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Chronic intermittent heroin produces locomotor sensitization and long-lasting enhancement of conditioned reinforcement

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ABSTRACT

In a previous study we showed that chronic intermittent heroin in rats enhanced responding with conditioned reinforcement and reversal learning of a conditioned magazine approach task when tested three days after the heroin treatment. Whether or not this enhanced appetitive learning persists after a protracted withdrawal period remains unknown and constitutes the aim of the present study. Forty-eight male Long Evans rats were each exposed to positive pairings of a light stimulus and food for 4 consecutive daily sessions. Then, two groups of rats received saline and two groups received heroin (2 mg/kg) injections before placement in activity monitors for 9 consecutive daily sessions. This was followed by testing in operant conditioning chambers where one lever produced the light stimulus previously paired with food and another no stimulus. For one saline and one heroin group this testing occurred after 2 days of withdrawal while for the other saline and heroin groups it occurred after 30 days of withdrawal. The results indicate that animals treated with heroin displayed progressively and significantly greater locomotor activity across sessions while animals treated with saline displayed locomotor activity that remained low and stable across sessions. In addition, the heroin groups in each withdrawal condition displayed significantly enhanced responding with conditioned reinforcement compared to their respective saline control groups. These results demonstrate that chronic intermittent heroin enhances appetitive learning for natural reinforcers and motivational processes and that this effect persists even after 30-days of withdrawal.

Chronic intermittent heroin, and other opiates, produces neural and behavioral adaptations in animal models. These include behavioral sensitization, such as progressively larger locomotor responses to a constant drug dose, as well as sensitization of neural activity. Because some of these neural changes occur in the mesolimbic DA system, a system implicated in learning and motivational (i.e., reward) processes, we hypothesize that chronic intermittent heroin may also affect learning and motivational processes for natural rewards.

The mesocorticolimbic DA system is implicated in drug and non-drug reinforcement, as well as in appetitive learning (Robbins and Everitt, 1992; Fibiger and Phillips, 1986; Wise and Bozarth, 1987; Everitt and Robbins, 1992; Wolterink et al., 1993; Berridge and Robinson, 1998; Wyvell and Berridge, 2001; Di Ciano and Everitt, 2001; Ito et al., 2000, 2004; Wise, 2004). Some researchers have reported long-term changes in the mesolimbic DA system after chronic opiate administration in the following ways: increases in extracellular DA in nucleus accumbens (Kalivas and Duffy, 1987), enhanced mesolimbic DA release in response to opiates (Spanagel et al., 1993; Spanagel and Shippenberg, 1993), enhanced cAMP functioning (Chao and Nestler, 2004; Nestler, 2004) and proliferation of GluR1 receptors on DA neurons (Carlezon and Nestler, 2005). Based on these facilitative changes in mesocorticolimbic DA functioning with chronic opiate treatment we hypothesized that repeated heroin injections in rats would enhance appetitive learning and motivational processes for natural rewards. We tested this hypothesis and found that animals receiving repeated injections of heroin not only demonstrated sensitization of locomotor activity but also, when tested three days after the last injection, significantly greater responding with conditioned reinforcement and accelerated reversal learning of a conditioned food trough approach task (Ranaldi et al., 2009) compared to saline-treated animals. Thus, chronic intermittent heroin enhanced motivational and learning processes involving a natural reward. This finding is in agreement with other reports (Taylor and Horger, 1999; Taylor and Jentsch, 2001; Wyvell and Berridge, 2001) that chronic administration of psychostimulants to rats enhances similar appetitive learning and motivational processes.

