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Heroin self-administration in rats under a progressive ratio schedule of reinforcement

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Abstract. Heroin self-administration behavior under a progressive ratio (PR) schedule of reinforcement was evaluated in rats. The schedule was designed to restrict drug intake, minimize opiate dependency, and quantify the number of responses emitted (final response ratio) in order to receive a limited number of heroin infusions. Final ratios were found to be stable and did not increase with chronic (31 days) PR reinforcement. The ability of the PR schedule to detect changes in heroin reinforcement was demonstrated by evaluating the effect of naltrexone pretreatment and unit dose alteration on final ratios. Naltrexone (0.4 mg/kg) reduced final ratios and an inverted U dose-response relationship was established for the unit heroin doses 12.5–100 μg/injection. Maximal final ratios occurred with 50 μg/injection heroin reinforcement. This PR schedule may provide a useful method for evaluating the effects of pharmacological manipulations or lesions on opiate reinforcement.

Key words: Self-administration – Progressive ratio – Heroin – Dose-response – Naltrexone

Many experiments have examined the effects of pharmacological pretreatments or neurotoxic lesions on drug self-administration behavior in laboratory animals in an attempt to define the neural mechanisms responsible for drug reinforcement. A number of neurotransmitter systems have been implicated by data showing that self-administration behavior is altered subsequent to perturbation of neural projections or systemic injections of receptor antagonists. Typically, these experiments use simple schedules of reinforcement (often a Fixed Ratio 1, FR1) and interpretations are necessarily based on changes in the rate of self-administration. However, changes in the rate of operant responding might be produced not only by a shift in drug reinforcement but also by a change in the ability or disposition of the organism to perform. It would seem that the role each neurotransmitter plays in drug self-administration should be assessed using a variety of dependent measures measured under different reinforcement schedules.

Unfortunately, the range of reinforcement schedules that have been used in self-administration studies in rats has been extremely limited. As an alternative to simple FR schedules, progressive ratio (PR) schedules of reinforcement have been adapted from implementations used with canine (Risner and Silcox 1981; Risner and Goldberg 1983) and primate subjects (Griffiths et al. 1975, 1978, 1979; Hoffmeister 1979). Cocaine self-administration reinforced under a PR schedule in rats has been shown to be sensitive to changes in the unit dose (Roberts et al. 1989b), to be influenced by manipulations of dopaminergic (Roberts et al. 1989b) and serotonergic systems (Loh and Roberts 1990), and to be altered by changes in the estrous cycle (Roberts et al. 1989a). With respect to the opiate drugs, however, we are aware of only two reports that have evaluated self-administration behavior reinforced by morphine under a PR schedule in rodents (Weeks and Collins 1987; Hubner and Koob 1990). The purpose of the present study was to characterize heroin self-administration behaviour in rats reinforced under a PR schedule designed to restrict drug intake and minimize opiate dependency.

Materials and methods

Subjects. Drug naive male Wistar rats (Woodlyn Labs, Guelph, Canada) weighing 300–330 g at the start of the experiment were used.

Apparatus. Animals were individually housed 24 h a day in testing chambers (25 cm wide × 30 cm long × 30 cm high) fitted with a removable lever (Coulbourne Instruments, model No. E23-07) and a 12V DC stimulus light located to the immediate left of and 1 inch above the lever. A water bottle was mounted on another side of the box and was available at all times. Rats were maintained on a

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reversed 12 h light/dark cycle with light offset beginning at 10: 00 A.M. Light on and offset was maintained by overhead house lights.

Procedure. Subjects were initially deprived of food and trained to depress a lever for food reinforcement (45 mg Noyes pellets). Food was delivered in a spout located 1 inch below the stimulus light. Once animals had acquired the lever-press response, food (Purina Rat Chow) was made freely available. Each animal was anaesthetised with sodium pentobarbital and implanted with an indwelling jugular cannula using a procedure described in detail elsewhere (Roberts and Goeders 1989). The cannula emerged at the mid-scapular level of the animal's back and was protected by a spring casing suspended above the subject by a counterbalance/swivel assembly, permitting the animal freedom of movement. Following surgery, rats were housed in test chambers identical to the food training chambers with the exception that the food spout was no longer present. Rats were allowed 1 day to recover from the surgery. Daily 5 h drug self-administration sessions began 1 h after light offset with the introduction of the lever into the cage. Animals were initially reinforced under a FR1 schedule of reinforcement. Each lever-press resulted in a single intravenous injection and in the 20-s illumination of the stimulus light. Responses made during this 20-s period had no programmed effect. Heroin (250 µg/ml) was dissolved in 0.9% saline and injected in a unit volume of 0.1 ml/injection from a Razel syringe pump over 5 s. This delivery corresponds to a unit injection of 25 µg/injection. Where changes in unit dose are indicated, the stock heroin solution was adjusted accordingly. The volume of drug delivered was not changed. During both the FR1 training sessions and subsequent PR test sessions, subjects never received a "free" priming injection prior to session initiation. Subjects were always drug-deprived at session onset.

