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Drug Intake is Sufficient, but Conditioning is not Necessary for the Emergence of Compulsive Cocaine Seeking After Extended Self-Administration

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Compulsive drug seeking, which is characterized by continued instrumental effort despite contingent punishment, has been shown to emerge after extended drug self-administration. Exactly what aspect of drug self-administration drives the appearance of addictive behavior is unclear, but the mechanistic explanations that have been offered differ in one key respect. On one hand, it has been suggested that dysfunctional conditioning during self-administration drives unrealistic reward expectations, ultimately producing resistance to punishment. If this is indeed the pathological process that drives compulsive behavior, then compulsivity should be apparent only in the presence of the pavlovian and instrumental stimuli that underwent frequent pairing with the drug reward. On the other hand, it has also been suggested that extended drug intake produces general changes to reward and decision-making circuits that manifest as compulsive drug seeking. Unfortunately, conditioning history and drug intake are generally intrinsically intertwined. However, here we used an animal model of compulsive cocaine seeking to selectively manipulate drug intake and the degree of conditioning in the test context, to investigate which of the two is more important for the emergence of compulsive cocaine seeking. The results show that extended drug intake alone is sufficient, but extended conditioning in the test context is not necessary for the emergence of compulsive cocaine seeking, resolving a fundamental question in addiction research.

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INTRODUCTION

Drug addiction is a complex behavioral disorder that is characterized by compulsive drug seeking, or continued instrumental effort despite contingent punishment (DSM-IV, 2000). Drug addiction does not manifest itself immediately after first use, but develops slowly with repeated drug consumption, as the interval between first cocaine use and diagnosis of cocaine dependence has been reported to lie between 4 to 12 months (Ridenour *et al*, 2006). Clearly, continued drug use has some effect on the individual that can lead to compulsive drug use, although exactly what aspect of the drug is producing this compulsive use is an important subject of debate. The explanations that have been offered to date are numerous and varied, but in one important sense they explain the emergence of drug addiction through two different mechanisms.

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On one hand, investigators have proposed that the abnormal activation of reward circuits by drugs of abuse may drive dysfunctional conditioning, such that the incentive or expected value of drug consumption is overestimated by neural motivational and hedonic circuits (Berke and Hyman, 2000; Di Chiara *et al*, 1999; Kauer and Malenka, 2007). This would then enable strong motivation for drugs in spite of contingent punishments that actually outweigh the rewarding effects of the drugs. A more specific proposal, based on the prediction error signal hypothesis of phasic dopamine signals in the brain (Schultz *et al*, 1997), posits that drug intake pharmacologically activates dopamine transients that function as prediction error signals, perpetually increasing the expected value of drugs even if the actual value of the drug outcome is lower than predicted (Redish, 2004). If this is indeed the pathological process that drives compulsive behavior, then compulsivity should be apparent only in the presence of pavlovian and instrumental stimuli that underwent frequent pairing with the drug reward.

On the other hand, it has been noted that drugs of abuse produce general changes to neural reward (Koob and Moal, 1997) and decision-making circuits (Jentsch and

Taylor, 1999) that do not necessarily depend on the drug conditioning context. Thus, prior administration of cocaine has been shown to reduce the functioning of brain reward circuits (Ahmed *et al*, 2002), to produce perseverative choice behavior on reversal learning tasks (Ersche *et al*, 2008; Schoenbaum *et al*, 2004), to increase the motivation to work for rewards (Olausson *et al*, 2006), and to impair the functioning of circuits for goal-directed control of instrumental behavior (Nelson and Killcross, 2006). Crucially, these impairments were observed while animals were working in novel contexts for rewards other than cocaine. The combination of decreased functioning of brain reward circuits, increased incentive for rewarding stimuli, and perseverative and more habitual behaviors could well explain the appearance of compulsive drug use as a function of extended drug consumption. If these drug-intake induced changes to decision-making circuits drive compulsive drug use, then compulsive drug use should transfer to alternative drug self-administration contexts once induced, as the compulsivity should be independent of conditioning. However, drug-induced changes to decision-making circuits also affect natural reward behaviors, and it remains to be determined if they would also produce truly compulsive seeking of high incentive natural rewards such as sucrose.

