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ORIGINAL INVESTIGATION

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Cocaine-seeking by rats: regulation, reinforcement and activation

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Abstract *Rationale:* In animal models of drug self-administration, response rates often decrease with dose suggesting that a regulative process may mask the reinforcing effects of the drug. *Objective:* The purpose of the present experiments was to dissociate the role of regulative and reinforcement processes in intravenous cocaine self-administration by rats using a paradigm that explicitly distinguishes between drug-seeking and drug-taking. *Methods:* Rats were trained to respond for intravenous cocaine (0.25 mg/infusion) under a heterogeneous chain (tandem FR1 RI 30 s) FR1 schedule of reinforcement using different levers in the first (seeking) and second (taking) links of the chain. After 10 days of training, rats were switched to one of three doses of cocaine (0.08, 0.25, or 0.5 mg/infusion) and self-administration patterns were recorded for a further ten sessions in experiment 1. In experiment 2, a time-out (TO) period (0, 4, or 12 min) was imposed between successive cycles of the chain schedule. Finally, the effect of allowing animals to perform a drug-taking response on subsequent drug-seeking was assessed in experiment 3. *Results:* Having verified that seeking responses for a conventional reinforcer (sucrose) were sensitive to changes in reward magnitude, experiment 1 demonstrated that the number of self-administered infusions was inversely related to dose whereas the latency to initiate drug-seeking increased with dose. Variations in the cocaine dose had no reliable effect on the number of drug seeking response per cycle of the chain schedule. The effect of dose on the latency to initiate drug-seeking was reversed in experiment 2 with increasing TO periods. Moreover, at the longest TO period, drug-seeking responses per cycle increased and the latency to initiate drug seeking decreased with dose. Experiment 3 showed that the latency to drug-seek for the low dose was reduced dramatically when the first drug-seeking response was preceded by a drug-taking response, even when this response did not produce a drug infusion. *Conclusions:* The overall pattern of results suggests that drug-seeking and drug-taking are controlled by three interacting processes: a regulative process depresses drug-seeking in the short-term; behavioral activation enhances drug-seeking and is sustained over longer intervals by higher drug doses; the reinforcing effect of cocaine increases with dose once the satiety producing effects of the drug dissipate.

Key words Drug-seeking · Addiction · Reward · Stimulant · Self-administration

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Introduction

Endogenous reinforcement systems appear to play an important role in drug addiction (Fibiger 1978; Wise 1980, 1982; Wise and Bozarth 1987) in that common neuropharmacological processes mediate the reinforcing properties of natural rewards, such as food, and addictive drugs, such as cocaine or heroin (Koob and Bloom 1988; Wong et al. 1991; Wise 1996). On the other hand, variations in reward magnitude sometimes produce differences in response-rate profiles for food and drugs. Increasing the magnitude of food rewards typically enhances the response rate maintained by these reinforcers, at least in open economies (Bonem and Crossman 1988). By contrast, the response rate profile under variations in the dose of self-administered stimulants, such as cocaine, is often biotonic, increasing from low to medium doses and then decreasing from medium to high doses (Goldberg et al. 1976; Yokel and Wise 1976; Caine et al. 1992). Some authors suggest that these behavioral differences reflect a fundamental dissociation in the mechanisms underlying the reinforcing effects of food and drugs (Katz 1989), whereas others argue that apparent

differences in reinforcing efficacy are an artifact of the schedule of reinforcement. For example, differences in response rates for food and drugs are most robust under continuous reinforcement schedules, but generally disappear under schedules which program long intervals between successive presentations of the reinforcer (progressive ratio, second order, time-out). Thus, the evidence that variations in reward magnitude have differential effects on response-rate profiles for drug and natural rewards is mixed.

The purpose of the present experiments was to re-examine the issue by directly comparing the dose-response function for cocaine and sucrose following identical training schedules. On ecological groups, we chose to use a schedule that explicitly distinguished between reward-seeking and reward-taking responses because, outside of the laboratory, the activities involved in procurement of a drug differ from those required to take it. This distinction can be modeled in the laboratory by a heterogeneous chain schedule in which the animal performs a seeking response in the first link of the chain in order to gain access to the second link in which a different, taking response delivers the drug. Moreover, the distinction between seeking and taking response may reflect a difference in the motivational control of behavior. There is evidence that food-taking responses are more sensitive to shifts in the level of food deprivation than are food-seeking responses (Balleine et al. 1995). As a consequence, a schedule that employs the same response for drug-seeking and drug-taking may well confound the control of drug intake by different motivational processes.

The first experiment verified that performance of the seeking response under this chain schedule is sensitive to parameters of a natural reward by varying the concentration of a sucrose solution. When sucrose concentration increased, sucrose-seeking responses increased and the latency to initiate responding decreased. In contrast, increases in cocaine dose had no effect on the rate of cocaine-seeking but increased the latency to cocaine-seek. Positive, dose-response functions for cocaine are often observed under schedules of reinforcement that programme relatively long intervals between each infusion (Goldberg 1973; Goldberg et al. 1981; Corrigall and Coen 1989; Howell and Byrd 1995). For example, response rates increase with cocaine dose when there is an enforced time-out (TO) period between successive opportunities to earn the drug (Balster and Schuster 1973; Winger and Woods 1985; Winger 1993). Moreover, on progressive ratio schedules (which are characterised by increasing delays between successive infusions), the maximum number of responses per cycle that the drug will support (Hodos 1961; Hodos and Kalman 1963) is positively correlated with dose (Yanigaita 1973; Griffiths et al. 1978; Risner and Goldberg 1983; Roberts and Bennett 1993). The second study, therefore, investigated the effects of dose on cocaine-seeking on the chain schedule when the TO period between successive cycles of the chain schedule was varied.

The final study investigated a possible priming effect on cocaine-seeking. In both experiments 1 and 2, the number of self-administered infusions was inversely related to dose when there was no TO period between a drug infusion and the next opportunity to cocaine-seek. As the TO period was increased in the second experiment, the number of self-administered infusions declined in the low dose group. This finding suggested that cocaine infusions produce behavioral activation effects, the duration of which is positively related to the dose of the drug. If this is true, the low dose of cocaine may have been unable to maintain behavior over long TO periods because any activating effect of the infusion dissipated during this interval. Experiment 3 tested this hypothesis directly by examining how self-administered infusions at the end of a long TO period affected the subsequent propensity to cocaine-seek.

