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Motivation of heroin-seeking elicited by drug-associated cues is related to total amount of heroin exposure during self-administration in rats

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Abstract

Conditioned stimuli (CSs) previously associated with heroin are critically involved in activating long-lasting relapse and compulsive drug seeking. This study examined the magnitude of heroin seeking induced by drug-related cues in relation to the total amount of drug exposure during training. Five groups of male Sprague–Dawley rats (n=6/group) were trained by the nose-poking response to self-administer different doses of heroin (0, 0.01, 0.025, 0.05, and 0.1 mg/kg per infusion respectively, one 4-h session daily, limited to 25 infusions per session) under an identical progressive ratio schedule with gradual incremental response requirements. All the rats established stable heroin self-administration within 14 days of self-administration training, and the time needed to obtain all the 25 heroin infusions decreased across sessions. After 14 days of abstinence, heroin seeking elicited by contextual cues (self-administration chamber) or discrete contingent CSs previously associated with heroin infusions was measured in two consecutive 1-h test phases. During both test phases, the rats trained with heroin even at the lowest dose (0.01 mg/kg) showed higher active responses than saline controls, and the active responses were also higher in rats trained with doses of 0.025, 0.05, and 0.10 mg/kg in comparison with those trained with a dose of 0.01 mg/kg. There was no observable dose-dependence increase of responses at doses above 0.025 mg/kg. The results suggested that an increased motivation to seek heroin induced by drug-related cues is associated with the total amount of heroin intake.

Keywords: Heroin; Self-administration; Relapse; Cue

1. Introduction

Drug addiction is a chronic relapsing disorder characterized by compulsive drug taking and drug seeking. The development of addiction often involves a gradual process of escalated drug intake, whereby the transition from controlled to uncontrolled drug use occurs with repeated and chronic exposure to a drug (Koob and Le Moal, 1997; Ahmed and Koob, 1998; Koob et al., 2004). Environmental stimuli previously associated with drug availability acquire conditioned reinforcement and a motivational property through Pavlovian conditioning, and can elicit craving and relapse in abstinent individuals (Childress et al., 1992). Mounting evidence has confirmed the role of environmental cues in precipitating relapse in animal models (Crombag and Shaham, 2002; Gracy et al., 2000; Di Ciano and Everitt, 2002; See, 2002; Weiss et al., 2001; Shalev et al., 2002). However, the behavioral characteristic of the motivating property of drug-related environmental stimuli in relation to the total amount of previous drug exposure has yet to be systemically clarified in animal models.

Currently, only a few studies have examined drug priming or stress-induced "relapse" intensity in relation to the total amount of drug self-administered. Ahmed et al. (2000) have demonstrated that rats trained for 11 h/day to self-administer heroin produce an enhanced response to footshock stress-induced reinstatement of heroin seeking compared with rats trained for 1 h/day. Studies on psychomotor stimulants also suggest that the magnitude of reinstatement is associated with the amount of drug

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intake during training (Deroche et al., 1999; Sutton et al., 2000; Baker et al., 2001). The purpose of the present study was to resolve the question of whether the strength of the motivating property of heroin-related environmental stimuli was correlated to the total amount of drug exposure during training. We trained rats by nose-poke responding to self-administer different doses of heroin, during which each heroin infusion was paired with compound conditioned stimuli (CSs). After a period of abstinence, heroin seeking elicited by the drug-related stimuli was measured when the rats were reintroduced into the self-administration chambers.

2. Materials and methods

2.1. Animals

Thirty male Sprague–Dawley rats (Zhejiang Experimental Animal Center, China), weighing between 280 and 300 g at the beginning of the experiment, were housed individually in stainless-steel mesh home cages (size 25×30×30 cm) in a temperature-controlled ventilated colony room with a 12-h light/dark cycle (lights on 7:00 a.m.–7:00 p.m.). Food and water were available ad libitum. Experimental sessions were performed between 8:30 a.m. and 5:00 p.m. All procedures were conducted in accordance with the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*.

