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Unreinforced responding during limited access to heroin self-administration

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ABSTRACT

These studies were designed to explore a peculiar behavior displayed by rats during the acquisition of heroin self-administration (0.05 mg/kg/infusion) on a fixed-ratio 1 schedule of reinforcement in limited access conditions (i.e. 3 h/day). Rats trained under these conditions develop a tendency to emit extra lever presses during the time of heroin infusions (unreinforced responses). We found that a similar behavior develops in animals responding for sucrose pellets, but not for intravenous infusions of cocaine (0.5 mg/kg/infusion, 3 h/day). In sucrose trained rats, unreinforced responses emitted during the delivery of sucrose pellets was enhanced by food deprivation. In heroin trained rats, development of unreinforced responding was accompanied by an increase in responding for heroin on a progressive ratio schedule, and by a reduction of the depressant action of heroin (3 mg/kg, SC) on locomotor activity.
On the basis of these findings, we concluded that unreinforced responding during heroin self-administration reflects a change in the motivation to obtain the drug, as well as a reduced sensitivity the motor impairing action of heroin. This suggests that acquisition of heroin self-administration is regulated by a balance between drug effects that promote and limit heroin intake.

1. Introduction

Substance dependence is characterized by a pathological pattern of drug intake (Diagnostic and Statistical Manual of Mental Disorders, 2000) that develops in 15–18% of people that use drugs (Anthony et al., 1994; Tjepkema, 2004). The process of transition from drug use to abuse and dependence is not fully understood, and different "transitional phases" have been proposed (Jellinek, 1946). Several factors are likely to modulate transitions between phases, and intravenous (IV) drug self-administration studies in laboratory animals have been useful in exploring them. For example, when the time period of drug self-administration is extended, drug-intake escalates, in some cases even leading to toxicity (Ahmed and Koob, 1998, 1999; Bozarth and Wise, 1985).

The present study in laboratory animals focuses on factors modulating self-administration behavior at the early stages of acquisition. Sex (Lynch and Carroll, 1999), sensitivity to novelty (Ambrosio et al., 1995; Piazza et al., 1989), stress (Shaham and Stewart, 1994), drug dose (Carroll and Lac, 1997), housing conditions (Alexander et al., 1978) and food deprivation (Carroll et al., 1981; Carroll and Meisch, 1981; Oei, 1983) have all been found to modulate rate of acquisition. But, little is known about how the behavioral and the motivational effects of the drug change during this period. In other words, acquisition of drug self-administration may be associated with the simultaneous development of tolerance to drug effects such as sedation that would limit intake, and sensitization to effects such as drug reward that would facilitate it (Bitran and Kalant, 1991; Grecksch et al., 2006; Horger et al., 1992; Morgan et al., 2006; Woolverton et al., 1984).

The present experiments were designed to explore a behavior displayed by rats during the acquisition of heroin self-administration on a fixed-ratio 1 (FR1) schedule of reinforcement in limited access conditions (i.e. 3 h/day). Leri and Stewart (2002) reported that rats trained under these conditions developed a tendency to emit extra lever presses during the time (10 s) of drug infusions, and this behavior was found to increase with additional self-administration training. At the time, it was speculated that the development of unreinforced responding during acquisition of heroin self-administration may have reflected a shift in the motivation to obtain heroin, but no additional experimental evidence was available to substantiate such interpretation.

Accordingly, four experiments were conducted to investigate unreinforced responding during the acquisition phase of limited access to heroin self-administration. The first experiment was designed to replicate the findings of Leri and Stewart (2002), but using a shorter infusion time (5 s). The second experiment was designed to test whether the development of unreinforced responding is specific to the self-administration of opiates. Thus, we studied the behavior of rats during IV self-administration of cocaine, under the same experimental conditions of heroin self-administration and using a cocaine dose known to produce similar rates of acquisition. In both experiments, we also compared drug loading (Campbell and Carroll, 2000; Garcin et al., 1977), another aspect of self-administration

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behavior that is known to change during acquisition of responding for heroin (Leri and Stewart, 2002) or cocaine (Sorge and Stewart, 2005). The objective of the third experiment was to determine whether unreinforced responding could be modulated by the motivational state of an animal. One way to test this is to study unreinforced responding during acute heroin withdrawal. However, acute spontaneous heroin withdrawal can result in depression of locomotor activity in rats (Leri et al., 2003), and this may interfere with operant behavior. As a result, the third experiment focused on rats responding for sucrose pellets because they also display high levels of unreinforced responding (Ghitza et al., 2006; Hood et al., 2007), and because their motivation to obtain the reinforcer can easily be manipulated by food deprivation. Finally, the fourth experiment was designed to assess whether acquisition and maintenance of heroin self-administration is associated with tolerance to the locomotor depressant action of heroin and with sensitization to its motivational properties as assessed by the progressive ratio schedule of reinforcement.