Materials and methods

Animals

Male Lister hooded rats (Olac, Bicester, UK), weighing between 300 and 350 g at the beginning of the experiment, were housed in pairs under natural lighting conditions. Following recovery from surgery, animals were placed on a restricted feeding schedule sufficient to maintain pre-operative body weight and growth by giving them free access to food (Purina rat chow) in their home cages for 1 h per day. Water was freely available at all times in the home cage. The experiments were undertaken in accordance with the UK 1986 Animals (Scientific Procedures) Act (Project License PPL 80/00668).

Intravenous catheterisation

The catheters were made using guide cannulae (C313G 5UP; Semat Technical Ltd, St Albans, Herts, UK), silicon tubing (STHT-C-030-0 and STHT-C-020-0; Osteotec Ltd, Christchurch, UK), dental cement (Simplex Rapid; Associated Dental Products Ltd, Wilstshire, UK) and silicon rubber (RS Components, Northants, UK).

The rats were anaesthetised with Avertin (10 g 99% 2,2,2-tribromoethanol; Sigma-Aldrich Company, Dorset, UK) in 5 mg tertiary amyl alcohol and 4.5 ml phosphate buffered saline (Dulbecco "A"; Unipath Ltd, Basingstoke, Hampshire, UK) in 40 ml absolute alcohol; 1 ml/100 g body weight injected IP). The catheters, previously sterilised in 70% alcohol, were implanted with the proximal end reaching the heart through the right jugular vein, continuing dorsally over the right shoulder and exiting between the scapulae (Caine et al. 1992). They were flushed daily for the first 5 days post-surgery with 0.1 ml of a strong antibiotic solution (Timentin 3.2 g: 200 g potassium clavulanate with 3 g ticarcillin (Beecham Research, Welwyn, Herts, UK); 65 mg of this powder was dissolved in 1 ml 0.9% sterile saline (Animalcare Ltd, Dunnington, UK)) and thereafter daily with 0.1 ml heparin solution (CP Pharmaceuticals Ltd, Wrexham, UK; 30 units/ml 0.9% sterile saline).

Apparatus

Training and testing took place in six operant chambers (26.5×22×20 cm), each housed in a sound attenuating box and fitted with two retractable levers 4.8 cm wide, positioned equidistantly on one wall, 17.5 cm apart and 9 cm from the grid floor (Med Associates Inc., St Albans, Vt., USA). The operant cham-

bers could be illuminated by a ceiling house light and signal lights above the levers, and external noise was masked by ventilating fans mounted on the side of the sound-attenuating boxes. Each box was equipped with a Razel infusion pump (Semat Technical Ltd, St Albans, Herts, UK) that delivered intravenous infusions of cocaine through a single channel liquid swivel (Stoelting, Wood Dale, Ill., USA) with connector attachments (Plastics One, Roanoke, Va., USA). A dipper delivered 0.01 ml of a sucrose solution to a recessed magazine (3.8 cm side and 5.5 cm from the grid floor) situated between the levers. The apparatus was controlled and data were collected by an Acorn Archimedes microcomputer (Acorn Computers Ltd, Cambridge, UK) running the control language ARACHNID (Paul Fray Ltd, Cambridge, UK).

Drugs

Cocaine hydrochloride (McFarlan-Smith, Edinburgh, UK) was dissolved in sterile 0.9% saline. All doses of cocaine were calculated as the salt and intravenous infusions were delivered in a 0.1 ml solution over 3.64 s.

Experimental procedures

Experiment 1: effects of varying reward magnitude

Twelve rats were trained under a heterogeneous chain schedule using a 5-s elevation of the dipper containing a 20% sucrose solution as the reinforcement cycle. Prior to instrumental training, these animals received two sessions of magazine training in which they received 30 non-contingent presentations of the sucrose solution on a random time (RT) 60-s schedule with both levers retracted. They were then trained under a heterogeneous chain schedule under which pressing the seeking lever in the first link gave access to the second link in which a press on the taking lever delivered the sucrose solution. The assignment of the left and right levers to the roles of seeking and taking levers was counterbalanced across animals.

In the first stage of training, the seeking lever was retracted and presses on the taking lever produced the sucrose reinforcement cycle on a fixed ratio (FR) 1 schedule. The taking lever retracted at the beginning of the reinforcement cycle and was reintroduced to the chamber at the end of the cycle. Once responding on the taking lever had been acquired, the chain schedule was introduced. Each cycle of the chain schedule started with the insertion of the seeking lever and the first press on this lever initiated a random interval (RI) schedule. The first seeking response meeting the RI contingency terminated the first link of the chain with the retraction of seeking lever and the insertion of the taking lever to initiate the second link. A single press on the taking lever retracted this lever and started the reinforcement cycle. The insertion of the taking lever and the reinforcement cycle was accompanied by switching off the house light and the illumination of the signal light above the taking lever. Immediately following the reinforcement cycle, the seeking lever was reinserted and the house light illuminated to start the next cycle of the chain. In summary, lever pressing was reinforced under a heterogeneous chain (tandem FR1 RI) FR1 schedule. The parameter of the RI link was increased from 2 to 15 to 30 s across successive sessions and training continued under this schedule for a further seven sessions. The rats were then divided into three equal groups (n=4) and each group was tested with one of the following sucrose concentrations: 8%, 16% or 32% for a further ten sessions. Throughout training and testing each session terminated after either 2 h or 100 reinforcement cycles, which ever occurred first.

Fourteen rats were trained under the heterogeneous chain schedule using an identical procedure except in two respects. The magazine training was omitted and the reinforcement cycle consisted of the infusion of 0.25 mg per cycle of cocaine. The rats were then divided into three groups and the performance of each group was tested for ten sessions under one of the following doses of cocaine: 0.08 mg (n=5); 0.25 mg (n=4); or 0.5 mg (n=5) per cycle.

Experiment 2: effects of varying the time-out (TO) period

Twelve rats were trained to respond for the 0.25 mg IV dose of cocaine under the heterogeneous chain schedule using the procedure employed in experiment 1. After 11 sessions of training, the rats were divided into three equal groups (n=4), each of which was then trained with one of the following doses of cocaine: 0.08 mg, 0.25 mg or 0.50 mg. In addition, a TO period was introduced between successive cycles of the chain schedule to generate a multiple (chain (tandem FR1 RI 30-s) FR 1) TO schedule. Thus, following the termination of each drug infusion the requisite TO period elapsed before the next cycle of the chain was initiated by the insertion of the drug-seeking lever.

