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The effect of heroin dependence on resumption of heroin self-administration in rats

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ABSTRACT

Background: It has been proposed that relapse vulnerability in previously dependent individuals results from augmentation of drug-induced reinforcement due to repeated associations between the interoceptive properties of the drug and reduction of acute withdrawal distress.
Methods: To test this hypothesis, male Sprague-Dawley rats self-administered 0.05 mg/kg/inf heroin on continuous reinforcement (CR) and progressive ratio (PR) schedules. During this period, they also received injections of vehicle or escalating doses of heroin. Following tests of naloxone-precipitated withdrawal, as well as a drug-free period (4 days), and extinction (9 sessions), they were pre-treated with vehicle or yohimbine (0.5 mg/kg, IV) and tested for resumption of heroin self-administration (0.05 mg/kg/inf) on CR and PR schedules, or tested for reinstatement in extinction conditions.
Results: Increased self-administration on the CR schedule was observed in the heroin-injected rats, but no group differences were observed on the PR schedule, in spite of greater signs of withdrawal precipitated by naloxone in the heroin-injected rats. More importantly, there were no group differences in resumption of heroin self-administration, and this was not altered by yohimbine.
Conclusions: These results suggest that relapse vulnerability cannot be uniquely ascribed to enhanced reinforcing action of drugs; contextual and other conditioning factors must play a role in modulating resumption of drug intake after abstinence.

1. Introduction

An interesting topic in drug addiction research is the role of physical dependence in relapse to drug use after the dissipation of clear signs of withdrawal. Early studies demonstrated that animals previously dependent on opiates are more likely to resume opioid intake after periods of abstinence (Hinson et al., 1986; Wikler and Pescor, 1967; Kumar and Stolerman, 1972; Garcin et al., 1977; Weeks and Collins, 1968). Wikler (1971) hypothesized that relapse vulnerability in previously dependent individuals results from augmentation of drug-induced reinforcement due to repeated associations between the interoceptive properties of the drug and reduction acute of withdrawal distress. To test this hypothesis, Miller et al. (1979) employed two protocols of morphine administration that equated for total amount of drug exposure and tolerance (Dougherty et al., 1979), but that differed in induction of withdrawal distress. One group of rats received single daily intravenous morphine injections (escalating up to 200 mg/kg) while another group received continuous intravenous morphine infusions at the same dose. A test of relapse 12 days after termination of treatment revealed greater consumption of etonitazene in rats given the single daily injections. It was reasoned that, in this group, the interoceptive effects of morphine (nausea, hypothermia, pruritis) acquired conditioned reinforcing properties because they were followed by alleviation of withdrawal (Wikler and Pescor, 1967) experienced every day, prior to the morphine injection. Therefore, during the test of relapse, consumption of the opiate was more reinforcing because these interoceptive stimuli acted as conditioned (secondary) reinforcers.

As far as the authors know, this hypothesis has never been explored in the context of drug self-administration and modern models of relapse. Therefore, experiment 1 was designed to test this putative mechanism of relapse vulnerability using a protocol in which all animals were trained to lever press for intravenous heroin infusions on a continuous reinforcement schedule (CR), prior to extinction and resumption of heroin self-administration. During acquisition, four hours after each self-administration session, some rats received additional injections (SC) of heroin to increase total level of heroin exposure and induce a state of withdrawal prior to the self-administration of heroin the following day. Prior to the test of resumption, some rats were

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also pre-treated with yohimbine, an alpha-2 adrenergic antagonist, to mimic some interoceptive features of opiate withdrawal (Stine et al., 2002). All tests of resumption were performed using a progressive ratio (PR) schedule of self-administration (Roberts and Bennett, 1989). To control for the effects of yohimbine on heroin seeking, a test of reinstatement (i.e., lever pressing in extinction conditions; Shaham et al., 2003) was also performed. On the basis of the results of experiment 1, an additional experiment was carried out. Experiment 2 specifically explored the interaction between CR and PR schedules during acquisition and resumption of heroin self-administration.

2. Materials and methods

2.1. Subjects

Subjects were adult male Sprague-Dawley rats (Charles River, QC) weighing 250–300 g at the beginning of the experiments. Rats were individually housed, maintained on a reverse light/dark cycle (7:00 AM lights off; 7:00 PM lights on) and behavioral testing occurred during their active cycle. All rats had free access to food and water except during testing. All procedures were approved by the Animal Care Committee of the University of Guelph and were carried out in accordance with the recommendations of the Canadian Council on Animal Care.

2.2. Intravenous surgery and self-administration and activity chambers

Details of surgical procedures and apparatus have been described in Leri et al. (2009).

2.3. Procedures experiment 1

The experimental phases, final group sizes and conditions/tests are represented in Table 1A. The design included 4 phases: self-administration, tests of precipitated withdrawal, extinction, and tests of resumption and reinstatement.

2.3.1. Phase 1: self-administration

A total of 110 rats were trained to self-administer heroin (0.05 mg/kg/inf) on a continuous reinforcement (CR) schedule for 10 consecutive days. Each day, self-administration sessions began at 8:00 AM and ended at 11:00 AM. Each session was initiated by the activation of the house light, the entry of the levers, and the illumination of the stimulus light located above the active lever for 30 s. Subsequently, each press on the active lever resulted in the delivery of a 150 μl infusion and illumination of the stimulus light for 5 s.