2. Methods and materials

2.1. Subjects

Subjects were 98 adult male Sprague–Dawley rats (Charles River, QC) weighing 375–400 g at the beginning of all experiments. They were singly housed and maintained on a reverse light/dark cycle (8:00 am lights off; 8:00 pm lights on) with free access to food (Teklad Global 14% Protein Rodent Maintenance Diet, Harlan Teklad, Madison, WI), and water except during behavioral testing, which always occurred during the dark cycle. Rats were allowed 6 days to habituate to the animal facility and were handled twice (10 min) before the experiments began. Experiments were approved by the Animal Care Committee of the University of Guelph and followed the guidelines of the Canadian Council on Animal Care.

2.2. Surgery

Rats were surgically implanted with intravenous silastic catheters (Dow Corning, Midland, MI) in the right jugular vein, under general anesthesia induced by a combination of sodium pentobarbital (18.5 mg/kg IP, MTC Pharmaceutical, Cambridge, ON), morphine (5 mg/kg SC, Ontario Veterinary College, Guelph, ON) and diazepam (1 mg/kg SC, Sabex Inc., Boucherville, QC). Rats were given atropine sulfate (4.5 mg/kg SC, Ontario Veterinary College, Guelph, ON) just before surgery and Depocillin (300,000 IU, 0.1 ml/rat IM, Intervet Canada, Whitby, ON) immediately following surgery. The catheter was secured to the vein with silk sutures and was passed subcutaneously to the top of the skull where it exited into a connector (a modified 22 gauge cannula; Plastics One, Roanoke, VA) mounted to the skull with jeweler's screws and dental cement. A plastic blocker was placed over the opening of the connector when not in use. Catheters were flushed daily with 0.1 ml of a saline–heparin solution (0.2 mg/ml Hepalean 1.000 IU, Organon, Toronto, ON).

2.3. Testing apparatus

2.3.1. Operant chamber

Twenty-six Plexiglas operant chambers (model ENV-008CT, Med Associates, Lafayette, IN) were used, each enclosed in larger sound-attenuating plywood chambers (model ENV-018 M, Med Associates). Each operant box had a house light (28 V) and two levers, one retractable and one stationary, located 10 cm apart and 8 cm above the floor of the box. The retractable lever (active lever) was connected to an infusion pump for the delivery of drugs (Razel Scientific Instruments, Stamford, CT), positioned outside the sound-insulating chamber. The stationary lever (inactive lever) served to control for baseline, non-reinforced operant behavior; pressing this lever had no consequence, but all presses were recorded. A white light (28 Watts) located 3 cm above the active lever served as a stimulus light. Six chambers were equipped with a hopper mounted on the exterior of the operant chamber that would deliver sucrose into a magazine feeder located between the active and inactive lever.

2.3.2. Activity chambers

Locomotor activity was monitored using 12 custom-made (University of Guelph) chambers (40 cm×40 cm×28 cm) constructed of semi-transparent Plexiglas and lit by individual LED lights (42 diodes). Each compartment was covered by black wire mesh to allow video tracking during testing. The tracking software employed was EthoVision (version 3, Noldus Information Technology, The Netherlands).

2.4. Drugs

Diacetylmorphine hydrochloride (Heroin; Pharmascience, Montreal, Qc) and cocaine hydrochloride (Dumex, Toronto, On) were dissolved in physiological saline. The doses used for IV self-administration were 0.05 mg/kg/infusions for heroin and 0.5 mg/kg/infusion for cocaine. These were selected because they have been shown to produce reliable acquisition of self-administration, as well as comparable levels of responding (Leri and Stewart, 2001, 2002). A heroin dose of 3 mg/kg (SC) was selected for the locomotion tests because we have found it to produce significant effects on locomotion (Leri and Rizos, 2005).

3. Procedures

3.1. Experiment 1: Unreinforced responding during acquisition of heroin self-administration

The goal of Experiment 1 was to study the development of unreinforced responding during the acquisition of heroin self-administration using a 5 s infusion time. Rats (n=44) were placed in the chambers and their connectors attached to the infusion lines. Prior to the start of each session, rats were given a 5 min period in which the chamber was dark and not activated. Each session started with the activation of the house light, the entry of the retractable lever and the illumination of the light stimulus for 30 s. Subsequently, lever presses on the active lever led to drug infusions according to a FR1 schedule of reinforcement. Drug was infused at a volume of 150 µl over a 5 s period, and during this period, the light stimulus was illuminated. Responses on the active lever made during the infusion were recorded, but did not lead to further infusions. Drug concentration was adjusted for differences in body weight. Rats were trained to self-administer 0.05 mg/kg/infusion heroin for 7 consecutive daily sessions, each lasting 3 h.

3.2. Experiment 2: Unreinforced responding during acquisition of cocaine self-administration

The goal of Experiment 2 was to study the development of unreinforced responding during the acquisition of cocaine self-administration. Thus, 27 rats were trained to self-administer 0.5 mg/kg/infusion cocaine in conditions identical to those used in Experiment 1.

