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ORIGINAL INVESTIGATION

Compulsive drug seeking by rats under punishment: effects of drug taking history

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Abstract
Rationale Abstinence from drug occurs in human addicts for several reasons, including the avoidance of adverse consequences.
Objectives To explore a model of drug use in the face of adverse consequences in rats through intermittent punishment of drug seeking and to investigate whether the ability to withhold seeking responses depends upon the duration of drug history.
Materials and methods Rats were trained under a seeking–taking chained schedule with sucrose or cocaine as reinforcer. Pressing the seeking lever gave access to the taking lever, and a single press on this lever delivered the reinforcer after which the seeking–taking chain recycled. During punishment, half of the seeking links terminated with a mild foot shock without access to the taking link.
Results After a moderate history of reinforcement, punishment of the terminal response in the seeking link suppressed both sucrose- and cocaine-seeking responses. By contrast, rats with an extended cocaine history were more resistant to punishment than those with a moderate cocaine history. This enhanced resistance to punishment was due to a sub-group of rats that showed minimal or no suppression of drug seeking. No differences in suppression of sucrose seeking were observed in animals with moderate versus extended sucrose histories.
Conclusions These results suggest that an extended drug self-administration history decreases the ability of vulnerable rats to suppress their drug seeking.

Keywords Drug addiction · Drug seeking · Cocaine · Punishment · Vulnerability · Compulsion

Introduction

There is as yet no generally agreed procedure for studying abstinence in animals self-administering drugs. Studies of relapse and reinstatement generally have withdrawn the drug reinforcer and, thereby, extinguished the instrumental self-administration response (for reviews, see Shaham et al. 2003; Kalivas and McFarland 2003). It is not clear, however, that performance under extinction has either face or ecological validity as a model of abstinence and addiction (Katz and Higgins 2003; Conklin and Tiffany 2002). The limited clinical data suggest that negative consequences directly related to use are a major reason for abstinence from cocaine taking (Waldorf et al. 1991), and indeed, one of the key characteristic features of addiction (or substance dependence) described in the *Diagnostic and Statistical Manual of Mental Disorders IV* is compulsive drug seeking or drug-taking despite aversive consequences (American Psychiatric Association 2000). The concept of suppression of drug seeking in the face of adverse consequences is one in which the drug user refrains from drug seeking and taking, although these behaviours would still procure the drug, usually because of the aversive and deleterious context or consequences of drug seeking and taking, such as illness, impoverishment, social dys-

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function and the threat of actual punishment and incarceration (Cunningham et al. 2000; Klingemann 1991; Burman 1997). Therefore, this analysis suggests that abstinence should be modelled by the suppression of self-administration by an aversive contingency rather than extinction and addiction by abnormal resistance to this suppression.

Although there have been demonstrations that drug self-administration is suppressed by aversive conditioned stimuli (e.g. Kearns et al. 2002) and by response-contingent punishment (e.g. Panlilio et al. 2003; for review Schuster 1986), two studies suggest that prolonged exposure to instrumental drug self-administration establishes an abnormal resistance to suppression by an aversive contingency that is characteristic of addiction. Vanderschuren and Everitt (2004) trained rats to self-administer cocaine on a seeking–taking chain schedule (Olmstead et al. 2000, 2001; Hutcheson et al. 2001). This schedule distinguishes between drug seeking and drug taking by training rats on a chained schedule under which responding on one lever in the initial seeking link is reinforced not directly by intravenous drug, but by access to the opportunity to press another lever in the taking link, which then directly administers the drug. The chain then recycles after a time-out (TO) period. Given that the TO period is sufficiently long to minimise short-term satiety effects, responding in the seeking link is positively related to the cocaine dose (Olmstead et al. 2000). The distinction between seeking and taking responses not only has face and ecological validity but is also important because reinforcer seeking and taking are controlled by different processes. Whereas performance of a food taking response is influenced by Pavlovian conditioning, Corbit and Balleine (2003) found that the seeking responses are more goal-directed in the sense of being determined by the current incentive value of the reinforcer. Indeed, Olmstead et al. (2001) confirmed the goal-directed nature of cocaine seeking on the chained schedule by demonstrating that independently extinguishing the taking response produced an immediate reduction in seeking.

To investigate the sensitivity of cocaine seeking to aversive contingencies, Vanderschuren and Everitt (2004) presented an aversive conditioned stimulus (CS) that had been independently paired with a shock to assess the extent to which the CS would suppress drug seeking. Whereas substantial suppression was observed after moderate drug self-administration training, the seeking responses were unaffected by the aversive CS after more extensive training. Moreover, this enhanced resistance to the impact of the aversive CS was specific to the use of cocaine as a reinforcer in that the CS produced comparable suppression of sucrose seeking trained on the same seeking–taking chain after moderate and extended training.

Deroche-Gamonet et al. (2004) also reported that extended training enhanced resistance of drug self-administration to aversive contingencies, which in this case, consisted of the discriminated punishment of a nose poke cocaine-taking response by foot shock. In contrast to the Vanderschuren and Everitt (2004) experiment, however, Deroche-Gamonet et al. (2004) found that only a sub-population of their rats was resistant to punishment after extended drug training. Given these divergent results, we re-examined the effect of extended cocaine self-administration on the sensitivity to aversive contingencies by training our rats on the seeking–taking chain employed by Vanderschuren and Everitt (2004) before assessing the suppression of seeking by a response-contingent punishment with a foot shock. Therefore, our aversive contingency was more similar to that studied by Deroche-Gamonet et al. (2004), whereas the cocaine self-administration schedule was the same as that employed by Vanderschuren and Everitt (2004).

