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Heroin self-administration: I. Incubation of goal-directed behavior in rats

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ABSTRACT

This study used heroin self-administration to investigate incubation of goal-directed heroin-seeking behavior following abstinence. Male Sprague–Dawley rats self-administered heroin on a fixed ratio 10 (FR10) schedule of reinforcement with licking of an empty spout serving as the operant behavior during 14 daily 3 h sessions. After this acquisition period, all rats received a 90 min extinction session following either 1 day or 14 days of home cage abstinence. When the extinction session occurred after only 1 day of home cage abstinence, rats with a history of heroin self-administration divided their responses equally between the previously "active" and "inactive" spouts. However, when the extinction session occurred following 14 days of home cage abstinence, the rats exhibited marked goal-directed heroin-seeking behavior by licking more on the previously "active" than "inactive" spout. These findings demonstrate that heroin-seeking behavior incubates over time, resulting in goal-directed heroin-seeking behavior in rats following 14 days but not 1 day of abstinence. Moreover, this facilitatory effect occurred in response to a different training schedule, lower total drug intake, and shorter periods of daily access than previously reported with heroin.

1. Introduction

Heroin is a highly rewarding drug with strong abuse potential. Use of the drug is currently on the rise, in part due to higher purity, which allows for intranasal rather than intravenous administration (NIDA Research Report Series — Heroin: Abuse and Addiction, 2005). In addition to the potent rewarding properties of the drug, the problem of addiction is further complicated by the fact that nearly 90% of all addicted individuals will relapse to drug use following even prolonged periods of abstinence (DeJong, 1994). Addiction, then, is recognized as a brain disease of chronic relapse (Leshner, 1996; Koob et al., 1998) and continued research is essential to understand the neurobiological basis of the disease.

Recent work has demonstrated that craving and relapse increase with increasing periods of abstinence. This phenomenon has been termed "incubation" and occurs in rats (Tran-Nguyen et al., 1998; Grimm et al., 2003; Lu et al., 2005) and in humans (Gawin and Kleber, 1986). Operationally, incubation is defined as an increase in drug-seeking as a function of the time since the last drug exposure. This increase in drug-seeking is positively correlated with the length of the withdrawal period. In rats, the strength of the correlation continues through an arbitrary period of abstinence, after which there is a gradual decrease in drug-seeking (Shalev et al., 2001).

Incubation has been observed in rats in several studies of cocaine self-administration (Grimm et al., 2001; Lu et al., 2004b; Lu et al., 2005), but in only one report involving heroin self-administration (Shalev et al., 2001). In that report, rats were trained to self-administer heroin (0.1 mg/kg, iv) for 9 h/day for 10 days. Rats were then withdrawn from heroin for varying numbers of days. All rats were then tested in repeated 60-min extinction sessions, spaced 5–10 min apart until they reached the extinction criterion (less than 15 responses/h). Heroin-seeking behavior during extinction was greater following 6, 12, or 25 days of withdrawal, than it was after just one day of withdrawal. In the present work, we re-examined this initial report using a lower dose of heroin (0.06 vs. 0.1 mg/kg iv), shorter daily access periods (3 h vs. 9 h), and a different operant behavior (spout licking vs. lever pressing) that supports a relatively high level of responding. Spout licking is an effective operant behavior for self-administration with the notable benefit that nearly all rats take drug during the first drug self-administration session (Jones et al., 2002; Grigson and Twining, 2002; Liu and Grigson, 2005). Moreover, we also measured responding on both an active and an inactive spout during acquisition training and during extinction testing in an effort to gauge the change in goal-directed heroin-taking and heroin-seeking behavior over time.

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2. Methods

2.1. Subjects

The subjects were 32 male, Sprague–Dawley rats (Charles River Laboratories, Raleigh, NC) weighing 250–300 g at the start of the experiment. They were individually housed in standard wire mesh cages in a colony room with controlled temperature, humidity, and ventilation. The rats were housed on a 12 h light/dark cycle (lights on at 0700 and lights off at 1900) and were provided food and water *ad libitum* except where otherwise noted. Tissues obtained from these subjects are analyzed in the companion paper that describes the effect of heroin administration (and the contingency of its delivery) on gene expression following 1 or 14 days of abstinence. All studies were conducted in accordance with The Pennsylvania State University Institutional Animal Care and Use Committee, strictly adhering to the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council, 2003).