Each rat received four sessions of testing with TO periods of 0 min, 4 min and 12 min each. For half of the rats in each group the TO periods were presented in an ascending order of duration, and for the remaining rats in a descending order. The maximum of number of cocaine infusions that could be received during each session was reduced to 20 in an attempt to ensure that each group received the same number of infusions at each TO period. This ensured that the effect of dose at each TO period would not be confounded by the amount of training on the chain schedule. Sessions were 6 h in length to accommodate 20 possible infusions at the 12-min TO period.

Experiment 3: effects of contingent cocaine infusions on drug-seeking

Eight rats were trained to respond for the 0.25 mg IV dose of cocaine under the heterogeneous chain schedule using the procedure employed in experiment 2. After seven to nine sessions of training, half of the animals continued to receive the 0.25 mg dose during testing, whereas the remaining rats were tested with the 0.08 mg dose. During testing the TO period was 24 min. The 24-min TO period was chosen in an attempt to minimise any effect of the preceding infusion, thereby allowing performance to be determined solely by the contingent cocaine infusion. Each test session contained a maximum of ten cycles of the chain schedule in a 6 h session. During the first four “prime” sessions of testing, half of the cycles of the chain schedule were preceded by a contingent cocaine infusion. On these drug cycles, the drug-taking lever was inserted on termination of the preceding TO period and the first lever press delivered an infusion of cocaine and retracted the lever. The drug-seeking lever was then inserted immediately following the infusion. On the remaining no-drug cycles, the preceding TO period terminated with the insertion of the drug-seeking lever. Drug and no-drug cycles were presented in a semi random order during these four sessions. The procedure was changed on the fifth, control session so that the drug-taking lever was inserted prior to both drug and no-drug cycles, but the drug-taking response produced a cocaine infusion only during drug cycles. Thus, the contingencies for a press on the drug-taking lever were identical for the two types of cycles except for the fact that no drug infusion occurred on the no-drug cycles. This control session, therefore, dissociated the effects of performing a drug-taking response and receiving a contingent cocaine infusions on the subsequent propensity to drug-seek. Testing concluded with a final “prime” session that reinstated the contingencies of the first four test sessions.

Statistical analysis

Because the different concentrations of sucrose cannot be matched to the different doses of cocaine, the performance of the groups trained with sucrose and cocaine in experiment 1 were analysed separately. The dependent variables for both analyses were the number of reward presentations per session, the number of seeking-responses per cycle, and the latency of the first seeking response in each cycle of the chain schedule. The latency measure was subject to a logarithmic transformation prior to analysis and presentation to enhance the homogeneity of variance. All

data sets were tested for homogeneity of variance using Levene's test (Levene 1960). This confirmed the need to log transform the latency data. The number of seeking responses and the latency of the first seeking response were assessed using a two-way analysis of variance (ANOVA) with reward magnitude as a between subjects factor and session as a within subjects factor. The number of rewards per session was assessed using a Kruskal-Wallis test because the lack of variance in the sucrose group precluded a parametric analysis. The between-subject variable of reward magnitude distinguished performance under the different doses of cocaine and concentrations of sucrose. We conducted separate analyses for the last three training (i.e., all animals responding for the same reward magnitude) and testing (different reward magnitudes) sessions. Data from the training sessions served as a baseline measure. Session was included as a within-subject factor to verify stability of responding during the final training and testing sessions.

In experiment 2, cocaine-seeking responses per cycle and latency of the first seeking response were analysed using a two-way ANOVA with cocaine dose as the between subject factor and the duration of the TO period as the within subject factor. Kruskal-Wallis tests were used to analyse the effect of these variables on the number of infusions per session. Experiment 3 examined how different priming doses of cocaine effected the latency to drug-seek using a three-way ANOVA with dose as a between subject factor, and session (prime versus control sessions) and cycle as within subject factors. Pairwise comparisons were evaluated by the Newman-Keuls procedure. Reliability in all three experiments was assessed against a type 1 error rate of 0.05.

Results

Experiment 1: effects of varying reward magnitude

Figure 1 and Fig. 2 illustrate the performance of the rats trained with sucrose and cocaine rewards, respectively, averaged across the last three sessions of baseline training and the last three sessions of testing. A comparison of these figures shows that varying the reward magnitude produced opposite effects for the sucrose and cocaine rewards on two of the three measures during the test sessions. Whereas the number of sucrose presentations increased with concentration (Fig. 1A; H=8.02, P<0.05), the number of cocaine infusions decreased with dose [Fig. 2A; $F(2,11)$=50.76, P<0.01]. Contrasting profiles were also observed for the latency of the first seeking response. This latency decreased with sucrose concentration [Fig. 1C; $F(2,9)$=6.86, P<0.05], but increased with cocaine dose [Fig. 2C; $F(2,11)$=6.86, P<0.05]. Although variations in reward magnitude did not produce contrasting effects on the number of seeking response per cycle, this measure was much more sensitive to variations in sucrose concentration than cocaine dose within the parameters of the study. Although the number of seeking response increased robustly with sucrose concentration [Fig. 1B; $F(2,9)$=126.26,P<0.001], the effect of cocaine dose on this measure was only marginally reliable [Fig. 2B; $F(2,11)$=4.25, P=0.05]. The interpretation of these effects was not compromised by systematic changes across the last three test sessions (F<1) nor by differences in performance during the baseline for which there were no significant effect in comparable analyses.