The study included two groups of rats: vehicle injection (initial sample size = 52) and heroin injection (initial sample size = 58). After each self-administration session, the groups received subcutaneous injections of vehicle (saline) or heroin at 3 PM, 5 PM and 7 PM. The drug doses employed in the heroin injection group escalated across the 10 days of self-administration according to the regimen: 1, 2, 3, 4, 4, 6, 6, 8, 8, and 8 mg/kg. Therefore, by the last day of operant training, rats in this group self-administered 0.05 mg/kg/inf heroin in the AM, and received 3 injections of 8 mg/kg heroin in the PM.

On day 11, rats self-administered 0.05 mg/kg/inf heroin between 8:00 AM and 11:00 AM on a progressive ratio (PR Test I) schedule of reinforcement. On this schedule, the number of responses required for each infusion progressively increased according to the ratio: 1, 5, 11, 19, 31, 49, 76,117, 177, 267, 401, 602, 900 (adapted from Roberts and Bennett, 1993). The breakpoint (BP) was the final ratio completed within the session (Richardson and Roberts, 1996).

2.3.2. Phase 2: tests of precipitated withdrawal

Within an hour following PR Test I on day 11 of self-administration, different groups of animals were injected with 0.01 mg/kg or 0.1 mg/kg naloxone prior to assessment of three indices of heroin withdrawal. The assignment of specific animals to specific tests of withdrawal was done randomly, but no rat received more than one injection of naloxone.

2.3.2.1. Locomotion. Vehicle and the heroin injection groups were injected with 0.1 mg/kg naloxone and placed in activity chambers. Horizontal and vertical activities were recorded. It should be noted that 10 and 8 rats in the vehicle and the heroin injection groups, respectively, received a lower test dose of naloxone (0.01 mg/kg) prior to locomotion testing. However, because on this measure of withdrawal, the effect of 0.01 mg/kg did not differ statistically from that of 0.1 mg/kg, the data from the animals were combined. At the conclusion of the experiment, it was confirmed that rats injected with 0.01 or 0.1 mg/kg naloxone did not differ on tests of extinction and resumption.

2.3.2.2. Loss of body weight. A subset of rats in the vehicle (n = 5) and the heroin (*n = 8*) Injection groups tested on locomotion were weighed prior to, and 2 h following, the injection of 0.1 mg/kg naloxone. Percent weight loss was calculated.

2.3.2.3. Wet dog shakes. A subset of rats in the vehicle (n = 14) and the heroin (*n = 13*) Injection groups tested on locomotion were videotaped for 2 h after the injection with 0.1 mg/kg naloxone. Subsequently, an observer blind to treatment scored instances of wet dog shakes.

2.3.3. Phase 3: extinction

Following a 4-day drug free period in home cages, rats received 9 sessions of extinction over 9 days (3 h each) during which responding on the active lever was not reinforced by heroin.

2.3.4. Phase 4: tests of resumption and reinstatement (PR test II)

Following extinction, different rats in the vehicle and heroin injection groups were randomly assigned to two different tests during which responding was assessed using the same PR schedule employed on day 11 of self-administration. For the test of resumption, animals were tested for 3 h in self-administration conditions; that is, each completed ratio resulted in an intravenous infusion of 0.05 mg/kg/inf heroin. Five minutes prior to this test, rats were injected with either 0 or 0.5 mg/kg yohimbine (see Table 1A). In contrast, for the test of reinstatement, animals were tested for 3 h in extinction conditions; that is, each completed ratio resulted in an intravenous infusion of vehicle. Five minutes prior to this test, rats were injected with either 0 or 0.5 mg/kg yohimbine (see Table 1A).

2.4. Procedures experiment 2

The PR tests in experiment 1 yielded unexpected findings. In fact, irrespective of vehicle or heroin injections, the BPs maintained by 0.05 mg/kg heroin on PR test II (assessed following a drug-free period and extinction) were much lower than BPs on PR test I (assessed on day 11 of heroin self-administration). This observation generated three research questions that were explored in experiment 2. First, it was asked whether the BPs observed on PR test II were within the range of BPs that would be observed in rats that never self-administered heroin. Second, it was verified whether acquisition of heroin self-administration would increase BPs on the particular PR schedule used in these experiments. Finally, and more importantly, it was asked whether the decrease in BPs observed from acquisition to tests of resumption was a by-product of having tested the rats on the PR schedule.

Table 1A
Experimental phases, final group sizes and conditions/tests of Experiment 1.

	Vehicle Injection group				Heroin Injection group			
Phase 1	*n* = 47				*n* = 40			
	0.05 mg/kg H				0.05 mg/kg H			
	SA CR x 10 sessions + V injections				SA CR x 10 sessions + H injections			
	SA PR x 1 session				SA PR x 1 session			
Phase 2	*n* = 47				*n* = 40			
	Tests of precipitated withdrawal				Tests of precipitated withdrawal			
Phase 3	*n* = 47				*n* = 40			
	Extinction x 9 sessions				Extinction x 9 sessions			
Phase 4	*Reinstatement test*		*Resumption test*		*Reinstatement test*		*Resumption test*	
	0 mg/kg H		0.05 mg/kg H		0 mg/kg H		0.05 mg/kg H	
	SA PR x 1 session		SA PR x 1 session		SA PR x 1 session		SA PR x 1 session	
	n = 10	*n* = 11	*n* = 13	*n* = 13	*n* = 6	*n* = 12	*n* = 9	*n* = 13
	0 YOH	0.5 YOH	0 YOH	0.5 YOH	0 YOH	0.5 YOH	0 YOH	0.5 YOH