, few studies have characterized the behavioral and autonomic correlates of this dysregulation following opiate dependence. Houshyar, Cooper, and Woods (2001) subjected rats with a history of opiate injections to a period of restraint stress 12 hr and 16 days after last opiate exposure. The restraint stress produced pronounced hyperthermia that was attenuated in the opiate-treated rats at both time points. Xu et al. (2004) subjected rats with a history of opiate exposures to a 15-min swim stress. The opiate history significantly increased the number of active responses (e.g., climbing) observed during swim stress. The functional consequences of opiate-induced dysregulation of stress circuits therefore remain poorly understood.

The present experiments studied vulnerability to stress in rats with a history of opiate exposures. Stressors have well-documented effects on behavior and autonomic function. These include increases in anxiety, arousal, blood pressure, and heart rate. The aim of the present experiments was to characterize these behavioral and autonomic effects in rats with a history of opiate exposures. Rats were pretreated with daily injections of morphine or saline for 10 days. They were then subject to a 30-min restraint stressor either 1 or 7 days after last injection. Experiment 1 used radiotelemetry to study autonomic responses, namely heart rate and mean arterial blood pressure, to restraint stress and during recovery from this stress 1 or 7 days after last exposure to opiate.

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Experiment 2 studied anxiety, as measured by the low-light, familiar apparatus test condition of the social interaction test (File & Seth, 2003) as well as behavioral activation following restraint stress 1 or 7 days after exposure to opiates. Experiment 3 studied the role of opiate dose in altering vulnerability to stress. Rats were treated with 0 mg/kg, 1 mg/kg, 5 mg/kg, or 10 mg/kg morphine for 10 days and subjected to restraint stress 7 days after last injection. They were tested for anxiety in the social interaction test as well as for behavioral activation. Experiment 4 studied the role of duration of opiate exposure in altering vulnerability to stress. Rats were treated with 10 mg/kg morphine for 0, 1, 5, or 10 days and subject to restraint stress 7 days after last injection. They were tested for anxiety in the social interaction test as well as for behavioral activation.

Method

Subjects

Subjects were male Wistar rats obtained from a commercial supplier (Gore Hill Research Laboratories, Sydney, Australia). Prior to the start of the experiment, rats were housed in groups of 8 in plastic cages (67-cm length \times 40-cm width \times 22-cm height) maintained under natural lighting. Food and water were freely available. They were handled for 5 days prior to the start of the experiment. At the commencement of the experiment, rats were singly housed in plastic cages (40-cm length \times 26-cm length \times 16-cm height) with food and water freely available. The procedures used were approved by the University of New South Wales Animal Care and Ethics Committee.

Apparatus

Restraint. Rats were restrained in Plexiglas tubes (7.5-cm diameter \times 21.5-cm length; Braintree Scientific, Braintree, MA) to induce autonomic and behavioral indicants of stress.

Telemetry. Individual restrainers (during restraint stress) or rat cages (during baseline and recovery) were placed on top of receivers for concurrent measurement of heart rate (HR) and mean arterial blood pressure (MAP). HR and MAP were extracted automatically from the pulsatile blood pressure signal by use of the Dataquest A.R.T. Gold software (Data Sciences International, St. Paul, MN). HR and MAP were sampled every 30 s from 3-s time windows.

Social interaction and activity. Social interaction and activity were observed in a plastic arena (40-cm length \times 26-cm width \times 16-cm height). The front of this arena was constructed of Perspex, and the walls and floor were constructed of plastic. The roof was constructed from stainless steel bars. The arena was located in a room illuminated by a 40-W red light. A camera in the experimental room relayed a video signal to a monitor and VCR in an adjacent laboratory.

Drugs. Rats were injected subcutaneously in the dorsal neck region daily for 10 days with morphine HCL (10 mg/kg; a generous gift from GlaxoSmithKline, Brentford, Middlesex, England) or sterile nonpyrogenic saline (0.9% wt/vol). This injection regime was chosen because previous work has shown that daily injections of 10 mg/kg morphine lead to pronounced and prolonged sensitization to the locomotor activating effects of the drug (Vanderschuren et al., 1997). All injections were in a volume of 1 mL/kg.

Procedure

Surgery. Rats in Experiment 1 were surgically implanted with radiotelemetry devices (Model TA11PA-C40, Data Sciences International, St. Paul, MN) as described by Carvive (2000). Briefly, a midline incision was

made in the abdomen, and the descending aorta was exposed at the level of the iliac bifurcation. The artery was punctured at this level, and the fluid-filled sensor catheter was inserted and fixed in place with tissue adhesive (3M, Animal Care Products, St. Paul, MN). The body of the probe was immobilized by suturing to the ventral abdominal wall. The abdomen was closed with suture clips. The rats were injected subcutaneously with 5 mg/kg of Carprofen to provide pain relief and 0.33 mL procaine penicillin to prevent infection and moved to individual plastic home boxes in which they were housed for the duration of the experiment. The rats were allowed 7 days recovery prior to start of the experiment.