A number of features of our training and testing procedure should be noted. First, we wanted to ensure that any response suppression produced by the punishment was mediated by the response-shock contingency to distinguish it from the general conditioned suppression studied by Vanderschuren and Everitt (2004). Consequently, we trained our rats to nose poke for a sucrose solution whilst responding on the seeking–taking chain for cocaine. If the introduction of the punishment produced a general suppression, not only should we have observed a reduction in the punished seeking response, but also a suppression in nose poking for sucrose. By contrast, a specific punishment effect would have been manifest by a selective reduction in cocaine seeking accompanied by maintained responding for sucrose.

The second feature of our procedure concerned the scheduling of the punishment. It is well established that, if a punisher and reward co-occur, the aversiveness of the punisher can be attenuated by counterconditioning through its association with the reward. For example, arranging a punishment contingency in which the response produces an immediate shock followed by the reward can reduce the effectiveness of the shock as a punisher (e.g. Dickinson and Pearce 1976). In this respect, it is notable that the Deroche-Gamonet et al. (2004) procedure arranged for just such shock–reward pairings by selectively punishing only those responses that delivered the cocaine. The possibility that the enhanced resistance to punishment observed in this study was mediated by counterconditioning is suggested by the fact that the reinforcing properties of the cocaine were augmented in the resistant animals. The resistant rats achieved higher ratios on a progressive ratio schedule and responded more during periods of extinction than the non-resistant rats after extended training. Consequently, the increment in the reinforcing properties of the cocaine may have attenuated punishment through enhanced counterconditioning of the aversiveness of the shock in the resistant rats. We also included progressive ratio and extinction

assays of changes in the reinforcing and motivational properties of the cocaine with extended training.

In addition, we negated any role for counterconditioning by punishing only non-reinforced seeking responses to determine whether extended cocaine self-administration produces a directed increase in resistance to punishment. On an unpredictable basis, half of the seeking links of the chained schedule terminated in a shock without access to the taking link, whereas performance in the remaining seeking links yielded access to the taking link as it did during training. This schedule ensured that the punishing shock and the cocaine reward were unpaired, thereby obviating any role for counterconditioning.

Finally, we conducted an assay to determine whether any resistance to punishment could be attributed to a general attenuation of the aversive properties of the shock brought about by extended exposure to cocaine self-administration. By using a conditioned freezing assay, Vanderschuren and Everitt (2004) demonstrated that such attenuation did not occur in their study and we used the same assay.

Materials and methods

Subjects

Male outbred Lister hooded rats (Charles River, Kent, UK), weighing 180–200 g at the start of the experiment were housed in pairs in polycarbonate cages (L=40 cm, W=25 cm, H=18 cm) and maintained under a reversed 12-h light/dark cycle (lights on at 7:00 P.M.) at a constant temperature (21± 1°C), with free access to laboratory chow (SDS Ltd, UK) and water. The experimental procedures were conducted in accordance with the UK's 1986 Animals (scientific procedures) Act (project licence PPL 80/1767).

Apparatus

Instrumental training and testing took place in 12 operant conditioning chambers (29.5×32.5×23.5 cm; Med Associates, Georgia, VT) equipped with two 4-cm wide retractable levers that were mounted in the intelligence panel 12 cm apart and 8 cm from the grid floor. Above each lever was a cue light (2.5 W, 24 V), and a red house light (2.5 W, 24 V) was located on the opposite wall. A dipper delivered 0.04 ml of a 20% (w/v) sucrose solution to a recessed magazine (3.8 cm side and 5.5 cm from the grid floor) situated between the levers. Entry into this magazine was detected by the interruption of an infrared source. The floor of the chamber was covered with a metal grid with bars separated by 1 cm and connected to a shock generator and scrambler (Campden Instruments, UK), which delivered 0.55-mA foot shocks. The grid was located 8 cm above an empty tray. The testing chamber was placed within a sound- and light-attenuating housing equipped with a ventilation fan that also screened external noise. Silastic tubing shielded with a metal spring extended from each animal's intravenous catheter to a liquid swivel (Stoelting, Wood Dale, IL) mounted on an arm fixed outside the operant conditioning chamber. Tygon tubing extended from the swivel to a Razel infusion pump (Semat Technical, UK) located adjacent to the housing. The operant conditioning chambers were controlled by software written in C++ using the Whisker control system (Cardinal et al. 2000).

Conditioned fear was assessed in a chamber (32×22×34 cm; Med Associates) consisting of four black walls and a black, hinged ceiling, which served as a door. The floor was a shock grid with bars separated by 1 cm and connected to a shock generator and a scrambler (Campden Instruments). The grid was located 2 cm above a black sawdust tray. The house light (2.5 W, 24 V) was located in the top right corner of the right wall. A camera (Watec) was attached to the centre of the ceiling so that behaviour of the rats could be recorded for analysis. The chamber was controlled by a RiscPC6000 Acorn computer with programmes written in Arachnid (Paul Frey, UK).

