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Withdrawal from IV cocaine "binges" in rats: ultrasonic distress calls and startle

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Abstract Human cocaine abusers report that they experience intense anxiety during withdrawal from chronic use or "binging". Because the symptoms of cocaine withdrawal are not easily observed, it has been difficult to develop an adequate animal model for cocaine withdrawal that det′ects anxiety-like behavior. The objective of the present study was to examine the effects of continuous access to IV self-administered cocaine on ultrasonic vocalizations (USVs) induced by startling tactile stimuli as a possible animal model for cocaine withdrawal. Five days after implantation of a jugular catheter, rats were placed into self-administration chambers with access to cocaine (0.25 mg/infusion). Once the animal had a stable response rate over 3 days, on a fixed schedule of reinforcement (FR5), they were given unlimited access to cocaine (0.25 mg/infusion) for 48 or 12 h. Subsequently, animals were exposed to 18 air puffs (20 or 10 psi) at 6, 24, 72 h, 7 and 14 days after the "binge". Rats that self-administered cocaine for 48 h and were subsequently startled with 20 psi stimuli increased the number of automatically recorded ultrasonic distress calls and showed an enhanced startle response at 6 h after the last cocaine infusion when compared to handled controls. Animals that self-administered cocaine for 48 h and were subsequently startled with 10 psi stimuli showed increased USVs and an enhanced startle reflex at both 6 and 24 h after the unlimited access. Animals that self-administered cocaine for 12 h also showed an increase in ultrasonic distress calls and enhanced startle responses to 10 psi tactile stimuli when compared to handled controls. USVs during cocaine withdrawal may be interpreted to reflect affective distress during the first 24 h after the last cocaine infusion of 12 or 48 of continuous access to drug.

Key words Cocaine · Ultrasound · Vocalization · Withdrawal · Psychomotor stimulant · Self-administration · Reinforcement · Anxiety · Startle · Binge · Intravenous

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Introduction

Termination of cocaine self-administration was historically thought to be innocuous (Freud 1884; Jaffe 1970). The recognition of cocaine withdrawal in the pharmacological and clinical literature is a relatively recent development (Thompson and Pickens 1970; American Psychiatric Association 1987). One proposal characterizes withdrawal from a cocaine "binge" in three phases: crash, withdrawal and abstinence (Gawin and Kleber 1986; Gawin 1991). In this model, the user first experiences a marked anhedonia which is followed by intense cravings for the drug, and the subsequent abstinence phase lasts until relapse.

The prevailing symptom throughout all phases, especially the "crash" phase or short term abstinence, are the feelings of anxiety and depression (Weddington et al. 1990; Gawin 1991). It is the anxiety of the "crash" phase that actually defines the withdrawal syndrome as classified in the DSM-IV. A systematic and quantitative investigation of anxious states, that are the hallmark features of cocaine withdrawal, appears to be a necessary prerequisite for understanding the critical determinants for relapse.

The characterization of the anxiety during cocaine withdrawal as "psychological" may contribute to difficulties in developing an appropriate animal model to study cocaine withdrawal. Investigations of the anxiogenic aspects of cocaine withdrawal have employed several methodologies for the study of anxiety-like behavior. After termination of repeated experimenter-administered cocaine, rats trained to discriminate the "anxiogenic" drug pentylenetetrazol (PTZ) from saline, generalized cocaine withdrawal to PTZ (Wood and Lal 1987; Wood et al. 1989). Other anxiety-like behaviors are prominent during withdrawal from cocaine; for example, animals spent more time in the dark compartment of a light-dark box (Costall et al. 1989), exhibited increased defensive burying of stimuli associated with shock (Harris and Aston-Jones 1993), and decreased punished licking (Fontana and Commissaris 1989). Disruption of operant behavior in both rodents and non-human primates has been observed after termination of both self- and experimenter-administered cocaine (Carroll and Lac 1987; Woolverton and Kleven 1988).

After unlimited access of self-administered cocaine, defined as a "binge", animals trained to press a lever that was reinforced by intracranial self-stimulation (ICSS) required higher current, particularly after 12 or 48 h of continuous access to cocaine (Markou and Koob 1991; Koob 1995). It appeared that the length of a continuous access and the resulting increase in the amount of cocaine correlates well with an increase in ICSS thresholds. Cocaine self-administration more closely resembles human cocaine use and may be advantageous when compared to experimenter-administered cocaine. Disrupted operant behavior or the amount of increase in ICSS thresholds quantify the time course of disturbances in the early "crash" phase. It would be useful to develop an animal model that directly measures the anxious or depressive states observed during cocaine withdrawal, rather than rely on indirect measures.

