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Behavioural consequences of cocaine withdrawal in rats

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Abstract—This study was designed to examine the impact of cocaine withdrawal on several behavioural parameters in rats. After 1 and 3 day withdrawal from continuous cocaine administration (50 mg kg^{-1} for 28 days, subcutaneous infusion via osmotic minipumps), rats showed significant changes in spontaneous locomotor activity, conditioned avoidance response and increased levels of anxiety. However, cocaine withdrawal did not alter the motor co-ordination, body weight, food and water consumption of these animals. The neurochemical effects of cocaine on central dopaminergic neuronal systems may account for locomotor deficit observed in these cocaine-withdrawal animals.

Considerable animal research has been conducted to delineate the acute and chronic effects of cocaine on central dopaminergic systems. These studies point to an important role of brain dopamine in the psychotropic action and the addictive properties of cocaine. However, limited information is available regarding the possible behavioural changes occurring immediately following the period of cocaine withdrawal. Furthermore, the mechanisms by which cocaine may act to elicit any of the alterations during abstinence are poorly understood. It is possible that the neurochemical disturbances precipitated by cocaine withdrawal are transient and vary with time. The present study was designed to enhance our understanding of the effects of cocaine withdrawal after chronic administration on central dopaminergic systems which are important in the regulation of locomotor activities, motivation and emotional behaviour.

Materials and methods

Animals. All experiments were conducted on male, albino Sprague-Dawley rats (Dominion Labs, Omaha, NE, USA), 180–200 g, housed in groups of three in a temperature and humidity-controlled environment under a normal 12-h light–dark cycle. Animals were allowed free access to food (Purina Rodent Chow) and water, and were acclimatized for 1 week before beginning experimentation.

Administration and withdrawal of cocaine. In all studies, rats were anaesthetized with a mixture of isoflurane/oxygen during implantation and removal of the osmotic minipump model 2ML4 (Alza Corp., Palo Alto, CA, USA). Each rat received a subcutaneous implant on the back with an osmotic minipump containing either 0·9% NaCl (saline) (control group) or cocaine (50 mg kg^{-1} day^{-1}). The incision was closed with wound clips and covered with an antibiotic and 5% lignocaine to relieve any discomfort and to prevent infection. Cocaine hydrochloride (Sigma Chemical Co., St Louis, MO, USA) was dissolved in saline. After 28 days, the administration of saline or cocaine was terminated by removal of the osmotic minipump. Different groups of rats (n = 6–8) were used for behavioural studies commencing at 1 and 3 days post-treatment (phase 1 of cocaine withdrawal in man).

The body weight, food and water consumption in all animals were measured daily during the entire time period of cocaine infusion (28 days) and for 14 days after cocaine withdrawal.

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Assessment of locomotor activity and rolling roller performance. Locomotor activity was measured with a Digiscan animal activity monitor (Omnitech Electronic, Columbus, OH, USA). Each animal was allowed to adapt to the monitor for 30 min. Locomotor activity was measured every 10 min up to 90 min. Horizontal movement sensors of the monitor direct 16 beams from front to back (x-axis) and 16 beams from side to side (y-axis). Interruption of these beams generated data that was recorded by an analyser with the results printed automatically at the end of each time period.

After the assessment of locomotor activity, each rat was assessed for its ability to remain on a rotating rod, employing the rolling roller performance test to assess neurological function (Weaver & Miya 1961). The roller was set to revolve at 10 rev min^{-1}. Each rat was allowed a maximum of three trials to remain on the roller for 1 min before a deficit (failure) of performance was recorded.

Acquisition of a conditioned avoidance response. Training for active avoidance tests was conducted by placing a rat in a clear T-shaped runway. The transverse section of this runway measured 130 × 26 × 30 cm (length × width × height) and the vertical section was 110 × 33 × 30 cm. The entire runway was fitted with a steel floor grid wired in series to a grid floor shocker which delivered a scrambled shock (constant current). A sliding door located at the end of the vertical section served as the starting point of the runway. A piece of plexiglass (15 × 15 cm) was placed on top of the grid floor at the right side of the transverse section of the runway to serve as a safe area. A hand-held switch was wired to a mechanical timer which initiated a buzzer followed by an electric shock. The animal must learn to avoid the shock by running to the safe area. Each rat was allowed to adapt to the T-shaped runway for 5 min. The conditioned avoidance procedure consisted of three parts. First, a rat was placed in the runway by lifting the sliding door and placing the animal on the floor at one end of the vertical section (environmental stimulus).

