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Measuring the incentive value of escalating doses of heroin in heroin-dependent Fischer rats during acute spontaneous withdrawal

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Abstract

Rationale/objectives—Although continued heroin use and relapse are thought to be motivated, in part, by the positive incentive-motivational value attributed to heroin, little is understood about heroin's incentive value during the relapse-prone state of withdrawal. This study uses place preference to measure the incentive value attributed to escalating-dose heroin in the context of heroin dependence.

Methods—Male Fischer rats were exposed chronically to escalating doses of heroin in the homecage and during place preference conditioning sessions. Conditioned preference for the context paired with escalating-dose heroin was tested after homecage exposure was discontinued and rats entered acute spontaneous withdrawal. Individuals' behavioral and locomotor responses to heroin and somatic withdrawal signs were recorded.

Results—Conditioned preference for the heroin-paired context was strong in rats that received chronic homecage exposure to escalating-dose heroin and were tested in acute withdrawal. Behavioral responses to heroin (e.g., stereotypy) varied widely across individuals, with rats that expressed stronger heroin preference also expressing stronger behavioral activation in response to heroin. Individual differences in preference were also related to locomotor responses to heroin but not to overt somatic withdrawal signs.

Conclusions—Escalating doses of heroin evoked place preference in rats, suggesting that positive incentive-motivational value is attributed to this clinically relevant pattern of drug exposure. This study offers an improved preclinical model for studying dependence and withdrawal and provides insight into individual vulnerabilities to addiction-like behavior.

Keywords

Heroin; Conditioning; Motivation; Withdrawal; Addiction; Locomotor activity; Rat; Behavior; Place preference; Individual differences

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Introduction

Heroin addiction is a chronic relapsing disorder associated with significant socioeconomic and public health consequences. In humans, heroin addiction is characterized by recurrent phases of chronic drug exposure, spontaneous withdrawal, and relapse to drug-seeking behavior. Continued drug use and high rates of relapse are thought to be motivated by the aversive state of withdrawal and/or the positive incentive-motivational value of the drug itself (Frenois et al. 2005; Jaffe 1992; Koob 2009; Koob and Kreek 2007; Koob and Le Moal 2001, 2008; Robinson and Berridge 1993, 2000; Hutcheson et al. 2001). While the aversive state of opiate withdrawal is well characterized (Azar et al. 2003; Dymshitz and Lieblich 1987; Harris and Aston-Jones 1993; Rothwell et al. 2009; Spanagel et al. 1994), little is known about the positive incentive-motivational value of heroin during withdrawal. An important goal of current psychopharmacology research is to identify how the incentive value attributed to heroin by a dependent individual contributes to heroin-seeking behavior that can persist across withdrawal and prolonged abstinence.

The present study uses a place preference paradigm to measure heroin's incentive value within the context of heroin dependence and withdrawal. Exposure to a drug-paired context is a major factor precipitating relapse-like behaviors in animal models of drug addiction (Crombag et al. 2008; Shaham et al. 2003; Shalev et al. 2002), yet little work has characterized place preference responses to a drug-paired context in dependent subjects. Place learning paradigms require an effortful approach toward a context and are sensitive to both preference and aversion, offering insight into both directional and activational aspects of drug-paired stimuli (e.g., Bardo and Bevins 2000). While place preference can be usefully applied to animal models of dependence, to our knowledge, only one study has measured heroin place preference in dependent rats (Wang et al. 2009). In that study, heroin injections were relatively infrequent (twice per day) and at low doses (up to 75mg/kg/day), limiting the ability to generalize findings to clinically relevant conditions.

In the present study, place preference conditioning has been integrated into a preclinical model of heroin dependence developed by our laboratory to reflect key aspects of human addiction. Rats received frequent, intermittent homecage injections of heroin at doses that increased progressively (to 75mg/kg/day) over a prolonged (10-day) period, mimicking the progressive increases in heroin consumption that characterize human addiction (e.g., Dole et al. 1966; Haertzen and Hooks 1969; Kreek et al. 2009). In parallel to this chronic homecage exposure to heroin, rats learned to associate regularly scheduled injections of heroin with a unique context. Conditioned preference for this heroin-paired context—thought to reflect the incentive-motivational value attributed to escalating-dose heroin—was then tested in a heroin-free state, once chronic homecage exposure to heroin was discontinued and rats entered acute spontaneous withdrawal.

As place preference studies often use constant, low doses of heroin to elicit conditioned preference for the heroin-paired chamber (Bardo et al. 1995; Tzschentke 1998, 2007), our first aim was to determine if rats would prefer a context paired with escalating doses of heroin. Recent work revealed that escalating doses of cocaine induce long-lasting conditioned place preference in mice (Y. Itzhak, personal communication), yet few studies have examined place preference for escalating doses of heroin. Conditioned preference for a heroin-paired context was first measured in subjects exposed to escalating doses of heroin during place preference conditioning but that received no other exposure to heroin. Preference for escalating-dose heroin was then measured in heroin-dependent rats, once their chronic homecage exposure to heroin was discontinued and they had entered acute withdrawal. We hypothesized that conditioned preference for escalating-dose heroin would

emerge in both groups but that preference would be stronger in the context of heroin dependence.

The present study will also identify the extent to which heroin preference can be predicted by behavioral patterns that emerge in response to heroin and/or during acute withdrawal. Extensive research has identified factors that may predict an individual's vulnerability to psychostimulant self-administration and addiction-like behavior (Allen et al. 2007; Belin et al. 2008, 2009; Blanchard et al. 2009; Lucas et al. 1998; Mandt et al. 2008, 2009; Nelson et al. 2009; Piazza et al. 1990, 1991). Limited work, however, has explored behavioral factors contributing to opiate reward or individual differences in the context of opiate dependence (Deroche et al. 1993; Gieryk et al. 2010; Glick et al. 1992; McNamara et al. 2010; Wang et al. 2009). Examining the extent to which behavioral responses to chronic heroin exposure vary between individual rats and/or predict heroin preference may offer insight into individual vulnerabilities to dependence and/or relapse.

Methods

Animals

Subjects ($n = 39$) were adult male Fischer-344 rats (90–120 days old; Charles River Laboratories, Wilmington, MA, USA) housed in a stress-minimized animal facility, accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, at The Rockefeller University. Subjects were housed individually in standard clear shoebox cages with bedding, nest material and food/water ad libitum, and were weighed and handled daily. Subjects adapted to a reverse 12:12 h light/dark cycle (lights off at 0500 hours) for >1 week before the study; dim red lights allowed observation. Animal care and experimental procedures were conducted according to “Principles of laboratory animal care” and the “Guide for the Care and Use of Laboratory Animals” (National Research Council 1996) and the Institutional Animal Care and Use Committee.

Chronic exposure to escalating doses of heroin and acute spontaneous withdrawal

Heroin (diacetylmorphine; National Institute on Drug Abuse, Bethesda, MD, USA) was dissolved in physiological saline (0.9%) solution. The schedule of heroin administration was designed to mimic patterns of heroin use often observed in human addicts (e.g., Dole et al. 1966; Kreek et al. 2009; Hser et al. 2008; e.g. Zhou et al. 2008) with continued, intermittent administration of escalating doses of heroin across the active period and into the inactive period of the circadian cycle (Table 1). Subjects ($n = 12$) received three daily intraperitoneal injections of heroin for ten consecutive days, starting 4 h into the dark cycle (0900 hours) and spaced 6 h apart thereafter to approximate the half-life of heroin's bioactive metabolites in rats (Cerletti et al. 1980; Ko and Dai 1989; Way et al. 1960). One subject died after injection on day 4 and was removed from all analyses. Controls ($n = 6$) received saline vehicle on an identical schedule. Starting on day 11, heroin was withheld to allow spontaneous withdrawal.

Subjects not given homecage exposure to heroin—A separate set of subjects ($n = 16$) did not receive any homecage exposures to heroin but received a single injection of heroin at increasing doses (2.5–25 mg/kg), as described above, every other day for 10 days during place preference conditioning (Table 2). These subjects were thus exposed to the same conditioning stimuli as chronically exposed subjects but received no other heroin exposure. Two subjects died after injection and were removed from all analyses. Vehicle-treated controls ($n = 5$) received vehicle injections on the same schedule.