2.2. Surgical procedure

The animals were implanted with chronically indwelling intravenous catheters under sodium pentobarbital (50 mg/kg ip) anesthesia. A silicon catheter (3.5 cm length, 0.5 mm inner diameter, 0.94 mm outer diameter) was inserted into the right external jugular vein and secured with thread so that the tip reached the right atrium. The other end of the catheter (10 cm length, PE20) exited from an incision on the back of the body. The catheters were flushed daily with 0.2 ml saline containing sterile benzylpenicillin sodium (60,000 units) and heparin (5 units), to prevent bacterial infection and maintain catheter patency, and capped daily. All the animals were allowed to recover for at least 7 days.

2.3. Self-administration apparatus

Training and testing were conducted in stainless-steel operant chambers (size 30×30×30 cm) placed in a sound-attenuated, temperature-controlled room; the light of the room was turned off during training. The apparatus consisted of 12 chambers equipped with two nose-pokes (ENV-114M, Med Associates, Lafayette, IN) in the back wall. There were three LED lights (green, red, and yellow) inside each nose-poke hole. A cue-light (28 V, 0.1 mA, ENV-215M, Med Associates) was situated on the wall above the nose-pokes. Drug solution was delivered through Tygon tubing, protected by a leash assembly (PHM-120, Med Associates) and suspended through the ceiling of the chamber from a plastic fluid swivel (PHM-115, Med Associates). The leash assembly was modified to fit a custom-made fluid connector fixed with an animal jacket. The Tygon tubing was attached to a syringe pump (PHM-100, Med Associates) that delivered fluid at a speed of 1.08 ml/min using a 10-ml syringe. The experimental events were controlled by an IBM-compatible PC using a MED Associates interface, running self-programmed software (OBSM v4.0, operant behavioral schedule manager) written in Borland Delphi 6.0. Diacetylmorphine HCl (heroin) was obtained from the Institute of Forensic Science Ministry of Public Security of the People's Republic of China and dissolved in physiological saline. The infusion duration was about 3–4 s with a volume of about 60–70 μl, depending on the unit injection dose, the drug concentration, the pump speed, and the body weight of the rat.

2.4. Self-administration procedure and conditioning protocols

All the rats underwent an identical sequence of behavioral training and testing. They were randomly divided into five groups (n=6 per group). Each rat was trained with one daily 4-h session for 14 consecutive days with either saline (100 μl) or heroin (10, 25, 50, and 100 μg/kg per injection, respectively) self-administration. The animals were transferred into the operant chambers before each training session, and put back in their individual home cages shortly after the session where food was available. Water was always available both in the test cages and home cages. Enough food was provided to maintain natural weight gain.

The reinforcement schedule was a modified progressive ratio schedule that involved incrementing response requirements in a relatively gradual manner. The response requirement increased in a linear pattern as calculated according to the following equation: response requirement=truncate (0.2*(step−1)+1), where the results were truncated to integer value. The step number is the number of ratios completed. So in each daily session, the response requirements were 1 for the first five heroin infusions, 2 for the second five infusions, 3 for the third five infusions, 4 for the fourth five infusions, and 5 for the last five infusions. Based on our preliminary experiment, this schedule supported reliable heroin self-administration across a range of heroin doses, and the response training was as easy as a FR1 schedule but maintained a relatively high rate of responding, so the schedule could be kept constant throughout the sessions.