Unfortunately, conditioning and drug intake are generally intrinsically intertwined during extended drug consumption, making it hard to disentangle the relative contribution of the two processes to the development of addiction. However, it is clearly possible to have subjects undergo extended conditioning in a distinct and separable secondary context, allowing for a test of the sufficiency of extended drug intake and the necessity of conditioning for the appearance of compulsive drug use. Several animal models of compulsive drug use after extended cocaine self-administration have recently been developed (Deroche-Gamonet *et al*, 2004; Pelloux *et al*, 2007), modeling persistent cocaine seeking despite intermittent and unpredictable punishment. These allow for the rigorous experimental control needed to manipulate drug intake and conditioning separately, while controlling other variables, thereby enabling the investigation of the separate contributions of these two processes to the appearance of compulsive drug seeking. In the following experiments, animals were allowed to self-administer cocaine in two totally distinct contexts with different instrumental responses and conditioned stimuli. There were three groups of animals with: (i) limited intake and few response-cocaine pairings in the test context; (ii) extended intake and few pairings in the test context; and (iii) extended intake and many pairings in the test context. The fourth combination of limited drug intake and many pairings is not practically feasible without varying the drug dose (which would affect the quality of conditioning trials), so it was not possible to test if extended conditioning was sufficient for compulsive drug use. After testing for compulsivity of cocaine seeking, all animals acquired a matched sucrose seeking task in a third distinct context with a separate instrumental response, and the compulsivity of sucrose seeking was assessed to see if prior drug intake influenced sensitivity to punishment of sucrose seeking.

MATERIALS AND METHODS

Subjects

Male outbred Lister hooded rats (Charles River, Kent, UK), weighing 275–325 *g* at the start of the experiments were housed individually in polycarbonate cages (L = 40 cm, W = 25 cm, H = 18 cm) and maintained under a reversed 12-hr light/dark cycle (lights on at 1900 hours) at a constant temperature (21 ± 1 °C) with free access to water. Each subject was given 18 *g* of laboratory chow (SDS, UK) per day, which was sufficient to maintain body weight at no less than 85% of free feeding weight. The experimental procedures were conducted in accordance with the UK's 1986 Animals (scientific procedures) Act (project licence PPL 80/2234).

Apparatus

Instrumental training and testing took place in 18 operant conditioning chambers (29.5 × 32.5 × 23.5 cm; Med Associates, Georgia, VT). In all boxes, 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 0.50-mA foot shocks. The 12 boxes that were used for cocaine self-administration had silastic tubing shielded with a metal spring extending 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 testing chamber was placed within a sound- and light-attenuating housing equipped with a ventilation fan that also screened external noise. The operant conditioning chambers were controlled by software written in C++ using the Whisker control system (Cardinal, 2001). In addition, the boxes were configured in three different ways to provide distinct contexts (see Figure 1 for both cocaine self-administration contexts).

Six boxes that were equipped for cocaine self-administration were designated context A, and were located in testing room A. These boxes contained two standard 4 cm

	Context A	Context B
Instrumental response	Press down (forepaw) 	Push side (snout) 
Conditioned stimulus	Light 	Tone 
Context	Empty tray Houselight off Test room A Morning / afternoon	Sawdust in tray Houselight on Test room B Afternoon / morning

Figure 1 Main differences between cocaine self-administration in context A and B.

wide retractable levers that were mounted in the intelligence panel 12 cm apart and 8 cm from the grid floor and that were activated by pressing downwards, which rats tend to do with their forepaws, and a cue light (2.5 W, 24 V) was located above each lever. There was no houselight present in these boxes and the tray underneath the floor grid was left empty. Animals that were run both in context A and B were run in the morning or afternoon in this context and opposite time in context B.

Six further boxes that were also equipped for cocaine self-administration were designated context B, and were located in testing room B. These boxes contained two 4 cm wide retractable levers that were rotated by 90°, such that they were activated by pushing the lever to the left, which rats tend to do with their snouts, and a speaker (3 kHz tone, 80 dB) was located above the left lever. A white house light (2.5 W, 24 V) was located on the wall opposite of the levers. The tray underneath the floor grid was filled daily with sawdust before the start of the sessions. Animals that were run both in context A and B were run in the morning or afternoon in this context, and opposite time in context A.

The last six boxes that were outfitted for sucrose pellet delivery were designated context C, and were located in testing room B (on the opposite wall). These boxes contained a chain (20 cm long) that was fixed at the center of the ceiling, and inserted into the chamber. In addition, a pellet dispenser delivered individual 45 mg food pellets, (Noyes dustless pellets; Sandown Scientific, Middlesex, England) into a recessed magazine (3.8 cm side and 5.5 cm from the grid floor) situated between the levers. A white house light (2.5 W, 24 V) was located on the wall opposite of the magazine. The tray underneath the floor grid was left empty.