Second, after 10 s, a buzzer was automatically sounded (conditioned stimulus). Third, following 10 s of the buzzer, the shock (unconditioned stimulus) (1·5 mA) was initiated while the buzzer remained on. If the animal did not respond by seeking the safe area located at the right side of the T-shaped runway within 10 s, the conditioned avoidance procedure would be terminated and the training cycle was restarted. Each training trial took 30 s. A performance score was assigned based on the initiating stimulus as follows: 4 for environmental response, 3 for conditioned response, 2 for unconditioned response and 1 for no response. The acquisition of this conditioned response was defined as the ability of the animal to seek the safe area 3 out of 3 consecutive trials in response to environmental stimulus. The number of trials required for the saline and cocaine withdrawal rats to reach this pre-determined criterion was recorded.

Assessment of anxiety level in animals. The method used for the detection of changes in anxiety in rats was similar to that described by Crawley (1981). Briefly, a closed black plexiglass box (41 × 21 × 25 cm) (length × width × height) with an opening (13 × 5 cm) (width × height) (Omnitech Electronic, Columbus, OH, USA) at the center of the box was placed in the left side of a clear plexiglass chamber (42 × 42 × 30 cm) in a quiet, darkened

room illuminated with a 30 W red light. The right side of the clear plexiglass box was brightly illuminated with a 60 W light source. Thus, the left side (dark compartment) and the right side (bright compartment) were of equal dimension. The entire clear plexiglass chamber was placed inside a Digiscan animal activity monitor RXYZCM-16 (Omnitech Electronic Inc., Columbus, OH, USA) equipped with photocells to detect behavioural changes. The data generated were collected by an analyser and the results printed automatically. Each rat was allowed 60 min to acclimatize to a quiet, darkened room. The animal was then placed in the center of the bright area of the testing chamber. The amount of time each rat spent in the left (dark) area was recorded for a period of 30 min.

Statistics. Results are presented as the mean and the standard error of the mean (s.e.m.). All data were analysed by analysis of variance followed by least significant difference test or Dunnett's *t*-test for multiple comparisons or by a two-tailed Student's *t*-test if only 2 treatment groups were being compared.

Results

Cocaine administration and withdrawal on body weight, food and water consumption in rats. We found a time-dependent gain in body weight among both control and cocaine-treated animal groups. However, the gain in body weight in saline- and cocaine-treated rats was at the same rate. No significant differences were detected in control and cocaine-treated animals in terms of their food and water consumption (data not shown).

Effect of cocaine withdrawal on locomotor activity and rolling roller test. Fig. 1 shows that following a 24-h withdrawal from cocaine, a significant reduction in locomotor activity was detected. However, the locomotor activity of cocaine withdrawal rats had returned to control levels on day 3. All animals passed the rolling roller performance test, suggesting that withdrawal from cocaine did not cause any loss in motor co-ordination in these animals.

Effect of cocaine withdrawal on the acquisition of a conditioned-avoidance response. Table 1 shows that a significant increase in the amount of time was required for the rats suffering cocaine withdrawal to learn this task on day 3 following cocaine withdrawal.

Effect of cocaine withdrawal on the level of anxiety in animals. Table 2 shows an anxiogenic effect of cocaine in animals on days 1 and 3 following its withdrawal. The cocaine withdrawal rats spent significantly more time in the dark compartment than did control animals.

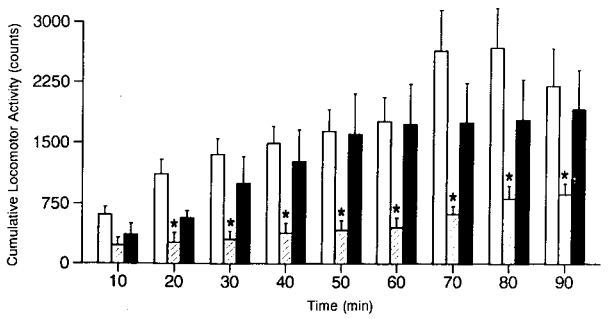

FIG. 1. Cumulative locomotor activity (monitored at 10 min intervals for 90 min) of saline-withdrawal rats (□), cocaine-withdrawal rats tested on day 1 after cocaine withdrawal(▨) and cocaine-withdrawal rats tested on day 3 after cocaine withdrawal (■). * $P < 0.05$ compared with saline controls.