3.3. Experiment 3: Effect of food deprivation on responding for sucrose

The purpose of Experiment 3 was to study the effect of food deprivation on unreinforced responding observed in animals responding for sucrose pellets. As part of another experiment, 6 rats were trained to press a lever for sucrose pellets for about 35 days (45 mg Dustless Precision Pellets; Bio-Serv, Frenchtown, NJ) while restricted to 85% of their body weight. When they were transferred to

Experiment 3, responding was assessed on FR1 schedule under free-feeding conditions for 7 days (i.e., baseline). Then, they were retested during gradual and progressive food deprivation that occurred over 9 days. More specifically, on the first day of food deprivation, rats were given 5 pieces of standard rat chow (approximately 5 g per pellet). For each following 3 days, one piece of chow was omitted, and for the remaining 5 days, pieces of rat chow were omitted in 1/4 amounts. Therefore, on the last day of food deprivation (i.e. Day 9) rats received 1/4 of a piece. After the behavioral test on Day 9, rats were returned to free-feeding conditions, and tested again for 4 additional sessions. All other conditioning parameters were identical to those used in Experiment 1, including a 5s time-out period following a response on the active lever whereby the cue light was illuminated and additional responses did not lead to the delivery of additional sucrose pellets.

3.4. Experiment 4: Effect of intravenous heroin self-administration on the effect of heroin on locomotion activity and on responding for IV heroin on a progressive ratio schedule of reinforcement

In this experiment, we investigated whether acquisition and maintenance of heroin self-administration is associated with tolerance to the depressant action of heroin on locomotion and with sensitization to its motivational properties. This experiment included two groups of animals, 7 rats that self-administered saline and 14 animals that self-administered 0.05 mg/kg/inf heroin. The purpose of the former group was to control for the effect of heroin exposure on locomotor activity. All rats underwent cycles of testing which included: 1) a test of locomotor reactivity to 3 mg/kg SC heroin challenge; 2) repeated sessions of heroin self-administration on a FR1 schedule; and 3) one session of heroin self-administration on a progressive ratio (PR) schedule. A total of 6 tests of locomotion were conducted, and each included one injection of saline and 1 h of behavioral observation, followed by one injection of heroin followed by an additional hour of observation. The parameters for the self-administration sessions on a FR1 were identical to those used in Experiment 1, and animals received a total of 24 sessions (3+3+3+3+12). Distributed within these sessions, rats received a total of 5 tests of PR responding during which responses required for each infusion escalated according to the equation Response ratio $=(5\times e^{(0.2\times \text{infusions number})})-5$, rounded to the nearest integer (Roberts and Bennett, 1993). The dose of heroin available on the PR sessions was also 0.05 mg/kg/infusion.

3.5. Statistical analyses

Independent, repeated-measure and mixed-design ANOVAs with one, two or three factors, as well as planned comparisons, were used as appropriate. Multiple comparisons were performed using the Tukey's Test method to identify individual mean differences (α=0.05) when significant interactions or significant main effects were found. The specific values of non-significant analyses are not reported. Two methods were used to index unreinforced responses: number of reinforced responses (infusions) was subtracted from the total number of responses made on the active lever; and a ratio was calculated by dividing the number of total responses on the active lever by the number of reinforced responses. Statistical analyses were conducted on both indexes. For the analysis of locomotion activity in Experiment 4, only behavior recorded during the initial 30 min of each test is reported because most indicative of the drug effect. All statistical analyses were performed using SigmaStat (version 3.0 for Windows, SPSS Inc).