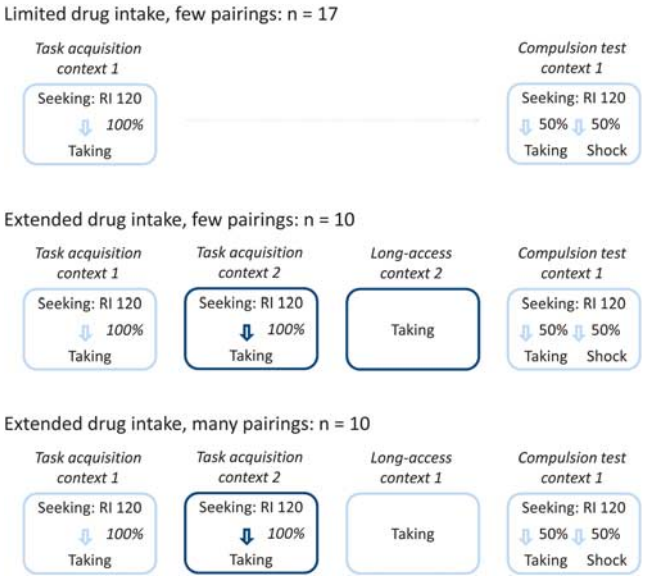

Figure 2 Experimental design and instrumental contingencies. The aim of these experiments was to tease apart the relative contribution of conditioning and drug intake in the appearance of compulsive drug seeking after extended self-administration. In the first experiment, rats were trained in the seeking taking task and the compulsivity of drug seeking was subsequently assessed in the same context. In the second experiment, animals went through training in two contexts, long-access cocaine self-administration in an alternative context, and were finally tested for compulsive drug seeking in the training context. In the third experiment, the animals went through training in two contexts, and long-access cocaine self-administration and testing for compulsive drug seeking in the same contexts. These experiments thus allow for investigation of the selective effects of conditioning history and drug intake on the emergence of compulsive responding. Contexts A and B were randomly assigned to context 1 and 2. RI: Random Interval schedule.

Surgery

Subjects were anaesthetized with ketamine (Ketalar, 90 mg/kg i.p.) and xylazine (Rompun, 6.7 mg/kg, i.p.) and implanted with an intra-venous catheter in the right jugular vein. Catheters were made from 22 gauge steel cannulae with elongated ends. Silastic tubing (0.012 inner diameter) was secured to one end of the cannula and the top was fixed to nylon mesh. The mesh end of the catheter was sutured subcutaneously between the scapulae. To prevent infection, rats were treated from the day before to 7 days after the surgery with 10 mg/kg Baytril (Bayer, Wuppertal, Germany) subcutaneously. The rats were allowed to recover for at least 7 days after surgery before behavioral testing.

Experimental Procedure

Experimental set-up. Based on the three different contexts described above and the various task phases described in detail below, three experiments were run (Figure 2) that selectively manipulated the number of response-cocaine pairings in the test context and the total self-administered cocaine intake, in order to assess their effect on compulsive cocaine seeking.

Limited cocaine intake and few response-cocaine pairings: The cocaine self-administration phase of this experiment was conducted in either context A or B (designated context 1). The animals ($n = 17$) were only run in one of these contexts, and went through the cocaine task acquisition and compulsivity test phases. Thereafter, all animals were shifted to context C and went through the sucrose task acquisition and compulsion test phases.

Extended cocaine intake and few response-cocaine pairings: The cocaine self-administration phase of this experiment was conducted in both contexts A and B for all animals ($n = 10$). Whichever context the rats were run in first was designated context 1 (context A or B) and the other was designated context 2 (context B or A). All animals went through a sequence of the following phases: cocaine task acquisition (in both contexts), long-access (in one of the two contexts), and compulsivity test (in the non-long-access context). All animals went through task acquisition in the second context to equate context-specific experience before long-access cocaine self-administration, and to allow for validation of context discrimination (see below). After testing for compulsive cocaine seeking, the animals were run for one more baseline session in the non-punished context to validate context discrimination. The order of testing in the two contexts was counterbalanced anew at all stages, based on previous seeking rates and drug intake during long-access. Thereafter, all animals were shifted to context C and went through sucrose task acquisition and compulsion testing.