Experiment 1: Effects of a history of opiate exposures on autonomic responses to restraint stress. The experiment employed a 2×2 factorial design. The first factor refers to drug history (saline vs. morphine), and the second factor refers to when restraint stress occurred (1 day or 7 days after last injection). Rats were randomly allocated to the four groups generated by the design: morphine–stress 1 day ($n = 3$), saline–stress 1 day ($n = 3$), morphine–stress 7 days ($n = 4$), saline–stress 7 days ($n = 4$). On the last day of injection, the rats were transported to the laboratory in their home cages, and they remained there for 120 min to habituate them to the laboratory. One day or 7 days after last injection, rats were again transported in their home cages to the laboratory. The animals remained in their home cages for 60 min. This period served as the baseline recording period. Rats were then placed in the Plexiglas restrainers for 30 min and then immediately returned to their home cages. Telemetry measurements were taken during the 60-min baseline, 30-min stress, and 120-min poststress periods.

Experiment 2: Effects of a history of opiate exposures on behavioral responses to restraint stress. The experiment employed a $2 \times 2 \times 2$ factorial design. The first factor refers to drug history (saline vs. morphine), the second factor refers to stress (stress vs. no stress), and the third factor refers to when stress occurred (1 day or 7 days after last injection). Rats were randomly allocated to the eight groups generated by this design: morphine–stress 1 day, saline–stress 1 day, morphine–no stress 1 day, saline–no stress 1 day, morphine–stress 7 days, saline–stress 7 days, morphine–no stress 7 days, saline–no stress 7 days. There were 8 subjects per group in all groups except morphine–no stress 7 days and saline–no stress 7 days, which had 7 subjects per group. On the day before stress (Day 10 of injections for the 1-day groups or 6 days after last injection for the 7-days groups), rats were transported to the laboratory and familiarized for 120 min with the testing arena. One day or 7 days after last injection, rats were transported to the laboratory and placed in the arena for a further 60-min habituation period. Rats were then removed and placed in the Plexiglas restrainers or home cages for 30 min according to group allocation. The social interaction test was conducted with untreated, unfamiliar, weight-matched partner male rats. We used untreated partners to facilitate comparison with published research on effects of central CRH administration on social interaction and with our concurrent research program in which we study effects of central CRH on social interaction in opiate-treated rats. This procedural variation has negligible effect on outcomes (File & Seth, 2003). The test commenced immediately after restraint stress and lasted for 10 min. Time spent in social interaction (sniffing, grooming, following, crawling over, crawling under, or passive contact with partner) was scored. In this test, a decrease in social interaction without a concomitant alteration in activity indicates an anxiogenic response (File & Seth, 2003). Partner rats were then removed, and the experimental rat was allowed 60 min of free exploration. The behavior of each experimental rat was scored every 4 s during this 60 min for grooming, locomotor activity, rearing, and sleeping.

Experiment 3: Effects of varying doses of opiate exposure on behavioral responses to restraint stress. The experiment employed a single-factor design. Rats were randomly allocated to four groups. Group 10 mg/kg ($n = 6$) received 10 daily injections of 10 mg/kg morphine. Group 5 mg/kg ($n = 6$) received 10 daily injections of 5 mg/kg morphine. Group 1 mg/kg ($n = 6$) received 10 daily injections of 1 mg/kg morphine. Group 0 mg/kg ($n =$

5) received 10 daily injections of saline. Six days after last injection, rats were transported to the laboratory and familiarized for 120 min with the testing arena. The following day (7 days after last injection), rats were transported to the laboratory and placed in the arena for a further 60-min habituation period. Rats were then removed and placed in the Plexiglas restrainers for 30 min according to group allocation. Immediately after this restraint, rats were returned to the chambers and tested for social interaction and activity as described for Experiment 2. We did not employ nonstressed controls in this experiment because the results of Experiment 2 indicated no significant effect of the history of opiate injections per se on either social interaction or behavioral activation at the 7-day test.

Experiment 4: Effects of varying durations of opiate exposures on behavioral responses to restraint stress. The experiment employed a single-factor design. Rats were randomly allocated to four groups. Group 10 days ($n = 6$) received daily injections of 10 mg/kg morphine. Group 5 days ($n = 6$) received 5 daily injections of saline followed by 5 daily injections of 10 mg/kg morphine. Group 1 day ($n = 6$) received 9 daily injections of saline followed by a single injection of 10 mg/kg morphine. Group 0 day ($n = 5$) received 10 daily injections of saline. Thus, all groups were equated on number of injections. Six days after last injection rats were transported to the laboratory and familiarized for 120 min with the testing arena. The following day (7 days after last injection) rats were transported to the laboratory and placed in the arena for a further 60-min habituation period. Rats were then removed and placed in the Plexiglas restrainers for 30 min according to group allocation. Immediately after this restraint, rats were returned to the chambers and tested for social interaction and activity as described for Experiment 2. Thus the interval between last opiate exposure and stress was the same for all opiate-treated rats. We did not employ nonstressed controls in this experiment because the results of Experiment 2 indicated no significant effect of the history of opiate injections per se on either social interaction or behavioral activation at the 7-day test.