Surgery

The rats trained with the cocaine reinforcer were anaesthetised with ketamine hydrochloride (100 mg/kg, i.p.; Ketaset) and xylazine (9 mg/kg, i.p.; Rompun) and supplemented with ketamine as needed (20 mg/kg). Rats were implanted with a single catheter in the right jugular aimed at the left vena cava. Catheters were made in-house from 22-g cannulae with elongated ends (CamCaths, Cambridge, UK). Silastic tubing (0.012 inner diameter) was secured to the bottom end of the cannula, and the top was fixed to nylon mesh with dental acrylic and silicone. The mesh end of the catheter was sutured sub-cutaneously on the dorsum. To prevent infection, rats were treated post-surgically with 10 mg/kg Baytril sub-cutaneously (Genus Express, Bury St. Edmunds, UK) for 7 days (Caine et al. 1992).

Procedure

Four groups of rats were distinguished by whether they were trained with the cocaine or sucrose reinforcer and by whether or not training on the seeking–taking chain was extended before the seeking response was punished, thereby yielding the moderate-cocaine group (n=19), the moderate-sucrose group (n=13), the extended-cocaine group (n=21), and the extended-sucrose group (n=12). A fifth, unpunished control group (n=14) received the same

initial training as the moderate-cocaine group before being shifted to the punishment schedule but with the delivery of the shock punisher omitted.

Figure 1 illustrates the timeline of the experimental procedure, which consisted of the stages detailed below.

Acquisition of the seeking–taking task

Acquisition of the taking response Behavioural training began 7–10 days after surgery. Each session began with the insertion of the taking lever with side of this lever counterbalanced across the rats in each group. Responding was reinforced under a fixed ratio (FR) 1 schedule so that each lever press produced either a 0.25-mg/kg infusion of cocaine at the rate of 0.1 ml/5 s (for the cocaine groups) or 0.2 ml of a 20% sucrose solution, which was delivered by presenting the dipper five times during 5 s at the rate of one presentation per second. The reinforcers were accompanied by the withdrawal of the taking lever, the extinction of the house light, and the illumination of the stimulus light above the lever for 20 s. The sessions terminated after either 30 cocaine infusions or 2 h or after either 30 sucrose reinforcers or 1 h, depending upon which criterion was met first. Training of the taking response continued for seven to ten sessions.

Training of the seeking–taking chain Each cycle of the seeking–taking chained schedule started with the insertion of the drug-seeking lever with the taking lever retracted, and the first press on the seeking lever initiated a random interval (RI) schedule. The first lever press after the RI had elapsed terminated the first link of the chain, resulting in the retraction of the seeking lever and insertion of the drug-taking lever to initiate the second link. One press on the taking lever was followed by the reinforcement event, either a drug infusion or sucrose presentations, accompanied by the same stimulus events as during the training of the taking response. There followed a TO period, which was 15 s following each sucrose reinforcer but progressively increased across sessions from 20 s to 10 min after each cocaine infusion. Thereafter, the seeking lever was reinserted to start the next cycle of the schedule. The RI parameter was progressively increased from 2 to 120 s. Consequently, at the end of this training, which took five sessions, the rats were responding on a heterogeneous chained (tandem FR 1 RI 120-s) FR 1 TO schedule allowing a maximum of 11 reinforcements. All groups then received three further sessions of training on this seeking–taking chain.

During these sessions, the rats in the moderate-cocaine and extended-cocaine groups were also trained to nose poke into the magazine for 0.04 ml of a 20% sucrose solution, which was delivered under an RI schedule, the parameter of which was progressively increased to 60 s. As this response was performed concurrently with the seeking and taking responses, performance of nose poking allowed an assessment of the specificity of response suppression when the punishment contingencies were introduced.

Extended training The extended training groups then received 14 further sessions of training on the seeking–taking chain. To increase the extent of reinforcer-taking experience, Vanderschuren and Everitt (2004) also gave their rats eight free access sessions with only the taking lever present in each of which the rats could earn 80 reinforcements (cocaine for extended-cocaine group and sucrose for extended-sucrose group) under an FR 1 schedule with a post-reinforcement TO of 20 s. As our intention was to use an extended training regime similar to that employed by Vanderschuren and Everitt (2004), we also interspersed eight FR 1 taking sessions among 11 days of additional sessions of training on the seeking–taking chain schedule. For the extended-cocaine group, sucrose remained available for nose poking during the additional seeking–taking and FR 1 taking sessions.

Motivation assessment To provide assessments of the reinforcing and motivational properties of the cocaine that were similar to those used by Deroche-Gamonet et al. (2004), we conducted progressive ratio and extinction. The assessment of performance on each of these tests was restricted to a single session to minimise any disruption of performance on the seeking–taking chain.