Extended cocaine intake and many response-cocaine pairings: The cocaine self-administration phase of this

experiment was conducted in both contexts A and B for all animals ($n = 10$). Whichever context the rats were run in first was designated context 1 (context A or B) and the other was designated context 2 (context B or A). All animals went through a sequence of the following phases: cocaine task acquisition (in both contexts), long-access (in one of the two contexts), and compulsivity test (in the long-access context). All animals went through task acquisition in the second context to equate context-specific experience before long-access cocaine self-administration, and to allow for validation of context discrimination. After testing for compulsive cocaine seeking, the animals were run for one more baseline session in the non-punished context to validate context discrimination. The order of testing in the two contexts was counterbalanced anew at all stages, based on previous seeking rates and drug intake during long-access. Thereafter, all animals were shifted to context C and went through sucrose task acquisition and compulsion testing.

Cocaine self-administration task phases.

Task acquisition: Each session began with the insertion of the taking lever and the assignment of the left or right lever as the taking lever was counterbalanced across rats. Responding on the taking lever was reinforced under a fixed ratio (FR) 1 schedule and each lever press produced a 0.25-mg/kg infusion of cocaine at the rate of 0.1 ml/5 s, accompanied by the withdrawal of the taking lever for 20 s, the extinction of the house light (if present), and the illumination of the stimulus light or activation of the speaker above the lever for 5 s. The sessions terminated after 2 h or before if 30 infusions had been earned. After five sessions with just the taking lever, the seeking lever was introduced and the schedule on the seeking lever was gradually increased from random interval 2 s (RI 2) to RI 15 s, RI 30 s, RI 60 s, and RI 120 s over 5 days, whereas the schedule on the taking lever remained at FR 1. Thereafter, the post-infusion timeout was increased from 20 s to 2 min, 4 min, and 10 min over 3 more days.

Long access: The animals were given 12 daily sessions of free cocaine self-administration, in which only the taking lever was presented and pressing that lever resulted in a cocaine infusion and the standard 20 s timeout. The sessions terminated after 6 h or earlier if 150 infusions had been earned.

Compulsion test: After four re-baseline sessions under the full seeking (RI 120 s)/taking (FR 1) 10 min timeout schedule, intermittent and unpredictable contingent punishment of the seeking response was introduced, to test whether drug seeking was compulsive. During each of four punishment sessions, half of the cycles contained no punishment and terminated with access to the taking lever and cocaine availability. In the remaining half of the cycles, the seeking response was punished: the first response that met the RI requirement in the seeking link delivered the foot shock (0.5 s, 0.5 mA) and led to a direct transition to the timeout period without the taking link. Reinforced and punished cycles were presented randomly within the daily sessions, except that the first cycle never terminated with punishment, and no more than two sequential cycles were punished.

Baseline seeking in non-punished context: After completion of compulsivity testing in one context, the animals in the two extended self-administration experiments ($n = 20$) were run for one more baseline seeking taking session in the alternative context to allow for confirmation of context discrimination. As the animals had not received shock in the alternative context, the latency to seek cocaine should be lower in this test compared with the session the previous day in the punishment context.

Sucrose seeking task phases.

Task acquisition: All animals received two 15 min magazine training sessions with the chain suspended: during these sessions sucrose pellets were freely delivered into the magazine on a random time 30 s schedule. Thereafter, the instrumental chain was introduced into the chamber at the start of the sessions and the schedule was gradually increased from random interval 2 s (RI 2) to RI 15 s, RI 30 s, RI 60 s, and RI 120 s over 5 days. As the instrumental chain was not automatically retractable and sucrose consumption does not produce long-lasting psychomotor effects like cocaine, there was no timeout after completion of a seeking cycle. The RI 2 s, RI 15 s, RI 30 s, and RI 60 s sessions terminated after completion of 30 cycles, whereas the RI 120 s sessions terminated after completion of 12 cycles.

Compulsion test: Next, after four baseline sessions under the RI 120 s schedule, intermittent and unpredictable contingent punishment of the seeking response was introduced, to test whether sucrose seeking was compulsive. During each of the four punishment sessions, half of the cycles contained no punishment and terminated with sucrose delivery. In the remaining half of the cycles, the seeking response was punished: the first response that met the RI requirement delivered the foot shock (0.5 s, 0.5 mA) and led to a direct transition to the next cycle without sucrose delivery. Reinforced and punished cycles were presented randomly within the daily sessions, except that the first cycle never terminated with punishment, and no more than two sequential cycles were punished.

Statistics

Statistical analysis was based on analysis of the number of seeking cycles completed per session. The data were analyzed using ANOVAs with appropriate between- and within-subject factors. All statistical tests used an α level of $P < 0.05$ for the rejection of the null hypothesis.