Behavioral observations

To minimize stress and disruption, observations were performed while subjects were in their homecages. The observer was not blind to condition.

Heroin administration—In rats given chronic homecage exposure to heroin, behaviors were recorded during visual checks once per minute for 40 min, starting after the first daily injection (at 0900 hours). Subjects not given homecage heroin exposure were placed into the place preference apparatus after receiving their only heroin injections and thus could not be observed. Typical behaviors included explore, rear, corporal groom, gnaw object, nest-build, walk, eat/drink, quiet wakefulness, and apparent sleep. Heroin-induced behavioral responses included pica (chewing/consumption of nonfood material, e.g., bedding), stereotypy of head/forelimbs, aberrant grooming (plucking/pulling at fur or digits), and hyperactivity. A point was awarded for each response present during each check (two points were awarded for responses that co-occurred, e.g., pica and stereotypy), and points were summed across each 40-min session. These responses were observed more frequently when stupor was mild (rated 1–2, see below) or absent, and toward the end of the 40-min session. The depth of behavioral stupor was rated on a graded scale based on volitional movement, orienting response, muscle rigidity, and stance (1 = little volitional movement, slowed orienting response, little/no muscle rigidity, upright stance; 5 = no orienting/startle response, severe muscle rigidity, prone position); regardless of its depth, stupor almost always coincided with exophthalmos. Duration of stupor (number of checks in which stupor was observed) was comparable to previous reports (Bloom et al. 1976; Havemann and Kuschinsky 1982; Mayo-Michelson and Young 1992; Young and Khazan 1986).

Somatic withdrawal—Somatic withdrawal signs were observed for 5 min, starting at 0900 hours, at 12, 36, and 60 h after the final heroin injection. The number of wet shakes, hops/darts, paw tremor, facial fasciculation/teeth chatter, and swallowing movements, as well as the presence of ptosis, piloerection, erection, abnormal posture, diarrhea (observed in homecage), and a loss of $\geq 1\%$ body weight, were recorded for each subject (Gellert and Holtzman 1978; Langerman et al. 2001). A point was awarded for each present sign and added to the total number of observed signs for a daily score.

Place preference apparatus

The place preference apparatus (Med Associates, St Albans, VT, USA) consisted of two equal-sized chambers (27.5 cm $W \times$ 21 cm $L \times$ 20.5 cm H) connected to a small central chamber with gray walls and solid gray flooring through manually operated doors. Chambers were dimly lit by small overhead white lights. One side chamber contained white walls and mesh flooring and the other side chamber contained black walls and bar flooring. The time spent in each chamber and the locomotor activity within each chamber were monitored by infrared photobeams traversing the floors. Each apparatus was contained within a larger sound-attenuating chamber.

Place preference paradigm

Pre-conditioning session—Prior to any drug exposure, each subject was allowed access to all three chambers of the place preference apparatus for 30 min. Subjects expressed a pre-existing bias for the black side chamber, so vehicle was assigned to that chamber and heroin to the white chamber (Bardo et al. 1995; Tzschentke 1998, 2007). Conditioning began on the next day.

Conditioning phase—heroin—Subjects received their second regularly scheduled heroin injection of the day (at 1500 hours) and were placed promptly in the white side

chamber for 30 min (Table 1). These sessions occurred every other day (i.e., days 1, 3, 5, 7, and 9) for 10 days and occurred on days in which the dose of all regularly scheduled heroin injections was increased. Thus, each consecutive session used a higher dose of heroin that matched the dose of the other homecage injections that day. After each session ended, subjects were returned to their homecage and remained undisturbed until their next heroin injection (at 2100 hours). Vehicle-treated controls received vehicle injections.

Conditioning phase—vehicle—On alternate days (days 2, 4, 6, 8, and 10), subjects were injected with saline vehicle (at 1300 hours) before being placed in the black side chamber for 30 min (Table 1). These sessions were timed so that they did not coincide with peak behavioral or physiological effects from earlier heroin injections (at 0900 hours) or the emergence of spontaneous withdrawal signs (prior to the first daily injection). After the session ended, subjects returned to their homecages for 90 min before receiving their second daily heroin injection to minimize its association with the end of this session.

Post-conditioning (test) session—Testing occurred 36 h after chronically exposed subjects received their final heroin injection (Table 1) and when somatic withdrawal signs peaked (data not shown).

Subjects could freely access all chambers for 30 min, and they entered each chamber at least once. Time spent in the heroin-paired chamber was compared to time spent in the vehicle-paired chamber.

Subjects not given homecage exposure to heroin—Subjects ($n = 14$) were exposed to an identical conditioning schedule, except that the increasing doses of heroin administered during conditioning constituted subjects' only exposure to heroin (Table 2). Controls ($n = 5$) received vehicle injections.

Locomotor activity—Breaks in the infrared beams within each place preference chamber, made by a subject's body, were recorded using MedPC-IV software (Med Associates, St Albans, VT, USA). The total numbers of beam breaks across each conditioning session were analyzed. Locomotor activity did not differ between vehicle-treated groups, so their data were pooled for analyses and presentation.

Statistics

Statistical analyses were performed using Statistica (version 5.5), with a significance level of $P < 0.05$. All ANOVAs were followed with a Newman–Keuls post hoc test or planned comparisons between treatment groups and/or across days. All t tests were two-tailed and followed a Levine's test for homogeneity of variance. Nonparametric tests were used as needed. Body weights across heroin exposure (days 1–10) were compared using a two-way ANOVA (day as repeated measure). During withdrawal (i.e., day 12), weights were compared using a one-way ANOVA. Homecage behaviors (e.g., total number of heroin-induced behavioral responses or mean depth of behavioral stupor across each 40-min period) were compared using one-way ANOVAs (days as repeated measure). Locomotor activity in each conditioning chamber was compared across sessions and treatment using two-way ANOVAs (day as repeated measure). Locomotor data averaged across all sessions was compared across treatment groups using a one-way ANOVA. Locomotor data failed to record during vehicle conditioning for seven subjects given no homecage exposure; data from these subjects were omitted from subsequent locomotor analyses. Withdrawal scores recorded at each time point were compared across heroin-treated groups using independent t tests. The time spent in each chamber of the place preference apparatus during the post-conditioning test session was compared across treatment groups using a two-way ANOVA.

(chamber as repeated measure). The difference in time that an individual rat spent in the heroin- versus vehicle-paired chamber during the test session was used to reflect the strength of an individual's preference for the heroin-paired chamber. Two criteria were used to identify a heroin preference (HP) or heroin non-preference (NP) in each rat. To be identified as HP, a rat must spend ≥ 5 min in the heroin-versus vehicle-paired chamber during the test session, and this difference in time must be >1 standard deviation above that of vehicle-treated controls. Rats that did not meet criteria were identified as NP. Behavioral data from HP and NP rats were compared in independent t tests and locomotor data compared using a two-way ANOVA. Correlations were performed using Pearson's tests and regressions calculated using GraphPad Prism v2.0.

Results

Body weight

There was a significant main effect of day [$F(9,288) = 14.19$, $P < 0.001$], treatment [$F(3,32) = 51.69$, $P < 0.001$], and day $\times$ treatment interaction on rats' body weight [$F(27,288) = 33.13$; $P < 0.001$] (Fig. 1). Post hoc tests revealed that weights decreased significantly between day 1 and days 4–10 ($P < 0.05$ to 0.001) and were lower in each heroin-treated group than its vehicle-treated controls (both $P < 0.001$). The weights of all vehicle-treated controls did not differ. Weights of rats given chronic homecage exposure to heroin were lower than weights of rats not given homecage exposure ($P < 0.001$), with planned comparisons revealing a highly significant difference on days 8–10 [$F(1,32) = 42.55$, $P < 0.001$]. On day 12, 36 h after chronic homecage exposure ended, body weights differed across treatment groups [$F(3,32) = 66.84$; $P < 0.001$]. Each heroin-treated group weighed less than its vehicle-treated control group (each $P < 0.001$) and rats given chronic heroin exposure weighed less than subjects not given homecage exposure ($P < 0.001$).

