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Withdrawal from Chronic Cocaine Administration Induces Deficits in Brain Reward Function in C57BL/6J Mice

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Abstract

Anhedonia is a major symptom of cocaine withdrawal, whereas euphoria characterizes the effects of acute administration of this drug in humans. These mood states can be measured quantitatively in animals with brain reward thresholds obtained from the intracranial self-stimulation (ICSS) procedure. Studies have previously reported the reward-enhancing effects of acute cocaine administration using the ICSS procedure in mice, but the effects of chronic cocaine administration and withdrawal on brain reward thresholds have not been widely investigated in this species. Cocaine withdrawal was induced in C57BL/6J mice by removal of intraperitoneal osmotic minipumps that delivered cocaine (90 or 180 mg/kg/day, salt) for 72 h. Mice were tested in the ICSS procedure 3–100 h post-pump removal. Anxiety-like behavior was assessed in the light-dark box 24 h post-pump removal. After an 18-day washout period, tolerance and sensitization to the reward-enhancing effects of cocaine were assessed by injecting bolus cocaine intraperitoneally (0, 2.5, 5, and 10 mg/kg). The results indicated that 72 h administration of 90 and 180 mg/kg/day cocaine significantly lowered brain reward thresholds. Withdrawal from 90 and 180 mg/kg/day of cocaine administration elevated ICSS thresholds to similar extents. No anxiety-like behavior was observed in the light-dark box during withdrawal from chronic cocaine administration, although the number of transitions between compartments and locomotion in the dark compartment markedly decreased. Chronic cocaine administration did not induce tolerance or sensitization to the reward-enhancing effects of acute cocaine. In conclusion, alterations in mood states induced by cocaine administration and withdrawal in mice can be measured using the ICSS procedure.

Keywords

Intracranial self-stimulation; light-dark box; anhedonia; anxiety

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Introduction

Anhedonia, defined as diminished interest or pleasure experienced from stimuli and situations that previously induced pleasure [1], and anxiety [2, 3] are two of the main symptoms of the early cocaine withdrawal syndrome in humans. By contrast, acute administration of cocaine resulted in mood elevation and euphoria in humans [4] and enhanced the rewarding effects of other stimuli in rats [5–9]. Quantitative measurement of the reward-enhancing and anhedonic effects of cocaine can be assessed in animals using brain reward thresholds derived from the intracranial self-stimulation (ICSS) procedure [10, 11]. These brain reward thresholds can be assessed by varying the current-intensity of a rewarding electrical stimulus that is delivered to the posterior lateral hypothalamus, a brain site that is part of the brain reward pathways. Thus, this procedure can be used to assess brain reward thresholds, defined as the value of the current-intensity above which the subject will perform an operant response to self-administer the electrical stimulus. Brain reward thresholds remain stable during repeated testing under baseline conditions. Deviations from an animal's baseline reward threshold value reflect changes in the animal's brain reward sensitivity and thus potentially its "mood" state. An animal with increased reward sensitivity will respond to less intense electrical stimuli, whereas an animal with decreased reward sensitivity will require more intense electrical stimuli before it perceives the stimulation as rewarding. Therefore, lowered brain reward thresholds reflect reward enhancement and potentially a hedonic mood state, whereas elevations in ICSS thresholds reflect anhedonia.

The reward-enhancing effects of acute and chronic cocaine administration on brain reward function have been widely studied in the rat (for review, see [12]). Moreover, studies have consistently and reliably demonstrated brain reward deficits during the early cocaine withdrawal syndrome using the ICSS procedure in rats [13–17]. The ICSS procedure has been used to investigate the reward-enhancing effects of acute cocaine administration in mice [18–20], but the effects of chronic cocaine administration and withdrawal on brain reward thresholds have not yet been investigated extensively in this species. Characterizing these phenomena in this species is important because mice offer unique opportunities for genetic engineering, thereby allowing for the investigation of specific gene products that may contribute to cocaine withdrawal and dependence.

