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Repeated heroin in rats produces locomotor sensitization and enhances appetitive Pavlovian and instrumental learning involving food reward

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ABSTRACT

We tested the hypothesis that sensitization to heroin enhances appetitive motivational processes involving food reward. In Experiment 1, sixteen rats were exposed to positive pairings of a light stimulus and food for 4 consecutive daily sessions. Then, the rats received either saline or heroin (2 mg/kg) injections before placement in activity monitors for 9 consecutive daily sessions. Rats were then tested in operant conditioning chambers where one lever produced the light stimulus previously paired with food and another lever produced a tone stimulus not paired with anything. Heroin produced both significant progressive increases in locomotor activity (sensitization) and significantly enhanced conditioned reinforcement of instrumental lever pressing by the food-associated stimulus. In Experiment 2, thirty-two rats were given Pavlovian discrimination training in a conditioned magazine approach task where one stimulus was associated with food and a second unpaired with food. Rats then received repeated saline or heroin injections as in Experiment 1, before being tested under extinction conditions with the two stimuli without the drug. Chronic heroin had no effect on performance in this test, but it facilitated learning of the reversed discrimination in a subsequent phase. These data suggest that sensitization to heroin enhances appetitive motivational processes involving food reward.

Chronic exposure to heroin produces neural and behavioral adaptations some of which occur in brain circuits that are important for learning and motivation. This raises the possibility that chronic exposure to heroin – specifically in the form of repeated intermittent doses as is experienced by heroin abusers – may result in changes in other more natural (i.e., non-drug) appetitive motivational processes. Investigating this possibility is important because not only would it provide insight into the changing motivational processes of heroin users but also into how these changes may contribute to the development and maintenance of addiction. The present study is an initial investigation of this possibility.

Repeated intermittent exposure to opiates leads to sensitization to at least some of their behavioral effects. Sensitization refers to the progressive augmentation of a behavioral response, such as locomotion, to a drug with repeated intermittent exposure to the drug. Sensitized responses have been observed weeks and even months after the final treatment (Vanderschuren and Kalivas, 2000), suggesting that it results from long-term changes in neuronal function. Repeated injections of morphine in rats produces sensitization to its locomotor-stimulant effects (Babbini and Davis, 1972) and this sensitized response has been demonstrated to persist for at least 2 weeks after the last treatment (Ranaldi et al., 2000). Sensitization to the behavioral effects of heroin has been studied considerably less than that to morphine. Although sensitization to the locomotor-stimulant effects of heroin has been reported (Pontieri et al., 1997), the effect is not well-characterized. Given that heroin is one of the more abused substances in the opiate class, as well as in all classes of abused drugs in general, it is important to investigate the behavioral effects of heroin specifically. Thus, another aim of this study is to begin to characterize the locomotor sensitization profile to heroin specifically.

Sensitization to opiates is associated with neural adaptations in the mesolimbic dopamine (DA) system (Vanderschuren and Kalivas, 2000). 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 hypersensitive nerve terminals (Spanagel et al., 1993; Nestby et al., 1997). At the level of the ventral tegmental area (VTA), the site of origin of the mesolimbic DA neurons, an increase in the number of GluR1 receptors is observed (Fitzgerald et al., 1996). Given that GluR1 receptors are located on DA

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cells and that their stimulation excites DA neurons, this latter finding is proposed as a possible mechanism through which sensitized responses to opiates occur (Carlezon and Nestler, 2002).

The mesolimbic DA system has also been implicated in natural reward (Wise, 2006). Enhanced DA neurotransmission in terminal regions of the mesolimbic system is observed in animals consuming natural rewards such as food and water or engaging in sex (Berridge and Robinson, 1998). The importance of the mesolimbic DA system in reward has been demonstrated in studies showing that reductions in DA neurotransmission in the nucleus accumbens (Aberman et al., 1998) or VTA (Sharf et al., 2005) attenuate responding maintained by food reinforcement. Mesolimbic DA is also implicated in conditioned reinforcement of instrumental behaviors by food-associated cues as well as in the acquisition and expression of conditioned magazine approach. In rats, injections of D-amphetamine directly into the nucleus accumbens enhance responding maintained by conditioned reinforcement and this effect is eliminated by 6-hydroxydopamine lesions of the nucleus accumbens (Taylor and Robbins, 1986). Also in rats, injections of DA antagonists into the nucleus accumbens reduce responding maintained by conditioned reinforcers for food (Wolterink et al., 1993). In conditioned magazine approach studies, injections of DA antagonists either systemically or directly into the nucleus accumbens impair both the acquisition (Di Ciano et al., 2001; Parkinson et al., 2002; Choi et al., 2005) and expression (Di Ciano et al., 2001; Parkinson et al., 2002) of Pavlovian approach responding.