Homecage behavior—behavioral stupor

There was a significant effect of day on the depth of behavioral stupor expressed by subjects given chronic homecage exposure to heroin [$F(9,90) = 4.56$, $P < 0.001$] (Fig. 2). Planned comparisons revealed that the depth of behavioral stupor increased significantly from the first day to the final days (9–10) of heroin exposure [$F(1,10) = 21.42$; $P < 0.001$].

Homecage behavior—somatic responses to heroin

There was a significant effect of day on the number of heroin-induced behavioral responses expressed by rats given chronic heroin exposure [$F(9,90) = 4.08$, $P < 0.001$] (Fig. 3). Post hoc tests revealed that more heroin-induced behavioral responses were observed on days 6, 8, 9, and 10 than on day 1 (all $P < 0.05$). The two most prominent responses, hyperactivity and stereotypy, were subsequently analyzed and also revealed a significant effect of day [respectively, $F(9,90) = 2.43$ and $F(9,90) = 2.36$; both $P < 0.05$], with more hyperactivity and stereotypy observed on days 9–10 than on day 1 [$F(1,10) = 10.82$ and $F(1,10) = 15.30$; both $P < 0.05$] (data not shown). Somatic responses increased when heroin was given at the same (low) dose for the second consecutive day [days 1–2, $t(10) = 2.651$; days 3–4, $t(10) = 2.587$; both $P < 0.05$].

Locomotor activity—heroin conditioning

There was a significant main effect of treatment [$F(2,33) = 6.03$, $P < 0.01$] and a treatment $\times$ session interaction that neared significance [$F(8,132) = 1.94$, $P = 0.058$] on locomotor activity in response to escalating doses of heroin administered prior to each conditioning session (Fig. 4a). Post hoc tests revealed that locomotor activity was higher in rats given chronic homecage exposure to heroin compared to rats not given homecage exposure

($P < 0.01$) and that locomotor activity increased significantly between the first and last conditioning session only in rats given chronic homecage exposure to heroin [$F(1,33) = 8.81$, $P < 0.01$]. To examine rats' overall locomotor response to heroin, data from all heroin conditioning sessions were pooled (Fig. 4b). Overall locomotor activity in response to heroin was lower in rats not given homecage heroin exposure than rats given chronic homecage exposure to heroin [$t(23) = 3.038$, $P < 0.01$] and vehicle-treated controls [$t(23) = 2.621$, $P < 0.05$].

Locomotor activity—vehicle conditioning

There was a significant main effect of treatment [$F(2,26) = 6.28$, $P < 0.001$] and session [$F(4,104) = 6.48$, $P < 0.001$] on locomotor activity during vehicle conditioning (Fig. 4c). Locomotor activity was lower in rats given chronic homecage exposure compared to vehicle-treated controls across all sessions ($P < 0.01$) and to rats not given homecage exposure during sessions 1–4 [$F(1,26) = 5.22$, $P < 0.05$]. Overall, locomotor activity was lower in rats given chronic homecage versus no homecage exposure [$t(16) = 4.266$, $P < 0.001$] and compared to vehicle-treated controls [$t(20) = 3.228$, $P < 0.01$] (Fig. 4d). Heroin-treated rats showed no evidence of being in an overt state of withdrawal before or after any vehicle-conditioning session.

Somatic withdrawal

Overt withdrawal signs were observed after heroin exposure ended in both heroin-treated groups (Fig. 5) but not vehicle-treated controls. Prior to place preference testing (36 h after chronic homecage exposure to heroin ended), chronically exposed rats expressed more overt withdrawal signs than subjects not given homecage exposure [$t(23) = 6.14$, $P < 0.001$].

Conditioned preference for escalating doses of heroin

There was a significant main effect of treatment [$F(1,15) = 5.20$, $P < 0.05$] and chamber [$F(2,30) = 6.18$, $P < 0.01$] and a treatment $\times$ chamber interaction [$F(2,30) = 3.48$, $P < 0.05$] in the time spent in each chamber of the place preference apparatus by rats given chronic homecage exposure to escalating-dose heroin or vehicle (Fig. 6). These heroin-treated rats spent more time in the heroin-paired chamber than did the vehicle-treated controls [$F(1,15) = 5.31$, $P < 0.05$] and compared to the vehicle-paired chamber [$F(1,15) = 8.07$, $P < 0.05$]. These comparisons were not significant in rats not given homecage exposure to heroin (Fig. 6, inset). Vehicle-treated controls spent similar amounts of time in each chamber.

Individual differences in heroin preference

The strength of conditioned preference for the heroin-paired chamber varied across individuals given chronic homecage exposure to heroin (Fig. 7a). Seven subjects were identified as having a relative heroin preference (HP), while the remaining four subjects did not meet criteria and were identified as non-preference (NP). HP subjects spent more time in the heroin-versus vehicle-paired chamber than did NP subjects [$t(9) = 4.36$, $P < 0.005$] (Fig. 7b). Heroin preference also differed across subjects not given homecage exposure to heroin (Fig. 7a, inset). Seven subjects were identified as HP and seven subjects as NP. Two NP subjects preferred the center chamber and spent little time in either side chamber; as it was unclear how to interpret this preference response, their data were omitted from subsequent analyses. HP subjects spent more time in the heroin- versus vehicle-paired chamber than NP subjects [$t(10) = 4.35$, $P < 0.005$] (Fig. 7b, inset).

Individual differences in heroin-related behaviors

Behavioral stupor induced by heroin did not differ between HP and NP subjects on any individual day (Fig. 8a). To examine rapid (24 h) behavioral tolerance in response to the

same doses of heroin, data were averaged across days that heroin doses were escalated (odd days) and days that heroin doses remained constant (even days); no differences emerged between HP and NP subjects (Fig. 8b). Heroin-induced somatic responses were also similar in HP and NP subjects on each day (Fig. 8c). To examine rapid sensitization in response to the same doses of heroin, data were averaged across odd and even days, as above. HP subjects expressed significantly more heroin-induced somatic responses on days that heroin doses remained constant (even days) compared to days that heroin doses increased (odd days) (Fig. 8d); this difference was not significant in NP subjects. On even days, there was a positive correlation between the frequency of heroin-induced somatic responses and the strength of heroin preference [all dependent subjects, $r(11) = .56$; $P = 0.07$], with these responses explaining a substantial proportion of variance in heroin preference [$R^2 = 0.32$, $F(1,9) = 4.234$, $P = 0.07$]. Locomotor activity during heroin conditioning was slightly higher in HP than NP subjects during sessions 3 and 4 (Fig. 8e) and overall (Fig. 8f), but was similar in nondependent HP and NP subjects (Fig. 8e, f, insets). Withdrawal scores in HP and NP subjects did not differ 12, 36, or 60 h after subjects received their last heroin injection (data not shown). No relationships emerged between overt withdrawal signs and the strength of heroin preference. Similar analyses could not be run in nondependent subjects as withdrawal signs were too infrequent (Fig. 5). Typical homecage behavior was similar in all subjects.

Discussion

The present study uses place preference to measure the incentive value attributed to heroin within a preclinical model of heroin dependence. Rats learned to associate escalating doses of heroin with a unique chamber while receiving chronic exposure to escalating doses of heroin in their homecages and, when tested during acute spontaneous withdrawal, preferred the heroin-paired chamber. Although conditioned place aversion has been used to examine the aversive state of withdrawal (Azar et al. 2003; Dymshitz and Lieblich 1987; Harris and Aston-Jones 1993; Rothwell et al. 2009; Spanagel et al. 1994), few studies have used conditioned place preference to measure heroin's incentive value across this relapse-prone period. Paired with chronic homecage exposure to heroin, the present place preference study offers insight into the incentive-motivational value attributed to heroin by a dependent rat, once heroin is discontinued and withdrawal begins.