Ultrasonic vocalizations (USVs) can communicate affective states. Both adult and young rodents emit USVs in several social situations (Miczek et al. 1995). Ultrasonic calls are emitted by intruder rats who are exposed to aggressive conspecifics (van der Poel and Miczek 1991) or a potential predator (Blanchard et al. 1990). Both rat and mouse pups emit USVs when separated from their mother and littermates (Nastiti et al. 1991; Olivier et al. 1992). The interpretation of these vocalizations as anxiety-like behavior is supported by the fact that anxiolytic drugs such as benzodiazepines and 5-HT_{1A} agonists decrease separation induced vocalizations in pups, while inverse agonists such as PTZ increased pup USVs (Gardner 1985; Insel et al. 1986; Nastiti et al. 1991). Vocalizations that are induced by acoustic "clicks" have been found to be sensitive to anxiolytics and to drug withdrawal (Kaltwasser 1991; Miczek et al. 1995). Rats exhibited higher rates of USVs during withdrawal from morphine and diazepam (Vivian and Miczek 1991; Miczek and Vivian 1993; Vivian et al. 1994), and the enhancement seen during diazepam withdrawal is reversed by anxiolytic drugs (Vivian and Miczek 1993). During withdrawal from orally self-administered cocaine, rats emitted a higher number of USVs 3 days after termination of daily orally self-administered cocaine (Barros and Miczek 1996). Some USVs are emitted during non-aversive situations, such as those emitted by male rats during the pre-ejaculatory phase; however, the characteristics of the USVs presently measured match those that are emitted in highly aversive situations (van der Poel and Miczek 1991). The objective of the present study was to investigate whether it was possible to measure withdrawal from IV self-administered cocaine with tactile startle-induced USVs. Specifically, how does the duration of cocaine "binges" affect startle reflex and rate of USVs?

Materials and methods

Animals

Subjects were male Long - Evans rats (Charles River, Wilmington, Mass., USA), weighing 320–350 g at the beginning of the experiment. The rats were housed individually in hanging, stainless steel cages in a vivarium with a reversed light cycle (12 h:12 h, lights on at 0800 hours, 35–40% humidity, 21 ± °C), with free access to water and food (Purina laboratory chow).

Training

After 3–5 days of acclimation to the laboratory, animals were separated into groups A, B, and C. Animals in groups A and B were food restricted to 85% of their original body weight. They were then placed into an operant chamber (30 × 26 × 31.5 cm) and trained to press a lever reinforced with food (0.1 ml of a 50:50 solution of condensed milk and water), until they responded reliably on the lever at least 100 times consecutively. Once trained, usually within three to four sessions, the animals were given free access to food and water until they reached a minimum of 350 g body weight. In order to study if prior food restriction affected the subsequent cocaine withdrawal, animals in group C were not food restricted and did not receive any food reinforced lever training. They were weighed and handled for the same number of days as those animals that received food training.

Surgery

All experimental animals received a permanently indwelling catheter (Silastic silicon tubing, ID 0.025 cm, OD 0.047 cm) that was implanted into the right jugular vein (Remie et al. 1990). The catheter was then threaded SC over the shoulders between the scapulae and through an incision in the scalp. Once the catheter was attached to a plastic pedestal (Plastics One, Roanoke, Va., USA) and capped, the pedestal was secured to the skull with stainless steel screws (Small Parts, Miami Lakes, Fla., USA) and dental cement. Rats recovered from surgery for 5 days in stainless steel hanging cages with free access to food and water, and were then moved to self-administration operant chambers. The animals were permanently housed in self-administration Plexiglas chambers (30 × 30.5 ×

24.5 cm) with a stainless steel tray covered with saw dust, illuminated by a 7 W white bulb, and enclosed in a light and sound attenuating box. The catheter was flushed daily with heparinized saline (20 IU/ml) and a brief pulse of saline was given every 30 min overnight in order to maintain the catheter's patency. Restriction of daily food intake was adjusted in order to allow minimal weight gain throughout the experiment (350–400 g). A regimen of 20 g chow a day does not significantly alter cocaine self-administration (Comer et al. 1995).