Thus, it appears that chronic intermittent exposure to opiates produces neural adaptations in the mesolimbic DA system, a system strongly implicated in food and other natural rewards and in appetitive motivational processes more generally. This raises the possibility that with chronic exposure to heroin there occur changes in appetitive motivational processes involving food reward. The present experiments tested this possibility. Specifically, we tested the hypothesis that repeated heroin injections in rats would produce sensitization to its locomotor-stimulant effect, and also enhance both instrumental and Pavlovian learning involving food reward. We investigated these hypotheses by examining the effects of chronic heroin on conditioned reinforcement of instrumental responding by a food-associated cue in Experiment 1, and on Pavlovian discriminative responding and reversal learning using a conditioned magazine approach task in Experiment 2.

1. Materials and methods

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

1.1. Experiment 1

1.1.1. Subjects

Subjects were sixteen male Long–Evans rats, facility-bred from males and females obtained from Charles Rivers Laboratories (Raleigh, NC), with initial weights ranging from 325 to 375 g. Each was kept on a 12:12 h light:dark cycle with the dark phase starting at 6 AM. All rats were tested during their active (dark) phase. Each rat had free access to water except when in activity or operant conditioning chambers. All rats were placed on a food restriction diet that maintained their weights to 85% of their free-feeding values through measured daily rations of Purina Rat Diet. All rats were maintained on the food restriction diet for the duration of the experiments.

1.1.2. Apparatus

1.1.2.1. Activity chambers. The activity chambers measured 50×32×20 cm. Each chamber was equipped with 8 photocells that recorded horizontal movements. Consecutive photo beam breaks were registered as locomotor movements while repetitive single photo beam breaks as stereotypy movements. Only locomotor movements were recorded.

1.1.2.2. Operant conditioning chambers. Operant conditioning sessions were conducted in operant chambers measuring 30×21×18 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. The floor of each chamber consisted of aluminum rods. Each operant conditioning 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 away from the edge of the wall and extended 2 cm from the wall. 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 a 1-kHz tone lasting 3 s while pressing the other lever turned on the white stimulus light above that lever for 3 s. The lever associated with the light-on 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.

1.1.3. Procedure

Each animal was exposed to a procedure consisting of 4 phases referred to as the pre-exposure, conditioning, treatment and test phases.

In the pre-exposure phase animals were placed in the operant conditioning 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 the tone stimulus. The number of responses made on each lever during each pre-exposure session was recorded. After completion of this phase, there was a 2-day rest period.

In the conditioning phase the animals were placed in the operant conditioning chambers for four consecutive daily 60-min sessions. The levers were removed prior to the start of the sessions. For each session, rats were exposed to 81 presentations of the 3-s light-on stimulus according to a random time 45-s schedule. A randomly selected one-third of these presentations (27 presentations) were paired with the delivery of two 45-mg food pellets. After completion of this phase, there was a 2-day rest period.

In the treatment phase all animals were exposed to the activity chambers for twelve consecutive daily 30-min sessions. Prior to the first 3 sessions (habituation) all animals received an intraperitoneal (IP) injection of saline. Prior to the remaining 9 sessions half 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) condition was randomly determined. Activity counts were measured during the entire 30-min period of each activity session. After the treatment phase there was a 2-day rest period.

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 the light-on stimulus for 3 s and presses on the other lever produced the tone stimulus for 3 s. Presses on both levers were counted.

1.2. Experiment 2

1.2.1. Subjects

Subjects were 32 male Long–Evans rats, bred at Brooklyn College from males and females obtained from Charles Rivers Laboratories (Raleigh, NC), with initial weights ranging from 345 to 465 g. Each was kept on a 14:10 h light:dark cycle with the dark phase starting at 9:30 PM. All rats were tested during their light phase. Each rat had free access to water throughout the experiment except during

experimental sessions, and was placed on a food restriction diet throughout the experiment that maintained their weights to 85% of their free-feeding values through measured daily rations of Purina Rat Diet.