Like humans, rodents allowed to self-administer heroin or morphine will increase their drug consumption over time (Ahmed et al. 2000; Chen et al. 2006; Dai et al. 1989; Kruzich et al. 2003). Here, subjects learned to associate a unique context with increasing doses of heroin that reached high levels, extending existing opiate place preference studies that used low doses that escalated modestly (Wang et al. 2009) or remained constant (see Bardo et al. 1995; Tzschentke 1998, 2007). Furthermore, the amount of drug that each subject received was standardized through the use of experimenter-administered injections. This design may thus be more amenable to investigating molecular mechanisms underlying behavioral changes across the addiction cycle compared to self-administration designs in which drug exposure can vary substantially across individuals.

It is thought that tolerance to the euphorogenic and/or sedative effects of a drug contribute to escalated drug consumption over time (e.g., Ahmed et al. 2000; Walker et al. 2003; Lenoir and Ahmed 2007; Le Merrer et al. 2009). Despite examples of rapid tolerance to sedative (stupor-like) response to repeated doses of heroin in the present study, however, behavioral stupor did not predict the strength of preference for the context paired with escalating-dose heroin. Given the inherent limitations of rat models, the role of euphoria in heroin preference remains unclear but is intriguing, as the subjective pleasure evoked by a drug is thought to contribute directly to the incentive value attributed to the drug (Robinson and

Berridge 1993, 2000) and clinical reports suggest that heroin's sedative and euphorogenic effects are dissociable (e.g., Haertzen and Hooks 1969).

In contrast to behavioral stupor, somatic behavioral responses to heroin increased across chronic heroin exposure, consistent with other works (Adler and Geller 1984; Larcher et al. 1998; Stewart and Badiani 1993). Examples of rapid behavioral sensitization also emerged in response to repeated heroin exposures, especially at low doses. As rats that expressed stronger heroin preference also expressed stronger behavioral responses to heroin given at a constant (repeated) dose, we speculate that the incentive value attributed to escalating-dose heroin may be influenced, in part, by the extent of physical dependence and/or degree of behavioral sensitization across relatively brief time periods.

Locomotor activity in response to heroin was elevated in dependent rats given chronic homecage exposure to heroin, as expected (e.g., Bailey et al. 2010), but was low and constant in the relatively nondependent group. Unlike dependent rats, nondependent rats displayed substantial behavioral stupor across the entire heroin conditioning session that may have competed with heroin's psychomotor effects. Mechanisms contributing to these differences might also contribute to distinct locomotor patterns that persist across withdrawal from chronic versus intermittent morphine (Le Marec et al. 2011). It is interesting to note that, in dependent rats, individuals that expressed stronger heroin preference displayed a trend toward stronger psychomotor responses to heroin compared to rats that expressed weak/no heroin preference. That psychomotoric and reinforcing properties of opiate drugs are thought to be mediated by partially distinct mechanisms (e.g., Bailey et al. 2010; Sharf et al. 2010) has interesting implications regarding presumed neurobiological differences in HP and NP rats. As this relationship between heroin-induced activity and preference did not emerge in the nondependent HP and NP rats, this emerging pattern may be specific to the state of dependence.

The incentive value attributed to heroin by dependent rats raises intriguing questions regarding the strength and persistence of heroin's value across subsequent stages of the addiction cycle. Preference for escalating doses of cocaine persists longer than preference for constant or decreasing doses (Y. Itzhak, personal communication), and it is likely that conditioned preference for escalating-dose heroin also persists over prolonged periods. This period would allow us to measure heroin's incentive value in dependent rats across drug abstinence and examine the extent to which potential pharmacotherapies, administered after intact conditioning, disrupt the expression of place preference at discrete points across this relapse-prone period. Although many existing rodent models designed to explore motivation across withdrawal incorporate aspects of behavioral extinction (e.g., Gerak et al. 2009; Hellems et al. 2002; Lu et al. 2002, 2005; Shaham et al. 1996; Shalev et al. 2001; van der Kam et al. 2009; Zhou et al. 2005), behavioral extinction can alter motivation-related systems (Bahar et al. 2003; Fuchs et al. 2006; Knackstedt et al. 2010; Self et al. 2004; Sutton et al. 2003) and has limited clinical relevance (O'Brien and Gardner 2005). Models that omit behavioral extinction and predictive cues that influence drug-seeking behavior (Shaham et al. 1996, 2003; Zhou et al. 2005), such as the present model, may offer relevant insight into motivational changes across withdrawal.

A major goal of this study was to determine if a state of acute withdrawal contributes importantly to heroin's incentive value. While the magnitude of heroin preference did not correspond to patterns of somatic withdrawal signs, extending limited self-administration analyses (Hellems et al. 2002), it is likely that negative emotional and stress states of withdrawal contributed to heroin preference. In rodents, withdrawal has been associated with increased anxiety- and depression-like behavior (Goeldner et al. 2011; Grasing et al. 1996; Harris and Aston-Jones 1993; Rothwell et al. 2009; Zhang and Schulteis 2008),

conditioned place aversions (Dymshitz and Lieblich 1987; Harris and Aston-Jones 1993; Mucha 1987; Rothwell et al. 2009; Spanagel et al. 1994) elevated self-stimulation thresholds (Schaefer and Michael 1985), and elevated and persistent heroin-seeking behavior (Hutcheson et al. 2001; Lenoir and Ahmed 2007; Gerak et al. 2009; Negus 2006; Negus and Rice 2009). We speculate that heroin place preference reported here was not driven by avoidance of withdrawal but rather the value attributed to heroin across cycles of withdrawal and re-exposure.

Although heroin withdrawal can be highly aversive, it is unlikely that the expression of heroin place preference in this study can be attributed to an avoidance of withdrawal. At no point during conditioning did subjects learn that entering the heroin-paired chamber would alleviate symptoms of withdrawal. The stimuli gained conditioned incentive value through Pavlovian contingencies but, unlike predictive or “seeking” CS cues in self-administration studies, never predicted subsequent heroin presentations. The contextual stimuli present in the heroin-paired chamber therefore lacked predictive value. Likewise, vehicle-conditioning sessions neither predicted subsequent heroin presentations nor the omission of expected heroin injections. The association between conditioning stimuli and symptoms of withdrawal was also minimized as much as possible. All conditioning sessions were timed to occur when bioactive metabolites are present at elevated levels in rat circulation (Cerletti et al. 1980; Ko and Dai 1989; Way et al. 1960), and no rat showed evidence of being in acute somatic withdrawal before or after any session. Although dependent rats were less active than vehicle-treated controls during vehicle-conditioning sessions, possibly reflecting a negative affective state coinciding in time with these sessions, rats spent moderate amounts of time in each chamber and did not actively avoid any chamber when tested. Furthermore, unlike forced-choice models, the third, central chamber was available as a neutral alternative to the heroin- and vehicle-paired chambers during testing. No dependent rat preferred the central chamber over the others, and the time spent in the vehicle (and central) chambers was similar across treatment groups, suggesting that neither conditioning chamber was associated with significant aversion, like that of acute withdrawal states.

It is more likely that recurrent episodes of withdrawal enhanced the incentive value attributed to heroin (Hutcheson et al. 2001; Frenois et al. 2005). Dysphoria and/or anhedonia associated with withdrawal are thought to establish a “deprived” motivational state that may be ameliorated by drug consumption (Koob and Le Moal 1997, 2008; Nader et al. 1994), and clinical studies report a clear link between negative emotional states of withdrawal and subsequent motivation to consume drug (Newton et al. 2003; Uslander et al. 1999). In an elegant self-administration study, Hutcheson et al. (2001) revealed that heroin-dependent rats that had experienced episodic withdrawal expressed stronger heroin-seeking behavior than dependent rats that had not experienced withdrawal; authors proposed that withdrawal enhanced the incentive value attributed to heroin, which manifested in heightened drug-seeking behavior (Hutcheson et al. 2001). Differences between somatic and affective aspects of withdrawal are clearly documented (e.g., Frenois et al. 2002; Goeldner et al. 2011; Harris and Aston-Jones 1993), and recent evidence suggests that anxiogenic and/or dysphoric states can precede overt, somatic signs of withdrawal (Rothwell et al. 2009). In this study, the onset of affective but not somatic aspects of withdrawal prior to conditioning may have modified the incentive value of subsequent heroin exposures. It is further possible that recurrent bouts of mild somatic withdrawal, observed sporadically in dependent rats before their morning injection, also contributed to heroin’s value and, together with conditioning exposures, enhanced preference.