Data Analysis

In Experiment 1, telemetry samples were averaged across 2-min bins. The average HR and MAP across the 60 min when rats were at rest in their home cage served as baseline. Subsequent MAP (mmHg) and HR (beats per minute [bpm]) across the 30-min period of restraint stress and the 120-min period of poststress in the home cages were expressed as differences from these baselines. The data were analyzed by repeated measures analyses of variance (ANOVAs). In Experiment 2, the amount of time spent in social interaction (s) across the 10-min test was recorded. For the subsequent 60-min test of behavioral activation in the experimental rats, the amount of time spent grooming and in exploration (locomotor activity and rearing) were analyzed with a $2 \times 2 \times 2$ ANOVA. Data from 2 rats (1 from the group saline 1 day—no stress and the other from the group morphine 1 day—no stress) in the behavioral activation test were lost because of a failure of the video recorder. In Experiments 3 and 4, time spent in social interaction (s) across the 10-min test and in grooming and exploration across the subsequent 60-min test was recorded. The data were analyzed with one-way ANOVAs.

Results

Experiment 1: Effects of a History of Opiate Exposures on Autonomic Responses to Restraint Stress

Figure 1 shows mean and standard error of the mean change in HR and MAP for rats tested 1 day (left panels) and 7 days (right panels) after last injection. Inspection of the figure indicates that the 30-min restraint stress provoked a pronounced increase in HR and MAP regardless of whether it occurred 1 day or 7 days after last injection. HR and MAP slowly returned toward baseline levels

during the 120-min recovery period. The history of morphine injections did not appear to influence the stress-induced tachycardia at either time point, but it did appear to reduce the pressor response at the 1-day time point.

There were no differences between groups in resting HR or MAP 1 day or 7 days after last injection, $F_s(1, 5) < 1, p_s > .05$ (group saline—1 day mean HR = 340 bpm, MAP = 97 mmHg; group morphine—1 day mean HR = 353 bpm, MAP = 104 mmHg; group saline—7 days mean HR = 333 bpm, MAP = 105 mmHg; group morphine—7 days mean HR = 332 bpm, MAP = 96 mmHg).

For rats subject to restraint stress at Day 1, analysis of stress HR failed to reveal any overall effect of drug, $F(1, 5) < 1, p > .05$; time, $F(14, 70) = 1.7, p > .05$; or a Drug \times Time interaction, $F(14, 70) < 1, p > .05$. Analysis of recovery HR failed to detect any overall effect of drug, $F(1, 5) = 1.8, p > .05$. There was a significant overall effect of time, $F(59, 295) = 7.8, p < .05$, indicating that HR decreased significantly across the recovery period. However, there was no Drug \times Time interaction, $F(59, 295) = 1.0, p > .05$. Analysis of stress MAP did reveal an overall effect of drug, $F(1, 5) = 8.0, p < .05$, so that rats with a history of morphine injections displayed significantly reduced pressor responses as compared with rats with a history of saline injections. However, there was no overall effect of time, $F(14, 70) = 1.5, p > .05$, and no Drug \times Time interaction, $F(14, 70) = 1.8, p > .05$. Analysis of recovery MAP failed to reveal a significant overall effect of drug, $F(1, 5) = 1.1, p > .05$. There was a significant overall effect of time, $F(59, 295) = 5.8, p < .05$, indicating that MAP decreased significantly across the recovery period. There was no Drug \times Time interaction, $F(59, 295) = 1.3, p > .05$, indicating that there was no difference in recovery of MAP as a function of drug history.

For rats subjected to restraint stress at Day 7, analysis of stress HR failed to reveal any overall effect of drug, $F(1, 6) = 4.7, p > .05$; time, $F(14, 84) = 1.3, p > .05$; or a Drug \times Time interaction, $F(14, 84) < 1, p > .05$. Analysis of recovery HR failed to detect any overall effect of drug, $F(1, 6) = 1.4, p > .05$. There was a significant overall effect of time, $F(59, 354) = 3.9, p < .05$, indicating that HR decreased significantly across the recovery period. There was no Drug \times Time interaction, $F(59, 354) = 1.0, p > .05$. Analysis of stress MAP failed to reveal any overall effect of drug, $F(1, 6) = 1.7, p > .05$; time, $F(14, 84) = 1.1, p > .05$; or a Drug \times Time interaction, $F(14, 84) = 1.1, p > .05$. Analysis of recovery MAP failed to detect any overall effect of drug, $F(1, 6) = 2.3, p > .05$. There was a significant overall effect of time, $F(59, 354) = 2.8, p < .05$, indicating that MAP decreased significantly across the recovery period. There was no Drug \times Time interaction, $F(59, 354) = 1.0, p > .05$.