1.2.2. Apparatus

1.2.2.1. Pavlovian conditioning chambers. The apparatus consisted of two sets of eight identical standard conditioning chambers, each of which was housed in a sound- and light-resistant shell. The conditioning chambers measured 30.5×24.0×25.0 cm. Two end walls were constructed of aluminum, and the sidewalls as well as the ceiling were made from clear Plexiglas. The floor consisted of 0.60-cm diameter stainless steel rods spaced 2.0 cm apart. In the center of one end wall 1.2 cm above the grid floor was a recessed food magazine measuring 3.0×3.6×2.0 cm (length×width×depth). Two 45-mg pellets (TestDiet) were dropped onto the magazine floor when the US was scheduled. On the inner walls of the recessed magazine were an infrared detector and emitter enabling the automatic recording of head movements inside the magazine. These were located 0.9 cm above the magazine floor and 0.8 cm recessed from the front wall. Located 3.0 cm to the right of the magazine and 8.0 cm above the floor was a lever (4 cm in width). This lever protruded into the chamber at all times, but access to the lever was prevented throughout the experiment by a sheet metal covering. A 6-W light bulb was mounted on the bottom of the sidewall of the outer chamber, below and behind the rear wall of the conditioning chamber. When activated, this light bulb flashed with approximately equal on–off pulse durations at a frequency of approximately 2/s. A speaker was mounted 22 cm behind the front wall of the conditioning chamber (where the food magazine was located), and was used to present a 1.5 kHz tone stimulus (generated by the computer and amplified by a Radio Shack audio amplifier). The tone measured 4 dB above a background level of 78 dB (C weighting). The chamber was dark except when the visual stimulus was presented. A fan attached to the outer shell provided for cross-ventilation within the shell as well as background noise. All experimental events were controlled and recorded automatically by Pentium-based PCs and interfacing equipment (Alpha Products) located in the same room as the equipment. The two sets of 8 chambers and controlling computers were located in different running rooms.

1.2.3. Procedure

1.2.3.1. Magazine training. The rats were initially magazine trained with the pellet US on each of 2 days. In each 20-min session, 20 USs were delivered according to a variable time 60-s schedule.

1.2.3.2. Pavlovian discrimination training. Starting on the following day, all rats received a standard Pavlovian discrimination learning procedure with the Flash and Tone stimuli, and this continued for 10 sessions. Each conditioning session was 54.5 min in duration and included 8 reinforced presentations of one of the stimuli (CS+) and 8 nonreinforced presentations of the other (CS−). Half of the rats were trained with the Flash stimulus as CS+ and the Tone stimulus as CS−, while the remaining rats were trained with the opposite assignments. The CS duration on both trial types was 20 s, and the food pellet US was presented at the offset of the appropriate stimulus. A different pseudo-randomly generated trial sequence was used in each session with the constraint that neither stimulus could occur more than two times in a row. The average inter-trial interval (measured from US offset to CS onset) was 3 min (ranging from 1 to 5). Rats were taken out of the chamber 30 s following the final trial of the session.

1.2.3.3. Heroin sensitization. Over the next 9 days half the animals received an IP injection of heroin (2 mg/kg) in their home cages, and the other half an IP injection of saline. Injections occurred close to the rats' normal running times, but rats were not exposed to the Pavlovian chambers during this time. Rats were assigned to the heroin or saline (vehicle control) conditions based on their performance during the Pavlovian discrimination training phase. Differences in the mean rates of responding to the reinforced and nonreinforced stimuli were matched between the two groups. After the treatment phase there was a 2-day rest period.

1.2.3.4. Extinction test. Subjects were given two test sessions on consecutive days. Each test was performed as in the acquisition sessions except that no food was presented.

1.2.3.5. Pavlovian reversal training. All subjects were given reversal training over the next 5 sessions. Each session was conducted as in the Pavlovian discrimination training phase except the reinforcement contingencies were reversed. For example, if a subject was trained initially with Tone reinforced and Flash nonreinforced, then during this phase this subject would be trained with Flash reinforced and Tone nonreinforced.

1.2.4. Drug and doses

All solutions were prepared prior to the commencement of the experiment. Heroin (NIDA, Bethesda, MD) was dissolved in saline to achieve a concentration of 2 mg/ml. Solutions were injected in 1 ml/kg volumes.

1.2.5. Data analysis

For the activity tests in Experiment 1 the data consisted of the total number of consecutive beam breaks (locomotor counts) per 30-min session. Only the data from sessions 4 to 12 were analyzed (habituation data [sessions 1 to 3] did not differ between groups). A 2×9, mixed design analysis of variance (ANOVA) with group (between-subjects) and day (within-subjects) as factors was conducted on these data. A significant group by day interaction was followed by a test of simple main effect of day at each level of the group factor.