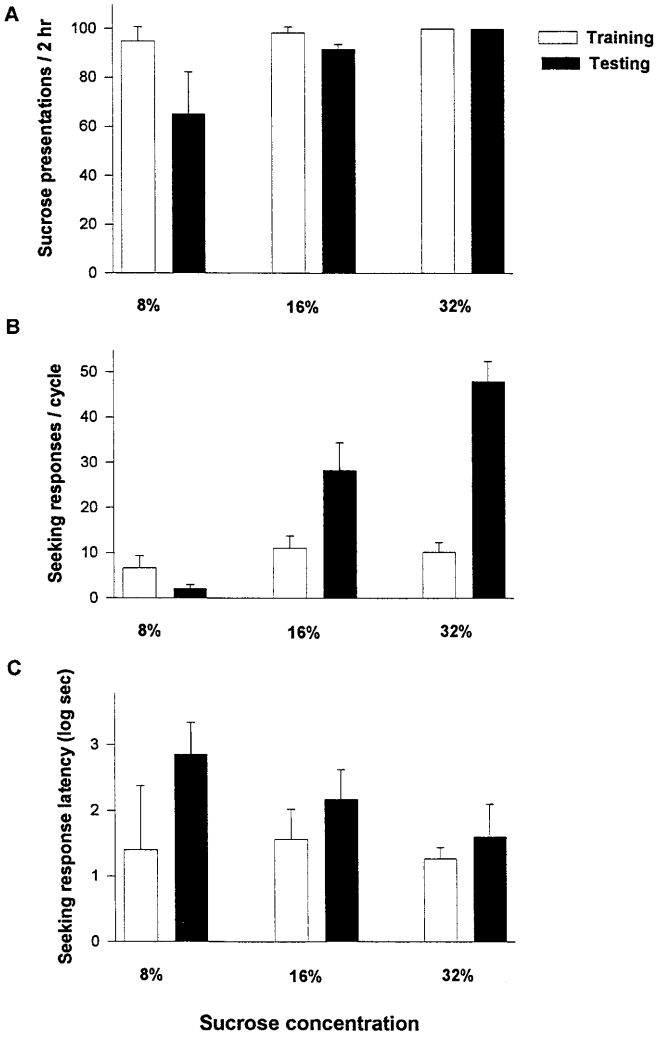

Fig. 1 Experiment 1: effects of sucrose concentration on the number of sucrose presentations (**A**), sucrose-seeking responses per cycle (**B**), and log latencies of the first sucrose-seeking response (**C**). Data are presented as the mean (+SEM) values for each group during the last three baseline and testing sessions. During baseline sessions, all animals were responding for a 10% sucrose solution. During ten testing sessions, animals responded for one of three concentrations of sucrose: 8%, 16%, or 32%

Experiment 2: effects of varying the time-out (TO) period

Figure 3 show the effects of varying the TO period between the drug infusion and the introduction of the drug-seeking lever on responding for different doses of cocaine. As can be seen in Fig. 3A, in the absence of a TO period, dose had little effect on the number of infusions when animals could receive a maximum of 20 infusions per session. The main effect of lengthening the TO period was to decrease the number of self-administered infusions by the low dose (0.08 mg) group. Although there was no significant effect of dose at the 0- and 4-min TO periods, this effect was reliable at the 12-min TO period (H=8.73, P<0.05).

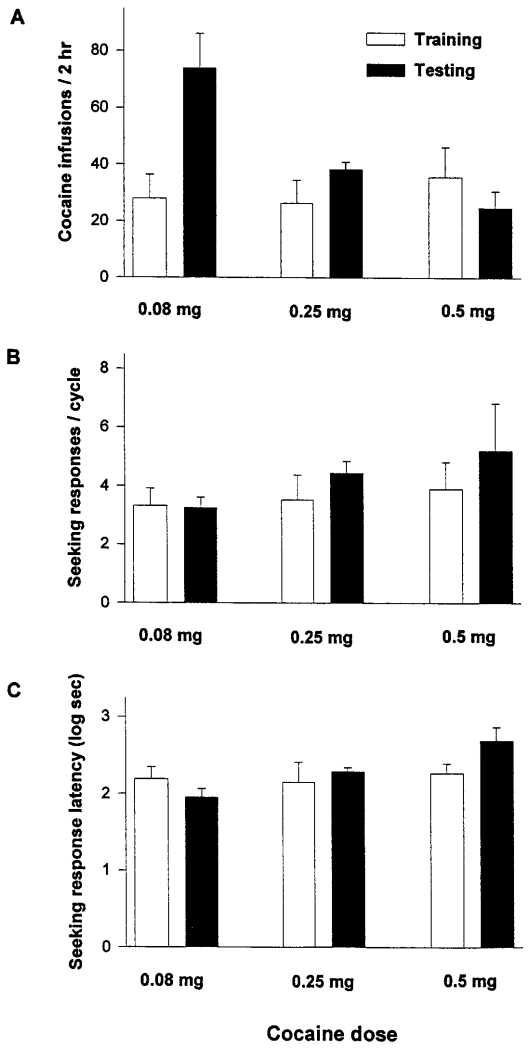

Fig. 2 Experiment 2: effects of dose on the number of cocaine infusions (**A**), cocaine-seeking responses per cycle (**B**), and log latencies of the first cocaine-seeking response (**C**). Data are presented as the mean (+SEM) values for each group during the last three baseline and testing sessions. During baseline sessions, all animals were responding for the medium dose (0.25 mg) of cocaine. During ten testing sessions, animals responded for one of three does of cocaine: 0.08 mg, 0.25 mg, or 0.5 mg per infusion