Experiment 2: Effects of a History of Opiate Exposures on Behavioral Responses to Restraint Stress

The top panel of Figure 2 shows the mean and standard error of the mean time spent in social interaction (top panel) across the 10-min period immediately following restraint stress. The middle and bottom panels of Figure 2 show the mean and standard error of the mean time spent in exploratory activity and grooming, respectively, across the 60-min observation period after the social interaction test. Inspection of the panels suggests that the restraint

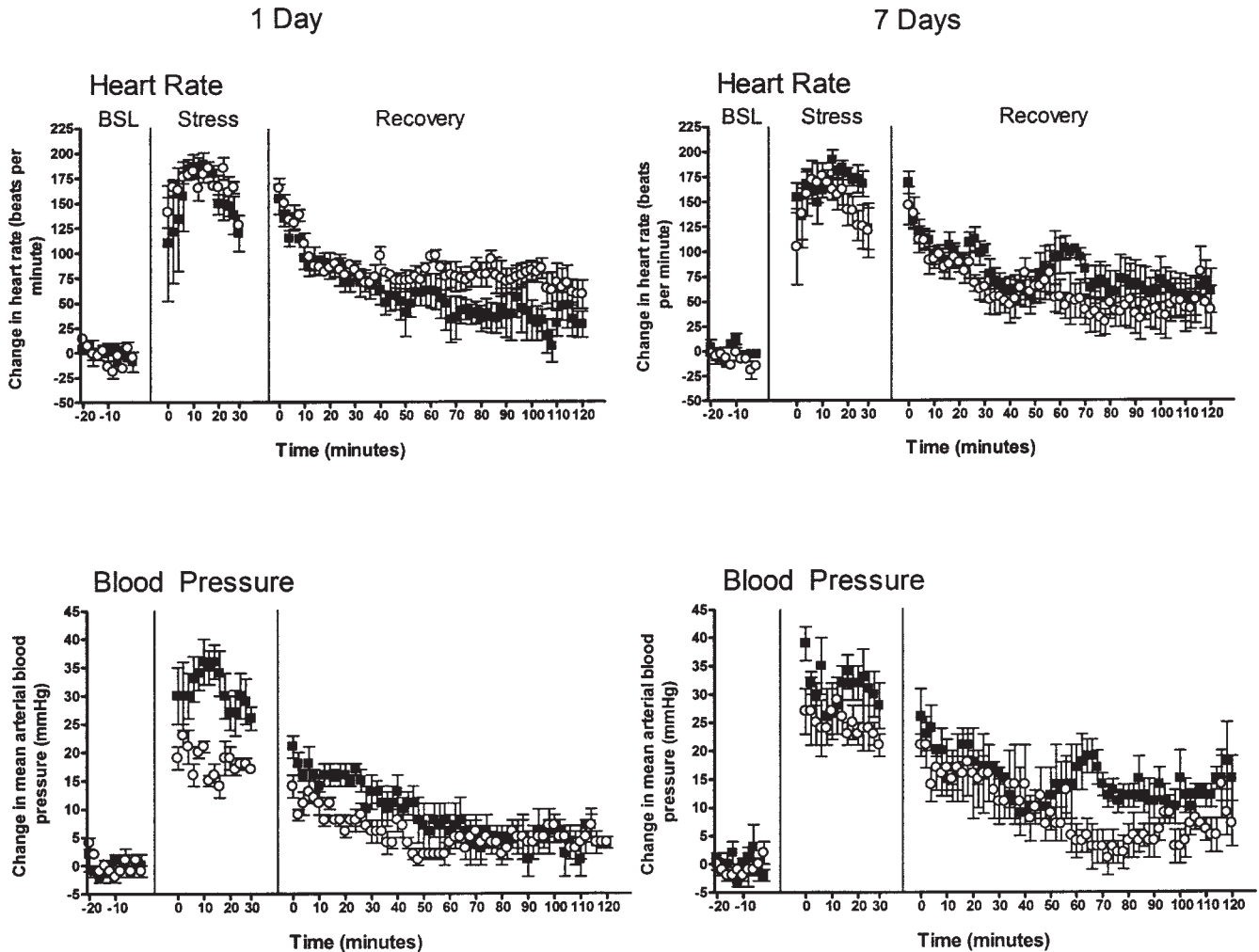


Figure 1. Experiment 1 mean and standard error of the mean heart rate and mean arterial blood pressure in rats with a history of saline (filled squares) or morphine (open circles) injections during baseline, 30-min restraint stress, and 120-min recovery 1 day or 7 days after last injection. Restraint stress provoked a pronounced increase in heart rate and mean arterial blood pressure regardless of whether it occurred 1 day or 7 days after last injection. The history of opiate exposures significantly reduced the pressor response 1 day but not 7 days after last injection. BSL = baseline.

stressor decreased both the time spent in social interaction and the time spent in subsequent exploration while concomitantly increasing the time spent in grooming. The history of morphine injections appeared to potentiate the effects of the restraint stressor on social interaction and on the subsequent grooming while leaving unaffected exploration.