Each trial started with the illumination of a green light inside the active nose-poke hole. The completion of a ratio requirement on the active nose-poke resulted in the delivery of one infusion. The infusion was either 10 μg/kg of heroin for the HER-10 group (heroin concentration 0.04 mg/ml),

25 μg/kg for the HER-25 group (heroin concentration 0.1 mg/ml), 50 μg/kg for the HER-50 group (heroin concentration 0.2 mg/ml), or 100 μg/kg for the HER-100 group (heroin concentration 0.4 mg/ml; in order to minimize the acute toxic effects of heroin at large doses, for the first week, heroin infusion doses were 50 μg/kg and then were changed to 100 μg/kg for the second week); or 100 μl of saline for the SALINE group. Each infusion was paired with 5 s illuminations of the cue-light and the red light inside the active nose-poke hole, in combination with the pump noise; all these stimuli served as discrete CSs. A timeout period was imposed for 30 s, during which the green light inside the active nose-poke hole was extinguished and responding produced no programmed consequences but was still recorded. Illumination of the green light signaled the end of the 30-s infusion-timeout period. Under all circumstances, responding at the inactive nose-poke produced no programmed consequences. The session ended after 25 infusions were earned or 4 hours had passed, whichever came first.

2.5. Abstinence and reinstatement

After 14 days of self-administration training, the rats were made to abstain from heroin for another 14 days during which they were confined to their individual home cages. The choice of abstinence duration was based on our previous work (Zhou et al., 2004). Using the same experimental procedure, we found that there were no significant differences of discrete CS-induced heroin seeking among the rats after 1, 2, and 4 weeks abstinence from self-administration.

The reinstatement testing lasted for 2 hours and each animal was tested only once. During testing, the animals still wore their jackets, but the leash assemblies were not connected, and water was available, although food was provided only after testing. The testing consisted of two consecutive 1-h phases. During the first phase, the rats were allowed to respond to the nose-pokes with all the conditioned stimulus lights kept off. The responses were recorded but no consequences were produced. This phase was used to measure contextual cue- (chamber environment) induced heroin seeking, and was generally regarded as an extinction phase.

Immediately after the first phase, the second phase began. This was signaled by one 5-s presentation of the CSs (the red nose-poke light, the cue light, and the pump noise), which was previously paired with each heroin infusion. During this phase, the green light inside the active nose-poke hole was turned on, and each active response resulted in another 5-s presentation of the CSs and the turning off of the green nose-poke light. After turning off the CSs, another trial began. This phase was used to measure discrete CS-induced heroin seeking.

2.6. Data analysis

Experimental data were given as mean±S.E.M. The differences in total active responses, heroin infusions,

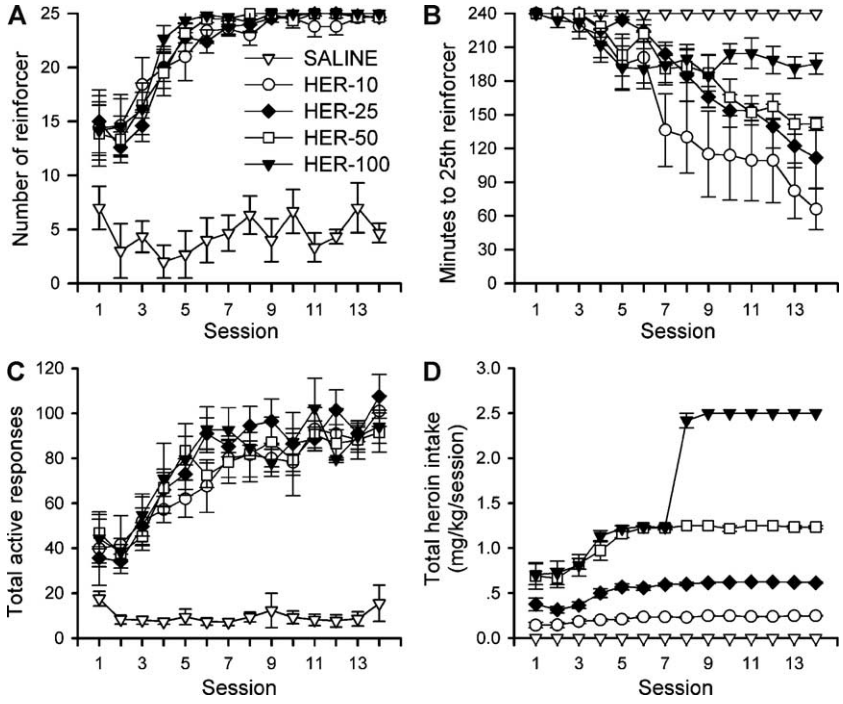

Fig. 1. Acquisition of intravenous heroin self-administration. Data were expressed with mean±S.E.M. of total infusions (A), time needed to obtain all 25 infusions (B), total active nose-poke responses (C), and total heroin intake (D) during each daily session.