RESULTS

Context Discrimination

For the experiments to truly test the influence of self-administration within a given context on compulsive cocaine seeking, the 20 animals in the two long-access experiments had to discriminate between the two contexts. To validate this, we compared the latency to seek drugs in the last punished session in the first context, and in the subsequent baseline session in the second context the next day. Successful discrimination should have resulted in expectancy of shock in context 1 but no expectancy of shock in context 2, as the animals had never received a shock in the second context. We compared the log of the latency to complete the second seeking cycle (because the animals

were never punished after completion of the first cycle). Within-subject one-way ANOVA of the two latencies showed a significant effect of test ($F_{(1,19)} = 6.2$, $P = 0.02$), demonstrating that the latency to complete the second seeking cycle was significantly shorter in the context where no shocks had been delivered. The latencies were 1743.4 s (± 348.0) in the last punished session and 1001.7 s (± 63.3) in the first session in the second context, and the log-transformed values were 3.15 (± 0.06) and 2.99 (± 0.02), respectively. This result thus validates discrimination between the two contexts.

Drug Intake and Conditioning Before Compulsivity Test

Cocaine intake at the time of testing for compulsivity. Total drug intake at the time of testing was significantly (more than sixfold) lower in the limited intake group compared with both extended intake groups but not different between the two extended intake groups (Figure 3a). In the overall ANOVA with three groups, there was a significant effect of group ($F_{(1,36)} = 315{,}5$, $P < 0.001$), and subsequent ANOVAs showed significantly lower drug intake in the limited intake group compared with both the extended intake few pairings group ($F_{(1,26)} = 402.1$, $P < 0.001$) and extended intake many pairings group ($F_{(1,26)} = 1481.3$, $P < 0.001$), whereas drug intake in the two extended intake groups was not significantly different ($F_{(1,19)} = 0.0$, $P > 0.05$).

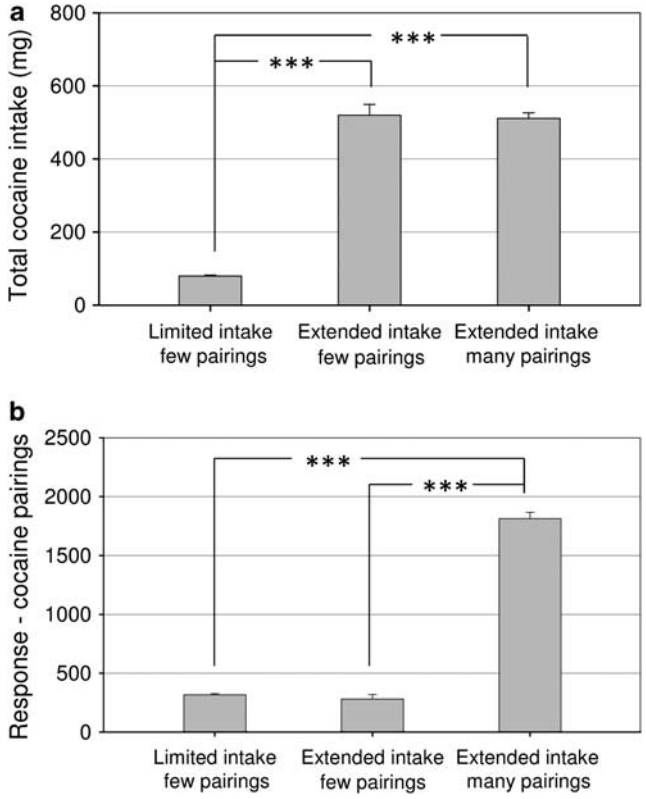

Figure 3 Cumulative drug intake and conditioning history in the test context at the time of testing for compulsivity. (a) Cocaine intake was more than sixfold lower in the limited intake group. (b) The number of response, cocaine pairings in the test context was more than fivefold higher in the extended intake many pairings group. ***$P < 0.001$.

Response-cocaine pairings in test context at time of testing for compulsivity. The number of pairings in the test context at the time of testing was significantly (more than fivefold) higher in the extended intake many pairings group compared with both the extended intake few pairings and limited intake groups but not different between the latter two groups (Figure 3b). In the overall ANOVA with three groups, there was a significant effect of group ($F_{(1,36)} = 807.9$, $P < 0.001$), and subsequent ANOVAs showed significantly higher number of pairings in the extended intake many pairings group compared with both the extended intake few pairings group ($F_{(1,19)} = 638.6$, $P < 0.001$) and the limited intake group ($F_{(1,26)} = 1495.4$, $P < 0.001$), whereas the number of pairings in the limited intake group and the extended intake few pairings group was not significantly different ($F_{(1,26)} = 1.6$, $P > 0.05$).