2.2. Surgery

Following one week acclimation to the colony room, intravenous catheters were implanted as previously described (Grigson and Twining, 2002). Briefly, each rat was anesthetized with the intramuscular administration of a ketamine (70 mg/kg)-xylazine (16 mg/kg) mixture. A chronic indwelling Silastic catheter was implanted into the right jugular vein and secured with a suture. The catheter was coupled to a cannula that exited between the rat's shoulder blades. Rats received one week to recover in their home cages with food and water available *ad libitum*.

2.3. Apparatus

Behavioral testing was conducted in 12 self-administration chambers (MED Associates, St. Albans, VT) constructed as previously described (Grigson and Twining, 2002). Each chamber was equipped with two retractable sipper tubes that entered the chamber through holes spaced 16.4 cm apart (center to center). A stimulus light was located above each tube. A lickometer circuit was used to monitor licking on both active and inactive spouts. Each chamber was also equipped with a houselight (25 W), a tone generator (Sonalert Time Generator, 2900 Hz, Mallory, Indianapolis, IN), and a speaker for white noise (75 dB). Heroin reinforcement was controlled by an electronic circuit that operated a syringe pump (Model A, Razel Scientific Instruments, Stamford, CT). Events in the chamber and collection of the data were controlled on-line with a Pentium computer that used programs written in the Medstate notation language (MED Associates).

2.4. Habituation and water-deprivation

Over nine days prior to the start of training, all rats were water deprived and placed in the self-administration chamber for a 20 min period each day. During the first 5 min of the 20 min session, the rats were given access to water in the chamber via the right spout. An additional 1 h access to water was provided in the home cage each afternoon. This water-deprivation regimen (1 h access to water each afternoon) was continued throughout the training and extinction portions of the study as described previously (Grigson and Twining, 2002).

2.5. Self-administration

All rats were given 14 daily, 3 h drug self-administration sessions. Sixteen rats self-administered heroin using licking on an empty spout as the operant behavior and were trained on a fixed ratio 10 (FR10) lick schedule of reinforcement. During acquisition, the rats were placed in individual operant chambers in which a house light was initially illuminated and white noise was broadcast. At the start of each session, two spouts extended into the chamber and a stimulus light was illuminated above the right-most (active) spout. Every 10 licks on the active spout resulted in an iv infusion of heroin (0.06 mg/0.2 ml infusion), extinguishment of the cue light located above the active spout, illumination of the house light, and retraction of the spouts for a 20 s timeout period indicated by onset of a 20 s tone. Licks on the left (inactive) spout had no consequence, but were recorded. With the exception of spout licking, these general parameters are similar to those used in previous studies of heroin self-administration (Martin et al., 1998; Zhou et al., 2005; Chen et al., 2006).

2.6. Forced abstinence

Following the 2-week self-administration period, half of the rats (Group 1 Day: $n=8$) were returned to their home cage for a 24 h period of forced home cage abstinence, while the other half underwent a 14 day period of enforced home cage abstinence ($n=8$). During this time, rats were handled and weighed daily, food was available *ad libitum*, and water was available for 1 h each afternoon.

2.7. Extinction session

Following either 1 or 14 days of abstinence, all rats were subjected to a 90-minute extinction session during which completion of the 10 lick contingency on the active spout resulted in all of the same consequences (e.g. light extinguishment, tone) as during the 2-week training period, but saline, rather than heroin, was infused. Catheters in all but one of the rats were patent during the extinction session.

2.8. Behavioral measures

Rats were weighed daily and licks on both spouts were recorded during acquisition and extinction. The latency to make the initial lick on each spout also was recorded, as was the total number of infusions (or infusion attempts) administered.

2.9. Drugs

Heroin HCl was provided by the National Institute on Drug Abuse (Research Triangle Institute, Research Triangle Park, N.C., USA). Drug was dissolved in sterile physiological saline at a concentration of 0.3 mg/ml.

2.10. Statistical analyses

The number of responses (licks) and the log 10 latency to initiate responding were averaged for the final two days of self-administration and then analyzed using 2×14 repeated measures ANOVAs varying spout (active, inactive) and training day (1–14). Post hoc tests were conducted, where appropriate, using Newman–Keuls tests with alpha set at 0.05. Additionally, the number of responses and the log 10 latency to initiate responding were averaged across the 90 min extinction session. The extinction lick and latency data were analyzed using 2×2 mixed factorial ANOVAs varying spout (active, inactive) and abstinence period (1 or 14 days). Post hoc tests were conducted, where appropriate, using Newman–Keuls tests with the alpha level set at $p<0.05$.