Anxiety can be measured in rodents using the light-dark box test [21]. This test is based on the motivational conflict of rodents between exploring a novel environment and avoiding brightly lit spaces [21]. In the present study, anxiety-like behavior in mice was assessed during cocaine withdrawal using the light-dark box. In rats, withdrawal from cocaine is associated with increased anxiety-like behavior in the elevated plus maze [22, 23]. Cocaine withdrawal, however, also suppresses locomotor activity [24, 25], which may yield a false-positive result in the light-dark box test. Therefore, locomotor activity was also assessed during the time spent in the dark compartment in the present study.

In humans, cocaine abuse typically occurs in prolonged binges that range in duration from several hours to days, followed by a period of withdrawal. A protocol previously applied for the assessment of ICSS thresholds during cocaine withdrawal in rats involves the administration of repeated cocaine injections, after which the rats undergo extensive testing in the ICSS procedure [13]. This procedure, however, is not well suited for mice because this species typically exhibits unstable behavior in the ICSS procedure upon repeated handling, injections, and testing (unpublished data from our laboratory). Thus, the present study assessed brain reward thresholds during cocaine administration via intraperitoneal osmotic minipumps and after removal of the minipumps, which induced a withdrawal state. Cocaine was administered for 72 h through the intraperitoneally implanted osmotic

minipumps, during which time the mice were tested daily in the ICSS procedure. Withdrawal was subsequently induced by removing the osmotic minipump. The mice were then repeatedly tested in the ICSS procedure and light-dark box 24 h post-pump removal. The effects of this cocaine administration regimen on tolerance and sensitization to the reward-enhancing effects of cocaine were assessed 18 days after the termination of chronic cocaine administration by administering acute bolus intraperitoneal injections of 0, 2.5, 5, and 10 mg/kg cocaine according to a within-subjects Latin-square design.

2. Materials and Methods

2.1. Subjects

Male C57BL/6J mice, 7–8 weeks old, were purchased from Jackson Laboratories (Sacramento, CA, USA). Mice were housed in a humidity- and temperature-controlled animal facility on a 12 h/12 h reverse light/dark cycle (lights off at 7:00 AM) with *ad libitum* access to food and water except during testing. Behavioral testing was conducted during the dark phase of the light/dark cycle (unless otherwise required by the experimental design). All experiments were conducted in accordance with the guidelines of the American Association for the Accreditation of Laboratory Animal Care and the National Research Council's Guide for Care and Use of Laboratory Animals and were approved by the University of California San Diego Institutional Animal Care and Use Committee.

2.2. Drugs

Cocaine hydrochloride (National Institute on Drug Abuse, Bethesda, MD, USA) was dissolved in sterile 0.9% saline solution and infused through intraperitoneal osmotic minipumps for 3 days (model 1003D, Alzet, Palo Alto, CA, USA) or injected intraperitoneally in a volume of 10 ml/kg. All drug doses are expressed as the salt.

2.3. Minipump implantation and removal

Mice were anesthetized with an isoflurane (1–3%) / oxygen vapor mixture, and osmotic minipumps that contained either a saline or cocaine solution were inserted intraperitoneally (IP) using aseptic surgery techniques. The abdominal muscle and skin layers were separately closed with surgical silk suture thread (Henry Schein, Melville, NY, USA). The minipumps were surgically removed from the intraperitoneal cavity 72 h after implantation.

2.4. Intracranial self-stimulation: surgery, apparatus, and procedure

Surgery—For electrode implantation, the mice were anesthetized by inhalation of 1–3% isoflurane in oxygen and positioned in a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA). Stainless steel bipolar electrodes (0.20 mm diameter; 6 mm length; Plastics One, Roanoke, VA, USA) were implanted into the medial forebrain bundle at the level of the lateral hypothalamus using the following coordinates: anterior/posterior, 1.58 mm; medial/lateral, 1.0 mm; dorsal/ventral, 5.3 mm; from flat skull [26]. Four stainless steel screws (3.2 mm long, Plastics One, Roanoke, VA, USA) were fixed to the skull to keep the electrode in place, together with the application of a resin ionomer (Den-Mat, Santa Maria, CA, USA) and dental acrylic (Ortho-Jet, Lang Dental, Wheeling, IL, USA). The mice were allowed seven post-surgery recovery days.