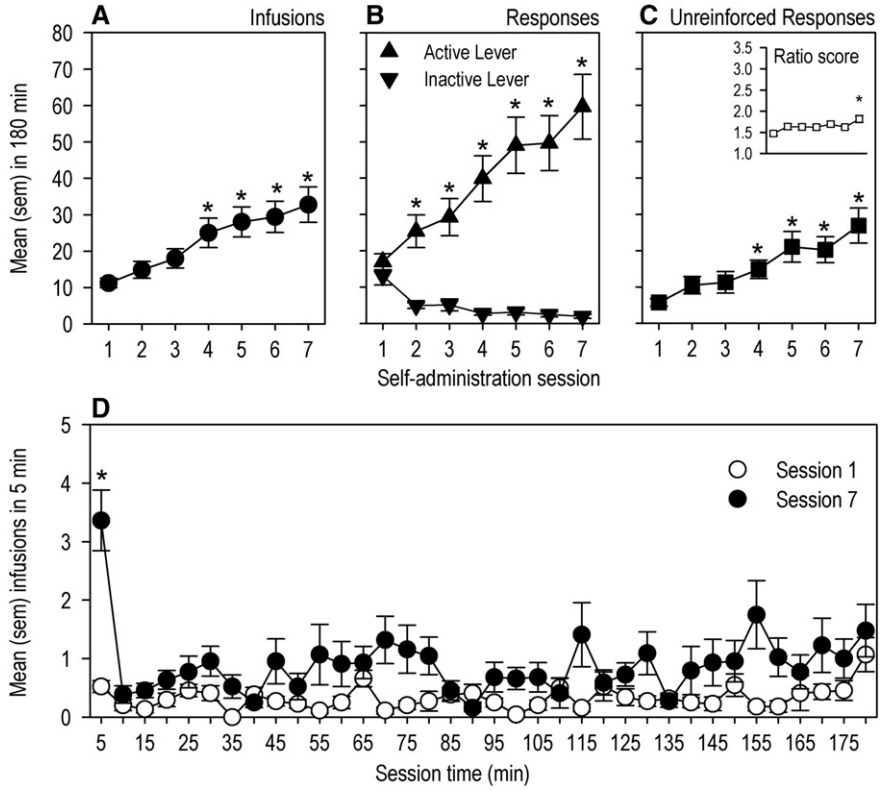

Fig. 1. Panel A — Mean (sem) heroin infusions across 7 self-administration sessions. Panel B — Mean (sem) responses made on the active and inactive levers across self-administration sessions. Panel C — Mean (sem) unreinforced responses calculated as difference between responses and infusions. Insert in Panel C — unreinforced responses calculated as ratio of responses to infusions. The * indicates a significant difference from session 1. Panel D — Mean (sem) heroin infusions taken in 5 min across self-administration sessions 1 and 7. The * indicates a significant difference between sessions, $p<0.001$.

4. Results

4.1. Experiment 1

4.1.1. Overall heroin intake, active and inactive lever responses

As displayed in Fig. 1A, the number of heroin infusions increased significantly across heroin self-administration sessions [$F(6,258)=20.7$, $p<0.001$]. Multiple comparisons revealed that heroin infusions increased significantly from self-administration session 1 to sessions 4–7 ($p<0.001$). Furthermore, rats displayed significant increases in responding on the active lever in parallel with significant decreases in responding on the inactive lever (Fig. 1B; Session by Lever interaction [$F(6,258)=25.3$, $p<0.001$] and main effect of Lever [$F(1,43)=34.7$, $p<0.001$] and of Session [$F(6,258)=14.1$, $p<0.001$]), and responding on these two levers differed significantly on each session after session 1.

4.1.2. Unreinforced responses

Unreinforced responses also increased across heroin self-administration sessions (Fig. 1C; difference score [$F(6,252)=12.4$, $p<0.001$] and ratio [$F(6, 252)=3.2$, $p<0.005$).

4.1.3. Heroin infusions and unreinforced responding over session time across acquisition

To explore the possibility that unreinforced responding reflected a form of drug loading characteristic of the initial stages of a drug self-administration session (Campbell and Carroll, 2000; Garcin et al., 1977), we compared heroin infusions obtained during the entire duration of the initial and final self-administration sessions, and significant differences were found (Fig. 1D; Time by Session interaction [$F(35,1505)=2.7$, $p<0.001$] and main effect of Time [$F(35,1505)=3.5$, $p<0.001$] and main effect of Session [$F(1,43)=30.6$, $p<0.001$]). Post-hoc comparisons revealed that the primary difference was during the initial 5 min of the sessions ($p<0.001$). Because of this, we compared infusions and unreinforced responses during the first 5 min of each self-administration session (data not shown) and, in both cases, significant increases over the acquisition period were found (infusions: [$F(6, 36)=15.3$, $p<0.001$]; unreinforced responding: difference score [$F(6, 36)=7.2$, $p<0.001$] and ratio score from session 1 to 7 [$t(16)=-3$, $p<0.01$]).

4.2. Experiment 2

4.2.1. Overall cocaine intake, active and inactive lever responses

As displayed in Fig. 2A, the number of cocaine infusions increased significantly across cocaine self-administration sessions [$F(6,156)=5.2$, $p<0.001$]. Multiple comparisons revealed that cocaine infusions increased significantly from self-administration session 1 to sessions 3–7 ($p<0.05$). Furthermore, results of the two way repeated measures ANOVA revealed a significant main effect of Lever (Fig. 2B; [$F(1,26)=31.6$, $p<0.001$]), but no significant effect of session, although there was a clear trend toward increases in responding on the active lever and decreases in responding on the inactive lever. Nevertheless, responding on the active and inactive levers differed significantly on each session.

4.2.2. Unreinforced responses

As displayed in Fig. 2C, unreinforced responses significantly decreased across cocaine self-administration sessions (difference score not significant; ratio score [$F(1,26)=3.4$, $p<0.01$]).