Cocaine self-administration

Both the experimental chamber and the outer enclosure had a circular hole in the ceiling to allow free movement of the tethered catheter (Plastics One, Roanoke, Va., USA) that was connected via a counterbalanced swivel to an infusion pump (Med Associates, St Albans, Vt., USA). One wall of the chamber accommodated interchangeable panels. One panel was equipped with two levers 5 cm from the floor with a cue light above each lever; red for the active lever and green for the inactive lever. The alternate panel provided access to a water bottle.

During acquisition, each lever press resulted in one infusion of 0.25 mg/0.1 ml cocaine over 5.6 s, corresponding to approximately 0.75 mg/kg per infusion. Acquisition was defined as obtaining 15 reinforcements within a 3-h session on two consecutive days, with each lever press being reinforced (0.25 mg/infusion). Throughout the rest of the experiment every fifth lever press (fixed ratio, FR5) was reinforced by a cocaine infusion. The start of the session was signaled by switching on the house light, and the session began by activating the syringe pump to fill the catheter with drug. Each infusion was accompanied by illumination of the red light above the active lever. The red light remained illuminated for 5.6 s during the infusion of cocaine. At the end of the infusion, both the house light and the red light were turned off for a 30-s time out period. At the end of the 30-s time-out, the house light was switched on again to signal the availability of drug. Lever presses on the inactive lever were counted, but were of no consequence. The experimental session terminated after rats were reinforced by 15 infusions of cocaine or after 3 h, whichever came first. In order to avoid possible contamination of the catheter and delivery line, rats received pulses of saline solution overnight (0.167 ml/30 min). All events were controlled and data were collected by an IBM compatible PC that interfaced (Med Associates, St Albans, Vt., USA) with the experimental chambers.

Experimental design

Once animals ($n = 40$) responded at a stable rate on an FR5 schedule for 3 consecutive days they were allowed continuous access to cocaine (0.25 mg/infusion) for 48 (groups A and B) or 12 h (group C). This continuous access period was defined as a "binge". Subsequently, the rats were subjected to tactile startle stimuli and their reflexes and ultrasonic vocalizations (USVs) were measured. Specifically, animals were exposed to 18 air puffs at either 20 (group A) or 10 psi (groups B and C) at 6, 24, 72 h, 7 and 14 days after the last cocaine infusion of the continuous access period. Additional non-surgical handled control animals ($n = 21$) were matched for weight, feeding, and testing schedule. In addition, the control animals for group C ($n = 10$) were housed in chambers identical to those used for IV self-administration.

Apparatus

Startle

Startle stimuli and responses were controlled by an IBM compatible PC and the SR-LAB startle response system (San Diego Instruments). The system included a clear acrylic cylinder (20 cm long; ID 8 cm) that was connected to an accelerometer which transduced the rat's movement into voltage changes. The startle apparatus was located inside of a sound and light attenuating chamber.

Startle stimulus and measurement

Startle stimuli were 50-ms bursts of compressed air delivered to the dorsal side of the animal through a copper pipe (0.4 cm in diameter) located 3 cm above the subject. Startle amplitude was defined as the maximal accelerometer voltage (V_{max}) measured during a 200-ms recording window.

Startle test

Rats were placed into the startle chamber; after a 5-min habituation period, 18 air puffs (10 or 20 psi) were delivered with varying time (25, 30, or 35 s) between puffs. After the 18th air puff, the animal remained in the enclosed cylinder for another 4 min during which ultrasonic vocalizations were recorded.

Ultrasound

USVs were detected with a 0.064 cm condenser microphone (Bruel and Kjaer Model 4135), preamplifier (B & K Model 2633), filter (Krohn-Hite Model 3550) and measuring amplifier (Bruel & Kjaer Model 2610). The condenser microphone was suspended 3 cm above the acrylic cylinder. Amplifier output was sent to: (1) an oscilloscope (Telequipment Model DM54) to allow visual verification of vocalizations and, (2) an automated MacIntosh II sound analysis system which digitized vocalizations that were longer than 0.075 s and separated by 0.05s or more and frequency filtered (15–35 kHz) the amplifier output to determine the rate and duration of vocalizations (Miczek and Vivian 1993).