Apparatus—ICSS training and testing were conducted in four Plexiglas operant chambers (30.5 × 24 × 27 cm; Med Associates, St. Albans, VT, USA). Each operant chamber was enclosed within a light and sound-attenuated chamber (40 × 60 × 63.5 cm). Intracranial stimulation was delivered by constant current stimulators (Stimtek model 1200c, San Diego Instruments, San Diego, CA, USA). The mice were connected to the stimulation circuit

through flexible bipolar leads (Plastics One, Roanoke, VA, USA) attached to gold-contact swivel commutators (model SL2C, Plastics One, Roanoke, VA, USA) mounted above the operant chamber. The operant response required by the subjects was a simple one-quarter turn of a wheel manipulandum (5.5 cm diameter, 4 cm width) that extended 1.5 cm from one wall of the operant chamber. The stimulation parameters, data collection, and all test session functions were controlled by a computer.

Procedure—The ICSS procedure was adapted from a discrete-trial current-intensity threshold procedure used in rats [11, 27] and has been described previously for mice [28, 29]. Initially, the mice were trained to turn the wheel manipulandum on a fixed-ratio 1 schedule of reinforcement. After successful acquisition of this schedule (i.e., two sessions in which the mouse received 200 reinforcers in less than 10 min), the mice were tested in the discrete-trial current-threshold procedure. Each trial began with the delivery of a noncontingent electrical stimulus followed by a 7.5 s response window within which the subject could make a response to receive a second contingent stimulus identical in all parameters to the initial noncontingent stimulus. A response during this time window was labeled a positive response, and the lack of a response was labeled a negative response. During a 2 s period immediately after a positive response, additional responses had no consequences. The intertrial interval that followed either a positive response or the end of the response window (in the case of a negative response) had an average duration of 10 s (ranging from 7.5 to 12.5 s). Responses that occurred during the intertrial interval were labeled time-out responses and resulted in a further 12.5 s delay of the onset of the next trial. During training on the discrete-trial procedure, the duration of the intertrial interval and delay periods induced by time-out responses were gradually increased until both reached a duration of 10 s (ranging from 1 to 10 s during training). The animals were subsequently tested in the current-threshold procedure, in which the stimulation current intensities were varied according to the classical psychophysical method of limits. A test session consisted of four alternating series of descending and ascending current intensities, starting with a descending series. Blocks of three trials were presented to the subject at a given stimulation intensity, and the intensity changed by 5 μ A steps between blocks of trials. The initial stimulus intensity was set approximately 30–40 μ A above the baseline current-threshold for each animal. Each test session typically lasted 30–40 min and provided two dependent variables for behavioral assessment: threshold and response latency. The threshold value of each series was defined as the midpoint in microamperes between the current intensity level at which the animal made two or more positive responses out of the three stimulus presentations and the level at which the animal made less than two positive responses. The animal's estimated current-threshold for each test session was the mean of the four series' thresholds. The response latency was defined as the average time in seconds that elapsed between the delivery of the electrical stimulus and the turning of the wheel manipulandum for all of the trials that led to a positive response.

2.5. Light-dark box: apparatus and procedure

The setup and experimental procedure for the light-dark box test have been described in detail previously [29]. Briefly, the light-dark box consisted of a dark compartment (27 $\times$ 15 cm) and a light compartment (27 $\times$ 29 cm) that were connected through an opening (10 $\times$ 10 cm). Testing was performed in a dark experimental room. A 40-watt light bulb was positioned above the light compartment, such that the light intensity in the middle of the light compartment was approximately 500 lux. At the beginning of the experiment, the mouse was placed in the dark compartment with its head facing away from the opening. A video camera directed at the dark compartment recorded all mouse behavior in the light-dark box for 5 min, during which time the experimenter left the room. The total time spent in the white compartment, latency to the first transition to the light compartment, and number of

transitions were scored during videotape observation at a later time. The mouse was considered to have entered the white compartment when all four paws were inside the white compartment. Similarly, the mouse was considered to have entered the dark compartment when all four paws were inside the dark compartment. To enable the assessment of locomotor activity in the dark compartment, this compartment was divided into six squares with black electrical tape. Line crossings were counted and divided by the time spent in the dark compartment. After testing each mouse, feces were removed, and the surface was wiped with a wet paper towel. In summary, the light-dark box test provided the following measures: time spent in white compartment, latency to the first transition, number of transitions, and line crossings in the dark compartment.