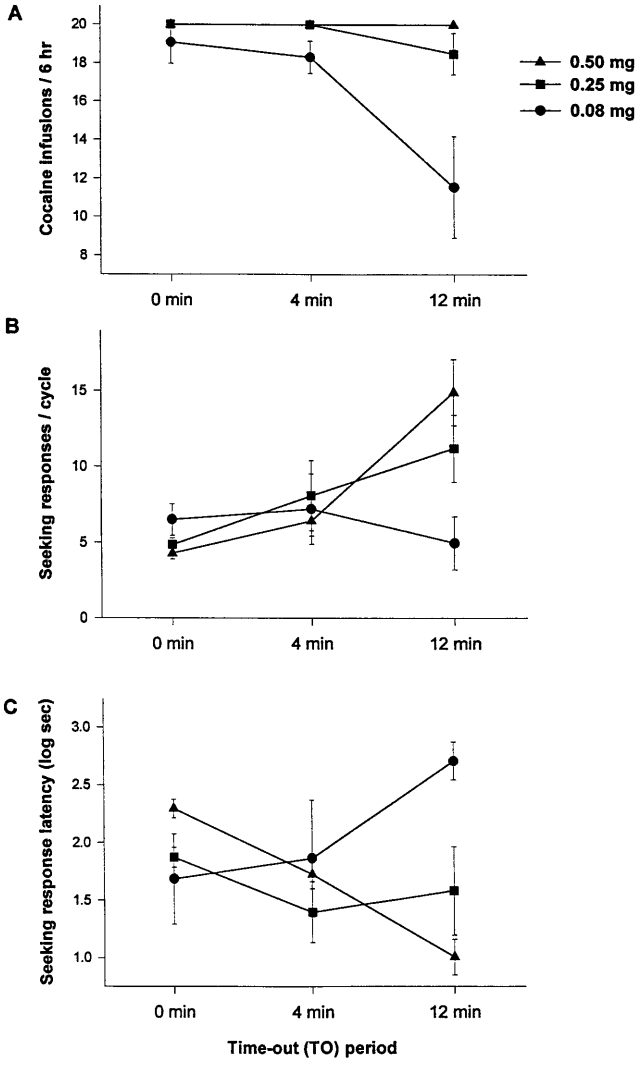

Fig. 3 Experiment 3: effects of a time-out (*TO*) period on the number of cocaine infusions (**A**), cocaine-seeking responses per cycle (**B**), and log latencies of the first cocaine-seeking response (**C**). Data are presented as the mean (+SEM) values for each group (0.08 mg, 0.25 mg, or 0.5 mg cocaine per infusion) at each TO period (0, 4, and 12 min). All animals underwent four test sessions at each TO period. The TO periods represent the latency to introduce the drug-seeking lever following each drug infusion

The analysis of the number of drug-seeking responses per cycle at different TO periods, shown in Fig. 3B, produced a significant dose×TO period interaction [$F(4,18)=9.91$, $P<0.001$]. This interaction arose from the fact that lengthening the TO period increased drug-seeking responses in the 0.50 mg [$F(2,18)=24.40$, $P<0.001$] and 0.25 mg groups [$F(2,18)=8.63$, $P<0.005$], but had no effect at the lowest dose ($F<1$). Moreover, the interaction yielded a significant, positive monotonic effect of dose at the longest TO period [$F(2,9)=7.96$, $P<0.01$] but not at the shorter periods. The 0.50 mg and 0.25 mg doses did not differ but both maintained more responding than the 0.08 mg dose ($P<0.05$) under the 12-min TO period.

Figure 3C shows that the decline in the number of infusions with increasing TO periods in the low dose group was due primarily to a lengthening of the latency to initiate drug-seeking. By contrast, the latency to drug-seek decreased with the duration of the TO period in the 0.50 mg group, yielding a significant dose×TO period interaction [$F(4,18)=10.15$, $P<0.001$]. An analysis of simple main effects revealed a significant effect of dose at the 12-min TO period [$F(2,9)=14.12$, $P<0.005$], but not at the two shorter periods. The mean latency for the 0.08 mg group was significantly longer than those of the other two groups under the 12-min TO period ($P<0.05$) which in turn did not differ. In addition, a significant effect of TO period on the latencies of both the 0.08 mg

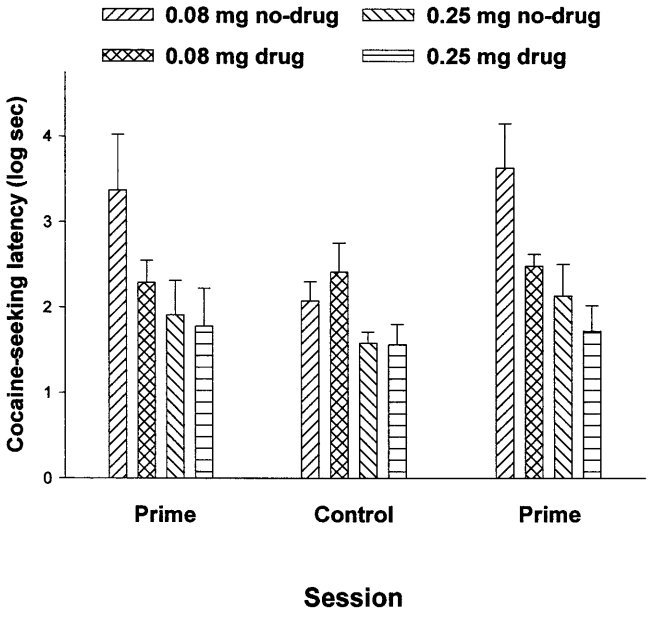

Fig. 4 Experiment 4: effects of contingent cocaine infusions on drug-seeking. Data are presented as the mean (+SEM) log latencies of the first drug-seeking response during drug and no-drug cycles of prime and control sessions. Animals were responding for 0.08 mg or 0.25 mg of cocaine per infusion. The time-out (*TO*) period following each drug infusion was 24 min. During prime sessions, the drug-seeking lever was inserted at the end of the TO period of each no-drug cycle. On drug cycles of the prime sessions, the drug-taking lever was inserted at the end of the TO period and the first response on this lever delivered a cocaine infusion and reintroduced the drug-seeking lever. During the control session, the drug-taking lever was inserted at the end of the TO period on both drug and no-drug cycles, but the drug-taking response produced an injection only during drug cycles

[$F(2,18)=8.00$, $P<0.005$] and the 0.50 mg groups [$F(2,18)=11.2$, $P<0.005$] confirmed the reliability of the contrasting profiles observed for the low and high doses.

Experiment 3: effects of contingent cocaine infusions on drug-seeking

Figure 4 displays the mean latencies of the first drug-seeking response by the 0.08 mg and 0.25 mg groups during sessions 4, 5, and 6 (prime, control, and prime, respectively). On the no-drug cycles of the prime sessions, the drug-seeking lever was inserted at the end of the TO period. On the no-drug cycles of the control session, and the drug cycles of all sessions, the drug-taking lever was inserted at the end of the TO period and the subsequent insertion of the drug-seeking lever was dependent upon a response on the drug-taking lever. However, the drug-taking response only produced an infusion during drug cycles. It is clear that the latency to drug-seek for the low (0.08 mg) dose was profoundly affected by whether or not the first drug-seeking response was preceded by a drug-taking response, even when that response did not produce a drug infusion. In the absence of a drug-taking response (no-drug cycles of prime sessions) the latencies were long, whereas when they were preceded by a drug-taking response but no injection (no-drug cycles of control session), the latencies of the first drug-seeking response were short. The fact that the latencies were as short on the drug as on the no-drug cycles of the control session suggests that the infusion of the drug itself has little effect on subsequent latencies to initiate drug-seeking. In contrast to this profile observed for the low dose group, the latencies of the first drug-seeking response were unaffected by whether or not the animals were required to make a preceding drug-taking response in the 0.25 mg group.