2.11. Goal-directed behavior

Goal-directed behavior is defined as the number of responses made on the active spout minus those made on the inactive spout (Fuchs et al., 1998; Lu et al., 2004a). This transformation also controls

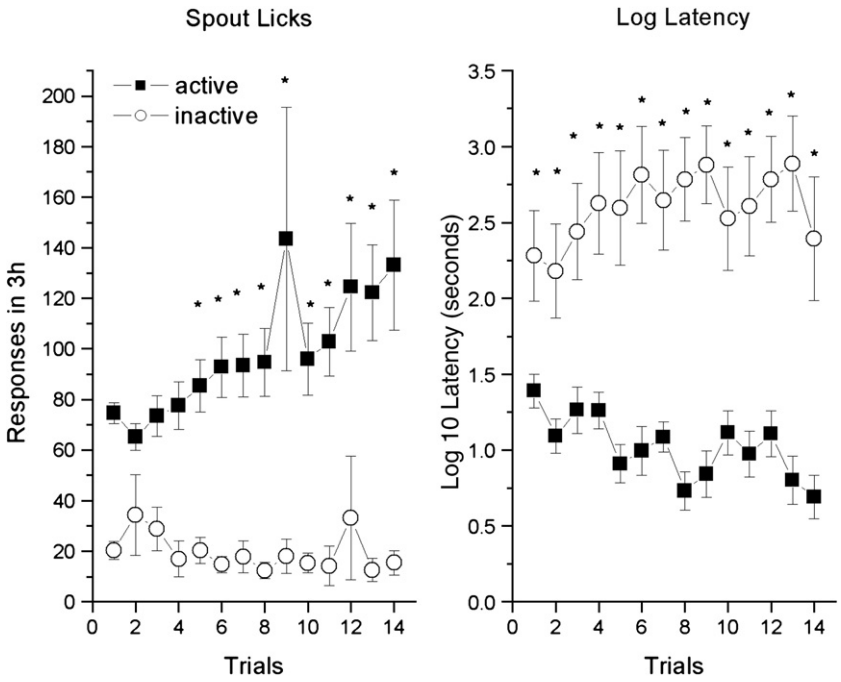

Fig. 1. Left panel. The mean (+/−SEM) number of licks emitted on the active and the inactive spout across 14 days of acquisition training. Right panel. Mean (+/−SEM) log latency (seconds) to make initial contact with the active and the inactive spout across 14 days of acquisition training. * denotes statistical significance.

for differences in overall activity. Goal-directed behavior exhibited during extinction testing was compared following either 1 or 14 days of home cage abstinence using an unpaired Students *t*-test.

3. Results

3.1. Number of responses

Rats clearly distinguished between the active and inactive spouts during the acquisition sessions (see Fig. 1, left panel).

A significant Spout×Training day interaction, F (13, 390)=2.12, $p<0.01$, was detected for spout licks and post hoc analysis demonstrated that the number of responses made on the active spout was significantly greater than the number of responses made on the inactive spout beginning with Trial day 5 (ps<0.05).

3.2. Log 10 latency to initiate responding

Rats also exhibited clear drug-taking behavior by initiating licking more quickly on the active than on the inactive spout (see Fig. 1, right