4.2.3. Cocaine infusions over session time across acquisition

Similarly to heroin, cocaine infusions obtained during the entire duration of the initial and final self-administration differed, primarily because of larger drug intake during the initial 5 min of the last session

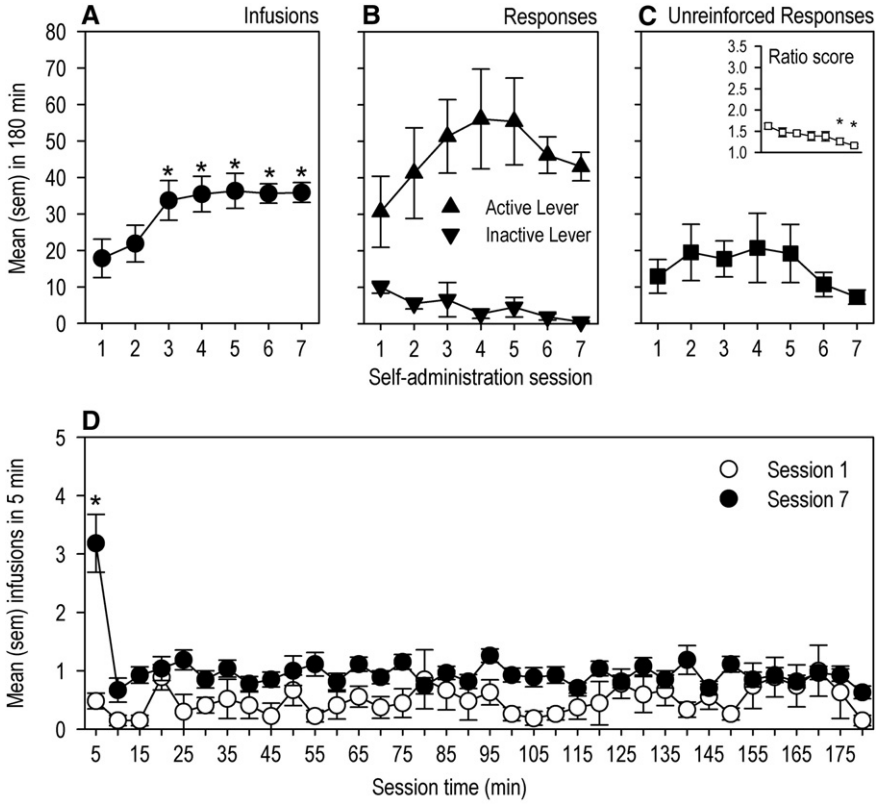

Fig. 2. Panel A — Mean (sem) cocaine infusions across 7 self-administration sessions. Panel B — Mean (sem) responses made on the active and inactive levers across self-administration sessions. Panel C — Mean (sem) unreinforced responses calculated as difference between responses and infusions. Insert in Panel C — unreinforced responses calculated as ratio of responses to infusions. The * indicates a significant difference from session 1. Panel D — Mean (sem) cocaine infusions taken in 5 min across self-administration sessions 1 and 7. The * indicates a significant difference between sessions, $p<0.001$.

(Fig. 2D – session 1 to 7 – Time by Session interaction [$F(35,910)=3.2$, $p<0.001$] and main effect of Time [$F(35,910)=3$, $p<0.001$] and main effect of Session [$F(1,26)=10.5$, $p<0.01$]).

4.3. Experiment 3

4.3.1. Effect of food deprivation on responding for sucrose

An average of the last 7 sessions under free-feeding conditions was used as a baseline. Gradual food deprivation induced a significant increase in number of pellets obtained (Fig. 3A; [$F(13,65)=8.4$, $p<0.001$]) as well as responses on the active lever (Fig. 3B; Session by Lever interaction [$F(13,65)=9.1$, $p<0.001$] and main effect of Lever [$F(1,5)=39$, $p<0.01$] and of Session [$F(13,65)=10$, $p<0.001$]). Responses on the active lever returned to baseline levels when food deprivation was terminated ($p<0.05$). Responding on the active and inactive levers differed significantly on each session.

4.3.2. Effect of food deprivation on unreinforced responses

Similarly to heroin self-administration, rats responding for sucrose displayed unreinforced responding and its magnitude was significantly enhanced by food deprivation across sessions (Fig. 3C; difference score [$F(13,65)=7.7$, $p<0.001$] and ratio [$F(13,65)=4.3$, $p<0.001$]).

4.4. Experiment 4

4.4.1. Overall heroin intake, active and inactive lever responses

The self-administration behavior of rats lever-pressing for saline was not included in the following analyses.