For social interaction, the 2 (drug) \times 2 (stress) \times 2 (day) ANOVA revealed significant main effects of drug, $F(1, 54) = 11.1, p < .05$; stress, $F(1, 54) = 129.1, p < .05$; and day $F(1, 54) = 10.4, p < .05$. It is important to note that the Drug \times Stress interaction was significant, $F(1, 54) = 4.2, p < .05$, indicating that stress-induced anxiety (as indexed by reductions in social interaction) was augmented in the opiate-treated rats. However, the Drug \times Day, $F(1, 54) = 1.5, p > .05$; Stress \times Day, $F(1, 54) < 1, p > .05$; and Drug \times Stress \times Day, $F(1, 54) = 1.3, p > .05$, interactions were not significant. This difference in social interac-

tion cannot be attributed to morphine-treated rats spending more time in exploration (e.g., locomotor activity and rearing). The 2 (drug) \times 2 (stress) \times 2 (day) ANOVA on exploration during the 10-min social interaction test failed to detect significant main effects of drug, $F(1, 54) < 1, p < .05$, or stress, $F(1, 54) < 1, p < .05$, but did reveal a significant effect of day, $F(1, 54) = 7.4, p < .05$, so that animals tested 1 day after last injection were more active than animals tested 7 days after last injection. There were no significant two-way or three-way interactions, $F(1, 54) = 2.1, p > .05$ (data not shown).

For exploration in the 60-min test, the 2 (drug) \times 2 (stress) \times 2 (day) ANOVA revealed a significant main effect of stress, $F(1, 52) = 9.6, p < .05$. Thus, exposure to restraint stress significantly reduced activity during the 60-min observation period. There was no main effect for day, $F(1, 52) < 1, p < .05$, and no main effect for drug, $F(1, 52) < 1, p < .05$. Neither the Drug \times Stress, $F(1,$

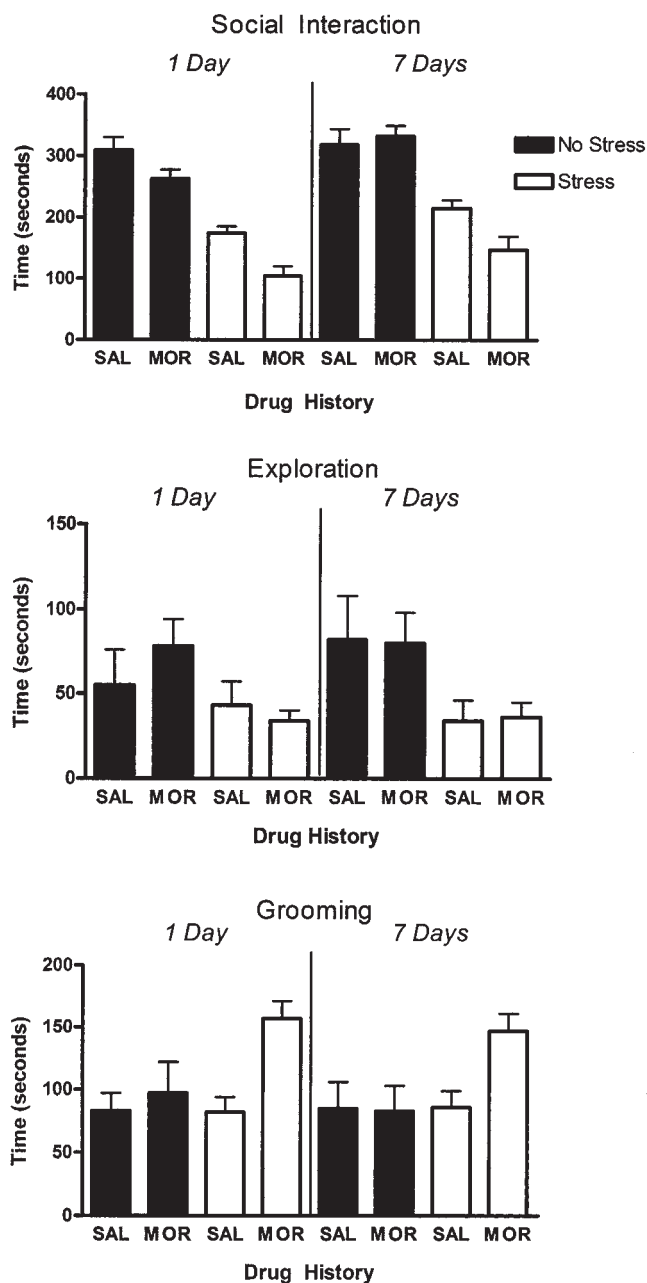


Figure 2. Experiment 2 mean and standard error of the mean social interaction (top panel), exploration (middle panel), and grooming (bottom panel) in rats with a history of morphine (MOR) or saline (SAL) injections subject to no stress or a 30-min restraint stress 1 day or 7 days after last injection. Restraint stress provoked significant increases in anxiety and grooming and significant reductions in exploration regardless of whether restraint occurred 1 day or 7 days after last injection. The history of opiate exposures significantly augmented the anxiogenic effects of restraint 1 day and 7 days after last injection.