For the conditioned reinforcement test in Experiment 1 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 each test session was averaged for each rat. Then, the mean number of responses on each lever in the test phase was divided by the mean number of responses on that lever in the pre-exposure phase [adding 1.0 to each value entering into the ratio in order to reduce the influence of numerically small values (see Winer, 1971)]. In this way, the data consisted of two values for each rat. A 3-way ANOVA with group (between-subjects), phase, and lever (within-subjects) was conducted on these data.

For the Pavlovian conditioning study, Experiment 2, the mean rates of magazine approach responding during each stimulus were calculated for each rat in each session. Conditioned responding was then calculated with a difference score subtracting Pre CS responding (in a comparable interval) from CS responding. Data from acquisition was assessed with a group×stimulus ANOVA applied to the final day of training. Data from the two extinction test sessions were then analyzed using a 3-way ANOVA with group, test session, and stimulus as the three factors. A similar 3-way ANOVA (with group, session, and stimulus as factors) was used to analyze the data from the reversal phase.

2. Results

2.1. Experiment 1

During the first treatment session animals that were injected with heroin demonstrated lower locomotor activity than those injected

with saline (see Fig. 1). During the second treatment session locomotor activity levels between heroin- and saline-injected groups appeared similar. During each of the third to ninth treatment sessions heroin-injected animals demonstrated greater locomotor activity than saline-injected animals. Also, animals treated with heroin demonstrated progressive increases in locomotor activity across most of the nine treatment sessions (Fig. 1). Locomotor activity in the heroin-treated rats was approximately 2.5 times greater in the last treatment session than in the first. Animals treated with saline failed to show progressive increases in locomotor activity. A two-way ANOVA revealed a significant session by group interaction [$F_{8,112}=4.526$, $P<.005$]. Tests of simple main effects revealed a significant session effect in the heroin group [$F_{8,112}=7.23$, $P<.005$].

During the pre-exposure phase (before any of the animals received any treatment) responding on the tone- and light-producing levers between groups was similar (see Fig. 2). During the test phase (after all animals were subjected to repeated saline or heroin treatment regimens) the saline-treated group demonstrated a small increase in responding on the tone-producing lever while the heroin-treated group demonstrated a small decrease in responding on this lever and both groups demonstrated large increases in responding on the light-producing lever. Furthermore, the increased responding on the light-producing lever was greater in the heroin- than in the saline-treated group (Fig. 2). A three-way ANOVA revealed a significant group×phase×lever interaction [$F_{1,14}=5.024$, $P<.05$]. Thus, both groups demonstrated a conditioned reinforcement effect but the heroin group demonstrated a significantly greater one.

2.2. Experiment 2

Both groups of subjects acquired the Pavlovian discrimination rapidly. The heroin group increased from a mean of 0.6 responses per minute on CS+ trials and −3.5 on CS− trials on session 1 to, respectively, 13.7 and −0.5 on session 10. The corresponding means for the vehicle group were 4.3 and −2.0 to CS+ and CS−, respectively, on session 1 to 14.5 and −0.4 on session 10. A group×stimulus ANOVA performed on the session 10 data only revealed a significant main effect of stimulus [$F_{1,30}=37.274$, $P<.05$], indicating that both groups had equally acquired the discrimination.

The extinction test data is displayed in Fig. 3. Both groups responded more to CS+ than CS− in each test session; however, the groups did not differ in this regard. A group×test×stimulus ANOVA revealed significant main effects of stimulus [$F_{1,30}=23.227$, $P<.05$], and test [$F_{1,30}=6.093$, $P<.05$], but no other main effects or interactions.

