ADOLESCENT EXPOSURE TO COCAINE INCREASES ANXIETY-LIKE BEHAVIOR AND INDUCES MORPHOLOGIC AND NEUROCHEMICAL CHANGES IN THE HIPPOCAMPUS OF ADULT RATS

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Abstract—Repeated exposure to cocaine during adolescence may affect both physical and psychological conditions in the brain, and increase the risk of psychiatric disorders and addiction behaviors in adulthood. Adolescence represents a critical development period for the hippocampus. Moreover, different regions of the hippocampus are involved in different functions. Dorsal hippocampus (dHP) has been implicated in learning and memory, whereas ventral hippocampus (vHP) plays an important role in emotional processing. In this study, the rats that were exposed to cocaine during adolescence (postnatal days, P28–P42) showed higher anxiety-like behavior in the elevated plus maze test in adulthood (P80), but displayed normal spatial learning and memory in the Morris water maze test. Furthermore, repeated exposure to cocaine during adolescence lead to alterations in morphology of pyramidal neurons, activities of astrocytes, and levels of proteins that involved in synaptic transmission, apoptosis, inflammation and addiction in both dHP and vHP of adult rats. These findings suggest that repeated exposure to cocaine during adolescence in rats may elicit morphologic and neurochemical changes in the hippocampus when the animals reach adulthood. These changes may contribute to the increased susceptibility for psychiatric disorders and addiction seen in adults. © 2015 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: adolescent exposure, anxiety-like behavior, changes in adulthood, cocaine, dorsal hippocampus, ventral hippocampus.

INTRODUCTION

Adolescence represents an important period for neuronal maturation, and adolescent brain is highly sensitive to neurobiological changes induced by internal and external stimuli, such as cocaine abuse (Paule, 2005; Realini et al., 2009). Epidemiologic and preclinical evidence suggests that adolescents are vulnerable to substance abuse (Chambers et al., 2003; Schramm-Sapyta et al., 2009; Wong et al., 2013). In humans, experiencing drug abuse during adolescence increases the risk of psychiatric disorders and addiction in adulthood (Realini et al., 2009; Rutherford et al., 2010; Staff et al., 2010; Hanson et al., 2011; Moss et al., 2014). Yet, the molecular mechanisms underlying these risks are still unclear.

The hippocampus is known to participate in a variety of addictive-related behaviors, such as enhanced drug memories and drug-seeking (Rogers and See, 2007; Lasseter et al., 2010; Noonan et al., 2010). Importantly, the hippocampus continues to undergo structural and functional changes throughout adolescence into adulthood. Thus, exposure to drugs such as cocaine during adolescence may impair hippocampal maturation and result in long-lasting changes in hippocampal neuronal function. Hippocampi are thought to be functionally subdivided into dorsal (posterior in primates) and ventral (anterior in primates) regions (Fanselow and Dong, 2010; Strange et al., 2014). Dorsal hippocampus (dHP) is thought to be mainly involved in spatial cognitive functions (White and Gaskin, 2006; Sannino et al., 2012). While, ventral hippocampus (vHP) has a preferential role in emotional processing related to stress, fear, and anxiety (Trivedi and Coover, 2004; Albrecht et al., 2013). Drugs of abuse affect behavior and brain function differently in adolescents and adults, and the hippocampus is highly sensitive to drug abuse during the developmental period (Collins and Izenwasser, 2004; Izenwasser, 2005). However, knowledge is lacking about morphologic and neurochemical changes that may occur in adult hippocampus after adolescent cocaine exposures.

In this study, we tested the hypothesis that repeated exposure of cocaine to adolescent rats may elicit morphologic and neurochemical changes in adult hippocampus, which may contribute to cognitive and emotional dysfunction in adult rats. To test this hypothesis, we first investigated animal behaviors, especially those related to spatial cognitive functions and anxiety, in adult rats with a cocaine exposure...
history during adolescence. We also examined morphologic structure, oxidative and antioxidant status, apoptotic and inflammatory status, and expressions of various addiction-associated proteins in dHP and vHP of adult rats.

EXPERIMENTAL PROCEDURES

All experiments were performed in accordance with the Nanjing Medical University Guide for the Care and Use of Laboratory Animals, China, and were approved by the Nanjing Medical University Institutional Animal Care and Use Committee. All efforts were made to minimize the number of animals used and to minimize their suffering.