Table 1. Effect of cocaine withdrawal on the acquisition of a conditioned-avoidance response.

Pretreatment	Days of cocaine withdrawal	Number of trials
Saline	1	15±1
Cocaine	1	16±1
Saline	3	16±1
Cocaine	3	24±1*

Animals were implanted with osmotic minipumps containing either saline or cocaine (50 mg kg^{-1} day^{-1}) for 28 days. The administration of saline or cocaine was terminated by the removal of the osmotic minipumps. On days 1 and 3 after withdrawal from saline or cocaine, the number of trials required for each rat to reach the pre-established criterion was recorded. Results are expressed as mean ± s.e.m. of six animals. * $P < 0.05$ compared with saline group.

Table 2. Effect of cocaine withdrawal on anxiety level in rats.

Pretreatment	Days of drug withdrawal	Time (s) spent in dark area
Saline	1	1393±48
Cocaine	1	1634±31*
Saline	3	1330±44
Cocaine	3	1548±43*

Animals were implanted with osmotic minipumps containing either saline or cocaine (50 mg kg^{-1} day^{-1}) for 28 days. The administration of saline or cocaine was terminated by the removal of the osmotic minipumps. On days 1 and 3 after withdrawal from saline or cocaine, each rat was assessed for the level of anxiety as described in the methods section. Results are expressed as mean ± s.e.m. of five to six animals. * $P < 0.05$ compared with saline control.

Discussion

Behavioural evidence indicates that the strong reward and dependence effects of cocaine are mediated, in part, by activation of the mesoaccumbens dopamine system projecting from the A10 neurons in the ventral tegmental area to the nucleus accumbens (Bozarth 1989; Johanson & Fischman 1989; Peris et al 1990; Rowbotham & Lowenstein 1990; Izenwasser & Cox 1990). Administration of cocaine has differential effects on rat striatal and mesolimbic (nucleus accumbens) dopaminergic systems. Daily administration of cocaine led to a decrease in the total amount of [^{3}H]dopamine uptake in rat nucleus accumbens with no change in its uptake in the striatum (Izenwasser & Cox 1990). Furthermore, daily injections of cocaine for 15 days caused a reduction in the density of dopamine D_2 receptor binding sites in the striatum and an increase in dopamine D_2 sites in the nucleus accumbens when these areas were examined shortly after the last injection of cocaine (Goeders & Kuhar 1987; Kleven et al 1990). Cocaine administration, however, failed to change the levels of dopamine in the striatum or the nucleus accumbens (Kleven et al 1988; Yeh & DeSouza 1991). Repeated administration of cocaine may exert a long-lasting effect on dopamine neurotransmission. Thus, the results of the present study are strongly influenced by the protocol of cocaine administration.

It has been suggested that dopaminergic activity in the nucleus accumbens is associated with the mediation of locomotor activity, while that in the striatum is involved with the initiation of stereotyped behaviour (Jackson et al 1975; Mogenson et al 1980; Grenhoff & Svensson 1988). Other studies have shown a

reduction in dopamine concentrations in the nucleus accumbens as well as decreased activity of spontaneously active A10 dopamine neurons following withdrawal from repeated cocaine administration in rats (Ackermann & White 1992; Weiss et al 1992). Therefore, decreased dopaminergic neuronal activity in the mesolimbic dopaminergic system immediately following cocaine administration may provide an explanation for the observed deficit in locomotor activity.

Conditioned avoidance has been one of the behavioural paradigms frequently used in the evaluation of psychoactive drugs and the disruption in performance after drug withdrawal. As for nicotine- and (+)-amphetamine-withdrawn rats, cocaine-withdrawn animals showed a deficit in the shock avoidance performance (Balfour 1990). However, this behavioural change was only evident on day 3 following cocaine withdrawal.