Once an animal had exhibited a stable pattern of drug intake on the FR1 schedule (range of less than 20%) over 3 days, the operant requirements were switched to the PR format. Under this schedule of reinforcement, the response requirements initially began at 1 and escalated through the PR steps: 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95.. This progression was calculated by the equation: Response ratio $=(5 \times e^{(0.2 \times \text{infusion number})})-5$ rounded to the nearest integer. Final ratios, the last ratio successfully completed by the end of the 5-h session, were recorded daily for each animal. On day 2, the first response requirement was set 2 PR "steps" below the final ratio completed on the preceding session. For example, if a rat received ten injections on the first test day (beginning at a response ratio of one), it would have reached the tenth PR "step" corresponding to a ratio requirement of 32 responses. The final ratio would be ten. During the next test session, the starting ratio would be set to the eighth PR "step" (a response ratio of 20). An animal would be required to make 20 responses before receiving its first injection of the day. Subsequent response requirements would follow the ratios outlined above using the new first ratio as an entry point into the series. Twenty-nine animals were trained to self-administer 25 µg/injection heroin and were used to establish the pattern of heroin self-administration under this PR schedule. Five of these rats were permitted to self-administer 25 µg/injection heroin for 31 days in order to assess the effects of chronic heroin self-administration on the stability of the dependent measure (final ratios).

The effect of opiate antagonism on final ratios was assessed using six rats trained to self-administer 25 µg/injection heroin. Subjects were pretreated 1 h before session onset for 7 days with an intraperitoneal injection of saline (1 ml/kg). Mean final ratios were established in the final 5 days of testing. Animals were subsequently pretreated 1 h before session onset for 7 days with an intraperitoneal injection of naltrexone HCL (0.4 mg/kg). Mean final ratios were established in the final 5 days of testing. Saline pretreatment, as described, was then resumed for an additional 7 days and mean final ratios calculated.

The effect of altering unit dose on final ratios was studied in six rats trained to self-administer 12.5 µg/injection heroin. Rats were permitted to self-administer three different heroin doses in the following order: 12.5, 25, 100, 50, 25 µg/injection. Rats were given access to each dose for seven days. Mean final ratios were calculated in the final 5 days of testing at each dose.

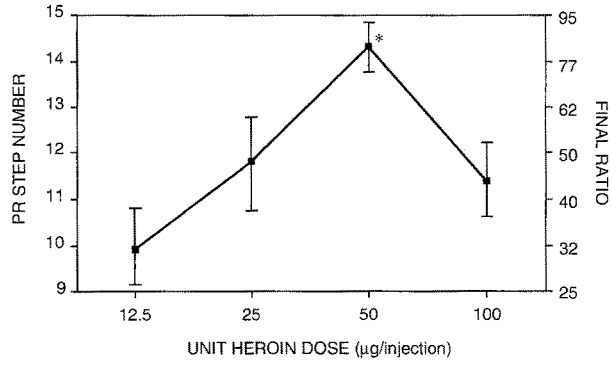

Fig. 1. The effect of changes in unit heroin dose on heroin self-administration behavior reinforced under a PR schedule. Points represent mean final ratios ±SEM ($n=5$). The PR step number is depicted on the left-hand axis and the actual ratio value is shown on the right-hand axis. The *asterisk* represents a statistically significant difference between the 50 and the 12.5 µg/injection doses (post hoc Tukey test, $P<0.05$)

Analyses. One-way repeated measures analyses of variance (ANOVAs) were performed on the ordinal values, of the final ratios (the PR step number) rather than the actual ratio values, since these latter numbers were derived from an exponential equation and might therefore have violated assumptions of homogeneity of variance. On days when animals failed to self-administer a single injection, the final ratio was assigned the value falling one ordinal step below the first PR response requirement of the session.

Results

The pattern of self-administration quickly stabilized on this PR schedule. Final ratios climbed for the first 5 days and then stabilized at a constant level. It may be noted that the operation of the PR schedule ensured that subjects received an average of three infusions per session, thus the average daily intake of heroin was restricted. Chronic access (31 days) to heroin (25 µg/injection) failed to effect the stability of the dependent measure, as no statistically significant increase or decrease in final ratio was observed.

A dose-response relationship was established for the five rats that self-administered 12.5, 25, 50, and 100 µg/injection heroin. Data are presented in Fig. 1. A repeated measures ANOVA revealed a statistically significant effect of dose ($F=5.9$; df 4,3; $P<0.05$). The highest final ratios occurred, on average, at the 50 µg/injection heroin dose. In order to rule out the effect of order of dose presentation, animals were returned to the 25 µg/injection dose. Final ratios determined at this dose following completion of the dose-response study were not statistically significantly different from the original values established 3 weeks earlier (one-tailed paired Student's $t=1.55$; $df=4$; $P>0.05$).