Statistical analysis

The rate of USVs were expressed as medians. The analysis focused on the 8-min startle session since USVs during the pre- and post-startle periods were less consistent. All other results were expressed as mean ± SEM and the criterion for significance was $P < 0.05$. The rate of ultrasonic vocalizations was analyzed using the Fisher Exact Probability test because the data were not normally distributed. Startle amplitude (V_{max}) and bodyweight data were analyzed using Planned Multiple Comparison *t*-tests. Differences in the rate of acquisition and stability of response rate were analyzed using the Kruskal-Wallis ANOVA on ranks.

Results

The rats in groups A and B were trained to press a lever reinforced by food and they acquired food prior to catheterization reinforced lever pressing within ca. 3 days. After 5 days of recovery from surgery, it took less than 3 days for the animals to acquire cocaine self-administration (see Table 1). After ca. 2 weeks, animals responded at a stable rate (group A 1.39 ± 0.18; group B 1.21 ± 0.12 resp./min.). At this time, the rats in group A and B were given free access to cocaine for 48 h,

Table 1 Acquisition of cocaine self-administration and "binges" (means ± SEM)

	N	Days of food training	Days to acquisition	Resp./min in acquisition	Days to cocaine binge	Duration of (h) cocaine binge	Resp./min in binge	Cumulative (mg/kg) cocaine in binge	Startle stimuli intensity (psi)
A	14	3.4 ± 0.31	2.86 ± 0.33	1.39 ± 0.18	12.21 ± 0.61	48	0.7 ± 0.06	213.1 ± 22.45	20
B	14	3.6 ± 0.36	2.06 ± 0.07	1.21 ± 0.12	11.4 ± 0.75	48	0.8 ± 0.08	266.0 ± 23.75	20
C	12	None	6.25 ± 1.59	1.37 ± 0.21	19.33 ± 2.48	12	0.94 ± 0.1	86.97 ± 5.68	10

during which the average rate of responding was 0.7 ± 0.06 and 0.8 ± 0.08 resp./min.

During the 48-h continuous access, animals self-administered an average of 213.1 ± 22.45 mg/kg cocaine in group A and an average of 266 ± 23.75 mg/kg cocaine in group B. After termination of the unlimited access, rats in group A were presented with 18, 20 psi tactile startle stimuli and rats in group B were presented with 18, 10 psi startle stimuli, at several time points. In both groups A and B, the rate of USVs was higher in rats that had self-administered cocaine than in controls at 6 h after termination of the unlimited access (Fisher Exact; $P = 0.053$; see Fig. 1A; Fisher Exact; $P = 0.005$; see Fig. 1B). In addition, in group B, the rate of USVs was higher in rats that had self-administered cocaine than in controls at 24 h after termination of the unlimited access (Fisher Exact; $P = 0.02$; see Fig. 1B). The differences between cocaine-discontinued and control animals are apparent both when examined as group medians and as individual data. There were no significant differences between experimental and control groups at any later time points.

In groups A and B, the maximal startle reflex response of experimental animals was significantly enhanced when compared to controls 6 h after termination of the continuous access [$t_{(35)} = 2.88$; $P < 0.005$; see Fig. 2A; $t_{(28)} = 2.62$; $P < 0.01$, see Fig. 2B]. In addition, in group B, the maximal startle reflex response of experimental animals was increased also at 24 h after termination of the unlimited access [$t_{(28)} = 1.7$; $P < 0.05$, see Fig. 2B]. The differences between cocaine-discontinued and control animals are apparent both when examined as group medians and as individual data.

Animals in group C, without any training for food reinforced lever pressing, acquired cocaine self-administration in ca. 6 days. After ca. 20 days, animals responded at a stable rate of 1.37 ± 0.21 min and were allowed continuous access to cocaine for 12 h, during which the average rate of responding was 0.94 ± 0.1 responses/min (Fig. 5). Animals in groups A and B, that were previously trained, acquired cocaine self-administration [$H(2) = 14.29$; $P < 0.001$] and responded at a stable rate [$H(2) = 8.732$; $P < 0.013$] significantly faster than the animals in group C. During the subsequent 12 h unlimited access, animals self-administered an average of 86.97 ± 5.68 mg/kg cocaine at a rate of 1.37 ± 0.21 min. At 6 h and 24 h (Fisher Exact; $P = 0.01$ and $P = 0.05$, respectively), tactile startle stimuli (10 psi) increased the rate of ultrasonic vocalizations, when cocaine-discontinued animals were compared to controls (see Fig. 3A). Twenty-four hours after termination of the unilimited access [$t_{(36)} = 1.7$; $P < 0.05$], the maximal startle reflex response of experimental animals was significantly enhanced when compared to controls (see Fig. 3B).