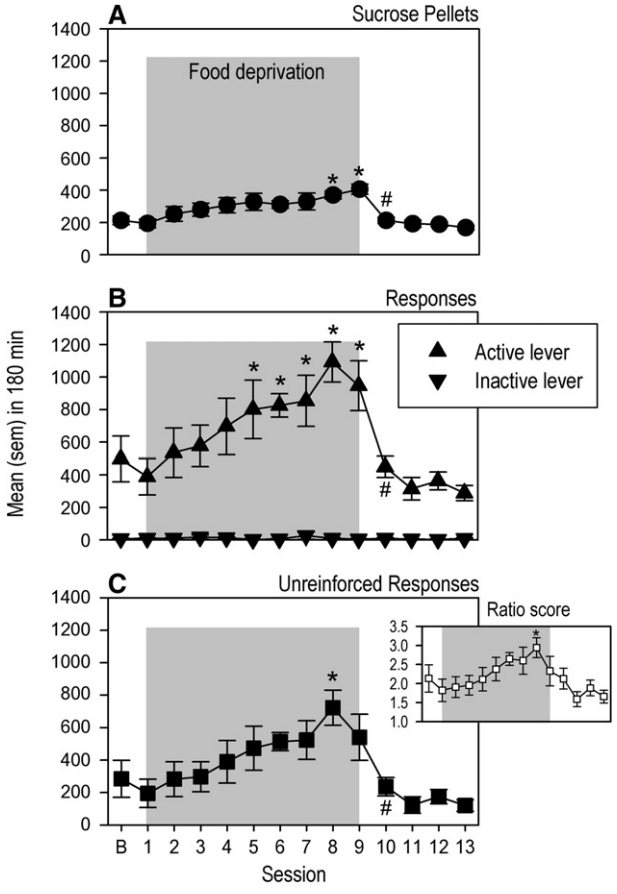

Fig. 3. Panel A — Mean (sem) sucrose pellets obtained across 14 sessions. Panel B — Mean (sem) responses made on the active and inactive levers across test sessions. Panel C — Mean (sem) unreinforced responses calculated as difference between responses and sucrose pellets. Insert in Panel C — unreinforced responses calculated as ratio of responses to sucrose pellets. The grey shaded area in each panel indicates the period of food deprivation. The * indicates a significant difference from the baseline session (B), $p<0.05$. The # indicates a significant difference between sessions 9 and 10.

As displayed in Fig. 4A, infusions increased significantly across heroin self-administration sessions [$F(23,299)=4.7$, $p<0.001$]. Multiple comparisons revealed that heroin infusions increased significantly from self-administration session 1 to 19–24 ($p<0.05$). Furthermore, rats displayed significant increases in responding on the active lever in parallel with significant decreases in responding on the inactive lever (Fig. 4B; Session by Lever interaction [$F(23,299)=4.6$, $p<0.001$] and main effect of Lever [$F(1,13)=61.9$, $p<0.001$] and of Session [$F(23,299)=4.3$, $p<0.001$]), and responding on these two levers differed significantly on each session after session 1.

4.4.2. Unreinforced responses

Unreinforced responses also increased significantly across heroin self-administration sessions (Fig. 4C; difference score [$F(23,299)=4.7$, $p<0.001$] and ratio score [$F(23,299)=4$, $p<0.001$]).

4.4.3. Heroin infusions and responses on the active lever during PR tests

A significant increase in heroin infusions across heroin self-administration sessions on the PR schedule was found (Fig. 5A; [$F(4,52)=7.2$, $p<0.001$]). Multiple comparisons revealed that heroin infusions increased significantly from PR test 1 to 2–5 ($p<0.05$). Similarly, rats displayed significant increases in responding on the active lever (Fig. 5B; [$F(4,52)=4.5$, $p<0.01$]) from PR test 1 to 5 ($p<0.05$).

4.4.4. Locomotion

Fig. 6 represents distance moved by animals that self-administered vehicle or heroin after acute saline and heroin (3 mg/kg) injections administered on tests 1 and 6. A three factors mixed-design ANOVA revealed a significant Group by Locomotion test by Injection interaction [$F(1,19)=31.8$, $p<0.001$], as well as significant Locomotion test by Injection interaction [$F(1,19)=128.8$, $p<001$], main effect of Group [$F(1,19)=18.3$, $p<0.001$] and main effect of Injection [$F(1,19)=351.4$, $p<0.001$]. Post-hoc tests revealed that, in comparison to a saline injection, acute 3 mg/kg heroin produced a significant decrease in locomotion in both groups on test 1. However, on test 6, the sedative effect of heroin was no longer observed in those animals that self-administered heroin.

5. Discussion

These experiments were designed to explore psychopharmacological factors modulating the development of unreinforced responding during the acquisition of heroin self-administration in limited access conditions. Four main results were obtained. First, the occurrence of this behavior during the acquisition of heroin self-administration was obtained even at a relatively short infusion time. Second, it was found that unreinforced responding is minimal in rats lever-pressing for the dose of cocaine used in our study. Third, unreinforced responding was observed in rats lever-pressing for sucrose and this behavior was sensitive to changes in food deprivation. Fourth, repeated heroin self-administration lead to increases in responding for heroin on a progressive ratio, and decreases in heroin-induced depression of locomotor activity.