Effect of Varying Cocaine Intake and Response-Cocaine Pairings on Compulsive Drug Seeking

Group level. The animals in the limited intake few pairings group were significantly less compulsive after introduction of contingent punishment than the animals in the extended intake many pairings group, thus validating earlier findings. In the first critical comparison of differing drug intake but equal conditioning history (the limited intake few pairings group and the extended intake few pairings group), the group with more drug intake completed significantly more seeking cycles on the third and fourth day after introduction of punishment. In the second critical comparison of differing conditioning histories but equal drug intake (the extended intake few pairings group and the extended intake many pairings group), there was no difference in drug seeking under punishment (Figure 4a).

In the overall two-way ANOVA with three groups, there was a significant effect of test day ($F_{(2,35)} = 103.5$, $P < 0.001$), a significant effect of group ($F_{(2,35)} = 3.4$, $P = 0.046$), and a significant interaction between these two factors ($F_{(2,35)} = 3.7$, $P = 0.004$). In the separate two-way ANOVA with the extended intake many pairings group and the limited intake few pairings group there was a significant effect of test day ($F_{(2,25)} = 111.7$, $P < 0.001$), a significant effect of group ($F_{(2,25)} = 9.1$, $P < 0.006$), and a significant interaction between these two factors ($F_{(2,25)} = 7.7$, $P < 0.001$). Separate one-way ANOVAs revealed a significantly higher number of cycles completed in the limited intake group during baseline testing on day 1 ($F_{(1,26)} = 4.7$, $P = 0.040$), but a significantly lower number of cycles completed in the limited intake group after introduction of punishment on days 1 ($F_{(1,26)} = 5.4$, $P = 0.028$), 2 ($F_{(1,26)} = 9.6$, $P = 0.005$), 3 ($F_{(1,26)} = 8.9$, $P = 0.006$), and 4 ($F_{(1,26)} = 8.2$, $P = 0.008$). In the separate two-way ANOVA with the extended intake few pairings group and the limited intake few pairings group there was a significant effect of test day ($F_{(2,25)} = 89.9$, $P < 0.001$), no significant effect of group ($F_{(2,25)} = 1.9$, $P > 0.05$), but a significant interaction between these two factors ($F_{(2,25)} = 3.8$, $P = 0.017$). Separate one-way ANOVAs revealed a significantly higher number of cycles completed in the limited intake group during baseline testing on day 4 ($F_{(1,26)} = 7.1$, $P = 0.013$), but a significantly lower number of cycles completed in the limited intake group after introduction of punishment on days 3 ($F_{(1,26)} = 5.0$, $P = 0.035$)