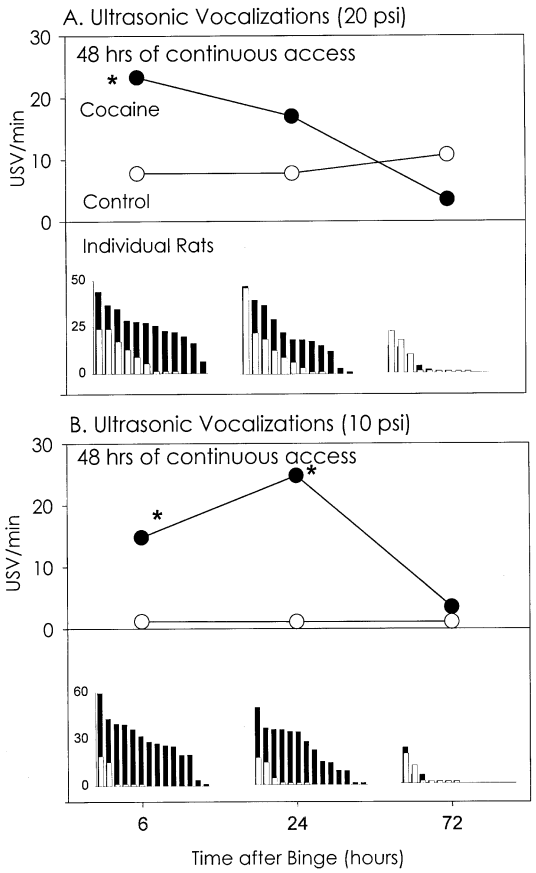

Fig. 1 **A** The group medians for the rate of ultrasonic vocalizations after 48 h of continuous access to cocaine. **B** The group medians for the rate of ultrasonic vocalizations after 48 h of continuous access to cocaine. The startle stimuli were 18, 20 psi air puffs. The *bottom half of both graphs* represent data, in a descending order of magnitude, from individual rats (*$P < 0.05$)

The animals that were allowed free access to self-administer cocaine for 48 h had a significant weight loss at 6 [$t_{(36)} = -4.5$; $P < 0.001$], 24 ($T = 378$; $P < 0.001$) and 72 h [$t_{(27)} = -3.04$; $P < 0.005$] after

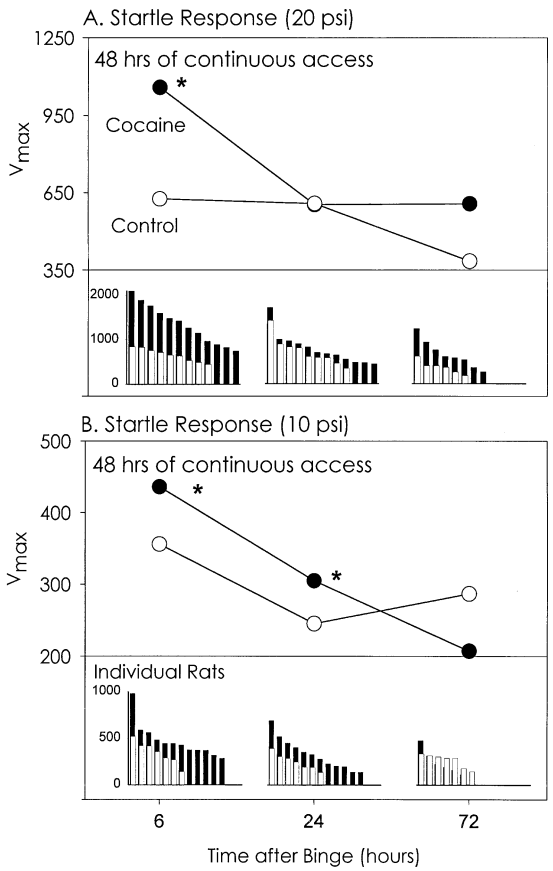

Fig. 2 **A** The group medians for the maximum startle reflex, after 48 h of continuous access to cocaine, in response to 20 psi stimuli. **B** The maximum startle reflex in response to 10 psi stimuli after 48 h of continuous access to cocaine. The *bottom half of both graphs* represents data from individual rats; see Fig. 1 for details (*$P < 0.05$)