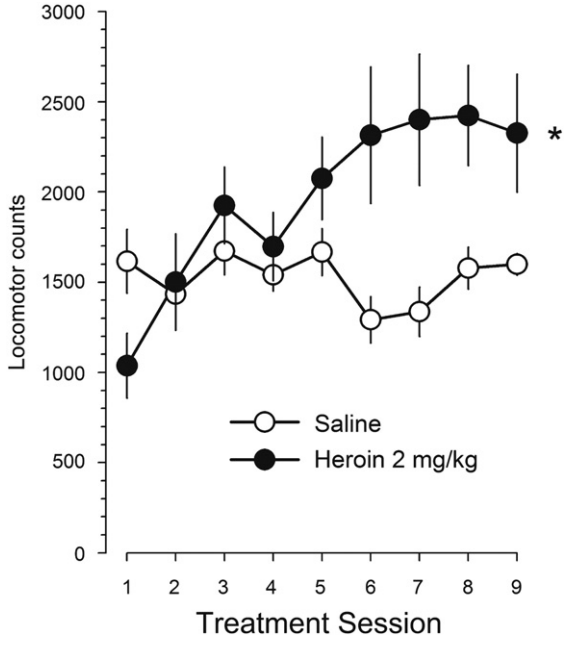

Fig. 1. Mean locomotor activity counts (measured as consecutive photo beam breaks in an activity chamber) in rats treated daily with heroin ($n=8$) or saline ($n=8$) for 9 consecutive sessions. Injections were administered intraperitoneally immediately prior to being placed in the activity chambers. * represents a significant session effect. Vertical lines represent the standard error of the mean.

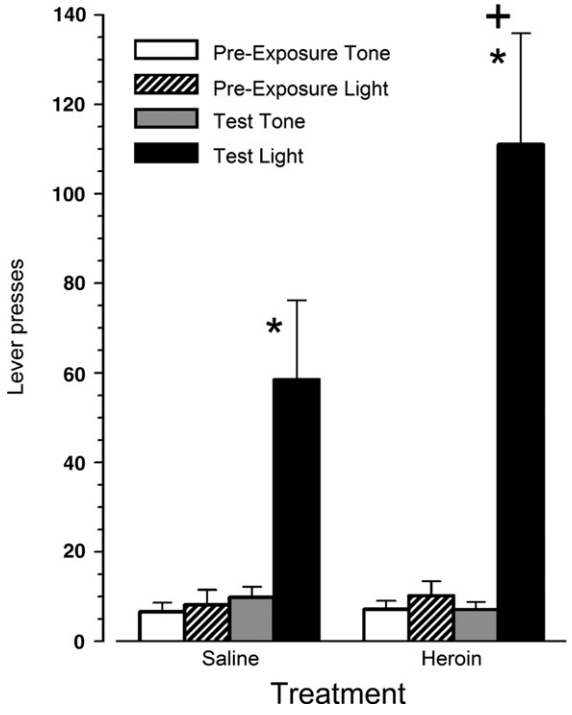

Fig. 2. Mean number of presses on a lever producing a light-on stimulus and one producing a tone stimulus during a pre-exposure phase (before any animal was subjected to a drug treatment regimen) and during a test phase (after all animals were subjected to their respective drug treatment regimen). Treatment regimens consisted of nine daily consecutive injections of saline or heroin (2 mg/kg). All operant conditioning sessions, pre-exposure and test, were conducted drug free. * represents a significant difference in light-on lever presses between phases (test and pre-exposure). + represents a significant difference in light-on lever presses between groups. Vertical lines represent the standard error of the mean.

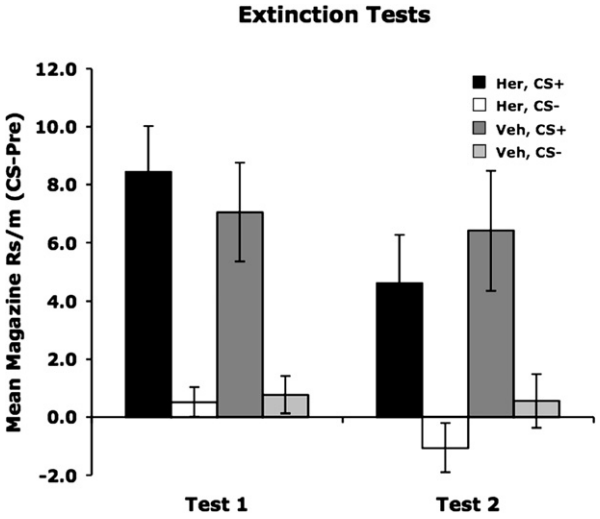

Fig. 3. Mean rate of magazine responses per minute during the extinction tests of Experiment 2 to previously reinforced and nonreinforced stimuli (CS+ and CS−, respectively) in groups given chronic heroin (2 mg/kg) or vehicle injections. These tests were conducted drug free. Vertical lines represent the standard error of the mean.