During the first progressive ratio test, the seeking lever was withdrawn, and the ratio requirement of taking response was increased after each reinforcer according to

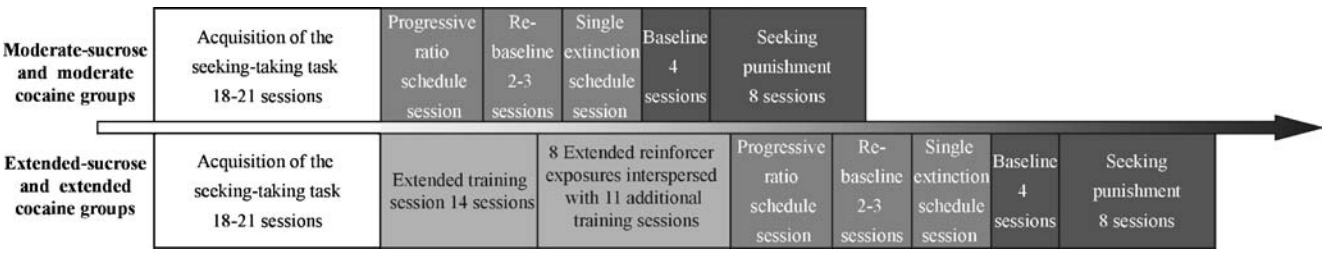

Fig. 1 Flowchart of the simplified experimental procedure. For details, see "Materials and methods"

the following progression: 1, 3, 6, 9, 12, 17, 24, 32, 42, 56, 73, 95, 124, 161, 208, 268, 346, 445, 573 and 737. The value of the last ratio completed was taken as the break-point (Hodos 1961). The session ceased after 4 h or after a period of 40 min elapsed since the previous reinforcer delivery.

After the progressive ratio session, baseline performance on the seeking–taking chain was re-established for two to three sessions before we assessed any effects of extended training on motivation and reinforcement using the seeking rather than taking response. During this test session, the seeking–taking chain was suspended and responding on the seeking lever alone was measured in extinction. Completion of the seeking link led to a direct transition to the TO period, without the taking link, after which the seeking chain restarted.

Punishment All rats received a further four sessions of training under the seeking–taking chain to establish a baseline against which to assess the effects of punishment. As noted in the "Introduction", punishement and reward were scheduled on separate cycles to ensure that the punishing effect of the shock was not modulated by counter conditioning. During each punishment session, half of the cycles contained no punishment and were identical to those in baseline training, i.e. terminated with access to the taking lever and, thereby, cocaine or sucrose availability. In the remaining cycles, the seeking response was punished: The first response that met the RI requirement in the seeking link delivered the 0.5-s foot shock and led to a direct transition to the TO period without the taking link. The reinforced and punished cycles were presented randomly within eight daily sessions. Because this schedule introduced a decrease in the frequency of reinforcement of the seeking response at the same time as the punishment contingency, an independent, unpunished control group received the same initial training as the moderate-cocaine group before being shifted to the intermittent schedule, but with the delivery of the shock punisher omitted.

Conditioned fear Finally, we used the contextual fear conditioning procedure (Lee et al. 2004) employed by Vanderschuren and Everitt (2004) to assess whether there was a relationship between the effectiveness of the punishment and the capacity of the shock to support aversive conditioning. Rats were individually placed in the conditioning chamber. The house light was illuminated, and after 2 min, one 2-s 0.6-mA foot shock was administered. After 1 min, the animals were removed and returned to their home cage. Freezing, defined as the lack of movements except breathing and counted at 5-s intervals to give a percentage freezing measure, was not observed before the presentation of the foot shock. Twenty-four hours later, the animals returned to the conditioning chamber for a 2-min test session in which animals expressed freezing as the results of aversive conditioning. The videos were analysed for freezing during the test session and also for locomotor activity during the shock as a measure of responsiveness to the shock. For locomotor activity measurements, the cage was divided virtually into four equal sectors, and the number of sectors crossed by the base of the tail was measured during the 2-s foot shock administration.

Results

Because the sucrose reinforcer maintained a higher rate of the seeking response than the cocaine reinforcer [352±41 and 231±22 responses per session, respectively; $F(1,61)=6.9$, $p=.011$], our initial analysis of responding under punishment employed a ratio of the number of seeking responses during the last punishment session to the number of responses during the last baseline session to minimise the contribution of baseline differences. Figure 2 shows the distributions of these suppression ratios for the punished groups in the form of a cumulative function. As the variance of the ratios increased with their mean, the ratios were log transformed before analysis, which yielded a significant interaction between reinforcer type and the amount of training [$F(1,61)=4.6$, $p=.036$] when assessed

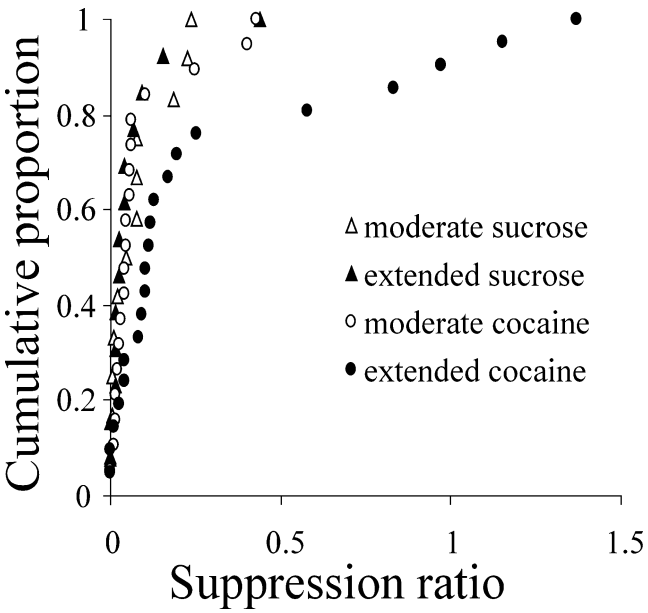

Fig. 2 Cumulative proportion of the populations with a moderate (*white*) or extended (*black*) sucrose (*triangles*) or cocaine (*dots*) seeking taking history according to suppression ratio during the last day of punishment

against a Type 1 error rate of 0.05. Pairwise comparisons established that, on average, rats with an extended history of cocaine self-administration had higher ratios than those with a moderate history (p=0.009), whereas the amount of training did not affect the sensitivity to punishment with the sucrose reinforcer.