An analysis of the latencies of the first drug-seeking response yielded a marginally significant dose×session× cycle interaction [$F(2,12)=3.80$, $P=0.053$]. Subsequent analyses revealed a significant cycle×session interaction [$F(2,6)=7.94$, $P<0.05$] for the 0.08 mg group but not for the 0.25 mg group. The drug-seeking latencies under the low dose on the no-drug cycles of the prime sessions were significantly longer than on any of the other test cycles ($P<0.05$).

The higher dose (0.25 mg) of cocaine sustained on average 14.0 (+1.7) drug-seeking responses per cycle whereas the lower dose maintained only 6.9 (+1.2) response per cycle, although this difference failed to reach the conventional level of significance [$F(1,6)=4.30$, $P<0.08$] probably due to the relatively low number of animals per group. In contrast to the latency of the first drug-seeking response, however, there was no difference between the number of response on primed and control cycles and this factor was not involved in any significant interactions. The average number of drug-seeking response per cycle was 11.0 (+1.7) for primed cycles and 9.9 (+1.6) for control cycles.

Discussion

The first study replicated the often observed finding that there is an inverse relationship between the number of self-administered cocaine infusions and dose. By contrast, the number of sucrose presentations per session increased with concentration. Increasing the reward magnitude also had different effects on seeking responses for sucrose and cocaine. Sucrose-seeking increased and the latency to initiate responding decreased with higher concentrations, whereas the higher cocaine doses were associated with longer latencies to initiate drug-seeking and no significant increase in seeking responses. In experiment 2, however, a response profile similar to that sustained by the sucrose reward was observed for cocaine when a TO period was imposed between the drug infusion and the opportunity to drug-seek. At the longest TO period, drug-seeking responses increased and latencies to drug-seek decreased with higher doses.

It should be noted, however, that there were some apparent inconsistencies between the results of experiments 1 and 2. The effect of dose on the number of cocaine in-

fusions in the first study, which employed no TO period, was not observed in experiment 2 under the same TO condition. The absence of a dose effect in the second study is probably due to the reduction in the number of available infusions per session, from 100 in the first study to 20 in the second. Secondly, there was reliable effect of cocaine dose on the latency of the first seeking response in experiment 1, but not in the 0-min TO condition of experiment 2. This difference is more apparent than real, however, for the means show the same ordering in the two experiments.

In this discussion, we examine the extent to which the interaction between cocaine dose and the spacing of infusions produced by varying the TO period can be explained in terms of the interaction of a regulative and reinforcement process. We also consider the role of a third, activation process to explain the selective increase in the latency of the seeking response for the lowest cocaine dose at the longest TO period in experiment 2, and the decrease in this latency produced by the opportunity to perform the drug-taking response in experiment 3.

Regulation

Regulation of cocaine intake was first demonstrated experimentally in monkeys (Wilson et al. 1971). Subsequent work confirmed that both rats and primates will adjust their response rate as the dose per injection is varied, resulting in stable rates of drug intake over several months (Johanson and Fischman 1989). A regulative account of these data argues that animals seek to maintain the rate of drug intake, or some internal consequence of the drug, at an optimal level (Yokel and Wise 1976; Wise et al. 1995; Bardo 1998). High drug doses, therefore, decrease response rates because the infusion produces a short-term satiety effect. This can be tested by introducing a delay between each infusion and the next opportunity to earn the drug. Using this manipulation, Winger (1993) reported that responding for a high dose injection increased with longer TO periods. Other studies showed that higher rates of responding are maintained on FR and PR schedules with increasing TO periods (Griffiths et al. 1979). Taken together, these results support a short-term regulation account of drug self-administration in that responding for a relatively high dose increases with the TO period. Our results confirm these data in the rat model of drug self-administration as the number of seeking responses per cycle was positively correlated with the length of the TO period for both the 0.5 and 0.25 mg infusions (see Fig. 3).

It is unlikely that the initial inhibition of responding following a high dose infusion reflects either aversive or motor-inhibiting properties of the drug. First, animals do not chose to self-administer low dose over high dose injections (Iglauer and Woods 1974; Johanson and Schuster 1975; Brady and Griffiths 1977) and, second, animals will lever press at high rates for intra-cranial stimulation between normally spaced lever-presses for psychomotor stimulants (Wise et al. 1977). More likely the TO period allowed the short-term satiety effect produced by the preceding infusion to dissipate before the animal had the opportunity to re-engage in drug-seeking.

Wise et al. (1995) have developed a neurobiological model of the regulative process based on the temporal correlation between cocaine self-administration and extracellular dopamine (DA) concentrations in the nucleus accumbens. They reported that each cocaine infusion elevated DA levels and that the next response was made whenever DA fell to a “trigger point” that was consistent across successive administrations for individual rats. Because the magnitude of DA release was positively related to the dose of the infusion, the time required for the concentration to fall to the trigger point increased with dose. This model, therefore, may account for variations in the latency to initiate drug-seeking observed in our studies. For example, it predicts that the latency of the first drug-seeking response will be positively related to dose, an effect that was observed in experiment 1. Enforcing a TO period between successive cycles of the chain in experiment 2 attenuated this relationship by allowing the DA concentration to decline towards the trigger point during the TO period. Indeed, the temporal profile of the change in DA accords with the effect of the TO manipulation observed in our second experiment. That is, DA levels return to the trigger point approximately 4 min after a 1.0–2.0 mg/kg infusion of cocaine (see Wise et al. 1996, Fig. 6). This temporal profile is compatible with the relatively short latencies to initiate drug-seeking for the 0.50 mg dose (approximately 1.5 mg/kg) following 4- and 12-min TO periods (see Fig. 3, bottom panel).

Behavioral activation

On the other hand, the regulative process by itself cannot provide a full account of the behavioral effects under variations in the TO period (experiment 2). The most pronounced feature of this profile is the increase in the latency to drug-seek for the low dose of cocaine when the TO period was lengthened. Winger (1993) also reported that responding for a low dose of cocaine decreased as the TO increased. One interpretation of these effects is that a cocaine infusion exerts short-term behavioral activation that decays after longer TO periods at the lower dose. In support of this interpretation, allowing the rats to self-administer a low dose infusion following a long TO period, and immediately prior to the insertion of the drug-seeking lever, decreased the latency of the first drug-seeking response in experiment 3. The latency to initiate drug-seeking for the medium dose of cocaine was unaffected by the priming manipulation suggesting that the 0.25 mg dose was sufficient to maintain a tonic level of behavioral activation, even when spaced across long TO periods.