There are several possible explanations as to why rats develop unreinforced responding during limited access to heroin self-administration. For example, it may be that heroin impedes their ability to learn the operant response, but rats in these experiments clearly learned to selectively respond on the lever that was associated with heroin infusions, and there is evidence that administration of heroin can actually facilitate learning (Castellano, 1980). It may also be that unreinforced responding reflects the development of superstitious behavior (Skinner, 1948), but this cannot account for the fact that the animals responding for cocaine did not develop unreinforced responding. Finally, it could be that unreinforced responding is not really "unreinforced" because animals actually receive IV infusions of heroin while emitting the extra responses. But, this explanation

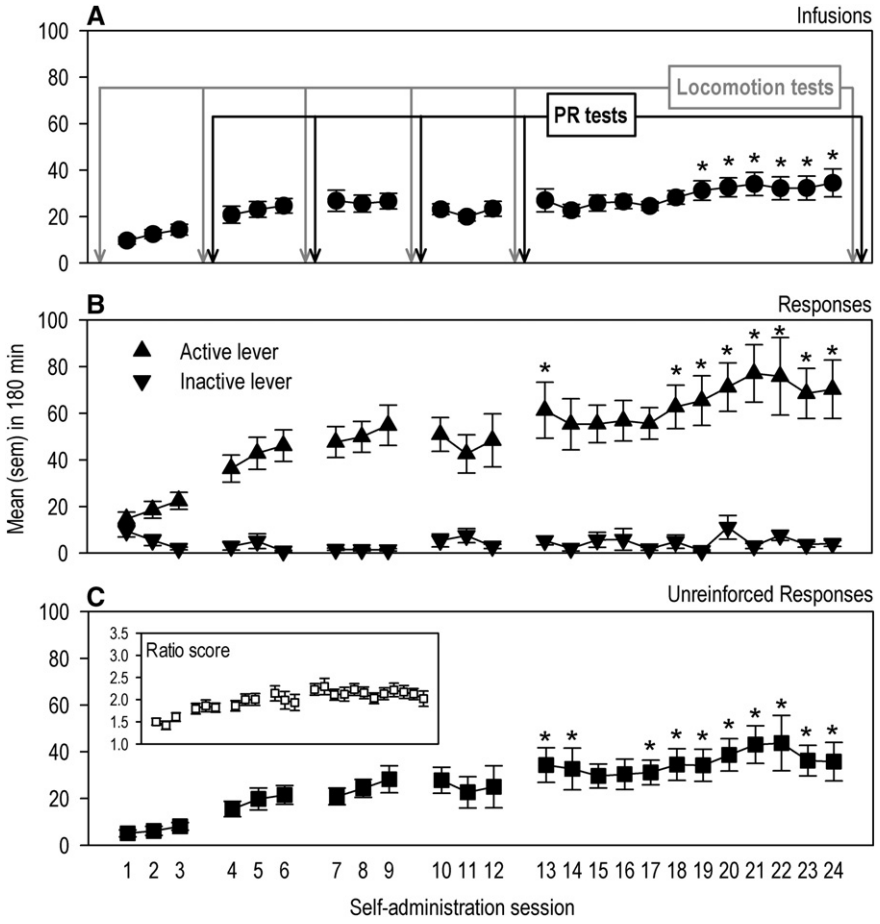

Fig. 4. Panel A — Mean (sem) heroin infusions across 24 self-administration sessions. Panel A also illustrates when animals received tests of locomotion and of self-administration on the PR. Panel B — Mean (sem) responses made on the active and inactive levers across self-administration sessions. Panel C — Mean (sem) unreinforced responses calculated as difference between responses and infusions. Insert in Panel C — unreinforced responses calculated as ratio of responses to infusions. The * indicates a significant difference from session 1.

cannot account for the increase and stabilization of this behavior with additional training, and it is not supported by the presence and absence of unreinforced responding in rats tested with sucrose and cocaine, respectively.

The question that arises from these studies is why unreinforced responding develops during self-administration of heroin, but not cocaine, a difference that has been observed even in individual rats given access to both heroin (0.025–0.1 mg/kg/infusion) and cocaine (0.25–2 mg/kg/infusion) on alternative days (Leri and Stewart, 2001). It should be noted that one study reported unreinforced responding during cocaine self-administration, but this was only observed in a specific strain of inbred rats (Fischer 344) administering a low dose (0.0625 mg/kg/inf) under an FR3 schedule and an extended time-out period (10 s infusion +5 s time-out; Kosten et al., 2007).

One answer may be that the motivation to self-administer heroin changes during the initial stages of self-administration, but it remains constant for cocaine. The results of experiments performed using

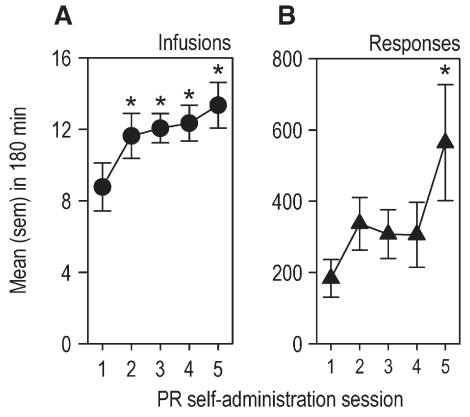

Fig. 5. Panels A and B — mean (sem) infusions and responses on the active lever during the five PR tests. The * indicates a significant difference from PR test 1, $p<0.05$.