However, inspection of Fig. 2 suggests that this overall analysis obscured an important variation in sensitivity to punishment. Whereas three of the punished groups yielded a relatively homogenous distribution of suppression ratios, the rats in the extended-cocaine group appeared to fall into two sub-groups, one sensitive to punishment and one resistant to punishment. Although the majority of the animals in the extended-cocaine group yielded a distribution of ratios similar to that of the other groups, five animals in this group had suppression ratios higher than the maximum value observed in the moderate-sucrose and cocaine groups and the extended-sucrose group. Indeed, an analysis of the population by the Kolmogorof–Smirnof test revealed that the distribution of suppression ratios of the extended-cocaine group differed from both the extended-sucrose group (Z=1.588, p=0.013) and the moderate-cocaine group (Z=1.425, p=0.034). Consequently, we divided the extended cocaine group into two sub-groups for further analysis: an extended cocaine-resistant group (n=5), which was composed of the rats whose suppression ratios were higher that any of those in the other punished groups, and an extended cocaine-sensitive group (n=16), whose suppression ratios fell within the range of the other groups.

Performance was then assessed by two measures: the total number of seeking responses per session and the number of seeking cycles completed, which are displayed in the top and bottom panels of Fig. 3, respectively. The effect of punishment was assessed by a within-subject variable contrasting the mean performance measures during the four baseline sessions with those during the eight punishment sessions. The first point to note is that the reduction in responding under punishment was due to the introduction of the shock rather than to the change in reinforcement contingency for the seeking response as illustrated in the bottom left part of Fig. 3. All of the rats in the moderate-cocaine group performed fewer seeking responses and completed fewer cycles during the punishment sessions than did any of the rats in the unpunished control group. Moreover, this suppression was specific to

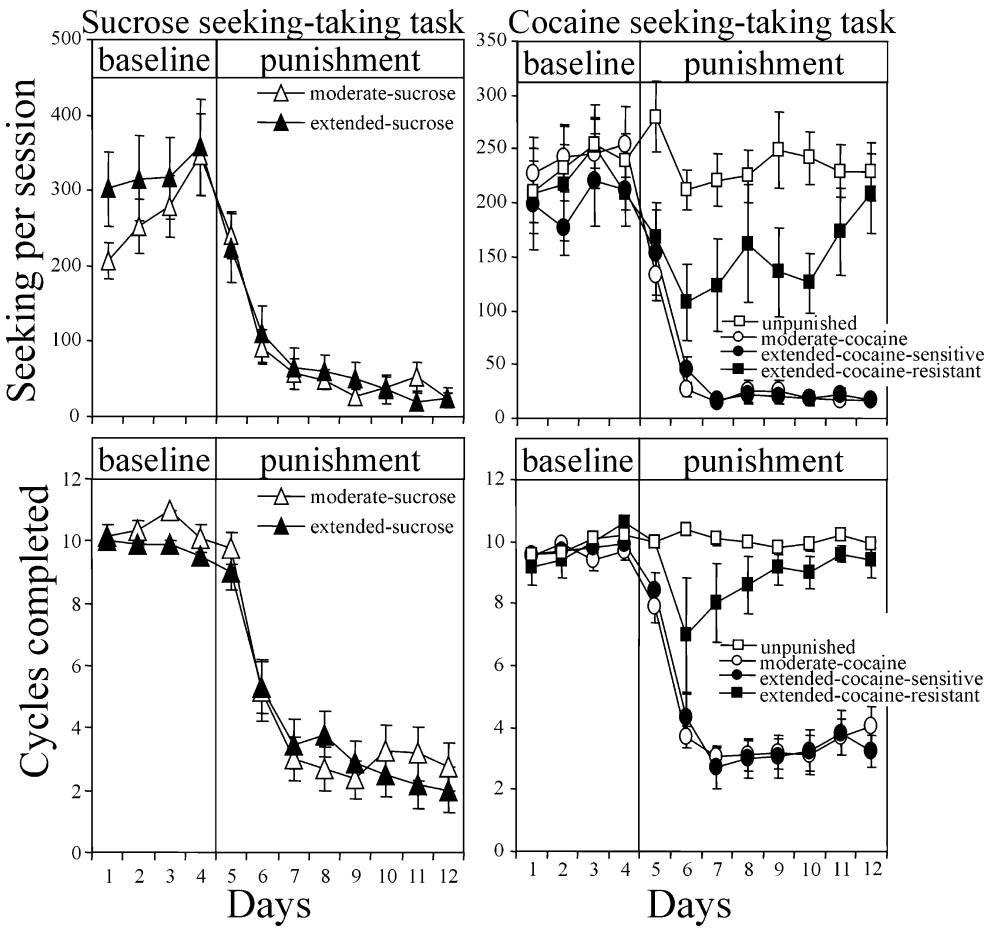

Fig. 3 Total number of seeking responses per session (*top panels*) and number of cycles completed (*bottom panels*) before (*baseline*) and during partial extinction (*white square*) or punishment of the seeking response (*punishment*) for sucrose (*left panels*) or cocaine (*right panels*) after a moderate (*white*) or extended (*black*) reinforcer history. The animals with an extended history of cocaine taking were divided according to their sensitivity or resistance to punishment of the seeking response. Average±SEM of 5 to 19 animals per group