In an effort to clarify the role of heroin dependence during conditioning, a contrast group was exposed to identical conditioning stimuli but otherwise remained heroin naive. These rats were not in an overt state of withdrawal and did not express an overall preference for the

heroin-paired context. That a subset of nondependent rats did express heroin preference suggests that certain nondependent individuals will attribute incentive value to escalating-dose heroin and possibly reflects a relatively vulnerable population. The predictive contingencies of the heroin-paired context were also much stronger in this nondependent group than in the dependent group (Rescorla and Wagner 1972; Miller et al. 1995), and interference by homecage heroin exposures may have reduced the magnitude of place conditioning in the dependent group (Jenkins 1984). As interference and contingency could only be controlled by altering conditioning parameters, vehicle exposure, etc., only limited comparisons can be made between these groups. The goal of the present study was not to maximize the magnitude of place preference but to build heroin preference into a chronic escalating-dose model of drug exposure, after which dependent rats expressed positive heroin place preference.

This study reveals that positive incentive value is attributed to a clinically relevant pattern of heroin exposure. Paired with chronic homecage exposure to heroin, this study offers an improved preclinical model that may be usefully applied to the study of incentive-motivational processes across drug withdrawal. Furthermore, the extent of behavioral variability across individuals of this inbred rat strain suggests that nongenomic factors, such as neuroendocrine stress responses (Koob and Kreek 2007; Piazza et al. 1991; Sinha 2008; Yap and Miczek 2008) or opioid function (Kreek and LaForge 2007; Le Merrer et al. 2009; Matthes et al. 1996; Sim-Selley et al. 2000) may drive specific vulnerabilities to heroin dependence and provides direction to future molecular studies.

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References

- Adler MW, Geller EB. Contributions of neuropharmacology to understanding mechanisms of tolerance and dependence. *NIDA Res Monogr.* 1984; 54:27–38. [PubMed: 6442759]
- Ahmed SH, Walker JR, Koob G. Persistent increase in the motivation to take heroin in rats with a history of drug escalation. *Neuropsychopharmacology.* 2000; 22:412–421.
- Allen RM, Everett CV, Nelson AM, Gulley JM, Zahniser NR. Low and high locomotor responsiveness to cocaine predicts intravenous cocaine conditioned place preference in male Sprague–Dawley rats. *Pharmacol Biochem Behav.* 2007; 86:37–44. [PubMed: 17250883]
- Azar MR, Jones BC, Schulteis G. Conditioned place aversion is a highly sensitive index of acute opioid dependence and withdrawal. *Psychopharmacology.* 2003; 170:42–50. [PubMed: 12783156]
- Bahar A, Samuel A, Hazvi S, Dudai Y. The amygdalar circuit that acquires taste aversion memory differs from the circuit that extinguishes it. *Eur J Neurosci.* 2003; 17:1527–1530. [PubMed: 12713656]
- Bailey A, Metaxas A, Al-Hasani R, Keyworth HL, Forster DM, Kitchen I. Mouse strain differences in locomotor, sensitization and rewarding effect of heroin; association with alterations in MOP-r activation and dopamine transporter binding. *Eur J Neurosci.* 2010; 31:742–753. [PubMed: 20384817]
- Bardo MT, Bevins RA. Conditioned place preference: what does it add to our preclinical understanding of drug reward? *Psychopharmacology.* 2000; 153:31–43. [PubMed: 11255927]
- Bardo MT, Rowlett JK, Harris MJ. Conditioned place preference using opiate and stimulant drugs: a meta-analysis. *Neurosci Biobehav Rev.* 1995; 19:39–51. [PubMed: 7770196]
- Belin D, Mar AC, Dalley JW, Robbins TW, Everitt BJ. High impulsivity predicts the switch to compulsive cocaine-taking. *Science.* 2008; 320:1352–1355. [PubMed: 18535246]

- Belin D, Balado E, Piazza PV, Deroche-Gamonet V. Pattern of intake and drug craving predict the development of cocaine addiction-like behavior in rats. *Biol Psychiatry*. 2009; 65:863–868. [PubMed: 18639867]
- Blanchard MM, Mendelsohn D, Stamp JA. The HR/LR model: further evidence as an animal model of sensation seeking. *Neurosci Biobehav Rev*. 2009; 33:1145–1154. [PubMed: 19497336]
- Bloom F, Segal D, Ling N, Guillemin R. Endorphins: profound behavioural effects in rats suggest new etiological factors in mental illness. *Science*. 1976; 194:630–632. [PubMed: 185694]
- Cerletti C, Keinath SH, Tallarida RJ, Reidenberg MM, Adler MW. Morphine concentrations in the rat after intraperitoneal or subcutaneous injection. *Subst Alcohol Actions Misuse*. 1980; 1:65–70. [PubMed: 7323944]
- Chen SA, O'Dell LE, Hoefer ME, Greenwell TN, Zorrilla EP, Koob GF. Unlimited access to heroin self-administration: independent motivational markers of opiate dependence. *Neuropsychopharmacology*. 2006; 31:2692–2707. [PubMed: 16452993]
- Crombag HS, Bossert JM, Koya E, Shaham Y. Review. Context-induced relapse to drug seeking: a review. *Philos Trans R Soc Lond B Biol Sci*. 2008; 363:3233–3243. [PubMed: 18640922]
- Dai S, Corrigan WA, Coen KM, Kalant H. Heroin self-administration by rats: influence of dose and physical dependence. *Pharmacol Biochem Behav*. 1989; 32:1009–1015. [PubMed: 2798525]
- Deroche V, Piazza PV, Le Moal M, Simon H. Individual differences in the psychomotor effects of morphine are predicted by reactivity to novelty and influenced by corticosterone secretion. *Brain Res*. 1993; 623:341–344. [PubMed: 8221119]
- Dole VP, Nyswander ME, Kreek MJ. Narcotic blockade—a medical technique for stopping heroin use by addicts. *Trans Assoc Am Physicians*. 1966; 79:122–136. [PubMed: 4162906]
- Dymshitz J, Lieblich I. Opiate reinforcement and naloxone aversion, as revealed by place preference paradigm, in two strains of rats. *Psychopharmacology*. 1987; 92:473–507. [PubMed: 3114801]
- Frenois F, Cador M, Caille S, Stinus L, Le Moine C. Neural correlates of the motivational and somatic components of naloxone-precipitated morphine withdrawal. *Eur J Neurosci*. 2002; 16:1377–1389. [PubMed: 12405997]
- Frenois F, Le Moine C, Cador M. The motivational component of withdrawal in opiate addiction: role of associative learning and aversive memory in opiate addiction from a behavioral, anatomical and functional perspective. *Rev Neurosci*. 2005; 16:255–276. [PubMed: 16323564]
- Fuchs RA, Branham RK, See RE. Different neural substrates mediate cocaine seeking after abstinence versus extinction training: a critical role for the dorsolateral caudate-putamen. *J Neurosci*. 2006; 26:3584–3588. [PubMed: 16571766]
- Gellert VF, Holtzman SG. Development and maintenance of morphine tolerance and dependence in the rat by scheduled access to morphine drinking solutions. *J Pharmacol Exp Ther*. 1978; 205:536–546. [PubMed: 566320]
- Gerak LR, Galici R, France CP. Self administration of heroin and cocaine in morphine-dependent and morphine-withdrawn rhesus monkeys. *Psychopharmacology*. 2009; 204:403–411. [PubMed: 19194694]
- Gieryk A, Ziolkowska B, Solecki W, Kubik J, Przewlocki R. Forebrain PENK and PDYN gene expression levels in three inbred strains of mice and their relationship to genotype-dependent morphine reward sensitivity. *Psychopharmacology*. 2010; 208:291–300. [PubMed: 19997907]
- Glick SD, Merski C, Steindorf S, Wang S, Keller RW, Carlson JN. Neurochemical predisposition to self-administer morphine in rats. *Brain Res*. 1992; 578:215–220. [PubMed: 1380861]
- Goeldner C, Lutz P-E, Darceq E, Halter T, Clesse D, Abdel-Mouttalib O, Kieffer BL. Impaired emotional-like behavior and serotonergic function during protracted abstinence from chronic morphine. *Biol Psych*. 2011; 69:236–244.
- Grasing K, Wang A, Schlussman S. Behavioral measures of anxiety during opiate withdrawal. *Behav Brain Res*. 1996; 80:195–201. [PubMed: 8905143]
- Haertzen CA, Hooks NT Jr. Changes in personality and subjective experience associated with the chronic administration and withdrawal of opiates. *J Nerv Ment Dis*. 1969; 148:606–614. [PubMed: 4388972]
- Harris GC, Aston-Jones G. Beta-adrenergic antagonists attenuate somatic and aversive signs of opiate withdrawal. *Neuropsychopharmacology*. 1993; 9:303–311. [PubMed: 7905738]

