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Working memory deficits and alterations of ERK and CREB phosphorylation following withdrawal from cocaine self-administration

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ABSTRACT

Background: The mechanisms underlying memory functions during withdrawal from the chronic drug use are poorly understood.
Methods: We assessed learning and spatial working memory using the delayed alternation assay (T-maze) in rats, previously subjected to cocaine self-administration. The T-maze training was conducted 1–5 weeks after cocaine cessation; working memory efficacy was assessed at 5–8 weeks of drug withdrawal. After behavioral training and testing, the rats were sacrificed and the levels of p-CREB/CREB and p-ERK2/ERK2 in several brain areas were measured. The same molecular assessment was performed in rats with cocaine injections, but forced to drug abstinence in home cages.
Results: After 5 weeks of cocaine withdrawal from self-administration, a significant impairment of working memory under increased working memory load (inter-trial delay extended to 30 s), with no changes at baseline conditions (inter-trial delay 10 s), was noticed. Neither acquisition phase nor working memory performance measured 6–8 weeks after the last drug intake differed between cocaine or saline pretreated rats. Upon T-maze training and 8-week withdrawal, cocaine-pretreated rats had higher levels of p-CREB/CREB in prefrontal cortex and dorsal striatum and lower in hippocampus compared to saline rats. Increased levels of p-ERK2/ERK2 were observed in dorsal striatum, hippocampus and decreased in nucleus accumbens. In cocaine-pretreated caged rats no changes in p-CREB/CREB levels were observed, while ERK2 levels either decreased (frontal cortex) or increased (nucleus accumbens).
Conclusion: Our results suggest that cocaine self-administration results in cognitive impairments and alterations in ERK/CREB signaling pathway long after discontinuation of drug use.

Introduction

Exposure to drugs of abuse interferes with normal reward and learning mechanisms leading to abnormal brain function and addiction [1,2]. However, little information is available about cognitive functions over the course of cocaine withdrawal. Neuropsychological testing in cocaine users reveals memory impairments of selective cognitive domains related mainly to attention and cognitive flexibility [3–5]. There is also some evidence from preclinical animal studies that cocaine self-administration produces performance deficits in several cognitive tasks, such as object recognition [6] or reversal learning [7] and working memory [8] that persist following cocaine cessation.

In this context, working memory is of particular interest as impaired working memory has been found in cocaine users [9] and it has been recognized as a factor contributing to drug craving and relapse [10]. Consistent with this idea, it has been proposed that

Abbreviations: CREB, cAMP-response element binding protein; ERK, extracellular signal-regulated kinase; p-CREB, phosphorylated cAMP-response element binding protein; p-ERK, phosphorylated extracellular signal-regulated kinase.

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restoration of cognitive function may be an effective strategy to treat drug addiction [11]. Therefore a better knowledge about learning and working memory capacity over a long-term withdrawal period from repeated cocaine exposure may facilitate the development of innovative treatment strategies aimed at cocaine dependence condition.

It is well-established that learning and memory processes are mediated by medial prefrontal cortex, hippocampus and striatum which are connected with the structures related to brain reward system such as the nucleus accumbens [12,13]. The molecular pathways regulating learning and memory processes depend on the activation of the transcription factor of cAMP-response element binding protein (CREB) [14,15]. One of the important regulators of CREB phosphorylation is ERK [16,17]. Changes in expression and activation of CREB/ERK pathway have been shown to underlie the prevalent and persistent neurobiological and behavioral changes associated with learning and memory as well as drug addiction processes [1,2].

The current study investigated the efficacy of learning and working memory using the delayed alternation assay (T-maze) in rats, previously subjected to cocaine self-administration, at 5, 6, 7 and 8 weeks of cocaine withdrawal. Furthermore, in the same rats the molecular substrates, such as CREB and ERK, activated in brain regions associated with learning, memory and the reward system were studied. To our knowledge, this is the first study which examined working memory-related activation of CREB and ERK in rats following cocaine self-administration experience.