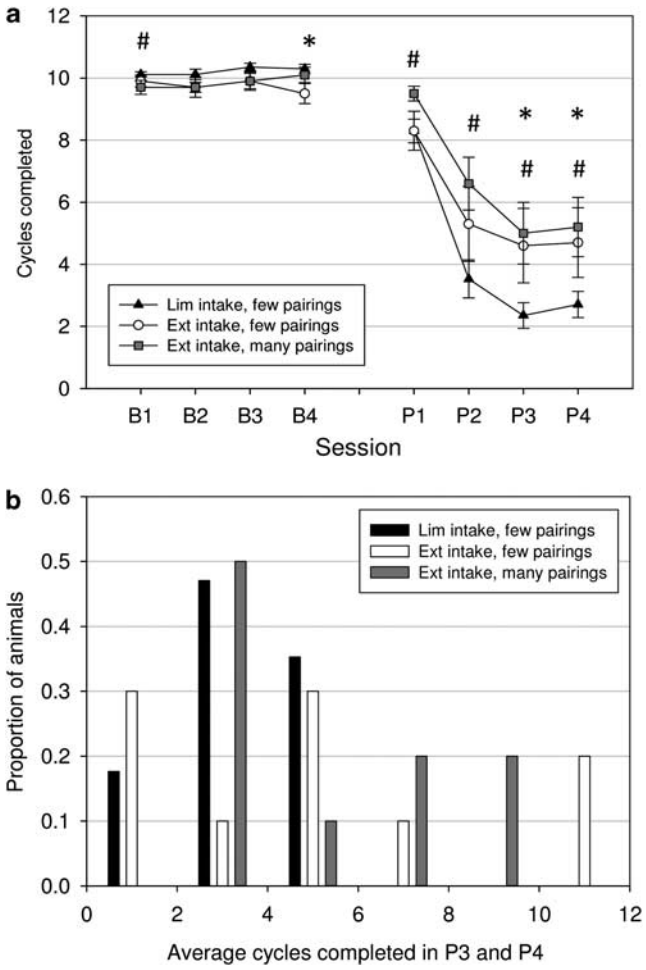

Figure 4 Effect of varying cocaine intake and conditioning on compulsivity of cocaine seeking. (a) At the group level, increased cocaine intake produced more compulsive drug seeking, while conditioning history did not affect drug seeking under punishment. $^{*}P<0.05$ between limited intake few pairings and extended intake few pairings groups. $^{\#}P<0.05$ between limited intake few pairings and extended intake many pairings groups. B = baseline session, P = punished session. (b) Distribution of compulsive drug seeking. The extended intake few pairings and extended intake many pairings groups contained three and four animals, respectively, that completed $\geqslant 6$ cycles under punishment, whereas the limited intake group contained none.

and 4 ($F_{(1,26)}=4.2$, $P=0.050$). Finally, in the separate two-way ANOVA with the extended intake many pairings group and the extended intake few pairings group there was a significant effect of test day ($F_{(2,18)}=31.7$, $P<0.001$), but no significant effect of group ($F_{(2,18)}=0.8$, $P>0.05$), and no significant interaction between these two factors ($F_{(2,18)}=0.5$, $P>0.05$).

Individual differences. Previous results have shown that truly compulsive drug seeking only occurs in a subset of animals, defined as a suppression ratio (average cycles completed in third and fourth punishment session divided by average cycles completed in third and fourth baseline session) of 0.6 or higher (Pelloux *et al*, 2007), roughly translating to $\geqslant 6$ cycles completed under punishment. So, to further validate that drug intake but not conditioning in the test context drove the emergence of compulsive drug seeking, we divided the animals in each group by how many cycles they completed on average on day 3 and 4 of punishment testing (Figure 4b). The results show that there were three and four animals in extended intake few pairings and extended intake many pairings groups, respectively, with $\geqslant 6$ cycles completed under punishment (and a suppression ratio of 0.6 or more), but no animals with more than 6 cycles completed (or a suppression ratio of 0.6 or more) in the limited intake group.

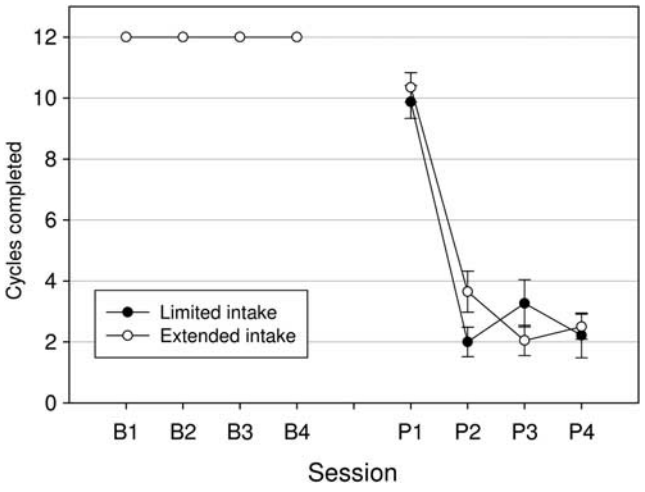

Figure 5 Effect of prior cocaine intake on compulsivity of sucrose seeking. After the animals in the limited and extended cocaine self-administration experiments were trained on sucrose seeking through chain-pulling in a third context, there was no difference in the response of the groups to punishment of sucrose seeking.

Punished Sucrose Seeking

For this analysis, the response to punishment of sucrose seeking was compared between the animals in both extended self-administration experiments combined ($n=20$) and the limited intake experiment ($n=17$), as prior conditioning history in the cocaine compulsion test context was not relevant to sucrose seeking in the new context. The between-subjects two-way ANOVA revealed a significant effect of test day ($F_{(2,35)}=341.9$, $P<0.001$), no overall significant effect of drug intake ($F_{(2,35)}=0.1$, $P>0.05$), and no significant interaction between these two factors ($F_{(2,35)}=1.9$, $P>0.05$). These results demonstrate that the previous cocaine intake that drove compulsive drug seeking did not produce compulsive sucrose seeking (Figure 5).