the punished seeking response. Thus, the mean(±SEM) number of nose pokes per second for sucrose during seeking cycles of the punishment sessions by the moderate-cocaine group (0.78±0.1) was very similar to that of the unpunished control group (0.70±0.1).

The amount of training had no effect on resistance to punishment of the sucrose-trained animals, which was confirmed by a significant effect of punishment for both measures [Fs(1,23)>82, ps<0.001] that did not interact with the amount of training for either measure. By contrast, analysis of the performance of the cocaine-trained animals revealed a significant training×punishment interaction for both measures [Fs(2,37)>3.6, ps<0.036], which reflected the fact that the amount of training and sensitivity to punishment affected performance under the punishment contingency [Fs (2,37)>14.7, p<0.001] but not during the baseline sessions. Whereas the performance of the three groups did not differ during baseline training, the extended cocaine-resistant group responded more during punishment sessions than the extended cocaine-sensitive (p<0.001) and moderate-cocaine groups (p<0.001), which again did not differ. Finally, it should be noted that there was no evidence that the punishment contingency reduced performance by either measure in the extended cocaine-resistant group, whereas there was a highly significant effect of punishment for both the extended cocaine-sensitive group [Fs(1,15)>39.9, p<0.001] and moderate-cocaine group [Fs(1,18)>79.9, p<0.001].

Figure 4 confirms that the suppression of responding produced by the punishment of cocaine seeking was specific to the punished response. The left panel reproduces the performance of the cocaine-seeking response during baseline and punishment sessions each when averaged across sessions and illustrates the relative resistance of the extended cocaine-resistant group to punishment. This response profile contrasts with that for concurrent nose poking for sucrose illustrated in the right panel of Fig. 4.

The first point to note is that the extended cocaine-sensitive group showed higher nose poke rates during baseline seeking than both extended cocaine-resistant (p=0.007) and moderate-cocaine group (p=0.008). More importantly, however, is the fact that the introduction of punishment did not produce suppression of nose poking, thereby demonstrating that the effect of punishment was specific to the seeking response and did not reflect a general suppression of responding. Finally, no differences during punishment in nose poke rate during seeking was observed between the groups demonstrating that the differential effects of the punishment of cocaine seeking was not secondary to group differences in competition from unpunished behaviour.

Whether or not extending training modulated the reinforcing and motivation properties of the cocaine is not entirely clear. Although there was no significant effect of group [F(2,37)=2.76, p=0.076] on the breakpoint under the progressive ratio schedule, Table 1 shows that the extended cocaine-resistant group had a higher breakpoint than the sensitive group [T(19)=2.5, p=0.03] but not the moderate cocaine group [T(22)=0.7] when assessed by simple pairwise comparisons. However, there was no reliable correlation between the resistance to punishment expressed as the suppression ratio and the breakpoint under the progressive ratio schedule [Spearman's r(19)=0.16]. Moreover, although the extended cocaine-resistant group responded on average more than the extended cocaine-sensitive and moderate-cocaine groups during the extinction test, the F ratio for the effect of groups was less than one [F(2,73)=0.97, p=0.39] showing that this difference was far from reliable.

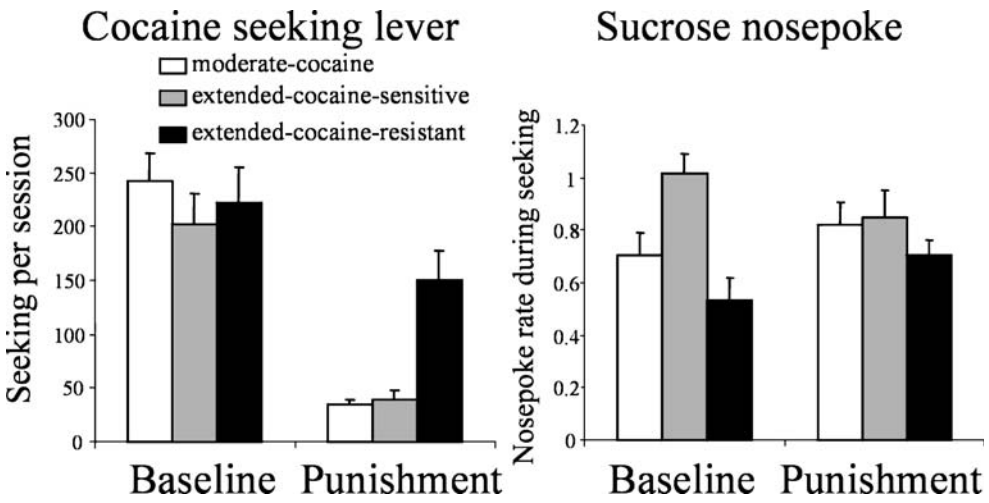

Fig. 4 Total number of cocaine seeking responses per session (*left panel*) and nose poke rate (nose poke per second) during the seeking schedule (*right panel*) during baseline and during punishment of seeking responses for moderate-cocaine (*white bars*), extended cocaine-sensitive (*grey bars*) and cocaine-resistant (*black bars*) groups. Mean±SEM of 5 to 19 animals per group