Materials and methods

Animals

Male Wistar rats (280–300 g) delivered by a licensed breeder (Charles-River Laboratories, Germany) were housed individually in standard plastic rodent cages in a colony room maintained at $20 \pm 1\ ^{\circ}C$ and at 40–50% humidity under a 12-h light-dark cycle (lights on at 06:00). Animals had free access to food (Labofeed pellets) and water during the 7-day habituation period. For the period of the self-administration procedures the rats were maintained on limited water during few initial training sessions. During the delayed alternation task the animals received limited amount of food (15 g/rat/day). All experiments were conducted during the light phase of the light–dark cycle (between 08:00 and 15:00) and were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and with approval of the Bioethics Commission as compliant with the Polish Law (21 August 1997). The animals were experimentally naive.

Drugs

Cocaine hydrochloride (Sigma–Aldrich, St. Louis, MO, USA) was used. Cocaine was dissolved in sterile 0.9% NaCl. During the self-administration procedure, cocaine was given *iv* (0.1 ml/infusion). Control group received *iv* infusions of sterile 0.9% NaCl.

Behavioral procedures

Rats were submitted to cocaine self-administration described elsewhere [18]. Briefly, rats were trained to press the lever of standard operant conditioning chambers (Med-Associates, USA). The rats were chronically implanted with a silastic catheter in the external right jugular vein and trained to lever press to fixed ratio 5 schedule. Subjects were then given access to cocaine during 2-h daily sessions performed 6 days/week (maintenance) and from that time they were given ad libitum water. Each completion of five presses on the "active" lever complex resulted in a 5-s infusion of cocaine (0.5 mg/kg per 0.1 ml). Response on the "inactive" lever never resulted in cocaine delivery. The control rats received the "yoked" *vs.* saline injections during the analogous 2-h daily sessions.

Following 14 daily cocaine self-administration session, when stable rates of responding were established, the rats were withdrawn from the drug self-administration and both groups, cocaine withdrawn and saline were assigned either to the T-maze trained group or untrained caged group. The final groups represent the initial treatment and subsequent behavioral procedure: (1) cocaine self-administration – T-maze training group; (2) saline self-administration – T-maze training group; (3) cocaine self-administration – caged untrained group; (4) saline self-administration – caged untrained group. The habituation to the T-maze started 48 h after the last cocaine intake. Pretraining and training sessions continued during 5 weeks of cocaine abstinence. The performance in the delayed alternation task (the efficacy of working memory of trained rats) was tested after 5, 6, 7 and 8 weeks of cocaine abstinence. The caged untrained groups were kept in home cages under the same conditions over 8 weeks. Twenty four hours after the last training session (T-maze trained group) or after 8 weeks in home cages (home cage untrained group), rats were decapitated, their brains were removed and the following brain structures were dissected: frontal cortex, prefrontal cortex, dorsal striatum, nucleus accumbens and thalamus; they were deeply frozen over dry ice. The schedule of the whole experiment is depicted in Fig. 1.

Delayed alternation task

Apparatus

The delayed alternation task was performed in the T-maze as described previously [19]. Briefly, T-maze consisted of 2 branch arms and a stem arm (dimensions: 68 cm length, 35 cm height and 17 cm width). Sucrose reward pellets (Noyes food pellets 45 mg) were used as reinforcement. Food pellets were presented in small cups placed at the end of each branch arm of the maze.

Acquisition of the delayed alternation

After 14 daily sessions, rats were withdrawn from cocaine self-administration and underwent spatial memory training in the T-maze. The procedure started 2 days after the last cocaine intake. Initially rats were habituated into the T-maze apparatus. During 5 days of habituation rats were placed in the central alley of the maze until they got accustomed to explore the maze and ate food pellets, scattered randomly throughout both arms. For the following 5 days the pre-training begun, when food cups were placed in both arms of the maze, each cup contained only one pellet. Rats were placed individually into the end of the central alley and were required to enter one of the arms and ate the food pellet. Rats were given 11 trials daily. The pre-training was followed by the alternation training. Again, each rat was given 11 trials, during the initial trial the food pellets were presented in both arms. During the next 10 trials, the rats were rewarded with food pellet for choosing the arm opposite to the one the animal had entered in the previous trial. The interval between the trials was 10 s. The acquisition training continued until a criterion of 80% correct choices over 11 trials was achieved. Data are given as the mean number of errors (incorrect choices) in 2-day blocks of training for both experimental groups.

Performance in delayed alternation task of trained rats

During the first of 11 trials, food pellets were presented in both arms. During the next 10 trials, the arm opposite to the one the animal had entered in the previous trial was baited. Entry into the non-baited arm was scored as an error. For each group of trained