Given that low doses of stimulants produce alerting responses consisting of an increase in exploration, locomotion, grooming and rearing (Scheel-Kruger 1971), the

0.08 mg dose could decrease latencies to respond by enabling the animal to detect the opportunity to engage in drug-seeking behavior shortly after the lever is presented. These infusions may also specifically increase behaviors directed towards obtaining the drug. For example, non-contingent drug administration reinstates responding following the extinction of cocaine or heroin self-administration (de Wit and Stewart 1981, 1983; Stewart and Wise 1992). The phenomenon that "the taste of a rewarding stimulus stirs up or amplifies an interest in additional exposure to the same reward" (Wise 1989) is referred to as priming. A recent study (Arroyo et al. 1998) reported that rats responding for a low (0.08 mg) dose of cocaine under a second-order schedule, respond more in the second than in the first interval of the schedule. The authors attributed this pattern to the priming effect of the infusion at the end of the first interval on performance in the second interval. This pattern matches the temporal profile observed in experiment 2 if it is assumed that responding in the first and second intervals of a second-order schedule is similar to drug-seeking after long and short TO periods under the chain schedule.

One problem with assessing the role of priming in self-administration studies is that these effects may be confounded by regulative mechanisms. That is, a single infusion may induce interest in the drug, but simultaneously satiate the animal. In support of this suggestion, priming (as measured by a decreased latency to respond and increased response rates once initiated) is increased with low doses and decreased with high doses of cocaine (Arroyo et al. 1998; Markou et al. 1999). Most importantly, the low dose priming effect is only apparent in the second interval of a second-order schedule (Arroyo et al. 1998); response patterns in the subsequent intervals resemble those in the first, drug-free, interval. This finding suggests that the infusion at the end of the first interval activates the animal and increases responding in the second interval, but over the session the cumulative effects of repeated infusions satiate the animal, thereby masking or overshadowing the priming effect of an individual infusion.

On the other hand, the fact that latencies in experiment 3 decreased in the low dose group when the drug-taking response was not reinforced suggests that behavioral activation observed in the present studies was not due entirely to priming. The critical condition for initiation drug-seeking in our study is the recent engagement in drug-oriented behavior, not the infusion of the drug. Results of a recent study (Markou et al. 1999) support our findings in that response-contingent cocaine decreased the latency to initiate drug-seeking under a second order schedule, relative to a non-contingent infusion. (It should be noted, however, that relative to the non-infusion baseline condition, the non-contingent infusion lengthened the latency to drug-seek whereas the contingent infusion produced no reliable change from the baseline.)

The fact that simply performing a drug-taking response promotes subsequent drug-seeking has implications for studies which employ a TO period (Balster and Schuster 1973; Griffiths et al. 1979; Winger 1993). In our experiments, animals were restricted from making responses during the TO period, whereas in other TO studies the end of the TO coincided with the presentation of a discriminative stimulus signaling the contingent availability of the drug. Similarly, under progressive ratio and second order schedules, lever pressing responses with no programmed consequences occur during the intervals between each infusions. Our results suggest that responding under these schedules could be partially maintained by the recent engagement in drug-oriented behavior.

Reinforcement

In our experiments, the reinforcing effect of the drug was measured as drug-seeking responses under the heterogeneous chain. The number of seeking responses in the first link of the chain was systematically related to the magnitude of a conventional reinforcer, sucrose, thereby validating this measure as an assay of reinforcement. In contrast, drug-seeking did not increase with dose, although the effect was close to significant (P=0.05). In experiment 2, however, the number of drug-seeking responses increased systematically with dose as the TO period lengthened. This finding is consistent with previous TO studies (Balster and Schuster 1973; Griffiths et al. 1979; Winger 1993) and again accords with the pattern of responding for cocaine under second-order schedules. That is, responding in the first interval (following a 20- to 24-h TO period) increases with dose, whereas the relationship is reversed in subsequent intervals (Markou et al. 1999). Thus, it is clear that when regulative mechanisms are controlled for, the reinforcing effect of the cocaine increases monotonically with dose.

In summary, the present experiments demonstrate that the control of cocaine seeking under a heterogeneous chain schedule is complex and depends upon the interaction of at least three different processes. Both the initiation and performance of seeking response are controlled by a regulative process that inhibits responding in the short-term. Secondly, the propensity to engage in drug-seeking appears to be enhanced by a tonic level of behavioral activation which is sustained over longer inter-infusion intervals by higher doses of the drug and by drug-oriented behaviors. Finally, the effectiveness of cocaine as a reinforcer of drug-seeking increases with the magnitude of the dose once the satiety producing effects of the drug have dissipated.

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References

Arroyo M, Markou A, Robbins TW, Everitt BJ (1998) Acquisition, maintenance and reinstatement of intravenous cocaine self-administration under a second-order schedule of reinforcement in rats: effects of conditioned cues and continuous access to cocaine. Psychopharmacology 140:331–344

Balleine B, Garner C, Gonzalez F, Dickinson A (1995) Motivational control of heterogeneous instrumental chains. J Exp Psychol 21:203–217

Balster RL, Schuster CF (1973) Fixed-interval schedule of cocaine reinforcement: effect of dose and infusion duration. J Exp Anal Behav 20:119–129

Bardo MT (1998) Neuropharmacological mechanisms of drug reward: beyond dopamine in the nucleus accumbens. Crit Rev Neurobiol 12:37–67

Bonem M, Crossman EK (1988) Elucidating the effects of reinforcement magnitude. Psychol Bull 104:348–362

Brady JV, Griffiths RR (1977) Drug-maintained performance and the analysis of stimulant reinforcing effects. In: Ellinwood EH, Kilbey MM (eds) Cocaine and other stimulants. Plenum Press, New York, pp 599–613

Caine SB, Lintz R, Koob GF (1992) Intravenous self-administration techniques in animals. In: Sahgal A (ed) Behavioural neuroscience: a practical approach. IRL Press, pp 117–143