- Havemann U, Kuschinsky K. Neurochemical aspects of the opioid-induced 'catatonia'. *Neurochem Int.* 1982; 4:199–215. Review. [PubMed: 20487869]
- Hellems KG, Shaham Y, Olmstead MC. Effects of acute and prolonged opiate abstinence on extinction behaviour in rats. *Can J Exp Psychol.* 2002; 56:241–252. [PubMed: 12491648]
- Hser Y-I, Huang D, Brecht M-L, Libo L, Evans E. Contrasting trajectories of heroin, cocaine, and methamphetamine use. *J Addict Dis.* 2008; 27:1–13.
- Hutcheson DM, Everitt BJ, Robbins TW, Dickinson A. The role of withdrawal in heroin addiction: enhances reward or promotes avoidance? *Nat Neurosci.* 2001; 4:943–947. [PubMed: 11528427]
- Jaffe, JH. Current concepts of addiction. In: O'Brien, CP.; Jaffe, JH., editors. *Addictive states*. New York: Raven; 1992. p. 1-21.
- Jenkins HM. Time and contingency in classical conditioning. *Ann NY Acad Sci.* 1984; 423:242–253.
- Knackstedt LA, Moussawi K, Lalumiere R, Schwendt M, Klugmann M, Kalivas PW. Extinction training after cocaine self-administration induced glutamatergic plasticity to inhibit cocaine seeking. *J Neurosci.* 2010; 30:7984–7992. [PubMed: 20534846]
- Ko W, Dai S. Plasma, cardiac tissue and brain morphine concentrations in acute and chronic morphine-treated rats. *Clin Exp Pharmacol Physiol.* 1989; 16:117–120. [PubMed: 2714019]
- Koob GF. Dynamics of neuronal circuits in addiction: reward, antireward, and emotional memory. *Pharmacopsychiatry.* 2009; 42:S32–S41. [PubMed: 19434554]
- Koob GF, Kreek MJ. Stress, dysregulation of drug reward pathways, and the transition to drug dependence. *Am J Psychiatry.* 2007; 164:1149–1159. [PubMed: 17671276]
- Koob GF, Le Moal M. Drug abuse: hedonic homeostatic dysregulation. *Science.* 1997; 278:52–58. [PubMed: 9311926]
- Koob GF, Le Moal M. Drug addiction, dysregulation of reward, and allostasis. *Neuropsychopharmacology.* 2001; 24:97–129. [PubMed: 11120394]
- Koob GF, Le Moal M. Addiction and the brain antireward system. *Annu Rev Psychol.* 2008; 59:29–53. [PubMed: 18154498]
- Kreek MJ, LaForge KS. Stress responsivity, addiction, and a functional variant of the human mu-opioid receptor gene. *Mol Interv.* 2007; 7:74–78. [PubMed: 17468387]
- Kreek MJ, Schlussman SD, Reed B, Zhang Y, Nielsen DA, Levrin O, Zhou Y, Butelman ER. Bidirectional translational research: progress in understanding addictive diseases. *Neuropharmacology.* 2009; 56:32–43. [PubMed: 18725235]
- Kruzich PJ, Chen ACH, Unterwald EM, Kreek MJ. Subject-regulated dosing alters morphine self-administration behaviour and morphine-stimulated [35S]GTPgammaS binding. *Synapse.* 2003; 47:243–249. [PubMed: 12539196]
- Langerman L, Piscoun B, Bansinath M, Shemesh Y, Turndorf H, Grant GJ. Quantifiable dose-dependent withdrawal after morphine discontinuation in a rat model. *Pharmacol Biochem Behav.* 2001; 68:1–6. [PubMed: 11274701]
- Larcher A, Laulin JP, Celerier E, Le Moal M, Simonnet G. Acute tolerance associated with a single opiate administration: involvement of N-methyl-D-aspartate-dependent pain facilitatory systems. *Neuroscience.* 1998; 84:583–589. [PubMed: 9539228]
- Le Marec T, Marie-Claire C, Noble F, Marie N. Chronic and intermittent morphine treatment differently regulates opioid and dopamine systems: a role in locomotor sensitization. *Psychopharmacology.* 2011
- Le Merrer J, Becker JAJ, Befort K, Kieffer BL. Reward processing by the opioid system in the brain. *Physiol Rev.* 2009; 89:1379–1412. [PubMed: 19789384]
- Lenoir M, Ahmed SH. Heroin-induced reinstatement is specific to compulsive heroin use and dissociable from heroin reward and sensitization. *Neuropsychopharmacology.* 2007; 32:616–624. [PubMed: 16641938]
- Lu L, Xu NJ, Ge X, Yue W, Su WJ, Pei G, Ma L. Reactivation of morphine conditioned place preference by drug priming: role of environmental cues and sensitization. *Psychopharmacology.* 2002; 159:125–132. [PubMed: 11862339]
- Lu L, Chen H, Su W, Yue W, Su F, Ma L. Role of withdrawal in reinstatement of morphine-conditioned place preference. *Psychopharmacology.* 2005; 181:90–100. [PubMed: 15739075]