and the time needed to obtain all 25 available heroin infusions per session during heroin self-administration were analyzed using two-factor analysis of variance (ANOVA) with *session* as a repeated within-subject factor and *heroin group* as a between-subject factor. Contextual cues and discrete CS-induced active responding during reinstatement testing were also analyzed using two-factor repeated ANOVA with *time block* (15 min) as a within-subject factor and *group* as a between-subject factor. The between-subjects dose–response curve and the total amount of heroin intakes during self-administration, as well as the total amount of active reinstatement responses, were analyzed for differences using one-factor ANOVA. Post hoc comparisons were conducted using Newman–Keuls tests and pairwise comparisons were conducted using LSD adjustments. P values less than 0.05 were considered significant. The values of the heroin satiety threshold and the heroin elimination half-life were generated using nonlinear regression analysis of the mean inter-injection intervals of all heroin-trained rats. Correlation tests between total heroin intake and reinstatement responding were also conducted using nonlinear regression analysis. Statistical analysis was conducted using SPSS software (SPSS, Chicago, IL) and nonlinear regression analysis was performed using Sigmaplot (SPSS, Chicago, IL).

3. Results

3.1. Heroin self-administration

All heroin-trained rats reached stable levels of heroin infusions within 14 days of heroin self-administration training without there being any differences between groups in total active responses and infusions per session. This was reflected by the nonsignificant main effects of heroin groups [$F(3,20)=1.19$, $F(3,20)=0.53$, respectively, for total responses and infusions per session; NS] and interactions between heroin groups and training sessions [$F(39,260)=1.21$, $F(39,260)=0.481$, respectively, for total responses and infusions per session; NS] (Fig. 1A and C). The time needed to obtain all the 25 infusions decreased across sessions with differences between heroin groups. The results of repeated ANOVAs showed significant main effects of group [$F(3,20)=0.53$, $P<0.01$] and interaction between group and session [$F(39,260)=5.73$, $P<0.001$] (Fig. 1B). The rats that were trained with saline could not establish stable self-administration; just a few infusions were made within each session (Fig. 1A). Only minimal responding was observed at the inactive nose-poke for all the training groups and during all the training sessions (data not shown).

An analysis of heroin intake during the first hour of the last days of heroin self-administration showed significant