Although enhanced appetitive processing after chronic intermittent heroin (Ranaldi et al., 2009) might be predicted based on the fact that similar treatments enhance DA functioning, the finding nevertheless

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appears contradictory to some others. Aston-Jones and Harris (2004) found that after chronic morphine administration rats exhibited a diminished preference for a food-associated chamber, suggesting a reduction in the motivational aspect of natural reward as opposed to the enhanced appetitive effect we observed. Perhaps the discrepancy between Aston-Jones and Harris (2004) and Ranaldi et al. (2009) is that the former tested for preference after protracted withdrawal periods of 14 and 35 days whereas we tested after three days. Thus, in the present study we tested whether or not our previous effect– enhanced responding with a food-associated conditioned reinforcement after intermittent heroin– would persist under conditions of protracted withdrawal (30 days from the last heroin injection).

1. Materials and methods

The protocols used in the present experiment were in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the Queens College Animal Care and Use Committee.

1.1. Subjects

Subjects were 48 male Long Evans rats, facility-bred from males and females obtained from Charles River Laboratories (Raleigh, NC), with initial weights ranging from 320 to 400 g. Each rat was kept on a 12:12 h light:dark cycle with the dark phase starting at 6 AM. All rats were tested during the active (dark) phase. Each rat had free access to water except when in experimental chambers. All rats were placed on a food-restricted diet, in which their weights were maintained at 85% of their free feeding values for the duration of the experiment in order to maintain a consistent motivational state across all phases of the experiment.

1.2. Apparatus

1.2.1. Activity chambers

The activity chambers measured $50 \times 32 \times 20$ cm. Each chamber was equipped with 8 photocells that recorded horizontal movements. Consecutive photo beam breaks were registered as locomotor movements.

1.2.2. Operant conditioning chambers

Operant conditioning sessions were conducted in operant conditioning chambers measuring $30 \times 21 \times 18$ cm. Each chamber consisted of an aluminum top and two aluminum sides. The front side, which served as the door, was made of transparent plastic, as was the back wall and the floor consisted of aluminum rods. Each chamber was equipped with two levers, two white stimulus lights and a food trough, all on the right wall. Each lever was positioned 2.5 cm from the edge of the wall and extended 2 cm into the chamber. Each white stimulus light was positioned 3 cm above a lever. The food trough measured 5×5 cm and was centered between the two levers at a height of 3 cm from the floor. Pressing one lever produced no consequence while pressing the other lever turned on the white stimulus light above that lever for 3 s. The lever associated with the light stimulus was on the right side for half of the chambers and on the left side for the other half. Each operant conditioning chamber was housed in a ventilated, sound-attenuating box.

1.3. Procedure

All rats were exposed to a procedure consisting of four phases referred to as the pre-exposure, conditioning, treatment and test phases.

1.3.1. Pre-exposure phase

In the pre-exposure phase, animals were placed in the operant chambers for five consecutive daily 40-min sessions. During this phase, pressing on one lever produced the light on stimulus and pressing on the other lever produced no stimulus. The number of responses made on each lever during each pre-exposure session was recorded. After completion of this phase there was a two-day rest period.

1.3.2. Conditioning phase

In the conditioning phase, animals were placed in the operant chambers for four consecutive daily 60-min sessions. The levers were removed prior to the start of the sessions. During each session, the rats were exposed to 81 presentations of the 3-s light stimulus according to a random time 45-s schedule. A randomly selected one-third of these presentations (27 in total) were paired with the delivery of two 45-mg food pellets. After completion of this phase, there was a two-day rest period.

1.3.3. Treatment phase

In the treatment phase all animals were exposed to the activity chambers for twelve consecutive daily 30-min sessions. Prior to the first three sessions (habituation) all animals received an intraperitoneal (IP) injection of saline. Prior to the remaining nine sessions half of the animals received an IP injection of heroin (2 mg/kg) and the other half an IP injection of saline. The assignment of rats to the heroin or saline (vehicle control) conditions was randomly determined. Activity counts were measured during the entire 30-min period of each activity session. Half of the saline- and half of the heroin-treated animals (the 2-Day Withdrawal groups) began the test phase two days following the last injection while the other half of the saline- and heroin-treated animals (the 30-day withdrawal groups) began the test phase 30 days following the last injection.