The method used for the detection of changes of anxiety was similar to that of Crawley (1981). This model is based on the natural tendency of rodents to explore a novel environment balanced against the aversive effect of a brightly-lit area; animals treated with an anxiolytic drug such as diazepam spent more time in the bright area, whereas rats spent more time in the dark area when treated with an anxiogenic drug such as pentetrazol (Kilfoil et al 1989). Our results suggest an anxiogenic effect of cocaine on days 1 and 3 following its withdrawal. This observation was in agreement with another study showing an anxiogenic effect of cocaine upon withdrawal from systemic administration (Costall et al 1989). It is possible that a change in 5-hydroxytryptaminergic function may underlie the anxiolytic effect of cocaine withdrawal (Costall et al 1989).

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References

Ackerman, J. M., White, F. J. (1992) Decreased activity of rat A10 dopamine neurons following withdrawal from repeated cocaine. Eur. J. Pharmacol. 218: 171–173

Balfour, D. J. K. (1990) A comparison of the effects of nicotine and (+)-amphetamine on rat behaviour in an unsignalled Sidman avoidance schedule. J. Pharm. Pharmacol. 42: 257–260

Bozarth, M. A. (1989) New perspectives on cocaine addiction: recent findings from animal research. Can. J. Physiol. 67: 1158–1167

Costall, B., Kelly, M. E., Naylor, R. J., Onaivj, E. S. (1989) The actions of nicotine and cocaine in a mouse model of anxiety. Pharmacol. Biochem. Behav. 33: 197–203

Crawley, J. N. (1981) Neuropharmacologic specificity of a simple animal model for the behavioral actions of benzodiazepines. Pharmacol. Biochem. Behav. 15: 695–699

Goeders, N. E., Kuhar, M. J. (1987) Chronic cocaine administration induces opposite changes in dopamine receptor in the striatum and nucleus accumbens. Alcohol Drug Res. 7: 207–216

Grenhoff, J., Svensson, T. H. (1988) Selective stimulation of limbic dopamine activity. Acta. Physiol. Scand. 133: 595–596

Izenwasser, S., Cox, B. M. (1990) Daily cocaine treatment produces a persistent reduction of [^{3}H]dopamine uptake in vitro in rat nucleus accumbens but not in striatum. Brain Res. 531: 338–341

Jackson, D. M., Anden, N. E., Dahlstrom, A. (1975) A functional effect of dopamine in the nucleus accumbens and in some other dopamine-rich parts of the rat brain. Psychopharmacology 45: 139–149

Johanson, C. E., Fischman, M. W. (1989) The pharmacology of cocaine related to its abuse. Am. Soc. Pharmacol. Rev. 41: 3–52

Kilfoil, T., Michel, A., Montgomery, D., Whiting, R. L. (1989) Effects of anxiolytic and anxiogenic drugs on exploratory activity in a simple model of anxiety in mice. Neuropharmacology 28: 901–905

Kleven, M. S., Woolverton, W. L., Seiden, L. S. (1988) Lack of long-term monoamine depletions following repeated or continuous exposure to cocaine. Brain Res. Bull. 21: 233–237

Kleven, M. S., Perry, B. D., Woolverton, W. L., Seiden, L. S. (1990) Effects of repeated injection of cocaine on D_1 and D_2 dopamine receptors in rat brain. Brain Res. 532: 265–270

Mogenson, G. J., Jones, D. L., Yim, C. Y. (1980) From motivation to action: functional interface between the limbic system and the motor system. Prog. Neurobiol. 14: 69–97

Peris, J., Boyson, S. J., Cass, W. A., Curella, P., Dwoskin, L. P., Larson, G., Lin, L. H., Yasuda, R. P., Zahniser, N. R. (1990) Persistence of neurochemical changes in dopamine systems after repeated cocaine administration. J. Pharmacol. Exp. Ther. 253: 38–44

Rowbotham, M. C., Lowenstein, D. H. (1990) Neurologic consequences of cocaine use. Ann. Rev. Med. 41: 417–422

Weaver, J. E., Miya, T. S. (1961) Effects of certain ataraxic agents on mice activity. J. Pharm. Sci. 50: 910–912

Weiss, F., Markou, A., Lorang, M. T., Koob, G. F. (1992) Basal extracellular dopamine levels in the nucleus accumbens are decreased during cocaine withdrawal after unlimited-access self-administration. Brain Res. 593: 314–318

Yeh, S. Y., DeSouza, E. B. (1991) Lack of neurochemical evidence for neurotoxic effects of repeated cocaine administration in rats on brain monoamine neurons. Drug Alcohol Depend. 27: 51–61