Daily naltrexone pretreatment (0.5 mg/kg) decreased mean final ratios when compared to saline pretreatment (Fig. 2). The final ratios determined on each day of naltrexone pretreatment were expressed as a percentage of

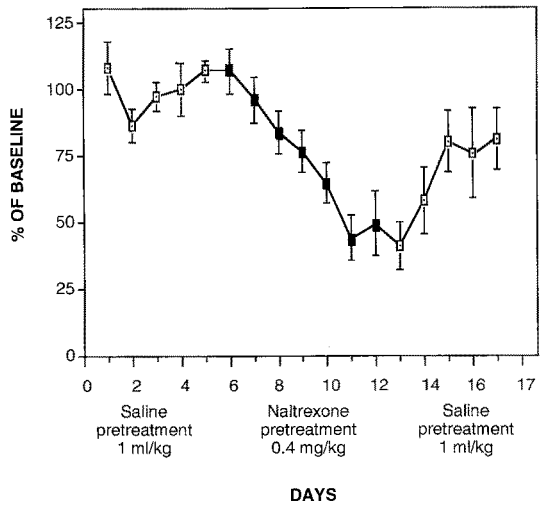

Fig. 2. The effect of naltrexone (0.4 mg/kg) pretreatment on heroin (25 μg/injection) self-administration behavior reinforced on a PR schedule. Data represent mean (±SEM) daily final ratios ($n=6$). Data are expressed as percentages of the mean final ratio established during 5 days of initial saline pretreatment. *White squares* represent final ratios after saline pretreatment. *Black squares* represent final ratios after naltrexone pretreatment

the saline pretreatment baseline measure (5 day mean). A repeated measures ANOVA, performed on these percentages, revealed a statistically significant "DAYS" effect ($F=10.7$; df 5,7; $P<0.01$). Although animals demonstrated a slight increased irritability to handling, they failed to exhibit the many other symptoms associated with a naltrexone-precipitated opiate withdrawal syndrome in dependent animals (including diarrhea, rhinorrhea, lacrimation, ptosis, urination, piloerection, teeth chattering, "wet-dog" shakes, salivation, ejaculation, and writhing).

Discussion

The present data confirm that rats will respond to relatively high ratios in order to obtain a limited number of heroin infusions and that the final ratios reached by these animals are a stable dependent measure that does not increase over time, is sensitive to pharmacological manipulations (i.e. opiate antagonism), and is dose-dependent.

The PR schedule used here was derived from an amalgamation of different PR implementations tested in primates and dogs (Griffiths et al. 1975, 1978, 1979; Hoffmeister 1979; Risner and Silcox 1981; Risner and Goldberg 1983). The ratio requirements for the first injection of each session was adjusted according to the performance of the animal during the previous session. If a subject failed to self-inject three drug infusions then the initial response requirements were lowered on the following day. If a subject self-injected more than three infusions in a single test session, then the initial response requirements were raised. By restricting heroin intake, we attempted to minimize the effects of drug intake (either stimulatory or debilitating) on a subject's performance and to minimize the degree of physical dependence that has frequently been observed in long-term opiate self-administration studies (Dai et al. 1989).

An inverted U-shaped dose-response relationship was observed within the dose range of 12.5–100 μg/injection, with peak final ratios occurring at 50 μg/injection. The observed dose-response relationship may, however, have been the result of an order effect since animals were tested in the series 12.5, 25, 100, and 50 μg/injection of heroin. Final ratios could have increased with repeated exposure to heroin reinforcement. This interpretation is unlikely, given that animals participating in the dose-response investigation were returned to 25 μg/injection heroin after completing the test series. Mean final ratios were comparable to the values determined at the same dose 3 weeks earlier. Furthermore, chronic exposure (31 days) to 25 μg/injection heroin reinforcement did not affect the stability of the dependent measure. Final ratios did not increase over time. While an order effect cannot be ruled out, these data argue against the premise that the sole determining factor of the observed dose-response relationship was the order of dose presentation.

FR studies have repeatedly demonstrated that prior administration of low to moderate doses of an opiate antagonist produces an increase in the rate of heroin self-administration in both heavily dependent and slightly dependent animals (Goldberg et al. 1971; Killian et al. 1978; Ettenberg et al. 1982; Koob et al. 1984; Corrigall 1987; Corrigall and Vaccarino 1988). Such increases in drug intake have been interpreted as a compensatory response towards reduced drug reinforcement. The present data are consistent with this interpretation. Final ratios were reduced across 7 days of naltrexone administration, indicating a diminished ability of the opiate to maintain responding. Following opiate receptor antagonism, animals apparently seek to increase heroin intake only when the response requirements governing the reception of the infusions are low. When these response requirements are elevated, animals fail to pay the high behavioral price for the reduced heroin reinforcement.

To summarize, the sensitivity of a PR schedule to changes in opiate reinforcement in rats was demonstrated by the reduction in final ratio following naltrexone pretreatment and by the establishment of a dose-response relationship. These data suggest that a PR implementation incorporating 1) a high first ratio into the schedule requirements dependent upon daily performance, and 2) a limitation upon drug intake, is sensitive to changes in opiate reinforcement. Modifying different reinforcement schedules, both PR and FR, should enable us to characterize different aspects of the motivation to receive a drug of abuse.

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