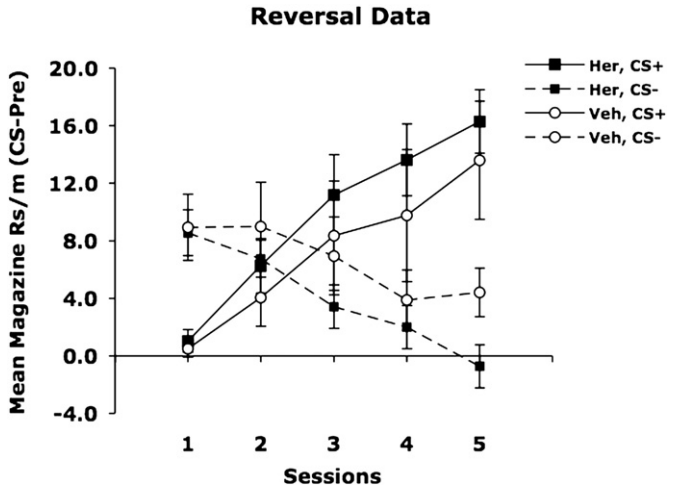

Fig. 4. Mean rate of magazine responses per minute during the reversal phase of Experiment 2 to reinforced and nonreinforced stimuli (CS+ and CS−, respectively) in groups previously given chronic heroin (2 mg/kg) or vehicle injections. These tests were conducted drug free. Vertical lines represent the standard error of the mean.

These data indicate that, overall, higher rates of responding were seen in Test 1 than Test 2, but, more importantly, CS+ responding was greater than CS− responding in both groups to the same degree.

The reversal phase data are depicted in Fig. 4. These data show that both groups began the reversal phase responding more to CS− than CS+ (because this reflected the contingencies in effect during the initial discrimination training phase). With additional training both groups acquired the reversed discrimination; however, the heroin group began to make this discrimination sooner and with greater accuracy than the vehicle group. A group×session×stimulus ANOVA revealed significant main effects of stimulus [$F_{1,30}$=9.938, P<.05] and session [$F_{4,120}$=3.295, P<.05], as well as significant stimulus×session [$F_{4,120}$=28.766, P<.05] and stimulus×group [$F_{1,30}$=6.443, P<.05] interactions. The stimulus×group interaction indicates that the heroin group was superior to the vehicle group in learning this reversal.

3. Discussion

Animals receiving repeated injections of heroin demonstrated progressively augmented locomotor responses to this drug in Experiment 1. Furthermore, when tested drug-free 3 days after the last treatment, the heroin-sensitized animals demonstrated significantly greater levels of lever pressing specifically for a food-associated conditioned stimulus than non-sensitized animals. In addition, in Experiment 2 animals receiving repeated injections of heroin following Pavlovian discrimination training, although not differing from vehicle controls in discriminative responding during an extinction test to CS+ and CS− stimuli, did acquire a reversal of this discrimination more rapidly and completely than vehicle controls. These demonstrations of enhanced locomotor, conditioned reinforcement, and Pavlovian discrimination reversal learning with food reward can be interpreted in a number of ways to which we now turn.

First, the findings in Experiment 2 are important in ruling out a trivial explanation of the effects found in Experiment 1. It is possible that chronic heroin did not actually enhance conditioned reinforcement per se, but merely increased lever pressing by raising baseline (re: heroin-free) locomotor activity. Although we observed in Experiment 1 that compared to controls heroin-treated subjects displayed a selective increase in reinforced lever pressing and no change in nonreinforced lever pressing, it is possible that such a putative locomotor effect could influence behaviors that have a higher baseline likelihood of occurrence more than behaviors with a lower baseline likelihood of occurrence. Thus, through such a putative locomotor effect a selective increase in reinforced as opposed to nonreinforced lever pressing could have occurred in the heroin-treated rats since in control rats reinforced lever responding was higher than nonreinforced lever responding.

While this sort of argument is always difficult to dismiss, we think the results from Experiment 2 do not support this account. If a locomotor effect were at work in this experiment then we should have observed poorer reversal learning in heroin-treated subjects, not better learning. At the point at which responding to CS− and CS+ was comparable during the reversal phase, then any general tendency to increase responding due to locomotor effects should have affected these stimuli equally. This would have made it difficult for heroin subjects to learn to withhold responding to CS− during the reversal phase, yet these subjects performed the task better than controls.