Animals and drug treatment

Male Sprague–Dawley rats (N = 60) were maintained on a reverse light/dark cycle with food and water available ad libitum. All animals were allowed to acclimate for 7 days before receiving any experimental manipulation. In rodents, the age range of postnatal day 28 (P28) to P42 is considered to be "adolescence" (Spear, 2000). Throughout the P28–P42 age range, rats were assigned to receive a single daily injection of cocaine hydrochloride (15 mg/kg in saline, i.p., cocaine group, Qinghai Pharmaceutical, China) or saline (saline group) for 15 consecutive days. From P43, these rats were maintained in their home cage (three rats in one cage) before behavior tests. On P80, brain tissue was collected for histology, western blot, and oxidative stress assays from rats that were not experienced any behavior test. Behavioral tests were carried out between P80 and P86. The experimental design scheme is shown in Fig. 1.

Behavioral tests

To assess spatial learning and memory, we evaluated the animals’ performance in a Morris water maze (MWM). The MWM consists of a circular water tank (120 cm in diameter, 50 cm in height) that is divided into four quadrants. A platform is hidden 1 cm below the water surface in the center of one of the quadrants. Animals were trained in four trials per day. For each trial, rats were placed into the water in one of the four quadrants, facing the wall. The time required for the animal to find the hidden platform was recorded as escape latency (EL). A trial was terminated once the rat found the platform. If the rat failed to find the platform within 90 s, it was guided to the platform and allowed to stay for 20 s, and a value of 90 s was assigned as the EL. The averaged EL over four trials on each day was used to assess spatial learning ability. At 24 h after the last training session (on P86), each rat was subjected to a probe test (90 s) in which the platform was removed. Time spent in the quadrant that had contained the platform was recorded as the retention latency. The probe test was used to assess the retention of spatial memory.

To assess anxiety-like behavior, we evaluated the animals’ performance in the elevated plus maze (EPM) on P80. The EPM apparatus consists of four elevated arms (70 cm above the floor) arranged in a cross pattern. It has two open arms bordered by clear plastic ledges (0.5 cm tall) and two closed arms bordered by black opaque walls (40 cm tall). A rat was placed in the center portion of the EPM facing an open arm and allowed to explore the maze for 5 min. Distance traveled the number of entries into open arms and the time spent in each arm were recorded and calculated by a computer.

Two separated lines of animals were subjected to MWM test and EPM test, respectively.

Histology

On P80, rats were anesthetized and perfused transcardially with saline followed by 4% paraformaldehyde in phosphate-buffered saline (pH 7.4). The brains were removed and embedded in paraffin. Coronal paraffin sections (5 µm) were deparaffinized, rehydrated, and stained with hematoxylin and eosin. The number of abnormally stained neurons was counted in 12 slices from four rats in each group.

Oxidative stress assay

Rats were anesthetized and decapitated on P80, and the brains were quickly removed. The dHP and vHP were immediately separated, dissected, and frozen in liquid nitrogen. Tissues were stored at −80 °C until use. All samples were homogenized individually. The homogenate was centrifuged and the supernatant used for the oxidative stress assay. We used commercial detection kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) to measure superoxide dismutase (SOD) activity, total antioxidative capacity (T-AOC), and the levels of glutathione (GSH) and...
malondialdehyde (MDA) according to the manufacturer's instructions. We used six rats in each group for these assays.

**Western blot analysis**

Rats were anesthetized and decapitated on P80, and the brains were quickly removed. The dHP and vHP were immediately separated, dissected, and frozen in liquid nitrogen. Tissues were stored at −80°C until use. Total protein was extracted from each sample in RIPA lysis buffer (Beyotime Institute of Biotechnology, Nanjing, China). Protein was separated by 10% SDS–PAGE and electrophoretically transferred onto polyvinylidene fluoride membranes. The membranes were incubated with one of the following target primary anti-mouse antibodies at 4°C overnight: neuronal class III β-tubulin (Tuj1, 1:100 dilution, Beyotime Institute of Biotechnology), glial fibrillary acidic protein (GFAP, 1:500 dilution, Santa Cruz Biotechnology, Dallas, TX, USA), S-100β (1:100 dilution, Boster Biotechnology, Wuhan, China), OX42 (1:200 dilution, Santa Cruz Biotechnology), synapsin I (1:100 dilution, Boster Biotechnology), synaptophysin (SYP) (1:100 dilution, Boster Biotechnology), brain-derived neurotrophic factor (1:100 dilution, BDNF, Santa Cruz Biotechnology), phosphorylated cyclic AMP-response element-binding protein 1 (1:100 dilution, p-CREB-1, Santa Cruz Biotechnology), ΔFOSB (1:100 dilution, Santa Cruz Biotechnology), caspase-3 (1:100 dilution, CAS3, Boster Biotechnology), caspase-8 (1:100 dilution, CAS8, Boster Biotechnology), Bcl-2 (1:100 dilution, Boster Biotechnology), Bax (1:100 dilution, Boster Biotechnology), interleukin 6 (1:100 dilution, IL-6, Boster Biotechnology), interleukin 1β (1:100 dilution, IL-1β, Boster Biotechnology), tumor necrosis factor α (1:100 dilution, TNFα, Boster Biotechnology), and COX-2 (1:50 dilution, Boster Biotechnology). Subsequently, the membranes were incubated with horseradish peroxidase-conjugated secondary antibody (1:10,000 dilution, Santa Cruz Biotechnology) at room temperature for 1 h. Protein bands were visualized by the ECL kit (Beyotime Institute of Biotechnology). β-actin (1:5000 dilution, anti-rabbit, Santa Cruz Biotechnology) was used as a loading control. We used stripping buffer (Beyotime Institute of Biotechnology) to remove the antibodies from the blotted membranes in order to re-probe for the loading control on the same membranes. Values for protein levels were calculated with Image J software (NIH, Bethesda, MD, USA) and normalized to β-actin. Target protein expression was calculated by dividing its band density by the corresponding normalized protein value of dHP tissue from the saline group. 4 rats in each group were used for western blot analysis.