DISCUSSION

These results confirm the previously published finding that extended compared with limited cocaine self-administration (differentiating both drug intake and conditioning) resulted in the emergence of compulsive drug seeking in rats (Pelloux *et al*, 2007). In addition, drug seeking by the animals that underwent extended cocaine intake but limited conditioning in the test context was significantly more compulsive compared to the limited access group but was not different from the group that underwent extended intake and extended conditioning in the test context. This

was true both at the whole group level and for the number of highly compulsive individuals. This conclusion would not hold if the animals that were tested in two contexts did not discriminate between these two contexts, but the results show that the animals still discriminated between the two contexts, even after testing for compulsivity. We were not able to test whether conditioning alone (without extended drug intake) might be sufficient for compulsive drug seeking, so it remains possible that extended conditioning alone could also produce compulsive drug seeking, although it is not clear that this condition is practically possible. Overall, the results show that extended drug intake alone is sufficient, but extended conditioning in the test context is not necessary, for the emergence of compulsive cocaine seeking, resolving a fundamental conundrum in addiction research.

These experiments discount an important role for dysfunctional conditioning in the appearance of compulsive drug seeking after extended self-administration. Strictly speaking, pavlovian and instrumental conditioning are clearly necessary for the drug self-administration that maintains drug intake until responding becomes compulsive. But it is not the specific pavlovian and instrumental associations that are formed during extended self-administration that are driving the appearance of compulsive cocaine seeking, even though they interact during the acquisition of drug seeking (Arroyo *et al*, 1998), the establishment of cocaine seeking habits (Vanderschuren *et al*, 2005) and when precipitating relapse (Cooper *et al*, 2007; Fuchs *et al*, 2006). Thus, these data are not consistent with a model of addiction that is based on aberrant conditioning (Redish, 2004), as indeed more evidence has accumulated recently to challenge this hypothesis (Marks *et al*, 2010; Panlilio *et al*, 2007).

In spite of the fact that compulsive drug use was immediately apparent in either context once induced, it did not transfer to subsequent sucrose seeking under highly similar contingencies in a third context (and with similar baseline response rates). Thus, although prior cocaine intake has significant effects on relatively subtle assays of associative control of food seeking and reversal learning, it does not induce frank compulsivity as measured by punished sucrose seeking, further enhancing the validity of this test as a model of truly compulsive behavior. In addition, prior experiments have shown that extended access to sucrose self-administration does not produce compulsive sucrose seeking (Pelloux *et al*, 2007; Vanderschuren and Everitt, 2004).

These results are relevant to the well-known study of opiate use of returning American veterans of the Vietnam war (Robins *et al*, 1975). These studies found that very few individuals who were addicted to opiates while on duty in Vietnam were later found to be addicted when they returned home to the United States. Although there could be many reasons why the former addicts did resume their use of opiates after returning home, the crucial finding here is that only a small minority of the returning veterans who did try opiates at least once back in the United States became re-addicted (Robins, 1993). These findings could be taken to suggest that the more extensive conditioning in the Vietnam context was driving addiction there. However, extended *vs* limited conditioning was certainly not the only difference between the two contexts in these studies, so these results do not conclusively favor one explanation over the other. In so far as opiate and cocaine consumption produce addiction through similar mechanisms, the present results suggest that conditioning history was not the primary reason why veterans that were addicted in Vietnam did not relapse to addiction in spite of opiate consumption back in the United States. Instead, the additional differences between the Vietnam War and United States home settings such as the stress of war, social acceptance of drug use, and drug availability might have been more important.

What neural mechanism might account for this pattern of results? One intriguing possibility is that the brain circuits that mediate the rewarding properties, pavlovian conditioned reinforcing properties, and associative instrumental regulation of drugs and natural rewards are partly separable (Cardinal and Everitt, 2004; Carelli *et al*, 2000), and that extended cocaine intake has a weaker effect on natural reward circuits, enough to produce observable behavioral effects on natural reward seeking (Nelson and Killcross, 2006; Olausson *et al*, 2006; Schoenbaum *et al*, 2004), but not sufficient to produce truly compulsive natural reward seeking. Drug induced changes to the striatum (Im *et al*, 2010; Jakub *et al*, 2007), prefrontal cortex (Crombag *et al*, 2005), and stress systems (Koob and Moal, 1997) would be prime anatomical candidates for such changes.

In conclusion, our results show that the resistance to punishment of cocaine seeking that emerged in a subset of animals after extended cocaine self-administration was not due to context specific associations, but was driven by more general adaptations to drug reward and drug seeking circuits, resolving a fundamental issue in drug addiction research. Future investigations should be directed to clarify more specifically which general drug-induced adaptations produce compulsive drug seeking.

DISCLOSURE

The authors declare no conflict of interest.

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