2.6. Experimental design

Mice were prepared with stimulating electrodes in the lateral hypothalamus and trained in the ICSS procedure. After establishment of stable ICSS performance, defined as less than 10% variation in thresholds over three consecutive days of testing, the mice were prepared with 3-day osmotic minipumps, implanted in the intraperitoneal cavity and delivering 90 ($n = 9$) or 180 ($n = 8$) mg/kg/day cocaine (salt) or saline ($n = 20$; nine saline-treated mice were tested concurrently with the mice that received 90 mg/kg/day cocaine, and 11 mice were tested concurrently with the mice that received 180 mg/kg/day cocaine), and tested once daily. ICSS thresholds and response latencies during cocaine/saline administration and withdrawal are expressed as the percentage of baseline (5-day average before cocaine administration). At 72 h of cocaine/saline administration, the pumps were removed, and the mice were tested in the ICSS procedure at 3, 6, 8, 12, 16, 24, 28, 48, 52, 72, 76, 96, 100, and 120 h post-pump removal. At the 24 h time-point post-pump removal, immediately after completion of the ICSS test, the mice were subjected to a 5-min light-dark box session. A three-week washout period was allowed before a Latin-square design of acute cocaine administration was implemented, during which cocaine (0, 2.5, 5, and 10 mg/kg) was injected intraperitoneally every other day.

2.7. Statistical analyses

All analyses were performed using the PASW 18 Statistical Package (SPSS, Chicago, IL, USA). Data were analyzed using mixed-design analysis of variance (ANOVA). Cocaine administration data were analyzed using a mixed-design ANOVA, with *Cocaine Dose* as the between-subjects factor and *Exposure Day* as the within-subjects factor. ICSS withdrawal data were analyzed using another mixed-design ANOVA, with *Cocaine Dose* as the between-subjects factor and *Withdrawal Time* as the within-subjects factor. The light-dark box data were analyzed with one-way ANOVA, with *Cocaine Dose* as the between-subjects factor. Finally, the effects of acute cocaine administration 18 days after cocaine exposure were analyzed using repeated-measures ANOVA, with *Cocaine/Saline Exposure History* as the between-subjects factor and *Acute Cocaine Dose* as the within-subjects factor. Newman-Keuls *post hoc* analyses followed statistically significant effects in the ANOVAs. For the ICSS withdrawal data, baseline thresholds were calculated as the average of thresholds and response latencies obtained during the last five daily sessions before pump implantation. For the evaluation of the effects of the acute bolus cocaine/saline injections, baseline was defined as the threshold obtained on the day preceding the cocaine/saline administration day. For analyses of ICSS withdrawal data, data from different time-points were combined for analysis to provide a robust and reliable estimate of the effects of cocaine/saline withdrawal. Specifically, the values entered into the analyses were the mean of the 3 h and the 6 h time-points, the mean of the 8 h and 12 h time-points, the mean of the 24 h and 28 h time-points, the mean of the 48 h and 52 h time-points, the mean of the 72 h and 76 h time-points, and the mean of the 96 h and 100 h time-points. The level of significance was set at 0.05.

3. Results

Effects of continuous cocaine/saline administration on ICSS thresholds

Continuous administration of 90 and 180 mg/kg/day cocaine for 3 days lowered ICSS thresholds in C57BL/6J mice (Fig. 1). Although the ANOVA revealed no significant interaction between *Cocaine Dose* and *Exposure Day* ($F_{4,68} = 1.129, p > 0.05$), significant main effects were found for *Cocaine Dose* ($F_{2,34} = 7.41, p < 0.01$) and *Exposure Day* ($F_{2,68} = 26.199, p < 0.001$). Pre-planned comparisons revealed statistically significant differences between saline- and 180 mg/kg/day cocaine-treated mice 4 h and 52 h after pump implantation ($p < 0.01$). Mice treated with 90 mg/kg/day cocaine exhibited significantly lower thresholds compared with saline-treated mice 52 h after minipump implantation ($p < 0.05$). Latencies to turn the wheel manipulandum did not differ between cocaine- and saline-treated mice (Table 1), and only a significant main effect of *Exposure Day* ($F_{(2,68)} = 27.459, p < 0.001$) was observed in an ANOVA.