A second explanation of our data rests on the idea that rather than influencing learning directly the heroin treatment influenced learning indirectly by increasing the value of food reward. According to this view the CS+ in Experiment 1 was a more effective secondary reinforcer in the heroin-treated animals because it evoked a representation of food that was more valuable than in controls. Further, if food reward was more valuable in heroin-treated rats, then reversal learning could proceed more rapidly as well because the reward magnitude is effectively larger and larger rewards have been shown to promote reversal learning (e.g., Kendler and Kimm, 1967; Mackintosh, 1974). One problem for this account, however, was our failure to find an effect of heroin treatment on discriminative Pavlovian responding during the extinction tests in Experiment 2. If CS+ were to evoke a representation of food that was more valuable than in controls, we would have expected this to promote more magazine responses than in the controls. The data provided no support for this. However, it seems possible that our reversal test was a more sensitive one at detecting differences in reward value than the extinction test, and so we cannot rule this explanation out.

Another explanation of our data is based on the idea that chronic heroin treatment may influence attentional processes. In particular, subjects given heroin may become more effective at processing conditioned stimuli, unconditioned stimuli, and possibly the sensory feedback provided by their own responses. If stimulus processing were more efficient in these animals, then we would expect to see improvements in both instrumental conditioned reinforcement and Pavlovian reversal learning. We might also have expected to observe an effect in our extinction test of Experiment 2, but, once again, this test may not have been as sensitive as our other tests at detecting between-group differences in processing efficiency. Nevertheless, this view suggests that heroin-treated subjects would be expected to perform better on a variety of tasks thought to engage attentional processes.

One final account of our results assumes that chronic heroin treatment results in changes in the neural substrates that mediate appetitive learning. It is of special interest that heroin treatment could potentially alter the circuitry involved in food reinforcement because it would suggest some overlap in the circuitries mediating food and drug reward. If chronic heroin treatment effectively changes this circuitry such that new associations, i.e., neural connections, can be formed more readily, then our findings of enhanced conditioned reinforcement in instrumental conditioning and enhanced reversal learning in a Pavlovian task would both be expected to occur. According to this view, we may have also expected new learning during extinction testing in Experiment 2 to have been facilitated by heroin treatment. This would have led to more rapid extinction to CS+ in heroin-treated rats, a result we did not observe. Nevertheless, although the circuitries of acquisition and extinction are liable to overlap to some degree, there are known differences as well (e.g., Quirk and Mueller, 2008), leading to the possibility that heroin treatment may affect acquisition and extinction circuits differently.

At a more molecular level of analysis, the enhancement in appetitive instrumental and Pavlovian learning observed here after

chronic intermittent heroin exposure may result from neural adaptations in the mesolimbic DA system. This system is implicated in both locomotion (Creese and Iversen, 1975) and reward (Wise and Rompré, 1989; Berridge and Robinson, 1998). Opiate injections in the VTA produce locomotor activating (Joyce and Iversen, 1979) and rewarding (Bozarth and Wise, 1981) effects, probably through a μ-receptor mediated hyperpolarization of local GABA neurons and consequent disinhibition of mesolimbic DA neurons (Johnson and North, 1992). Repeated intermittent exposure to morphine is associated with elevations in GluR1 receptors in the VTA (Fitzgerald et al., 1996), probably on DA neurons, as well as hypersensitivity of DA terminals in the nucleus accumbens (Spanagel et al., 1993; Nestby et al., 1997). These neural adaptations may directly enhance, or foster the enhancement, of activity in the mesolimbic DA system and this enhanced activity may serve as the underlying neural substrate of locomotor sensitization. Because the mesolimbic DA system is implicated in reward and incentive motivation, enhanced functioning of this system may constitute a mechanism whereby chronic heroin exposure enhances conditioned reinforcement of instrumental responses as well as Pavlovian reversal learning.

Our results are consistent with a broad literature reporting that chronic psychostimulant treatment enhances various indices of appetitive learning and motivation. For instance, in Pavlovian conditioning studies it has been shown that repeated systemic amphetamine injections in rats that produce sensitization to the drug are followed by enhanced acquisition of a Pavlovian approach response to a food-associated CS+ in the absence of effects on the response to a CS− (Harmer and Phillips, 1998). An enhanced acquisition of Pavlovian approach was also demonstrated after repeated injections of cocaine or methylenedioxymethamphetamine (MDMA or Ecstasy) and with water as the unconditioned stimulus (Taylor and Jentsch, 2001). Additional studies have shown that both systemic injections of amphetamine (Wyvell and Berridge, 2001) and local infusions into the nucleus accumbens shell (Wyvell and Berridge, 2000) amplify the stimulating effects of a Pavlovian CS for pellets on pellet-reinforced lever pressing. Furthermore, in conditioned reinforcement studies it has been found that chronic intermittent exposure to cocaine in rats is associated with a potentiation of intra-accumbens amphetamine-produced enhancement of responding for a CS previously paired with water reward (Taylor and Horger, 1999). In all of these studies, various pharmacological treatments that enhance the DA system have similar enhancing effects in different learning paradigms involving natural reward.