Corrigall WA, Coen KM (1989) Fixed-interval schedules for drug self-administration in the rat. Psychopharmacology 99:136–139

de Wit H, Stewart J (1981) Reinstatement of cocaine-reinforced responding in the rat. Psychopharmacology 75:134–143

de Wit H, Stewart J (1983) Drug reinstatement of heroin-reinforced responding in the rat. Psychopharmacology 79:29–31

Dickinson A, Balleine B (1994) Motivational control of goal-directed action. Anim Learn Behav 22:1–18

Fibiger HC (1978) Drugs and reinforcement mechanisms: a critical review of the catecholamine theory. Annu Rev Pharmacol Toxicol 18:37–56

Goldberg SR (1973) Comparable behavior maintained under fixed ratio and second-order schedules of food presentation, cocaine injection or *d*-amphetamine injection in the squirrel monkey. J Pharmacol Exp Ther 186:18–30

Goldberg SR, Morse WH, Goldberg M (1976) Behavior maintained under a second-order schedule by intramuscular injection of morphine or cocaine in rhesus monkeys. J Pharmacol Exp Ther 199:278–286

Goldberg SR, Kelleher RT, Goldberg DM (1981) Fixed-ratio responding under second-order schedules of food presentation or cocaine injection. J Pharmacol Exp Ther 218:271–281

Griffiths RR, Brady JV, Snell JD (1978) Progressive ratio performance maintained by drug infusions: comparison of cocaine, diethylpropion, chlorphentermine, and fenfluramine. Psychopharmacology 56:5–13

Griffiths RR, Bradford LD, Brady JV (1979) Progressive ratio and fixed ratio schedules of cocaine-maintained responding in baboons. Psychopharmacology 65:125–136

Hodos W (1961) Progressive ratio as a measure of reward strength. Science 134:934–944

Hodos W, Kalman J (1963) Effects of increment size and reinforcer volume on progressive ratio performance. J Exp Anal Behav 6:387–392

Howell LL, Byrd LD (1995) Serotonergic modulation of the behavioral effects of cocaine in the squirrel monkey. J Pharmacol Exp Ther 275:1551–1559

Iglauer C, Woods JH (1974) Concurrent performances: reinforcement by different doses of intravenous cocaine in rhesus monkeys. J Exp Anal Behav 22:179–196

Johanson CE, Fischman MW (1989) The pharmacology of cocaine related to its abuse. Pharmacol Rev 41:3–52

Johanson CE, Schuster CR (1975) A choice procedure for drug reinforcers: cocaine and methylphenidate in rhesus monkey. J Pharmacol Exp Ther 193:676–688

Katz JL (1989) Drugs as reinforcers: pharmacological and behavioural factors. In: Cooper SJ, Liebman JM (eds) The neuropharmacological basis of reward. Clarendon Press, Oxford, pp 164–213

Koob GF, Bloom FE (1988) Cellular and molecular mechanisms of drug dependence. Science 242:715–723

Levene H (1960) Robust tests for equality of variance. In: Olkin I (ed) Contributions to probability and statistics. Stanford University Press, Palo Alto

Markou A, Arroyo M, Everitt BJ (1999) Effects of contingent and non-contingent cocaine on drug-seeking behavior measured using a second-order schedule of cocaine reinforcement in rats. Neuropsychopharmacology 20:542–555

Risner ME, Goldberg SR (1983) A comparison of nicotine and cocaine self-administration in the dog: fixed-ratio and progressive ratio schedules of intravenous drug infusion. J Pharmacol Exp Ther 224:319–326

Roberts DCS, Bennett SAL (1993) Heroin self-administration in rats under a progressive ratio schedule of reinforcement. Psychopharmacology 111:215–218

Scheel-Kruger J (1971) Comparative studies of various amphetamine analogues demonstrating different interactions with the metabolism of the catecholamines in the brain. Eur J Pharmacol 14:47–59

Stewart J, Wise RA (1992) Reinstatement of heroin self-administration habits: morphine prompts and naltrexone discourages renewed responding after extinction. Psychopharmacology 108:79–84

Wilson MC, Hitomi M, Schuster CR (1971) Psychomotor stimulant self-administration as a function of dosage per injection in the rhesus monkey. Psychopharmacologia 22:271–281

Winger G (1993) Fixed-ratio and time-out changes on behavior maintained by cocaine or methohexital in rhesus monkeys: 1. Comparison of reinforcing strength. Exp Clin Psychopharmacol 1:142–531

Winger G, Woods JH (1985) Comparison of fixed-ratio and progressive ratio schedules on maintenance of stimulant drug-reinforced responding. Drug Alcohol Depend 15:123–130

Wise RA (1980) Action of drugs of abuse on brain reward systems. Pharmacol Biochem Behav 13:213–223

Wise RA (1982) Common neural basis for brain stimulation reward, drug reward, and food reward. In: Hoebel BG, Novin D (eds) Neural basis of feeding and reward. Haer Institute, Brunswick, pp 445–454

Wise RA (1989) The brain and reward. In: Liebman JM, Cooper SJ (eds) The neuropharmacological basis of reward. Oxford University Press, Oxford, pp 377–424

Wise RA (1996) Neurobiology of addiction. Curr Opin Neurobiol 6:243–251

Wise RA, Bozarth MA (1987) A psychomotor stimulant theory of addiction. Psychol Rev 94:469–492

Wise RA, Yokel RA, Hansson P, Gerber GJ (1977) Concurrent intracranial self-stimulation and amphetamine self-administration in rats. Pharmacol Biochem Behav 7:459–461

Wise RA, Newton P, Leeb K, Burnette B, Pocock D, Justice JB (1995) Fluctuations in nucleus accumbens dopamine concentration during intravenous cocaine self-administration in rats. Psychopharmacology 120:10–20

Wong LS, Eshel G, Dreher J, Ong J, Jackson DM (1991) Role of dopamine and GABA in the control of motor activity elicited from the rat nucleus accumbens. Pharmacol Biochem Behav 38:829–835

Yanigaita T (1973) An experimental framework for evaluation of dependence liability in various types of drugs in monkeys. Bull Narc 25:57–64

Yokel RA, Wise RA (1976) Attenuation of intravenous amphetamine reinforcement by central dopamine blockade in rats. Psychopharmacology 48:311–318