1.3.4. Test phase

In the test phase all rats were placed in the operant chambers for two consecutive daily 40-min sessions. During this phase, presses on one lever produced a light stimulus for 3 s and presses on the other lever produced no stimulus. Presses on both levers were counted.

1.4. Drug and doses

All solutions were prepared prior to the commencement of the experiment. Heroin (NIDA through RTI International, Research Triangle Park, NC) was dissolved in saline to achieve a concentration of 2 mg/ml. Solutions were injected in 1 ml/kg volumes.

1.5. Data analysis

For the activity tests, the data consisted of the total number of consecutive beam breaks (locomotor counts) per 30-min session. A 2-way, mixed-design analysis of variance (ANOVA) with treatment (between-groups) and session (within-subjects) was conducted on these data. A significant treatment by session interaction was followed by a test of simple main effect of day at each level of the treatment factor.

For the conditioned reinforcement test the number of responses made on each lever during each of the five pre-exposure sessions was averaged for each rat. The number of responses made on each lever during the two test sessions was averaged for each rat. A 3-way ANOVA with treatment, withdrawal condition (between-subjects) and phase (within-subjects) was conducted on the light lever responding data. Significant interactions were followed by tests of simple main effects of treatment at each level of phase and withdrawal condition at each level of phase.

2. Results

Both the heroin- and saline-treated animals showed similar levels of locomotor activity on day one (see Fig. 1). However, on days two to

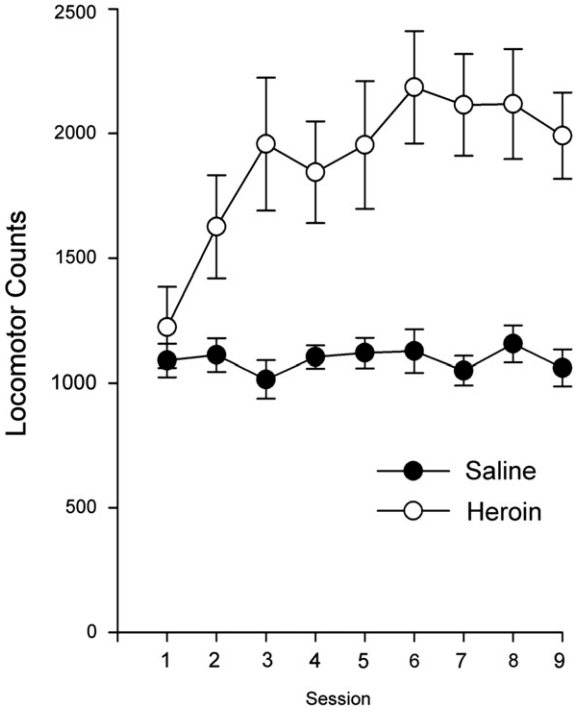

Fig. 1. Mean (±SEM) locomotor activity counts (measured as consecutive photo beam breaks) during the treatment phase of the experiment in rats treated daily with heroin ($n=18$) or saline ($n=15$) for 9 consecutive sessions. Injections were administered intraperitoneally immediately prior to being placed in the activity chambers.