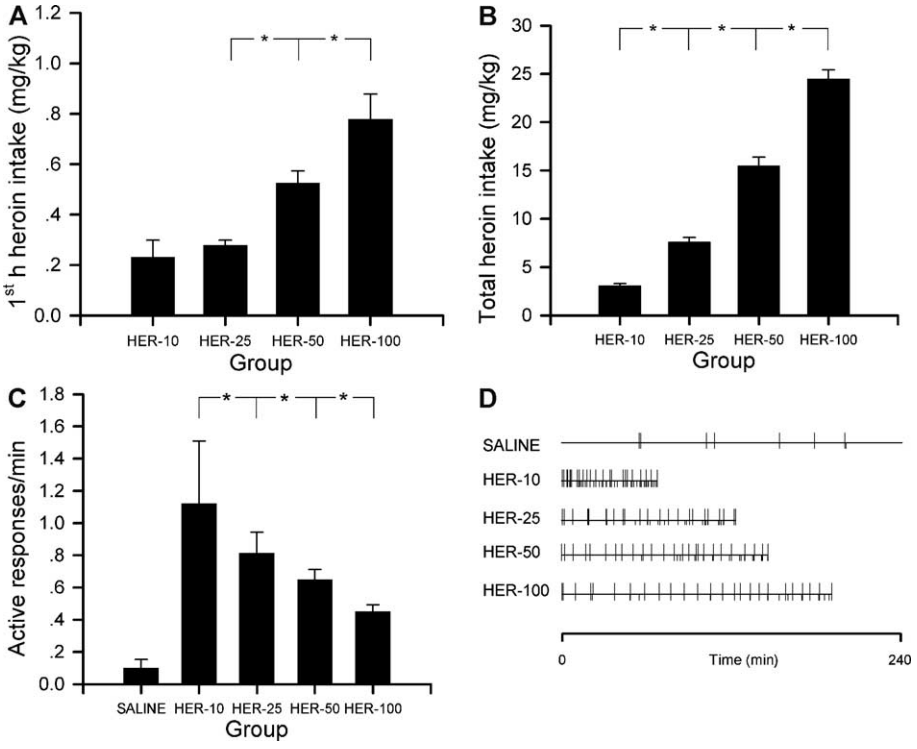

Fig. 2. (A) Mean heroin intake during the first hour of the last three sessions of self-administration. (B) Total heroin exposed during 14 days of self-administration training. (C) Between-subjects dose–response function of the last three days of heroin self-administration. (D) Representative event record on the last day of heroin self-administration. Each bar above the horizontal line represents one earned infusion, whereas each bar below the line represents a response to the active nose-poke. *P<0.05.

differences in drug levels between groups [$F(3,23)=148.6$, $P<0.001$] (Fig. 2A). Newman–Keuls post hoc test comparisons revealed that the rats infused more heroin with increasing doses at 25, 50, and 100 μg/kg ($P<0.05$), but that there was no significant difference between doses of 10 and 25 μg/kg (NS). The total amounts of heroin intakes during the 14 days of self-administration were different between heroin groups [$F(3,23)=1086.8$, $P<0.001$] (Fig. 1D, Fig. 2B). The between-subjects measure of the dose–response curve (Fig. 2C) of heroin self-administration showed that the response rate was negatively correlated with the heroin infusion dose. One way ANOVA on response rate revealed a significant effect of the dose [$F(3,11)=5.476$, $P<0.05$]. Heroin infusion and the response patterns during self-administration are illustrated in Fig. 2D.

The effect of the unit dose of heroin on the mean inter-infusion interval was analyzed by nonlinear regression using a model described by Tsibulsky and Norman (1999). The calculated mean heroin satiety threshold (D_{ST}) was 3.8±1.6 μg/kg, and the mean elimination half-life of heroin was 1.6±1.2 minutes (Fig. 4A).

3.2. Heroin-related environmental cue-induced drug seeking

Heroin cue-induced reinstatement of active responding occurred mainly in the first 15 min blocks and decreased across blocks during both reinstatement testing phases (Fig. 3A). Repeated ANOVAs revealed significant main effects of block [$F(3,75)=31.52$, $F(3,75)=213.02$, respectively, for contextual and discrete CSs, $Ps<0.001$] and group [$F(4,25)=14.98$, $F(4,25)=35.09$, respectively, $Ps<0.001$], and also a significant interaction between block and group [$F(12,75)=3.10$, $F(12,75)=15.90$, respectively, $Ps<0.05$]. Newman–Keuls post hoc test comparisons revealed significant differences in active responding between the groups of heroin-and saline-trained rats ($Ps<0.05$). Responses were also higher in heroin-trained rats at doses of 25, 50, and 100 μg/kg/infusion than at doses of 10 μg/kg/infusion (all $Ps<0.05$). However, there were no significant differences between doses of 25, 50, and 100 μg/kg/infusion ($Ps>0.05$). Responding at the inactive nose-poke was minimal during both testing phases (Fig. 3B).