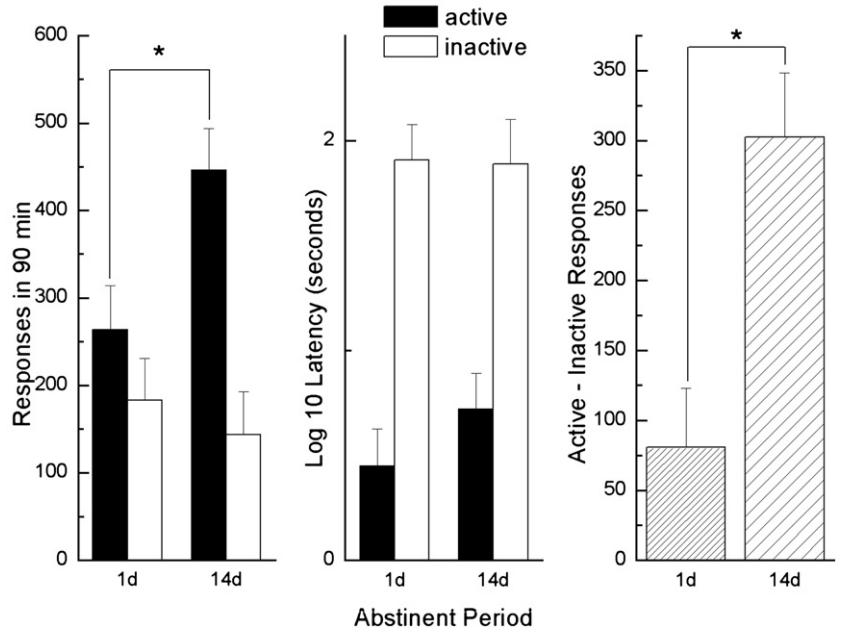

Fig. 2. Left panel. Mean (+/−SEM) number of licks emitted on the active vs. the inactive spout during the 90 min extinction test conducted after either 1 or 14 days of home cage abstinence. Middle panel. Mean (+/−SEM) log 10 latency (seconds) to make initial contact with either the active or the inactive spout during the 90 min extinction test conducted after either 1 or 14 days of home cage abstinence. Right panel. Mean (+/−SEM) goal-directed behavior (number of licks made on the active spout minus the number of licks made on the inactive spout) during the 90 min extinction test conducted after either 1 or 14 days of home cage abstinence. * denotes statistical significance.

panel). Support for this conclusion was provided by a significant main effect of spout, F (1,28)=143.58, $p<0.001$. The Spout×Training day interaction, however, was not statistically significant, F (13,364)=1.12, $p=0.34$. Thus, rats initiated licking on the active spout more quickly than on the inactive spout and this difference persisted throughout training.

4. Extinction

4.1. Number of responses

During the 90-min extinction session, rats responded more on the active spout than on the inactive spout after 14 days of abstinence compared to 1 day of abstinence (Fig. 2, left panel).

Post hoc tests of a significant Spout×Abstinence Period interaction, F (1,28)=5.14, $p<0.03$, verified that responses on the active spout were significantly higher after 14 days of abstinence than they were after 1 day of abstinence ($p<0.05$). Additionally, responses were significantly higher on the active than the inactive spout during the extinction session after 14 days of abstinence ($p<0.05$). The number of responses emitted on the active vs. the inactive spout did not differ when tested after only 1 day of abstinence ($p>0.05$). Finally, after 1 day of abstinence, most saline infusions were earned during the first 45 min of the extinction session. After 14 days of abstinence, saline infusions were spread relatively evenly throughout the 90-min extinction session.

4.2. Log 10 latency to initiate responding

Post-abstinence latency scores were analyzed using a 2×2 ANOVA varying spout and abstinence period (Fig. 2, middle panel). Unlike the lick data, the latency measure was not sensitive to incubation. Thus, the latency to initiate responding on the active vs. the inactive spout was very short, whether the extinction test occurred following 1 or 14 days of abstinence. This may represent a floor effect (i.e., the latency to lick cannot get much shorter). This conclusion was supported by a significant main effect of spout, F (1,28)=50.9, $p<0.0001$, and a non-significant Spout×Abstinence Period interaction, F (1,28)=0.61, $p=0.44$.

4.3. Goal-directed behavior

Goal-directed behavior (i.e., the number of responses emitted on the active spout minus those on the inactive spout) was significantly higher when tested following 14 days, as compared to 1 day, of abstinence (t(14)=−3.54, $p<0.003$, Fig. 2, right panel).

5. Discussion

During training, rats exhibited clear heroin self-administration behavior using spout licking as the operant behavior. Licking on the active spout was initiated more quickly than on the inactive spout, and goal-directed behavior was evident with rats making more responses on the active than on the inactive spout. The water-deprivation regimen (i.e., having a history of water intake on the right-most "active" spout) may have contributed to responding on the active spout during training, but cannot, alone, account for the behavior. This is because (a) water was never available in the chambers during either acquisition or testing and (b) saline-administering control rats, that were maintained on the same water-deprivation regimen, did not exhibit goal-directed behavior for the right-most "active" spout (see companion manuscript by Kuntz et al. and Grigson and Twining (2002)). Responding, then, was driven by the opportunity to self-administer the highly addictive agent, heroin.