52) < 1, $p > .05$; Drug \times Day, $F(1, 52) < 1, p > .05$; nor Stress \times Day, $F(1, 52) < 1, p > .05$, two-way interactions were significant. The Drug \times Stress \times Day three-way interaction also was not significant, $F(1, 52) < 1, p > .05$.

For the time spent grooming in the 60-min test, the 2 (drug) \times 2 (stress) \times 2 (day) ANOVA revealed a significant main effect of drug, $F(1, 52) = 8.8, p < .05$; a significant main effect of stress, $F(1, 52) = 6.0, p < .05$; and no significant main effect for day, $F(1, 52) < 1, p < .05$. Thus, a history of morphine injections or exposure to restraint stress increased grooming. The Drug \times Stress interaction was also significant, $F(1, 52) = 6.2, p < .05$, indicating that stress-induced grooming was augmented in the opiate-treated rats. However, the Drug \times Day, $F(1, 52) < 1, p > .05$, and Stress \times Day, $F(1, 52) < 1, p > .05$, two-way interactions were not significant. The Drug \times Stress \times Day three-way interaction also was not significant, $F(1, 52) = 2.2, p > .05$.

Experiment 3: Effects of Varying Doses of Opiate Exposures on Behavioral Responses to Restraint Stress

The top panel of Figure 3 shows the mean and standard error of the mean time spent in social interaction (top panel) across the 10-min period immediately following restraint stress. The middle and bottom panels of Figure 2 show the mean and standard error of the mean time spent in exploratory activity and grooming, respectively, across the 60-min observation period after the social interaction test. Inspection of the panels suggests that vulnerability to the restraint stressor was a linear function of the dose of opiate used during exposure. For social interaction, the one-way ANOVA revealed an overall difference between groups, $F(3, 22) = 5.3, p < .05$. A follow-up test indicated that the amount of social interaction decreased significantly as a linear function of dose of morphine, $F(1, 22) = 12.6, p < .05$. Thus, as the dose of morphine increased, so too did vulnerability to stress-induced anxiety. The one-way ANOVA failed to detect any overall differences between groups in exploration, $F(3, 22) = 2.2, p > .05$, or grooming, $F(3, 22) < 1, p > .05$.

Experiment 4: Effects of Varying Durations of Opiate Exposure on Behavioral Responses to Restraint Stress

The top panel of Figure 4 shows the mean and standard error of the mean time spent in social interaction (top panel) across the 10-min period immediately following restraint stress. The middle and bottom panels of Figure 2 show the mean and standard error of the mean time spent in exploratory activity and grooming, respectively, across the 60-min observation period after the social interaction test. Inspection of the panels suggests that vulnerability to the restraint stressor was a linear function of duration of opiate exposure. For social interaction, the one-way ANOVA revealed an overall difference between groups, $F(3, 22) = 4.5, p < .05$. A follow-up test indicated that the amount of social interaction decreased significantly as a linear function of morphine duration, $F(1, 22) = 11.7, p < .05$. Thus, as the duration of morphine exposure increased, so too did vulnerability to stress-induced anxiety. The one-way ANOVA failed to detect any overall differences between groups in exploration, $F(3, 22) < 1, p > .05$, or grooming, $F(3, 22) < 1, p > .05$.

Discussion

These experiments studied the impact of a history of opiate exposures on behavioral and autonomic responses to restraint

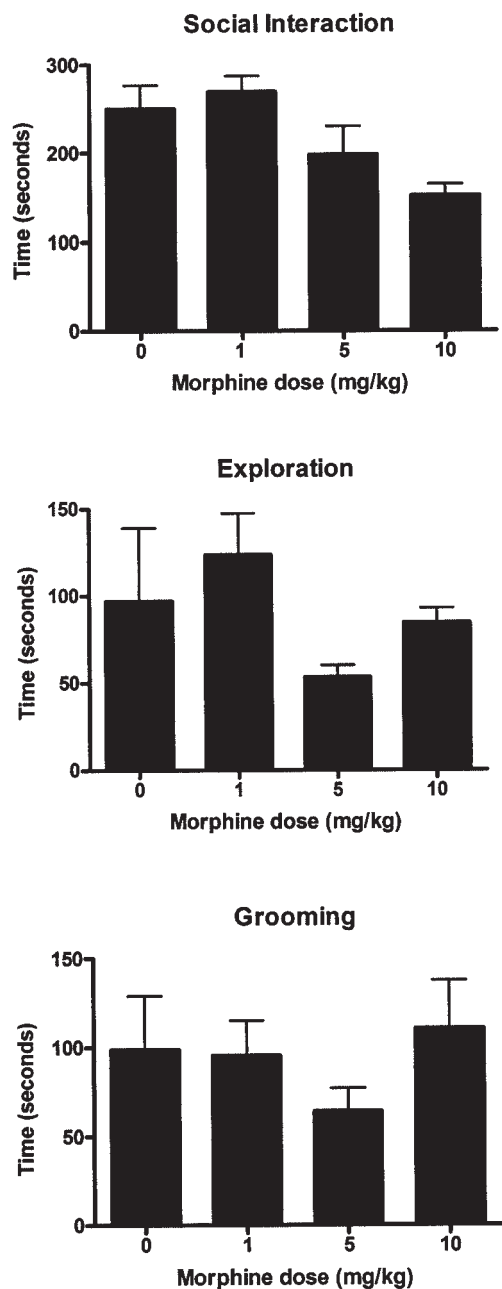


Figure 3. Experiment 3 mean and standard error of the mean social interaction (top panel), exploration (middle panel), and grooming (bottom panel) in rats that had a history of exposures to 0, 1, 5, or 10 mg/kg morphine and were subjected to a 30-min restraint stress 7 days after last injection. All rats were subjected to a 30-min restraint stress 7 days after last injection. There was evidence for significant alterations in stress-induced anxiety as a function of dose of opiate exposure.