When the total amount of active responses were analyzed, one-way ANOVA revealed significant effects of group for both contextual cues [$F(5,35)=12.88$, $P<0.001$] (Fig. 3C). Furthermore, discrete CSs [$F(5,35)=35.37$, $P<0.001$] (Fig. 3D) testing phases and total responses were significantly higher in all doses among heroin-trained rats than among saline-trained rats (all $Ps<0.05$). The total amount of responses was also higher for rats trained at doses of 25, 50, and 100 μg/kg/infusion than for those trained at a dose of 10 μg/kg/infusion. No differences were found between doses of 25, 50, and 100 μg/kg/infusion (NS).

Correlation tests were performed to assess the relationship between total heroin intake during self-administration and drug cue-induced active responding. A nonlinear

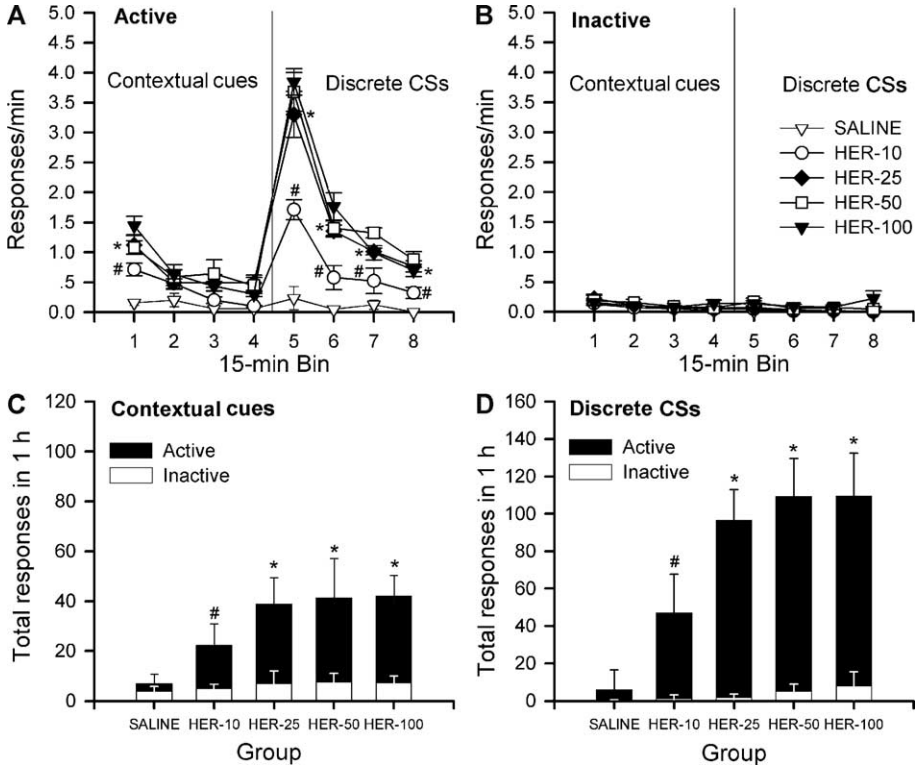

Fig. 3. Contextual cue-induced or discrete CS-induced heroin seeking after 2 weeks of abstinence from self-administration. Data were expressed as mean±S.E.M. of active (A) and inactive (B) response rates in 15-min blocks, contextual cue-induced total responses in 1 h testing phase (C), and discrete CS-induced total responses in 1 h testing phase (D). $^{\#}P<0.05$, different from SALINE. $^{*}P<0.05$, different from HER-10.

regression showed an exponential relationship between reinstatement responses and total heroin intake for both contextual cues ($r = 0.8220$, see Fig. 4B) and discrete CSs ($r = 0.9369$, see Fig. 4B). A linear positive correlation existed between doses of 0, 10, and 25 μg/kg ($r = 0.899$, $r = 0.963$, respectively, for contextual cues and discrete CSs), but not between doses of 25, 50, and 100 μg/kg ($r = 0.038$, $r = 0.222$, respectively).