Effects of cocaine/saline withdrawal on ICSS thresholds

Withdrawal from chronic administration of 90 and 180 mg/kg/day cocaine resulted in elevations in ICSS thresholds (Fig. 2). The ANOVA revealed a significant interaction between *Cocaine Dose* and *Exposure Day* during continuous cocaine/saline administration ($F_{10,170} = 2.102, p < 0.05$) and significant main effects of *Cocaine Dose* ($F_{2,34} = 5.039, p < 0.05$) and *Exposure Day* ($F_{5,170} = 6.030, p < 0.001$). *Post hoc* analyses revealed that withdrawal from 90 mg/kg/day cocaine significantly elevated ICSS thresholds on day 2 ($p < 0.05$) and day 3 ($p < 0.01$) after pump removal, whereas withdrawal from 180 mg/kg/day cocaine significantly elevated thresholds on day 1 ($p < 0.01$), day 2 ($p < 0.01$), and day 3 ($p < 0.05$) after pump removal compared with saline-treated mice. No differences in response latencies were observed among cocaine- and saline-treated mice (Table 1), and only a significant main effect of *Exposure Day* ($F_{(5,170)} = 6.879, p < 0.001$) was observed in an ANOVA.

Effects of cocaine/saline withdrawal on behavior in the light-dark box

ANOVAs revealed that mice withdrawn from cocaine exhibited a decreased number of transitions between the light and dark compartments of the light-dark box ($F_{2,34} = 12.186, p < 0.001$) and decreased locomotor activity in the dark compartment ($F_{2,34} = 8.507, p < 0.01$). However, no differences were observed in the time spent in the light compartment or latency to enter the light compartment (Fig. 3).

Effects of acute cocaine administration on ICSS thresholds after continuous cocaine/saline treatment

An ANOVA performed on ICSS thresholds revealed no interaction between the subjects' previous *Cocaine/Saline Exposure History* and *Acute Cocaine Dose*, no main effect of *Cocaine/Saline Exposure*, and a main effect of *Acute Cocaine Dose* ($F_{3,93} = 7.813, p < 0.001$). Therefore, data from the three treatment groups were combined for further evaluation of the effects of an acute bolus cocaine injection on ICSS thresholds (Fig. 4). Consistent with the multifactor ANOVA reported above, a oneway ANOVA revealed a significant effect of *Acute Cocaine Dose* ($F_{3,99} = 7.524, p < 0.001$). *Post hoc* tests indicated similarly lowered thresholds after administration of 2.5, 5, and 10 mg/kg/day cocaine ($p < 0.01$).

4. Discussion

Chronic 3-day cocaine administration enhanced the rewarding effects of ICSS in C57BL/6J mice, reflected by lowered ICSS thresholds. This cocaine-induced threshold-lowering effect

was similar in magnitude independent of the cocaine dose administered (90 or 180 mg/kg/day). The reward-enhancing effects of continuous or bolus cocaine administration, reflected by lowered ICSS thresholds, are consistent with previously reported results with bolus cocaine administration in rats [5–9], C57BL/6J mice [18, 20], C57BL/6N mice [19], Swiss-Webster mice [30], and DBA/2J mice [18] (for review, [31]). The present study, however, provides one of the first investigations of the effects of continuous cocaine administration on brain reward thresholds in mice. Consistently lowered brain reward thresholds were observed in C57BL/6J mice on the final day of cocaine administration. Whereas lowered reward thresholds were observed on the first day of 180 mg/kg/day cocaine administration, the thresholds of saline-treated mice were markedly elevated from their baseline thresholds. Thus, this short-lasting lowering of thresholds in cocaine-treated mice, which was not seen on day 2 of cocaine exposure, may have resulted from the analgesic effects of the high cocaine dose at the wound site that were not apparent with the low cocaine dose.