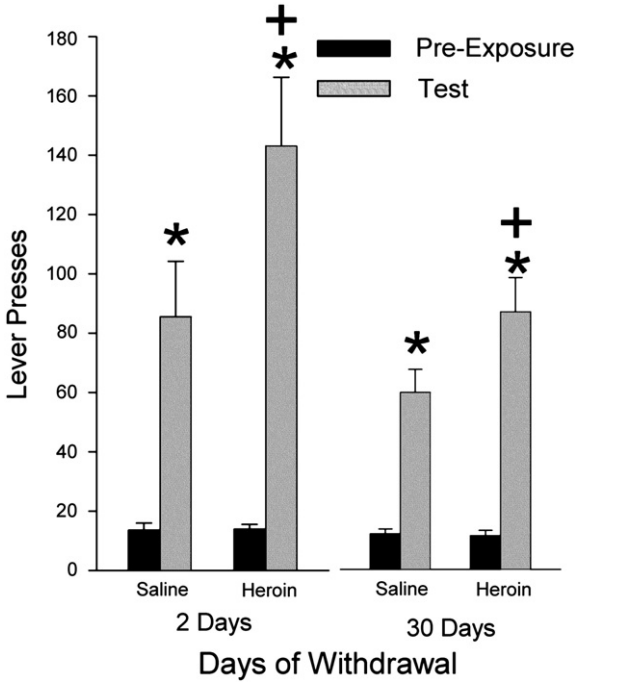

Fig. 2. Mean (±SEM) number of presses on a lever producing the light stimulus during the pre-exposure and test phases for the same rats as in Fig. 1. All operant conditioning sessions were conducted drug free. * Represents a significant phase effect. + Represents a significantly enhanced conditioned reinforcement effect compared to the corresponding saline group.

nine, the heroin-treated animals displayed progressive increases in locomotor activity while the saline-treated animals did not. A two-way ANOVA conducted on these data revealed a significant treatment × session interaction [$F_{8,336}=3.145$, $p<.002$]. Tests of simple main effects revealed a significant session effect for heroin [$F_{3,336}=3.156$, $p<.002$] but not for saline.

Fig. 2 shows the effects of chronic intermittent heroin on responding with conditioned reinforcement. Responding on the light lever was greater in the test than in the pre-exposure phase regardless of saline or heroin treatment and regardless of the number of days of withdrawal (Fig. 2). Furthermore, responding on the light lever in the test phase was greater in the heroin-treated than in the saline-treated groups and this relation was observed in both the 2-Day and the 30-Day Withdrawal conditions (see Fig. 2). Similar to what we observed in our previous report (Ranaldi et al., 2009) responding on the no stimulus lever did not change across phases or treatments (data not shown). A 3-way ANOVA with phase (pre-exposure and test), treatment (saline or heroin) and withdrawal condition (2 or 30 days) on light lever responding revealed significant phase × treatment [$F_{1,39}=9.142$, $p<.005$] and phase × withdrawal condition [$F_{1,39}=4.24$, $p<.05$] interactions but no significant phase × treatment × withdrawal condition interaction [$p>.48$]. Tests of simple main effect of treatment at each level of phase revealed a significant treatment effect at the test phase [$F_{1,39}=8.357$, $p<.01$]. Tests of simple main effect of withdrawal condition at each level of phase revealed a significant withdrawal condition effect at the test phase [$F_{1,39}=8.170$, $p<.01$].

3. Discussion

Animals that received repeated injections of heroin showed progressive increases in locomotor activity compared to animals that received saline injections. In addition, when tested drug-free after 2 or 30 days of withdrawal, animals treated with heroin showed a significantly greater increase in pressing on the lever reinforced by the food-associated CS compared to saline-treated controls, demonstrating an enhancement of conditioned reinforcement. Therefore, chronic intermittent heroin administration enhances responding with conditioned reinforcement and this effect persists into a protracted withdrawal period, suggesting that it is relatively long lasting.

Because this study was aimed at investigating the effects of chronic intermittent heroin on food-related learning and motivation we used a food restriction protocol in order to enhance motivation for food. Others have shown that a food restriction diet enhanced the behavioral and neural effects of food and psychostimulants (see Carr, 2007). This might suggest that the food restriction protocol used here may have enhanced the effects of heroin, raising the possibility that animals not on a food restriction diet may not have shown enhanced responding with conditioned reinforcement after chronic intermittent heroin treatment. However, we have demonstrated locomotor sensitization after repeated opiate injections in animals not on food restriction diets (Ranaldi et al., 2000) indicating that behavioral and neural adaptations brought about by chronic opiate treatment do not require food restriction protocols. Furthermore, at least one study indicates that food restriction results in a diminished DA response to morphine (Pothos et al., 1995). Nevertheless, the possibility of a food restriction/heroin treatment interaction is an interesting one that will be tested in future work.