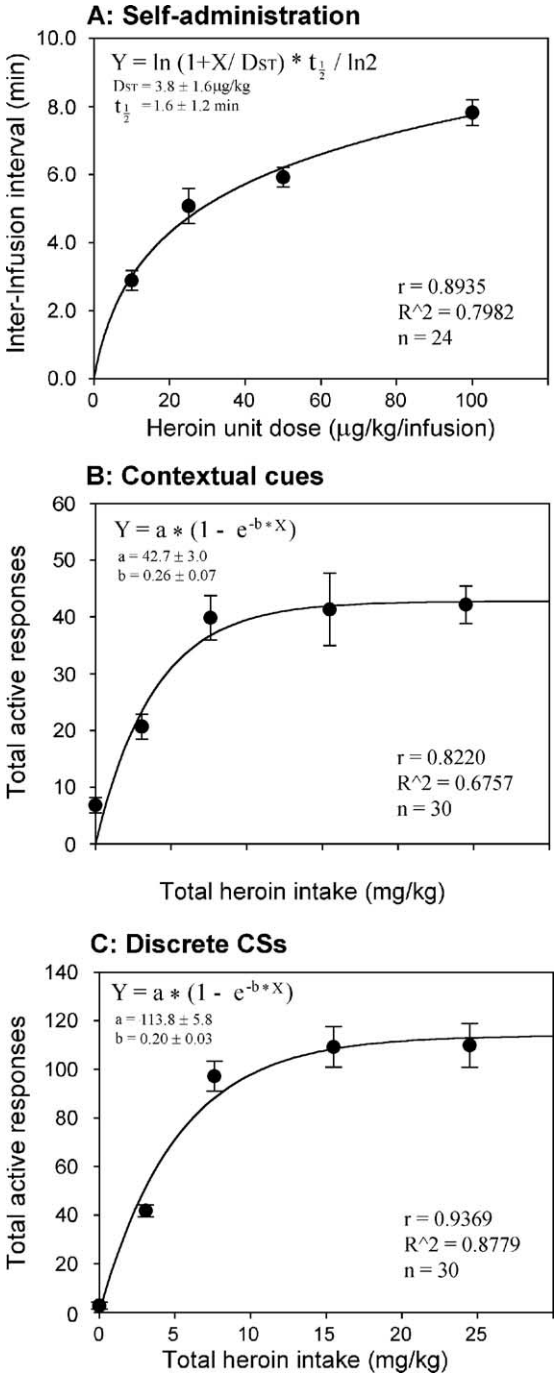

Fig. 4. Correlation between heroin intake and drug cue-induced heroin seeking. Data were expressed as mean±S.E.M. (A) Nonlinear regression of the unit dose of heroin and the inter-infusion interval using a model described by Tsibulsky and Norman (1999) to calculate the mean satiety threshold (D_{ST}) and the elimination half-life of heroin ($t_{1/2}$). (B [contextual cues] and C [discrete CSs]) Nonlinear regression of total heroin infused and drug cue-induced heroin seeking.

4. Discussion

The results demonstrated that contextual cues and discrete CSs previously associated with the availability and subjective effects of heroin elicit robust heroin-seeking behavior after prolonged abstinence, and that the magnitude of heroin seeking was related to the total amount of heroin exposure during self-administration. Even at a very low dose (10 μg/kg/infusion) of heroin self-administration with limited availability (a maximum of 25 infusions daily), the cue-induced recovery of responding reached significant levels relative to saline controls. The strength of cue-induced responding was increased with an increasing dose at 25 μg/kg/infusion. Such responding induced by heroin-related cues cannot easily be attributed to nonspecific effects, because the responding was specific to the active nose-poke hole. Meanwhile, novelty seeking might not account for the increased responding induced by the discrete CSs, because the CS-induced active responding was only slightly observable in the saline-trained rats. Of particular interest is the fact that the quantitative relationship between the total amount of heroin exposure and cue-induced heroin seeking was observed with doses of 10 to 25 μg/kg, but not with doses of 25 to 100 μg/kg.