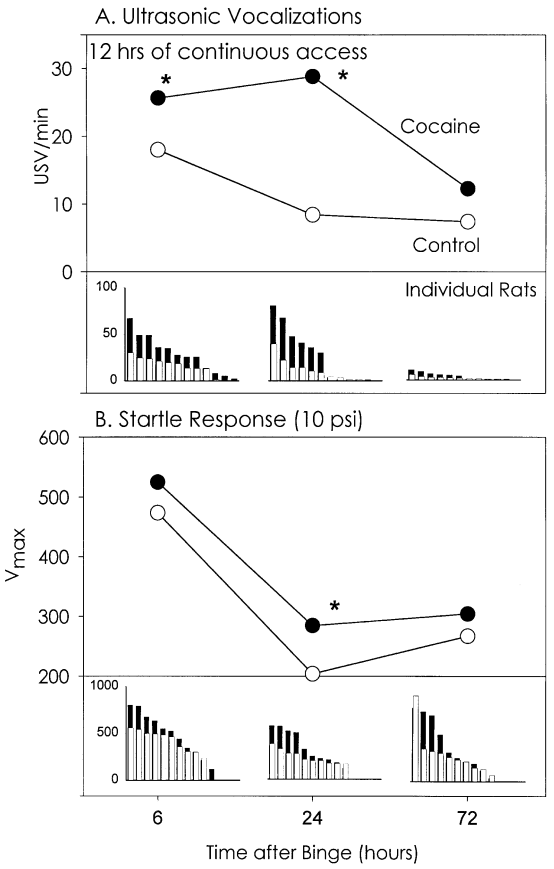

Fig. 3 **A** The group medians for the rate of ultrasonic vocalizations after 12 h of continuous access to cocaine. **B** The group medians for the maximum startle reflex, after 12 h of continuous access to cocaine, in response to 18, 10 psi air puffs. The *bottom half of both graphs* represent data from individual rats; see Fig. 1 for details (*$P < 0.05$)

termination of the continuous access when compared to controls (see Fig. 4). Because bodyweight did not differ among control groups, the data from all controls were collapsed for this comparison.

Discussion

The present findings show how termination of IV cocaine self-administration results in vocal and reflexive responses that appear to reflect distress. Within 6–24 h after termination of unlimited access to IV cocaine, rats emitted higher rates of ultrasonic vocalizations and exhibited an enhanced startle reflex when compared to controls. One may attribute an affective quality to the increase in the number of USVs after termination of drug, and draw parallels to human anxiety of fear during the crash phase of cocaine withdrawal (Gawin and Kleber 1986). Converging evidence indicates that USVs, in the currently measured frequency range and temporal bouts, can reflect anxiety-like states such as those detected in situations of social conflict, maternal separation or pain (Gardner 1985; Insel et al. 1986; Nastiti et al. 1991; van der Poel and Miczek 1991; Winslow and Insel 1991; Miczek et al. 1995).

The enhanced startle response may be interpreted as an index of the hyperexciteability and psychomotor agitation observed in humans after a long duration "binge" (Gawin and Kleber 1985; Gawin 1991). This increase in startle during cocaine withdrawal is in accordance with other studies that report increased startle amplitude during withdrawal from other stimulants such as nicotine (Helton et al. 1993; Rasmussen et al. 1996). The time course of the changes in both the startle response and the rate of ultrasonic calls showed a rapid onset (i.e. within 6–24 h) and disappeared within 3 days of termination of continuous access to IV cocaine. Clinically, the intense withdrawal symptoms, that have a similar short duration and time course, have only been associated with the binge pattern of cocaine use, which is not observed until the addiction has progressed to the point of uncontrolled drug use (Gawin and Kleber 1986; Gawin 1991). Many