stress. Rats were treated daily with morphine or saline for 10 days then subjected to restraint either 1 or 7 days after last injection. Exposure to restraint stress provoked increases in heart rate, blood pressure, anxiety, and grooming and reductions in exploration. A history of opiate exposures significantly altered some, but not all, of these responses. A history of opiate exposures attenuated the

pressor response to restraint stress when rats were subject to restraint 1 day, but not 7 days, after last opiate exposure. These exposures also increased vulnerability to the anxiogenic effects of restraint stress so that rats subject to restraint stress 1 day or 7 days after last opiate exposure displayed increased anxiety compared

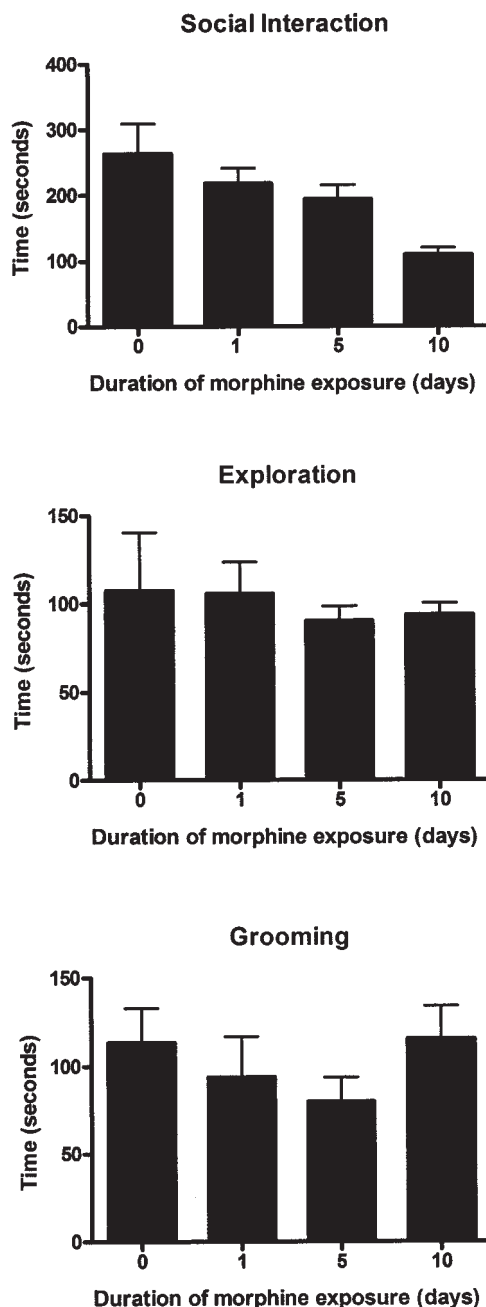


Figure 4. Experiment 4 mean and standard error of the mean social interaction (top panel), exploration (middle panel), and grooming (bottom panel) in rats with differing durations of opiate exposures (10 days, 5 days, and 1 day) or saline (0 days). All rats were subject to a 30-min restraint stress 7 days after last injection. There was evidence for significant alterations in stress-induced anxiety as a function of duration of opiate exposure.

with control rats treated with saline. A history of opiate exposures also increased stress-induced self-grooming 1 day or 7 days after last opiate exposure in Experiment 2. However, there was no evidence from Experiments 3 or 4 for differences between morphine- and saline-treated rats in grooming, so this finding should be treated with caution. Stress-induced tachycardia and reductions in exploration were unaffected by the history of opiate exposures. The influences of opiate exposure on the anxiogenic effects of restraint stress were a function of duration as well as dose of opiate exposure: The longer the duration or the higher the dose, the greater the difference in anxiety between morphine- and saline-treated rats. Together, these results show for the first time that a history of opiate exposures increases vulnerability to stress.