During extinction testing, rats with a history of heroin self-administration were as likely to lick on the active as the inactive spout when tested after just 1 day of home cage abstinence. When tested after 14 days of abstinence, however, rats with a history of heroin self-administration made 3 times as many licks on the active than on the inactive spout. The longer period of abstinence, then, resulted in the development of directed heroin-seeking behavior. Indeed, the unexpected loss of reward (i.e., extinction) shifted the behavior of these rats from an adaptive "search-like" strategy across the two spouts (Day 1) to persistent drug-seeking on the previously active operandum (Day 14).

The incubation behavior described in this manuscript extends reports where the study of incubation is limited to an evaluation of active responses (Sorge and Stewart, 2005). Difference scores can provide a measure of seeking behavior and have been reported in studies assessing cocaine-seeking behavior (Tran-Nguyen et al., 1998). Lu et al. (2004b) reported on both active and inactive responding for cocaine. In that case, the rats clearly responded on the active lever more than the inactive lever when tested even after only 1 day of home cage abstinence. In the present heroin study, rats showed a preference for the active lever, but only following 14 days of abstinence. Possible explanations for the difference between the previous data (Lu, et al., 2004b) and those reported here include differences in drug, FR schedule, and operant behavior. The difference in drug seems an unlikely explanation as incubation has been reported with both cocaine and heroin (Shalev et al., 2001; Freeman et al., 2007). Regarding the schedule of reinforcement, rats in the present study were trained on a FR10 schedule of reinforcement, while the rats in the Lu et al. study were trained on an FR1 schedule of reinforcement. Relative to continuous reinforcement, partial reinforcement (i.e., an FR greater than 1) during training can elicit greater persistence in responding when tested during extinction (Valles et al., 2006). This difference, however, also seems an unlikely explanation because spout licking was both less and more goal-directed in the present study, depending only upon the length of the abstinence period. Finally, in reference to the operant response, lever pressing has a potential advantage over spout licking because it offers a unique behavior that becomes exclusively associated with drug taking. Even so, spout licking readily supports operant responding for sweets (Sclafani and Ackroff, 2003; Hajnal et al., 2007) and for drugs (Liu and Grigson, 2005; Jones et al., 2002). Like lever pressing, spout licking for drug is orderly (Grigson and Twining, 2002) and, apparently, also is sensitive to incubation. We believe, then, that spout licking is a useful operant that may be even more sensitive to small changes in the strength of the goal-directed behavior than lever pressing. Further studies are required to test the accuracy of this conclusion and the merits of the resulting hypothesis that incubation involves a shift from an adaptive search strategy to a less plastic, tightly honed drug-seeking strategy.

Incubation occurred in rats in the present experiment despite the lower total daily intake of heroin and shorter daily exposure. As such, this study also extends the initial report by showing incubation of heroin-seeking behavior following increasing periods of abstinence (Shalev et al., 2001). Importantly, rats self-administered using an FR1 schedule of reinforcement in Shalev's study and therefore his study utilized not only a different operant behavior, but also a different reinforcement schedule. Incubation has traditionally been associated with an increased response with time following prior exposure to some aversive stimulus (McAllister and McAllister, 1967; Eysenck, 1968; Houston et al., 1999). Recent reports suggest that the incubation phenomenon also occurs with rewarding stimuli. Cue-induced sucrose-seeking (Grimm et al., 2005), cocaine-seeking (Lu et al., 2005), and heroin-seeking (Shalev et al., 2001) increase when tested over increasing periods of abstinence. Incubation, then, is an adaptive phenomenon that, when engaged by drugs of abuse, could contribute to drug relapse following abstinence. Increased craving with the abstinence period has been reported in humans (Gawin and Kleber, 1986). This may help explain the increased likelihood of relapse, not only to the explicit cues and contexts that were previously associated with the drug, but also to cues and contexts that are remotely similar

(Thiele et al., 1996; Houston et al., 1999). Incubation, in rats, also occurs with drug priming (Tran-Nguyen et al., 1998, but see Lu et al., 2004b) or following exposure to a stressor (Sorge and Stewart, 2005).

Studies focusing on the phenomenon of incubation are crucial to understanding this relapse model (Shepard et al., 2004). We can now conclude that the phenomenon of incubation, which develops after even relatively modest heroin self-administration, serves to narrow behavior to focus upon the goal. Further investigation of increased goal-directed behavior with abstinence will be needed to distinguish between learning and memory effects vs. physical craving. Additionally, characterization of the molecular underpinnings of this phenomenon is addressed in the companion paper, and ultimately may contribute to effective treatments for relapse prevention.

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