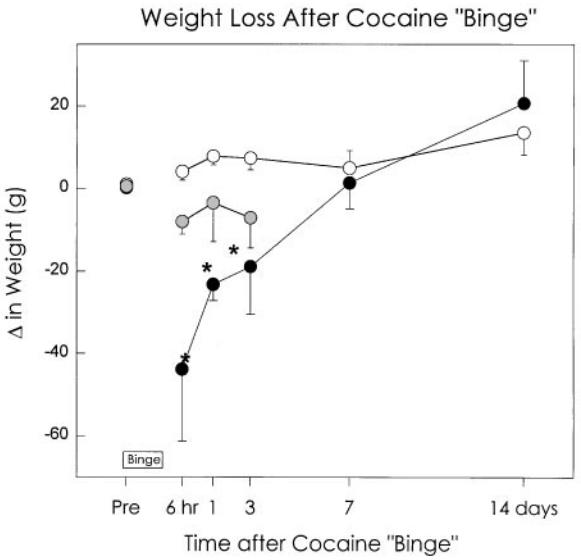

Fig. 4 The change in bodyweight for the control group, animals exposed to 48 h of continuous access to cocaine, and animals exposed to a 12-h binge. The "pre" on the x-axis represents the mean bodyweight of each group on the day before continuous access to cocaine. Because there was no significant difference in bodyweight between control groups, all controls are grouped together for this measurement (*$P < 0.05$). ○ Controls (12, 48 h), ◍ 12-h continuous access, ● 48 h continuous access

individuals may use cocaine recreationally or on a more regulated basis without the rapid onset of the symptoms that are experienced after termination of drug use (Gawin 1991). Similarly, the pattern of drug administration appears to affect the time course of withdrawal symptoms after termination of IV cocaine in animals, as also supported by the prolonged weight loss after 48 h, but not 12 h of cocaine self-administration (see Fig. 4).

The present results of an increase in the rate of USVs and startle reflex response was observed after animals were allowed continuous access to cocaine for varying durations. The presently observed time course show some similarity to that of increased ICSS thresholds during withdrawal from unlimited access to IV cocaine, which also peak between 6 and 24 h after termination of drug access (Markou and Koob 1991). These and the current studies more closely model what is observed clinically when cocaine use progresses to the point of uncontrolled drug use. Since clinical diagnosis emphasizes the "psychological" aspect of the symptoms during the crash phase, the present experimental measurements attempted directly to measure behaviors that are associated with affective expressions that may reflect the anxiety and tension that are observed in this phase (Gawin 1991). Because the onset of increased USVs emerges so rapidly, it is imperative to delineate more precisely when the increase in anxiety-like behavior begins after the termination of unlimited access to cocaine. Ongoing studies suggest that immediately after unlimited access to cocaine, the rate of USVs is actually lower than after 24 h of cocaine withdrawal. This pattern of results suggests that the increase in USVs indicates withdrawal from cocaine and not simply a direct or rebound effect of the drug (Mutschler and Miczek, in preparation). In our current studies, issues of designing an active and passive control have also been addressed more comprehensively. In these ongoing studies the controls are trained, surgically prepared and handled in the exact manner as experimental animals.

Most previous studies of withdrawal from cocaine have focused on disrupted behavior after termination of continuous passive or self-administered cocaine. The time course of behavioral disruption in these studies

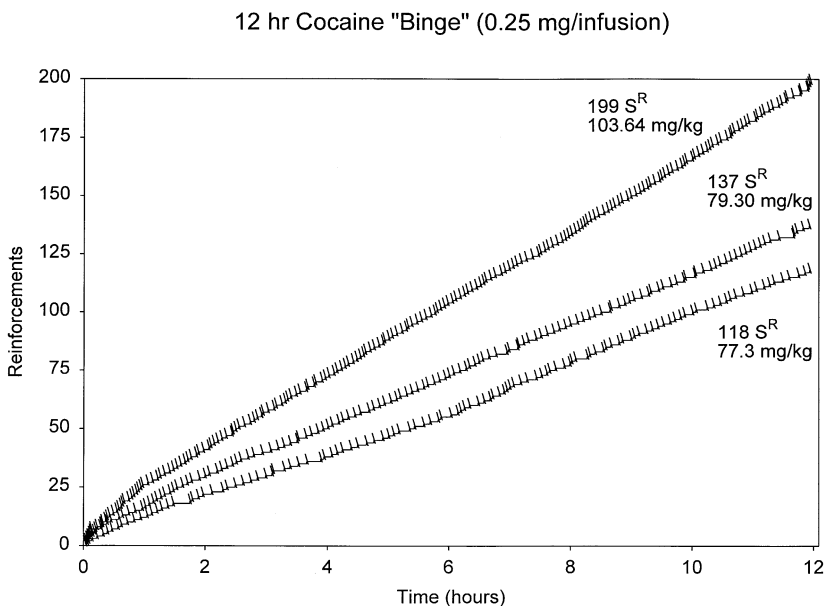

Fig. 5 Cumulative records of cocaine reinforcements from rats that were given unlimited access to cocaine for 12 h. The *middle record* is representative of the mean response rate and cumulative intake of cocaine. The *top record* is taken from the rat with the highest number of cocaine infusions, and the *bottom record* represents the data from the rat with the lowest number of cocaine infusions. Each upward deflection represents a cocaine reinforcement