Table 1 Motivation indices (break points under a progressive ratio schedule of reinforcement, number of seeking responses per session [see "Material and Methods" for procedure], shock sensitivity [activity during shock presentation of the fear conditioning period] and freezing in the context previously associated with shock) for the animals with either moderate or extended history of cocaine. Mean±SEM of 5 to 19 animals per group

		Breakpoint	Number of seeking responses per session	Number of lines crossed during shock	Percentage of time freezing
Moderate-cocaine (n=19)		177±32	175±19	6±1	45±5
Extended-cocaine	Sensitive to seeking punishment (n=16)	98±16	173±30	5±1	51±4
	Resistant to seeking punishment (n=5)	193±49	237±26	4±1	55±11

The animals with an extended history of cocaine taking were divided according to their sensitivity or resistance to punishment of the seeking response.

Finally, it is clear that the variation in resistance to punishment did not reflect corresponding differences in the sensitivity to the aversive properties of foot shock nor in aversive conditioning. Table 1 also shows that the groups did not differ in shock reactivity as assessed by the number of line crossings during shock administration [F(2,37)=1.4], nor in the level of aversive contextual conditioning as assessed by the percentage of time spent freezing in the test session, which did not differ between groups [F(2,37)=0.7].

Discussion

This experiment yielded two main conclusions. The first is that both drug and food seeking were suppressed by direct punishment of the seeking response after a moderate training history. Second, and more importantly, extended cocaine self-administration significantly enhanced resistance to punishment due to the fact that there was a sub-population of rats for which the punishment of drug seeking failed to suppress responding, but only after extended cocaine self-administration experience. We address each of these findings.

In the procedures used in these experiments, seeking and taking responses, if meeting the schedule requirement, continued to result in intravenous cocaine self-administration. However, the addition of a punishment contingency for the seeking response induced a progressive suppression in the number of drug-seeking cycles completed and the total number of seeking responses for those rats that had a limited history of cocaine self-administration. The suppression was not due to partial extinction associated with the punishment schedule because the unpunished control group continued to respond at the baseline rate during sessions in which there was a decrease in reinforcement probability similar to that experienced under the punishment schedule. Thus, under these conditions, intermittent punishment of seeking responses resulted in suppression of cocaine self-administration. Moreover, drug and sucrose seeking showed a comparable sensitivity to punishment, at least after limited training on the chained schedule, indicating that cocaine functions like a natural reinforcer in this respect.

By contrast, the behaviour maintained by the two reinforcers diverged after more extended training. Whereas the sensitivity of sucrose seeking to punishment was unaffected by the amount of training, this variable had a profound effect on a sub-group of rats trained for the cocaine reinforcer. Although all the sucrose-trained rats and those that received limited training with the cocaine reinforcer showed a clear suppression under punishment, there was a sub-group of rats, which received extended training with the cocaine reinforcer, that failed to show significant suppression. Although the comparison between sucrose and cocaine reinforcement was confounded by the presence of a concurrent nose poke for sucrose task for the cocaine-reinforcement animals, it is unlikely that the presence of the opportunity to nose poke for sucrose during punishment of cocaine seeking would have enhanced resistance to cocaine-seeking punishment—the presence of an alternative reinforcer, if anything, facilitates punishment-induced suppression (Thompson et al. 1999). Therefore, the resistant animals failed to show any suppression even with the presence of an alternative reinforcer.

In a number of respects, the profile of responding under punishment was very similar to the shock-induced response suppression observed by Vanderschuren and Everitt (2004) after comparable training on the seeking–taking chain. To recap, they too found that extended training with cocaine, but not sucrose, enhanced the resistance to response suppression without a concomitant change in sensitivity to shock or general aversive conditioning. However, there are two important factors that differentiate our findings from those of Vanderschuren and Everitt (2004). First, their suppression was induced by a response-independent aversive CS rather than by response-contingent punishment, and we can be certain that the response reduction observed in the present experiment was not a general form of

conditioned suppression. To the extent that the introduction of the punishment produced a general behavioural suppression, the concurrent nose poking for sucrose should also have been reduced. At variance with this prediction, the rate of nose poking was unaffected by the introduction of the punishment of cocaine seeking.