Other studies have investigated the effects of repeated experimenter- or self-administered psychostimulants or opiates on subsequent reward value of self-administered drug using progressive ratio procedures. Thus, when animals are pre-exposed to a repeated dosing regimen of amphetamine that results in sensitization to the drug they subsequently demonstrate higher break points for amphetamine (Mendrek et al., 1998; Lorrain et al., 2000) or cocaine (Suto et al., 2002, 2003) self-administration under progressive ratio schedules of reinforcement. Enhanced break points for cocaine self-administration also have been demonstrated in experienced drug-taking rats after being subjected to a drug-free deprivation period (Morgan et al., 2005). Interestingly, these same researchers have demonstrated that experienced cocaine self-administering rats who are switched to heroin self-administration for 10 days, when switched back to cocaine show higher break points for cocaine than previous to chronic heroin exposure (Ward et al., 2006). Thus, in addition to being the first demonstration that heroin sensitization is associated with enhanced appetitive learning involving food reward our findings are consistent with a growing literature demonstrating enhanced appetitive learning and motivational processes with drug sensitization.

One literature that is not entirely consistent with the findings we report here concerns the effects of chronic cocaine administration on reversal learning in appetitive tasks involving food reward. Schoenbaum and colleagues (Stalnaker et al., 2007a,b) have reported that chronic cocaine impairs reversal learning in an instrumental appetitive go/no go task with rats. Furthermore, these investigators report that the impairment is related to dysfunctional interactions between orbitofrontal cortex and basolateral amygdala caused by chronic cocaine. However, Ersche, Roiser, Robbins, and Sahakian (Ersche et al., 2008) have recently reported that human chronic cocaine, but not heroin or amphetamine, users were impaired on a probabilistic reversal learning task. Moreover, reversal deficits produced by serotonin, but not DA, depletion have been reported in marmosets (Clarke et al., 2007). These data suggest that cocaine's effect on reversal learning may be mediated by cocaine's disruptive effect on serotonin transport. Our findings that heroin facilitates reversal learning may, therefore, reflect a purer influence of the DA system on appetitive learning processes than can be observed with cocaine. Nevertheless, it remains to be seen whether cocaine and heroin would have opposite effects in the particular Pavlovian discrimination reversal task used here which differs in a number of ways from those reported above.

One remaining area where results are not entirely consistent with those reported here concerns the effects of continuous morphine on reward. Aston-Jones and Harris (2004) report that protracted withdrawal from continuous morphine reduces preferences for food-associated places. The authors suggest that opiate withdrawal is associated with anhedonic responses. The discrepancy between their findings and the present one is likely due to one or more procedural differences: intermittent versus continuous drug administration, short versus protracted withdrawal and heroin versus morphine. Our procedures were designed to test the relation between heroin sensitization and food reward at a time when the heroin intake is current rather than historical. Furthermore, they were designed to investigate *intermittent*, rather than continuous, heroin exposure because (1) locomotor sensitization may be uniquely produced by intermittent exposure and (2) intermittent dosing is similar to human heroin use. Nevertheless, further research will be needed to address the apparent discrepancy between our two data sets.

The present findings may also have important implications for understanding the development of heroin addiction. Our data show that, at least in the short term and before any significant period of withdrawal, chronic intermittent heroin exposure "sensitizes" reward-related behavioral processes. It is possible that this enhanced sensitivity to reward, in general, further facilitates seeking of reward stimuli such as heroin itself or heroin-related stimuli, in particular, by also increasing their reinforcing and conditioned reinforcing effectiveness. Furthermore, while our data suggest that chronic drug exposure can have effects on appetitive learning systems more generally, it is not known to what extent non-drug appetitive systems may influence drug reward systems in drug-sensitized animals. It seems possible that habitual drug-taking and seeking behaviors may be partly maintained not exclusively by processes involving drug reward, but by processes involving other forms of appetitive reward, as well, including food. Clearly, it is important to better understand the relation between chronic intermittent heroin exposure and appetitive motivational processes involving rewards other than drugs in order to better understand the nature of addiction.

In conclusion, repeated intermittent injections of heroin in rats produces sensitization to its locomotor-stimulant effects and enhances appetitive learning involving food reward both in instrumental conditioned reinforcement and Pavlovian discrimination reversal learning.

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