was somewhat longer than in the present experiments. For example, both non-human primates and rodents showed the most disruption of schedule-controlled behavior at 72 h after termination of several days of continuous experimenter- or self-administered cocaine (Carroll and Lac 1987; Woolverton and Kleven 1988). In another experimental approach, pentylenetetrazol (PTZ) was established as a discriminative stimulus, and then animals were injected IP with cocaine every 8 h for 7 days. Most animals did not generalize cocaine withdrawal to the PTZ stimulus until 5 days after termination of drug (Wood and Lal 1987; Wood et al. 1989). Other behavioral measures of cocaine withdrawal in rodents such as increased defensive burying (Harris and Aston-Jones 1993) and decreased exploration (Costall et al. 1989) were not measured until 48 h after termination of 14 days of IP injections of cocaine. The latter behavior was affected up to 96 h after termination of drug (Costall et al. 1989). It appears that in animals, a continuous pattern of cocaine administration causes a slower onset of withdrawal symptoms. It is also possible that these indirect behavioral measurements did not readily capture the rapid onset of the anxiety of the early abstinence or crash phase.

Previous studies have measured changes in startle reflex as an index of withdrawal from chronically administered nicotine, opiates and ethanol for which somatic symptoms of withdrawal are readily observed. After 14 days on an ethanol liquid diet, animals exposed to acoustic startle stimuli showed an enhanced reflex response 8 h after withdrawal from ethanol (Rassnick et al. 1992). However, after 3 days of receiving morphine via implanted morphine pellets, experimental animals exhibited a *decreased* startle reflex during naloxone-precipitated withdrawal (Mansbach et al. 1992). Interestingly, the changes in startle responses to loud acoustic stimuli during withdrawal from both morphine and ethanol were the opposite to those while animals were under the influence of the drug (Mansbach et al. 1992; Rassnick et al. 1992). Large doses of cocaine decrease startle responses (Davis 1985). The cumulative cocaine doses in the present experiment (see Table 1) would be considered very large doses. If the magnitude of the startle responses during cocaine withdrawal were the opposite to that after a large dose of IP cocaine, then one would expect that during cocaine withdrawal the startle response would be enhanced, and immediately after a unlimited access to cocaine, the response would be diminished. Preliminary results from ongoing research suggest that immediately after termination of unlimited access to cocaine for 16 h, animals that self-administered and those that were yoked and passively received cocaine, startle less than animals that received saline (Mutschler and Miczek, in preparation). This pattern of changes in startle response also suggest that cocaine withdrawal-induced behaviors can be reversed by unlimited access.

No significant changes in startle reflex were detected after neither daily unlimited access to oral cocaine nor 12 h of unlimited self-administered cocaine (Mansbach et al. 1994; Barros and Miczek 1996). One may trace the differences between this study and the present to the intensity of the tactile stimuli. One conclusion from the current startle protocol is that the use of a more subtle stimulus tends more easily to reveal enhanced responses to stimuli that would normally be less startling.

The time-dependent nature of the present results suggest that the termination of a cocaine binge causes a cascade of long-lasting neurochemical changes. For example, basal levels of dopamine in the nucleus accumbens were decreased 24 h after 12 h of unlimited access to cocaine (Weiss et al. 1992). More recently, rapid and transient kinetics of immediate early gene expressions (IEG) point to molecular changes due to acute administration and withdrawal from drugs such as cocaine (Ennulat et al. 1994). The rapid onset and short-term effects of the presently observed measurements may trigger IEG expression associated with dopamine depletion. Presently, we are comparing levels of the IEG zif268 after 16 h of unlimited access in animals that self-administered or passively received cocaine or saline (Mutschler et al., in preparation). The results of these studies may reveal which molecular mechanisms are the critical starting points in the presently observed crash-like behaviors.

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