To date, there have not been any studies examining the effects of chronic intermittent heroin on natural appetitive learning and motivational processes except for one other study conducted in this lab. Ranaldi et al. (2009) found that after chronic intermittent heroin injections and when tested after 2 days of withdrawal, heroin-treated rats showed enhanced lever pressing reinforced by a food-associated CS compared to saline controls. The current study replicates that finding, and extends it to a protracted withdrawal period.

One explanation for the enhancement of conditioned reinforcement observed after chronic intermittent heroin could be that the treatment produces neural adaptations that enhance activity in the mesolimbic DA pathway, a pathway involved in appetitive learning and motivation for natural, as well as drug, reward. In animals treated repeatedly with morphine, a morphine challenge given after a short withdrawal period–a few days after the last treatment–is associated with greater extracellular levels of nucleus accumbens DA than in animals not treated repeatedly with morphine (Kalivas and Duffy, 1987). Functional investigations of the mesolimbic DA system after protracted periods of withdrawal–typically three or more weeks from the last morphine treatment–reveal enhanced mesolimbic DA release in response to morphine (Spanagel et al., 1993; Spanagel and Shippenberg, 1993). At the level of the ventral tegmental area, the site of origin of the mesolimbic DA neurons, an increase in the number of GluR1 receptors is observed (Fitzgerald et al., 1996). Given the involvement of the mesolimbic DA system in the appetitive effects of food-associated CSs (see Zellner and Ranaldi, 2010 for a review), it might be expected that enhanced functioning of this system, after chronic intermittent heroin administration, might result in enhanced control over behavior by such CSs.

Apart from heroin, other drugs of abuse, when administered intermittently, enhance natural appetitive processes. Taylor and Horger (1999) examined the effects of repeated cocaine injections on acquisition of a novel response using a conditioning procedure similar to the one used here. They found that chronic cocaine-treated animals pressed significantly more on a lever producing a reward-associated CS than chronic saline-treated animals. Taylor and Jentsch (2001) also found that five days of cocaine or *d*-amphetamine injections resulted in more presses on a reward-associated CS lever than repeated saline injections did. Finally, Wyvell and Berridge (2001) examined the effects of amphetamine on learning a novel Pavlovian approach behavior. They found that previous amphetamine treatment enhanced responding on a CS+ lever for sucrose compared to a CS− lever, a finding that is in line with the interpretation that pre-exposure to psychostimulants enhances appetitive learning with natural rewards.

However, some studies have shown that chronic drug administration impaired, rather than enhanced, motivational processes for natural reward. Harris and Aston-Jones (2003a, 2003b) found that animals treated with continuous morphine, using subcutaneous morphine tablets, demonstrated less preference for a food-associated chamber and delayed acquisition of a food-reinforced lever press response compared to saline-treated rats. Such findings suggest that chronic morphine leads to a depression in other reward-related behaviors and does not coincide with our findings with intermittent heroin or those of others with intermittent psychostimulant treatments. Perhaps the discrepancies between studies showing enhancement of natural reward appetitive processes and the studies by the Harris and Aston-Jones lab are due to the differences in drug administration methods (intermittent vs. continuous) and the behavioral and neural effects that these different drug administration procedures might produce. For instance, it seems that sensitization only occurs after intermittent exposure to drugs (Spanagel et al., 1993; Louk et al., 1997; Lee et al., 1996, 1998; King et al., 1994, 1992), raising the possibility that drug administration procedures that produce sensitization are associated with enhanced natural appetitive processes whereas those that do not produce sensitization are not.

To our knowledge this is the first study examining the long-term effects of chronic intermittent heroin on appetitive learning processes involving natural rewards. In summary, the present study found that chronic intermittent heroin treatment in rats produces behavioral sensitization to its locomotor-stimulant effects and enhances responding with food-associated conditioned reinforcement. This finding suggests that repeated heroin intake produces relatively long-lasting enhancement of appetitive learning and motivational processes involving natural rewards.

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