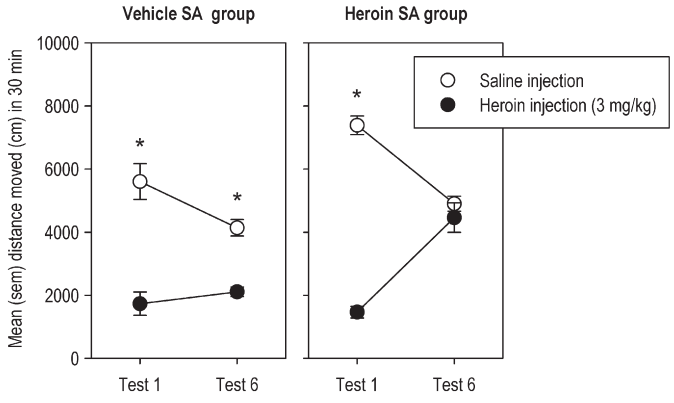

Fig. 6. Mean (sem) distance (cm) moved by animals that self-administered vehicle or heroin (0.05 mg/kg/infusion) after acute saline and heroin (3 mg/kg) injections administered on locomotion tests 1 and 6. The * indicates a significant difference from test 1 to 6, $p<0.05$.

sucrose as reinforcer partially support this interpretation. In fact, Hood et al. (2007) and Ghitza et al. (2006) reported increases in unreinforced responding during acquisition of the operant response, and the results of Experiment 3 indicate that level of unreinforced responding is highly sensitive to changes in motivation for the reward, in this case produced by food deprivation. In addition, it was found that responding for heroin on a progressive ratio schedule increased over the course of heroin self-administration on a FR1. This is not likely to result from increased familiarity/learning of the schedule because repeated PR testing with non-drug rewards leads to reduced breakpoints (Gulley, 2007). Rather, the increase in responding found in Experiment 4 is likely to reflect sensitization to the rewarding properties of heroin (for review see Ahmed, 2005; Lett, 1989). However, it is unlikely that the motivational properties of cocaine remained unchanged as other studies have found clear evidence of sensitization, even after limited access (Liu et al., 2007, 2005; Morgan et al., 2006). And, although we did not analyze PR responding in cocaine-trained rats, we did observe the development of 'drug loading' in the initial 5 min of the self-administration sessions, which can be viewed as a mild version of cocaine loading associated with escalation of intake following longer periods of access to cocaine self-administration (Ahmed and Koob, 1998, 1999; Tornatzky and Miczek 2000; Mantsch et al., 2004).

A further factor possibly implicated in the difference between heroin, sucrose and cocaine self-administration behavior may be ability to respond on the active lever. That is, even if unreinforced responding reflects a transition from goal-directed to habitual responding (Everitt and Wolf, 2002) that could occur regardless of the nature of the reinforcer, the delivery of heroin, sucrose and cocaine will differentially modulate the ability to press the lever. That is, while sucrose intake may not significantly alter ability to respond, heroin and cocaine could, and these effects may change in different directions as a result of drug self-administration. In the case of heroin, for example, although we did not assess activity within the operant chambers, we did measure the acute effect of 3 mg/kg heroin on horizontal activity at different stages self-administration, and found significant tolerance to its depressant action (see Fig. 6). Hence, it is possible that animals became progressively less sedated during self-administration (Madden et al., 1983) and therefore progressively more capable of responding on the lever. Cocaine, however, induces hyperactivity and stereotypic behaviors (Randrup and Munkvad, 1969; Fowler et al., 2007), which are generally focused away from the operant lever (see Zernig et al., 2007; Segal, 1975), and are more pronounced immediately following IV infusions (Fowler et al., 2007). Therefore, the ability to emit unreinforced responses may get progressively more impaired by the delivery of standard doses of cocaine during self-administration.

Finally, it is possible that the differential development and maintenance of unreinforced responding during heroin and cocaine self-administration may reflect differences in the way these two drugs activate neural centers involved in incentive motivation (Di Chiara, 1995). That is, although both heroin and cocaine can increase dopamine concentrations in the ventral striatum (Di Chiara and Imperato, 1988; Gratton, 1996; Wise et al., 1995a,b), they do so via different mechanisms (Gratton, 1996; Johnson and North, 1992; Joyce and Iversen, 1979; North, 1992; White, 1996, 1990), and a differential involvement of central dopaminergic systems in heroin and cocaine self-administration has been reported (Chang et al., 1998; De Vries et al., 1999; Ettenberg et al., 1982; Gerrits and Van Ree, 1996; Hemby et al., 1995, 1999; Pettit et al., 1984).

In conclusion, the results of these experiments indicate that the development of unreinforced responding during the acquisition of heroin self-administration in limited access conditions reflects parallel shifts in the motivation and the ability to obtain heroin. These data are consistent with other studies in animals suggesting that drug intake is regulated by both incentive and aversive drug effects (Goldberg et al., 1983; Panlilio et al., 2003; Pickens et al., 1969; Spealman and Kelleher, 1979). Extrapolating these results to humans, it is possible to conclude that the balance between factors that promote and limit drug intake may be an important determinant of the transition from occasional drug use to abuse and dependence.

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