In this study, we controlled several experimental parameters. Only the heroin dose of each infusion was manipulated; other parameters such as the reinforcement schedule, daily infusion times, and the duration of drug availability were controlled. So the increased responding could be accounted for by the total amount of heroin exposure. Consistent with drug-priming and footshock stress on reinstatement (Deroche et al., 1999; Sutton et al., 2000; Baker et al., 2001; Ahmed et al., 2000), the present results further confirmed that the amount of drug intake during training can also influence the effect of drug cue-induced reinstatement.

During self-administration training, the inter-injection interval is one of the most commonly measured dependent variables, and it is generally believed that rats change the infusion rate or the injection interval to maintain a constant average intake of the drug. According to a model proposed by Tsibulsky and Norman (1999), the calculated satiety threshold of heroin in this study was 3.8±1.6 μg/kg. The calculated mean elimination half-life of heroin was 1.6±1.2 min, a little shorter than the value of 3.0 min reported by the normal pharmacokinetic method (Inturrisi et al., 1984; Jenkins et al., 1994). This discrepancy could result from the tolerance of heroin's reinforcing effect, as Tsibulsky and Norman's model was mostly based on the animal's behavior, and the latter reflects the drug's reinforcing effect and satiety. We observed that at low doses (10 and 25 μg/

kg) of heroin self-administration, the rats could titrate their intake. Heroin intake during the first hour of the training session was similar between doses of 10 and 25 μg/kg (Fig. 2A). At these two doses, the rats could maintain similar levels or a similar concentration of heroin by adjusting the inter-injection intervals, as the heroin satiety thresholds were similar. But at the two larger doses (50 and 100 μg/kg), the rats increased their heroin intake with increasing unit doses. So the rats maintained higher levels of heroin during self-administration. This could have been caused by an increased heroin threshold. A similar nonlinear relationship with the unit heroin dose and the injection rate has been found with cocaine (Dai et al., 1989; Pettit and Justice, 1991). Because increased heroin seeking induced by drug cues was only observed when heroin doses were within the range between 10 and 25 μg/kg, but not beyond 25 μg/kg, the drug level was not relevant to the increased heroin-seeking induced by heroin-associated environmental cues.

The response rate during training is another dependent measurement of drug self-administration. Although it has been argued that the response rate during training is not a major methodological concern in reinstatement studies (Shalev et al., 2002), we observed a negative relationship between the response rate and cue-induced heroin seeking, a finding that is consistent with a report by Kruzich et al. (1999). Because in both the present study and Kruzich et al.'s study the response rate was not manipulated experimentally, further studies are still needed to elucidate the relationship between the response rate and reinstatement testing by comparing rats trained under different schedules of reinforcement.

Tolerance of heroin's reinforcing effects might account for the lack of a relationship between drug intake and cue-induced heroin seeking with doses of 25, 50, and 100 μg/kg/injection. Heroin dependence and withdrawal symptoms are other conditions that are thought to play important roles in relapse to heroin (O'Brien et al., 1986). However, in the present study, even when the largest dose (100 μg/kg) was used, because of the limited daily infusion times, no dependence could be developed during self-administration training (Dai et al., 1989). Because withdrawal from heroin might function as a motivational state that enhances the incentive value of the drug and its reward effectiveness for self-administration (Hutcheson et al., 2001), further studies are also needed to reveal the role of withdrawal in cue-induced heroin seeking by training rats with doses large enough to produce withdrawal syndromes.

In conclusion, the present study confirmed that only after a very limited amount of exposure to heroin, heroin-seeking behavior could be renewed after a prolonged period of abstinence by re-exposing the rats to the drug-associated environment. The intensity of the heroin-seeking behavior induced by drug-associated cues was related to the total amount of heroin exposure. These results also suggest that the amount of drug exposure plays an important role in the development of drug addiction and might have predictive validity for relapse propensity long after abstinence.

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