- Lucas LR, Angulo JA, Le Moal M, McEwen BS, Piazza PV. Neurochemical characterization of individual vulnerability to addictive drugs in rats. *Eur J Neurosci*. 1998; 10:3153–3163. [PubMed: 9786209]
- Mandt BH, Schenk S, Zahniser NR, Allen RM. Individual differences in cocaine-induced locomotor activity in male Sprague–Dawley rats and their acquisition of and motivation to self-administer cocaine. *Psychopharmacology*. 2008; 201:195–202. [PubMed: 18685831]
- Mandt BH, Allen RM, Zahniser NR. Individual differences in initial low-dose cocaine-induced locomotor activity and locomotor sensitization in adult outbred female Sprague–Dawley rats. *Pharmacol Biochem Behav*. 2009; 91:511–516. [PubMed: 18817807]
- Matthes HW, Maldonado R, Simonin F, Valverde O, Slowe S, Kitchen I, Befort K, Dierich A, Le Meur M, Dolle P, Tzavara E, Hanoune J, Roques BP, Kieffer BL. Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. *Nature*. 1996; 383:819–823. [PubMed: 8893006]
- Mayo-Michelson L, Young GA. Effects of chronic morphine administration and naloxone on EEG, EEG power spectra, and associated behavior in two inbred rat strains. *Pharmacol Biochem Behav*. 1992; 42:815–821. [PubMed: 1513864]
- McNamara R, Dalley JW, Robbins TW, Everitt BJ, Belin D. Trait-like impulsivity does not predict escalation of heroin self-administration in the rat. *Psychopharmacology*. 2010; 212:453–464. [PubMed: 20689939]
- Miller RR, Barnett RC, Grahame NJ. Assessment of the Rescorla–Wagner model. *Psychol Bull*. 1995; 117:363–386. [PubMed: 7777644]
- Mucha RF. Is the motivational effect of opiate withdrawal reflected by common somatic indices of precipitated withdrawal? A place conditioning study in the rat. *Brain Res*. 1987; 418:214–220. [PubMed: 3676713]
- Nader K, Bechara A, Roberts DC, van der Kooy D. Neuroleptics block high- but not low-dose heroin place preference: further evidence for a two-system model of motivation. *Behav Neurosci*. 1994; 108:1128–1138. [PubMed: 7893405]
- National Research Council. Guide for the care and use of laboratory animals. Washington: Institute of Laboratory Animal Resources; 1996.
- Negus SS. Choice between heroin and food in nondependent and heroin-dependent rhesus monkeys: effects of naloxone, buprenorphine, and methadone. *J Pharmacol Exp Ther*. 2006; 317:711–723. [PubMed: 16456085]
- Negus SS, Rice KC. Mechanisms of withdrawal-associated increases in heroin self-administration: pharmacologic modulation of heroin vs food choice in heroin-dependent rhesus monkeys. *Neuropsychopharmacology*. 2009; 34:899–911. [PubMed: 18704098]
- Nelson AM, Larson GA, Zahniser NR. Low or high cocaine responding rats differ in striatal extracellular dopamine levels and dopamine transporter number. *J Pharmacol Exp Ther*. 2009; 331:985–997. [PubMed: 19729579]
- Newton TF, Kalechstein AD, Tervo KE, Ling W. Irritability following abstinence from cocaine predicts euphoric effects of cocaine administration. *Addict Behav*. 2003; 28:817–821. [PubMed: 12726795]
- O'Brien CP, Gardner EL. Critical assessment of how to study addiction and its treatment: human and non-human animal models. *Pharmacol Ther*. 2005; 108:18–58. [PubMed: 16183393]
- Piazza PV, Deminiere J-M, Maccari S, Mormede P, Le Moal M, Simon H. Individual reactivity to novelty predicts probability of amphetamine self-administration. *Behav Pharmacol*. 1990; 1:339–345. [PubMed: 11175418]
- Piazza PV, Maccari S, Deminiere J-M, Le Moal M, Mormede P, Simon H. Corticosterone levels determine individual vulnerability to amphetamine self-administration. *Proc Natl Acad Sci USA*. 1991; 88:2088–2092. [PubMed: 2006148]
- Rescorla, RA.; Wagner, AR. A theory of Pavlovian conditioning: variations in the effectiveness of reinforcement and nonreinforcement. In: Black, AH.; Prokasy, WF., editors. *Classical conditioning II: current research and theory*. New York: Appleton-Century-Crofts; 1972. p. 64-99.
- Robinson TE, Berridge KC. The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res Rev*. 1993; 18:247–291. [PubMed: 8401595]

- Robinson TE, Berridge KC. The psychology and neurobiology of addiction: an incentive-sensitization view. *Addiction*. 2000; 95:S91–S117. [PubMed: 11002906]
- Rothwell PE, Thomas MJ, Gerwitz JC. Distinct profiles of anxiety and dysphoria during spontaneous withdrawal from acute morphine exposure. *Neuropsychopharmacology*. 2009; 34:2285–2295. [PubMed: 19494807]
- Schaefer GJ, Michael RP. Effects of opioid antagonists and their quaternary derivatives on locomotor activity and fixed ratio responding for brain self stimulation in rats. *Pharmacol Biochem Behav*. 1985; 23:797–802. [PubMed: 4080766]
- Self DW, Choi KH, Simmons D, Walker JR, Smagula CS. Extinction training regulates neuroadaptive responses to withdrawal from chronic cocaine self-administration. *Learn Mem*. 2004; 11:648–657. [PubMed: 15466321]
- Shaham Y, Rajabi H, Stewart J. Relapse to heroin-seeking in rats under opioid maintenance: the effects of stress, heroin priming, and withdrawal. *J Neurosci*. 1996; 16:1957–1963. [PubMed: 8774462]
- Shaham Y, Shalev U, Lu L, De Wit H, Stewart J. The reinstatement model of drug relapse: history, methodology and major findings. *Psychopharmacology*. 2003; 168:3–20. [PubMed: 12402102]
- Shalev U, Morales M, Hope B, Yap J, Shaham Y. Time-dependent changes in extinction behavior and stress-induced reinstatement of drug seeking following withdrawal from heroin in rats. *Psychopharmacology*. 2001; 156:98–107. [PubMed: 11465640]
- Shalev U, Grimm JW, Shaham Y. Neurobiology of relapse to heroin and cocaine seeking: a review. *Pharmacol Rev*. 2002; 54:1–42. [PubMed: 11870259]
- Sharf R, Guarnieri DJ, Taylor JR, DiLeone RJ. Orexin mediates morphine place preference, but not morphine-induced hyperactivity or sensitization. *Brain Res*. 2010; 1317:24–32. [PubMed: 20034477]
- Sim-Selley LJ, Selley DE, Vogt LJ, Childers SR, Martin TJ. Chronic heroin self-administration desensitizes mu opioid receptor-activated G-proteins in specific regions of rat brain. *J Neurosci*. 2000; 20:4555–4562. [PubMed: 10844025]
- Sinha R. Chronic stress, drug use, and vulnerability to addiction. *Ann NY Acad Sci*. 2008; 1141:105–130. [PubMed: 18991954]
- Spanagel R, Almeida OF, Bartl C, Shippenberg TS. Endogenous kappa-opioid systems in opiate withdrawal: role in aversion and accompanying changes in mesolimbic dopamine release. *Psychopharmacology*. 1994; 115:121–127. [PubMed: 7862883]
- Stewart J, Badiani A. Tolerance and sensitization to the behavioral effects of drugs. *Behav Pharmacol*. 1993; 4:289–312. [PubMed: 11224198]
- Sutton MA, Schmidt EF, Choi KH, Schad CA, Whisler K, Simmons D, Karanian DA, Monteggia LM, Neve RL, Self DW. Extinction-induced upregulation in AMPA receptors reduces cocaine-seeking behaviour. *Nature*. 2003; 421:70–75. [PubMed: 12511956]
87. Tzschentke TM. Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. *Prog Neurobiol*. 1998; 56:613–672. [PubMed: 9871940]
- Tzschentke TM. Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. *Addict Biol*. 2007; 12:227–462. [PubMed: 17678505]
- Uslaner J, Kalechstein A, Richter T, Ling W, Newton T. Association of depressive symptoms during abstinence with the subjective high produced by cocaine. *Am J Psychiatry*. 1999; 156:1444–1446. [PubMed: 10484960]
- van der Kam EL, De Vry J, Tzschentke TM. 2-Methyl-6-(phenylethynyl)-pyridine (MPEP) potentiates ketamine and heroin reward as assessed by acquisition, extinction, and reinstatement of conditioned place preference in the rat. *Eur J Pharmacol*. 2009; 606:94–101. [PubMed: 19210976]
- Walker JR, Chen SA, Moffitt H, Inturrisi CE, Koob GF. Chronic opioid exposure produces increased heroin self-administration in rats. *Pharmacol Biochem Behav*. 2003; 75:349–354. [PubMed: 12873626]
- Wang C, Chen H, Hao W. Novel environmental response and heroin-dependent susceptibility in Sprague–Dawley rats. *Zhong Nan Da Xue Xue Bao Yi Xue Ban*. 2009; 34:277–281. [PubMed: 19411741]

- Way EL, Kemp JW, Young JM, Grassetti DR. The pharmacological effects of heroin in relationship to its rate of biotransformation. *J Pharmacol Exp Ther.* 1960; 129:144–154. [PubMed: 13843200]
- Yap JJ, Miczek KA. Stress and rodent models of drug addiction: role of VTA-accumbens-PFC-amygdala circuit. *Drug Discov Today Dis Models.* 2008; 5:259–270. [PubMed: 20016773]
- Young GA, Khazan N. Differential protracted effects of morphine and ethylketocyclazocine challenges on EEG and behavior in the rat. *Eur J Neurosci.* 1986; 125:265–271.
- Zhang Z, Schulteis G. Withdrawal from acute morphine dependence is accompanied by increased anxiety-like behavior in the elevated plus maze. *Pharmacol Biochem Behav.* 2008; 89:392–403. [PubMed: 18308382]
- Zhou W, Zhang F, Tang S, Liu H, Gu J, Yang G. The dissociation of heroin-seeking patterns induced by contextual, discriminative, or discrete conditioned cues in a model of relapse to heroin in rats. *Psychopharmacology.* 2005; 181:197–206. [PubMed: 15830224]
- Zhou Y, Leri F, Cummins E, Hoeschele M, Kreek MJ. Involvement of arginine vasopressin and V1b receptor in heroin withdrawal and heroin seeking precipitated by stress and by heroin. *Neuropsychopharmacology.* 2008; 33:226–236. [PubMed: 17443128]

Fig. 1.