The second major difference between the drug-induced resistance to punishment observed in the present study and conditioned suppression in the Vanderschuren and Everitt (2004) study is the population of rats that showed the effect. Although both studies used the same strain of rats from the same supplier and trained them in the same apparatus, the resistance to *conditioned* suppression produced by extended cocaine training on the seeking–taking chain was a general characteristic of these rats, whereas the resistance to punishment, seen in the present experiment, was manifested by only a sub-population. Whether this difference represents an important difference in the mechanisms mediating conditioned suppression and response-contingent punishment or a parametric and procedural difference is unclear. As previous studies on punishment of drug-related behaviours have shown that suppression of such behaviours depends on the intensity of the punishment applied after moderate training (Grove and Schuster 1974), we assume that there is a shock magnitude at which response-contingent punishment would have suppressed cocaine seeking by all rats. Moreover, Vanderschuren and Everitt (2004) assessed conditioned suppression of drug seeking in a single extinction test that may have been insufficiently sensitive or long enough to reveal a resistant sub-group. Nevertheless, the present experiment and the Vanderschuren and Everitt (2004) study are importantly similar in that the failure of instrumental punishment and conditioned aversive stimuli to suppress cocaine seeking was only seen in rats with an extended history of cocaine self-administration.

In a number of respects, the profile of responding that we observed is more similar to that reported by Deroche-Gamonet et al. (2004) in spite of major differences in the cocaine self-administration regime and schedule. They also identified a comparable sub-population of rats for which cocaine self-administration was not suppressed by response-contingent foot shock after prolonged self-administration training. The present findings not only replicated their result but also extended it in at least two important respects. First, Deroche-Gamonet et al. (2004) demonstrated the developing resistance to punishment using a within-subject procedure by testing the same rats repeatedly after different amounts of training. Consequently, it is conceivable that the variation in resistance to punishment after extended training reflected a corresponding variation in adaptation to the shock, as it is well established that sensitivity to punishment varies with prior shock exposure (for review, see Church 1963). By contrast, the ineffectiveness of punishment after extended cocaine training in our study could not have been secondary to differences in exposure to the foot shock because the effect of punishment was assessed in different groups of animals having different cocaine self-administration histories. Moreover, we observed that neither the unconditioned response to the foot shock nor its ability to condition the freezing response was affected by the amount of training on the seeking–taking chain for cocaine.

Second, as noted in the "Introduction", the punishment procedure in Deroche-Gamonet et al. (2004) arranged shock–cocaine pairings that could have counterconditioned the effectiveness of the shock as a punisher. Consequently, the enhanced resistance to punishment could have been due to an increment in the positive reinforcing effects of cocaine brought about by extended training in the resistant rats, and in this respect, it is notable that these rats had higher breakpoints on a progressive ratio schedule and responded more in the extinction periods. Therefore, the resistance to punishment could not only have been augmented by counterconditioning, but also by establishing a stronger baseline level of self-administration.

Although our punishment procedure precluded counterconditioning by arranging for unpaired presentation of the shock and cocaine, the effect of extended training on the motivating and reinforcing effects of the cocaine are not entirely clear. In the Deroche-Gamonet et al. (2004) study, the sub-population of punishment-resistant rats expressed higher breakpoints for cocaine under a progressive ratio schedule and higher response rates during non-drug period after extended training relative to both the performance of the same resistant rats after limited training and relative to the punishment-sensitive rats after extended training. By contrast, the only evidence that extended training enhanced the motivating properties of the cocaine in our study was that resistant rats had higher breakpoints than the sensitive rats after extended training. However, we do not believe that the resistance to punishment was secondary to an increase in the reinforcing and motivating properties of the cocaine with extended training for three reasons. First, there was no correlation between the breakpoint on the progressive ratio schedule and the suppression ratio under punishment. Second, the locus of the difference in progressive ratio performance lies with the extended cocaine-sensitive group, which had low breakpoints not only relative to the extended-cocaine resistant group, but also relative to the moderate-cocaine group. Moreover, it was the extended cocaine-sensitive group that showed the abnormally high levels of baseline concurrent nose poking for sucrose. However, the most compelling evidence that the resistance to punishment was not secondary to the effect of extended training on the motivating and reinforcing

properties of the cocaine comes from the baseline levels of the seeking response. It is well established that the rate of cocaine seeking under the schedule parameters used in the present experiment is sensitive to the drug dose (Olmstead et al. 2000), and therefore, we should have expected resistant rats to have shown higher baseline levels of the seeking response if extended training had selectively enhanced the cocaine reinforcement and motivation in these animals. However, the baseline rates of cocaine seeking by the extended cocaine-resistant group were very similar to those of the other groups.

Finally, we should note that, because the experimental protocol involved both extended training of the seeking response and extended cocaine taking, it remains unknown whether both or just one of these variables enhanced the resistance to punishment of the sub-population of animals. In the case of oral drug taking, prolonged intake cannot only render drug consumption resistant to the effects of adulteration of the solution with an aversive substance (Heyne and Wolffgramm 1998) but can also enhance the reinforcing properties of both drug and non-drug rewards (Miles et al. 2004). Therefore, it is possible that the resistance to punishment is induced simply by extended drug administration rather than by prolonged training of the seeking response.

Whatever the critical variable may be, however, the present results suggest that the punishment-resistant subgroup of rats may provide an animal experimental model of the *Diagnostic and Statistical Manual of Mental Disorders* criteria, which define addiction in terms of compulsive drug seeking that occurs despite adverse consequences. Understanding the neural basis of this compulsive form of drug seeking, as well as its individual and experiential (e.g. drug history) determinants, may shed light on the vulnerability to developing a compulsive mode of drug seeking behaviour that is recognised clinically as the addicted, or substance-dependent, state.

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