The restraint stressor produced a pronounced and prolonged pressor response and tachycardia. These were similar in magnitude and duration 1 and 7 days after last injection and were also similar to the responses previously observed during restraint stress in normotensive rats (e.g., McDougall, Lawrence, & Widdop, 2004). Control rats and opiate-treated rats did not differ in resting HR or MAP and showed similar tachycardia during restraint stress. However, the opiate-treated rats showed reduced pressor responses to restraint stress when they were subject to restraint at 1 day, but not 7 days, after last injection. This is the first demonstration of altered pressor responses in rats with a history of opiate exposures, and it is consistent with prior research identifying interactions between opiates and autonomic function in rodents. For example, acute administration of opiates increases blood pressure (e.g., Chan, Irvine, & White, 1999), and prolonged administrations of opiates sensitize the responses of locus coeruleus neurons to hypotensive stress (Xu et al., 2004). The opiate-treated rats did not differ from saline-treated rats in stress-induced tachycardia. The reasons for the discrepancy between HR and MAP are unclear. In rats, acutely administered opiates such as morphine produce a pronounced and prolonged tachycardia and pressor response (McNally, personal communication, September 9, 2003), and both HR and MAP were clearly sensitive to restraint stress. It is unlikely that differences in sensitivity between the two measures can explain the results. Regardless of the reason for these differences, comparison of the present results with findings in human opiate addicts is potentially informative. Himmelsbach (1941) studied the pressor response to cold stress in opiate addicts during various stages of opiate addiction and abstinence. The key finding from this study was that addicts show altered pressor responses to stress during addiction and short-term abstinence but not after a prolonged period of abstinence (Himmelsbach, 1941). Thus, in both human opiate addicts and rats exposed to opiates, there is evidence for short-term alterations in autonomic vulnerability to stress. The mechanisms for this vulnerability warrant further investigation.

The present experiments also showed, for the first time, that a history of opiate exposures produces a long-lasting increase in vulnerability to the anxiogenic effects of stress as indexed by the social interaction test. It is worth emphasizing that behavioral and autonomic responses to stress were measured across different experiments in separate groups of animals. The social interaction test is sensitive to a variety of anxiogenic drugs, including benzodiazepine receptor antagonists and inverse agonists, yohimbine, and caffeine, as well to the anxiogenic effects of benzodiazepine, nicotine, and ethanol withdrawal (File & Seth, 2003). Exposures to noise stress (File, 1994), predator odors, or the blood of conspe-

cifics (Zangrossi & File, 1992) are also anxiogenic in the social interaction test. The 30-min restraint stress used here produced a clear anxiogenic response in the social interaction test. This finding supports recent demonstrations of the anxiogenic effects of restraint in this measure (Breese, Overstreet, Knapp, & Navarro, in press). We find it important that rats with a history of opiate exposures displayed increased vulnerability to this anxiety. This vulnerability was observed regardless of whether stress occurred 1 day or 7 days after last injection. Vulnerability to anxiety was dependent on both the duration and dose of opiate exposure. As either the duration of opiate exposure or the dose of opiate used during exposures increased, so too did vulnerability to stress-induced anxiety.

It is worth emphasizing that performance in the social interaction test cannot be attributed to any anxiogenic effects of opiate exposure or opiate withdrawal per se (e.g., Harris, Hanes, & Gewirtz, 2004). In Experiment 2, increased anxiety was not observed in control rats with a history of opiate dependence not subject to restraint stress, and it was preserved when restraint occurred 7 days after last injection. The reduction in social interaction also cannot be attributed to alterations in exploratory behavior, because there were no other differences in behavior between morphine and saline-treated rats during the social interaction test. Instead, the increased vulnerability anxiety was contingent on the interaction between opiate exposures and restraint stress. One candidate for this interaction is the neuropeptide CRH. Intracerebral infusions of CRH are anxiogenic in the social interaction test (Dunn & File, 1987). Rats with a history of opiate exposures show sensitized CRH gene expression to restraint stress (e.g., Houshyar et al., 2004). Moreover, opiate exposures sensitize neuronal noradrenergic systems to CRH (Xu et al., 2004). It is possible, therefore, that the opiate exposures used in the present experiments primed or sensitized the neural CRH and/or noradrenergic systems to restraint stress and that this priming or sensitization underpins the long-lasting increased vulnerability to anxiety. Current research in our laboratory is testing this possibility.

The present experiments may provide insights into emotionality and stress responsivity in human opiate users. Anxiety disorders are prevalent among heroin users. For example, in a recent large-scale, longitudinal treatment outcome study, 41% of heroin users met diagnostic criterion for posttraumatic stress disorder (Mills, Lynksey, Teesson, Ross, & Darke, 2005). Evidence reviewed in the introduction shows that alteration of central stress circuits is an important consequence of drug dependence. Our experiments show for the first time that a history of opiate exposures increases vulnerability to the anxiogenic effects of stress. Thus, prolonged opiate use may increase vulnerability to stressors in humans, which in turn causes increased anxiety and negative affect. Further research is needed to test this possibility.

In conclusion, the present experiments show that a history of opiate exposures alters autonomic and behavioral vulnerability to stress. This vulnerability is characterized by a short-lasting reduction in the pressor response to stress and a longer lasting increase in stress-induced anxiety. The alterations in vulnerability to the anxiogenic effects of stress are a function of both dose and duration of opiate exposure. Our results show that prolonged exposures to opiates have significant effects on responsivity to stress that persist beyond last exposure of the drug and may have important

implications for understanding coping, anxiety, and emotionality in current and former opiate users.

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