Body weight of subjects, shown as percent of weight in grams, over days. Subjects received escalating doses of heroin ($n = 25$; *black shapes*) or vehicle ($n = 11$; *white shapes*) during heroin place preference conditioning. One subset of these subjects also received chronic, daily exposure to escalating doses of heroin ($n = 11$, *black circles*) or vehicle ($n = 6$, *white circles*) in their homecages; see Table 1 for details. A second subset of subjects was not given homecage exposure to heroin ($n = 14$, *black squares*) or vehicle ($n = 5$, *white squares*); see Table 2 for details. Arrow denotes the first day in which all injections were withheld. * $P < 0.05$ compared to subjects not given homecage exposure to heroin; # $P < 0.05$ compared to matched vehicle-treated controls; † $P < 0.05$ compared to day 1 (all groups). Data are represented as mean $\pm$ SEM

Fig. 2.

Behavioral stupor in response to chronic (10-day) homecage exposure to escalating doses of heroin, observed during daily 40-min observation sessions. The depth of behavioral stupor was recorded after subjects received injections of 2.5 (**a**), 5 (**b**), 10 (**c**), 15 (**d**), and 25 mg/kg of heroin (**e**). *Black shapes* represent the first day that each dose was administered, and *white shapes* represent the second day that the same dose was administered. # $P < 0.05$ compared to day 1; * $P < 0.05$ in response to the same dose. Data are represented as mean $\pm$ SEM

Somatic Responses to Heroin

Fig. 3.

Total number of heroin-induced somatic responses, including hyperactivity, stereotypy, pica, and aberrant grooming, expressed during the daily 40-min observation session. *Dark gray bars* represent the first day that each dose was administered, and *light gray bars* represent the second day that this same dose was administered. $P < 0.05$ in response to the same dose (*asterisk*) or compared to day 1 (#) or day 3 (†). Data are represented as mean ± SEM

Fig. 4.

Locomotor activity recorded during each conditioning session with heroin (**a, b**) and with saline vehicle (**c, d**), expressed by subjects given chronic home cage exposure to heroin ($n = 11$; *black shapes*), subjects not given home cage exposure to heroin ($n = 14$; *white shapes*), and controls that received saline vehicle on an identical schedule (*gray shapes*). **a** Heroin was administered at an increased dose (2.5–25 mg/kg) during each conditioning session. These injections constituted a continuation of subjects' home cage exposures to heroin (*black shapes*) or subjects' only exposures to heroin (*white shapes*). Control subjects received vehicle injections (*gray shapes*). **b** Mean locomotor activity across all five heroin conditioning sessions. **c** Locomotor response during each vehicle conditioning session. **d**

Mean locomotor activity across all five vehicle conditioning sessions. [†] $P < 0.05$ between the first and last session; * $P < 0.05$ between subjects given chronic homecage exposure to heroin versus subjects not given homecage exposure; # $P < 0.05$ between heroin- and vehicle-treated groups. Data are represented as mean $\pm$ SEM

Fig. 5. Somatic withdrawal signs observed immediately prior to place preference testing. $*P<0.05$ between treatment groups. Data are represented as mean $\pm$ SEM

Place Preference for Escalating-Dose Heroin

Fig. 6. Conditioned preference for a chamber paired with escalating doses of heroin, expressed by rats given chronic homecage exposure to heroin (*black bars*) or vehicle (*white bars*). Preference was tested 36 h after heroin-treated rats received their final heroin injection (Table 1). *Inset* Conditioned preference for a chamber paired with escalating doses of heroin, expressed by subjects not given homecage exposure to heroin (*black bars*) or vehicle (*white bars*) but that were conditioned and tested on the same schedule as their chronically treated counterparts. * $P < 0.05$ between treatment groups; # $P < 0.05$ between conditioning chambers. Data are represented as mean $\pm$ SEM

Individual Differences in Conditioned Place Preference

Fig. 7.

a The strength of heroin preference varied across individual subjects given chronic home cage exposure to escalating doses of heroin (*black shapes*). Individuals were identified as having a strong heroin preference (HP, $n = 7$) or heroin non-preference (NP, $n = 4$), based on criteria (see Methods section). *Inset* Subjects not given home cage exposure to heroin were also identified as HP ($n = 7$) and NP ($n = 5$). **b** The strength of heroin preference differed significantly between HP (*black bars*) and NP (*hatched bars*) subjects given chronic home cage exposure to heroin. *Inset* The strength of heroin preference also differed between HP and NP subjects not given home cage exposure. $*P < 0.05$ between preference groups. Data are represented as mean $\pm$ SEM

Fig. 8. Behavioral responses of subjects expressing strong conditioned preference for the heroin-paired chamber (heroin-preference, HP; *black bars*) or weak/no preference for the heroin-paired chamber (non-preference, NP; *hatched bars*) following chronic homecage exposure to heroin. All data are represented as mean $\pm$ SEM. **a, b** The depth of behavioral stupor induced by heroin in HP and NP subjects on each day (**a**) and across days (**b**) that the dose was escalated (odd days) or remained constant (even days). **c, d** The frequency of heroin-induced behavioral responses in HP and NP subjects on each day (**c**) and across days (**d**) that the dose was escalated (odd days) and remained constant (even days); * $P < 0.05$ in HP subjects. **e** Locomotor activity recorded during heroin-conditioning sessions in HP (*black*

shapes) and NP subjects (*white shapes*) given chronic homecage exposure to heroin or (*inset*) HP and NP subjects not given homecage exposure to heroin. **f** Locomotor activity across all heroin-conditioning sessions in HP (*black bars*) and NP (*hatched bars*) subjects given chronic homecage exposure to heroin and (*inset*) HP and NP subjects not given homecage exposure to heroin

Table 1
Schedule of exposure to escalating doses of heroin during place preference conditioning sessions and in the homecage

Dose (mg/kg) per injection ^a												
Day ^b	1	2	3	4	5	6	7	8	9	10	11	12
Time (h)												
0900	2.5	2.5	5	5	10	10	15	15	25	25	–	Test
1300	0	0**	0	0**	0	0**	0	0**	0	0**	–	–
1500	2.5*	2.5	5*	5	10*	10	15*	15	25*	25	–	–
2100	2.5	2.5	5	5	10	10	15	15	25	25	–	–
Daily total	7.5	7.5	15	15	30	30	45	45	75	75	–	–

^a Administered via intraperitoneal injection by the experimenter
^b Conditioning sessions with heroin (*) or vehicle (**) occurred on alternating days and lasted 30min. Conditioned place preference was tested on day 12 (Test) and lasted 30 min

Table 2

Schedule of exposure to escalating doses of heroin during place preference conditioning sessions but not in the homecage

Dose (mg/kg) per injection ^a												
Day ^b	1	2	3	4	5	6	7	8	9	10	11	12
Time (h)												
0900	-	-	-	-	-	-	-	-	-	-	-	-
1300	-	0**	-	0**	-	0**	-	0**	-	0**	-	-
1500	2.5*	-	5*	-	10*	-	15*	-	25*	-	-	-
2100	-	-	-	-	-	-	-	-	-	-	-	-
Daily total	2.5	0	5	0	10	0	15	0	25	0	-	-

^a Administered via intraperitoneal injection by the experimenter

^b Conditioning sessions with heroin (*) or vehicle (**) occurred on alternating days and lasted 30min. Conditioned place preference was tested on day 12 (Test) and lasted 30 min