Importantly, the results of the present study provide one of the first demonstrations that discontinuation of continuous cocaine administration in C57BL/6J mice results in a withdrawal state characterized by elevated ICSS thresholds and decreased locomotion assessed in the light-dark box. These elevations in thresholds during cocaine withdrawal indicate an anhedonic mood state and are consistent with results from previous studies in rats [13–17]. This anhedonic-like mood state lasted for approximately 4 days, after which thresholds returned to baseline levels. These results are analogous to reports of anhedonia during the early cocaine withdrawal syndrome in humans, which are reported to be similar in duration [1]. Again, the magnitude of the effects was similar in mice treated with 90 or 180 mg/kg/day cocaine, with no statistically significant differences between these two groups of mice. Importantly, neither continuous cocaine administration nor cocaine withdrawal resulted in any changes in response latencies, suggesting that the threshold elevations cannot be attributed to the motoric effects of the manipulations.

The anxiogenic-like effects of cocaine withdrawal have been described previously in rats using the light-enhanced startle paradigm, the conditioned suppression of drinking conflict procedure and a drug discrimination procedure with the anxiogenic agent pentylenetetrazol [32–35], and also during withdrawal from spontaneous nicotine in mice in the light-dark box test in our laboratory [29]. However, the present study did not observe anxiogenic-like effects in mice withdrawn from continuous cocaine administration in the light-dark box. The light-dark box test did not reveal any anxiety-like effects 24 h after discontinuation of continuous cocaine administration for 3 days, with no differences in the latency to enter the light compartment or time spent in the light compartment between saline- and cocaine-treated mice. Interestingly, however, cocaine-treated mice, independent of cocaine dose, exhibited a decrease in the number of transitions between compartments and decreased line crossings in the dark compartment adjusted for time spent in the dark compartment. The decrease in transitions between compartments observed during cocaine withdrawal in these mice does not likely indicate anxiety-like behavior and more likely reflects a decrease in locomotor activity during cocaine withdrawal. Decreases in locomotor activity have been previously reported during cocaine withdrawal in rats [25, 36]. The decrease in locomotor activity in the light-dark box test, however, did not affect the latency to turn the wheel manipulandum during withdrawal testing in the ICSS procedure (data not shown), suggesting that this decrease in locomotion did not affect the ability of the mice to perform the ICSS task. Thus, detection of possible anxiogenic-like behavior during cocaine withdrawal in C57BL/6J mice may require a behavioral procedure that is not affected by this decrease in locomotor activity, such as the light-enhanced acoustic startle that revealed anxiogenic-like behavior during cocaine [32] and nicotine [37] withdrawal in rats.

Acute bolus administration of 2.5, 5, and 10 mg/kg cocaine, which was administered 18 days after the termination of continuous cocaine/saline administration, lowered ICSS thresholds similarly in mice previously treated with cocaine or saline, indicating that there was no development of tolerance or sensitization to cocaine-induced reward enhancement. Similarly, repeated daily administration of 10 mg/kg cocaine did not induce tolerance or sensitization to the reward-enhancing effects of cocaine in rats [38]. These findings indicate that the reward-enhancing effect of cocaine, remaining unaltered during the initial cocaine exposure and with continued cocaine use, may contribute significantly to the perpetuation of cocaine dependence in humans.

In summary, the present data demonstrate that continuous cocaine administration via intraperitoneal osmotic minipumps is an excellent tool for the assessment of the effects of cocaine administration and withdrawal on various behavioral measures. Continuous cocaine administration enhanced reward, whereas its discontinuation resulted in anhedonia and decreased locomotor activity. In addition to expanding our knowledge of the cocaine withdrawal syndrome, the present findings have important implications for the future exploration of the neurobiology of the cocaine withdrawal syndrome using genetically modified mouse strains.

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Figure 1. Brain reward thresholds during continuous cocaine/saline administration

Effects of chronic administration of saline and 90 and 180 mg/kg/day cocaine on ICSS thresholds in C57BL/6J mice. Data are expressed as a percentage of baseline thresholds (mean $\pm$ SEM). Asterisks indicate significant differences between 180 mg/kg/day cocaine- and saline-treated mice (** $p < 0.01$). The pound sign indicates a significant difference between 90 mg/kg/day cocaine- and saline-treated mice (# $p < 0.05$). The Delta sign ($\Delta p < 0.05$) indicates a significant main effect of *Cocaine Dose* in the ANOVA (see text for details).

Figure 2. Brain reward thresholds during saline/cocaine withdrawal

Effects of withdrawal from saline and 90 and 180 mg/kg/day cocaine on ICSS thresholds in C57BL/6J mice. Data are expressed as a percentage of baseline thresholds (mean $\pm$ SEM). Asterisks indicate significant differences between 180 mg/kg/day cocaine- and saline-withdrawing mice ($*p < 0.05$, $**p < 0.01$). Pound signs indicate significant differences between 90 mg/kg/day cocaine- and saline-withdrawing mice ($\#p < 0.05$, $\##p < 0.01$). Data time-points were combined for analysis (see 2.7. *Statistical analyses* for detail). Two time-points are displayed for the first day of withdrawal: day of withdrawal 1(1) depicts the combined data of 3 and 6 h post-pump removal, and 1(2) represents the combined data of 8 and 12 h post-pump removal.

Figure 3. Performance in the light-dark box 24 h after termination of continuous cocaine or saline administration

Effects of withdrawal from saline and 90 or 180 mg/kg/day cocaine on anxiety-like behavior in the light-dark box assessed as (A) time spent in the light compartment and (B) latency to enter the light compartment. Locomotion measured as (C) line crossings / time in the dark compartment and (D) transitions between the light and the dark compartments were also assessed as measures of locomotor activity. Data are expressed as mean $\pm$ SEM. Asterisks indicate significant differences between cocaine- and saline-withdrawing mice (* $p < 0.05$, ** $p < 0.01$).

Figure 4. Effect of acute bolus administration of 0, 2.5, 5, and 10 mg/kg cocaine on ICSS thresholds

Effects of acute bolus cocaine administration on ICSS thresholds after continuous saline or cocaine treatment (the 90 and 180 mg/kg/day data were combined for analysis; see text for details) 18 days after the termination of continuous cocaine/saline treatment. Data are expressed as a percentage of baseline thresholds (mean $\pm$ SEM). Asterisks indicate significant differences from the saline treatment condition (** $p < 0.01$).

Table 1

Response latencies to turn the wheel manipulandum during cocaine/saline administration and withdrawal.

	Saline	90 mg/kg/day Cocaine	180 mg/kg/day Cocaine
Cocaine/ Saline	Exposure Day ***		
Day 1	120.03 ± 3.23	116.49 ± 2.46	112.46 ± 3.41
Day 2	103.19 ± 1.59	103.91 ± 1.53	98.85 ± 4.17
Day 3	101.82 ± 2.18	102.46 ± 3.32	100.59 ± 4.35
Cocaine/ Saline	Withdrawal Day ***		
Day 1 (1)	111.03 ± 2.79	107.17 ± 3.50	122.73 ± 10.04
Day 1 (2)	106.40 ± 2.19	103.40 ± 4.38	107.90 ± 8.72
Day 2	104.15 ± 2.55	102.89 ± 4.63	114.08 ± 6.40
Day 3	102.65 ± 2.23	105.46 ± 6.11	105.05 ± 4.50
Day 4	94.71 ± 3.10	102.57 ± 3.90	105.70 ± 4.19
Day 5	99.69 ± 1.25	96.82 ± 7.15	104.09 ± 4.11

Latencies are expressed as percentage of baseline latencies (mean ± SEM).

Asterisks (***) indicate a significant main effect of *Exposure Day* or *Withdrawal Day* in an ANOVA. Data time-points were combined for analysis (see 2.7. *Statistical analyses* for details). ICSS behavior was assessed at four time-points on the first day of withdrawal: day of withdrawal 1(1) depicts the mean response latencies at the 3 and 6 h post-pump removal time-points, and 1(2) depicts the mean response latencies at the 8 and 12 h post-pump removal time-point.