**Statistical analysis**

Statistical analysis was carried out with GraphPad Prism 6 (GraphPad Software, USA). All results are expressed as mean ± standard deviation. For the MWM, we compared ELs from different days in individual groups using an analysis of variance (ANOVA). We compared data from the saline and cocaine groups by Student’s t-test. p < 0.05 was considered significant in all tests.

**RESULTS**

**Adolescent cocaine exposure did not affect spatial learning and memory behaviors in adult rats by MWM tests**

In the MWM test, ELs decreased progressively in both groups over the four consecutive days of testing (p < 0.01, ANOVA, n = 8 rats/group, Fig. 2A), indicating a cumulative learning process. However, there are no significances on the ELs between the saline and cocaine groups in each day (p > 0.05, Fig. 2A). Likewise, on P86, the probe test showed no significant differences in spatial memory retention between the two groups (p > 0.05, Fig. 2B).

**Adolescent cocaine exposure increased anxiety-like behaviors in adult rats by EPM tests**

In the EPM tests, there was no significant difference on the distance traveled by rat between cocaine and saline groups (p > 0.05, n = 8 rats/group, Fig. 3A), indicating no locomotive difference between the rats in two groups. However, rats in the cocaine group spent less time in open arms and more time in close arms than controls (p < 0.01, Fig. 3B). Furthermore, the number of entries into open arms is significantly less in rats of cocaine group, compared with that in saline control (p < 0.01, Fig. 3C). These data indicate an increased level of anxiety in adult rats in the cocaine group.

**Histology in the hippocampus of adult rats**

In adult rats that had received saline during adolescence, neurons in different subregions of dHP and vHP were well aligned with distinct structure, clear outline, transparent cytoplasm, round or oval nuclei, and clear nucleoli. However, in rats that had received cocaine during adolescence, hippocampal neurons showed morphologic damage. The pyramidal neurons in CA3 of dHP and in both CA1 and CA3 of vHP exhibited shrunken and irregular appearances, and some nuclei were heavily stained with hematoxylin (Fig. 4A). The percentage of abnormal neurons was significantly increased in these regions in cocaine rats, compared to that in saline controls (p < 0.01, n = 12 slices/4 rats/group, Fig. 4B).

**Oxidative stress in the hippocampus of adult rats**

SOD enzyme activity was significantly lower in dHP and vHP of adult rats in cocaine-exposed groups during adolescence than that in saline-treated groups (p < 0.05, n = 6 rats/group, Fig. 5A). However, there were no significant differences on T-AOC and levels of GSH and MDA between the two groups in both dHP and vHP (p > 0.05, Fig. 5B–D).
Protein analysis in the hippocampus of adult rats

Tuj1, GFAP, and OX42 were used to label neurons, astrocytes, and microglia, respectively, in the hippocampus, and S-100β is a biomarker for activated astrocytes (Fig. 6A). The levels of GFAP and S-100β in adult dHP and vHP were significantly higher in cocaine rats than in saline controls (p < 0.01, n = 4 rats/group, Fig. 6C, E). However, the levels of Tuj1 and OX42 showed no significant change between groups (p > 0.05, Fig. 6B, D).

Synapsin I and synaptophysin are proteins in the synaptic structure. Their expression levels may reflect functionality of synaptic transmission in the hippocampus. A significant increase in the levels of synapsin I and SYP was examined in dHP and vHP of adult rats treated with cocaine during adolescence, as compared with those in saline rats (p < 0.05, n = 4 rats/group, Fig. 7A–C).

The levels of CAS3, CAS8, Bcl-2, and Bax were evaluated to assess the apoptotic status in the hippocampus. The levels of all four proteins were elevated in dHP and vHP of the cocaine group (p < 0.01, n = 4 rats/group, Fig. 8A–E), compared with those in saline group, and the ratio of Bcl-2 to Bax was decreased (p < 0.01, n = 4 rats/group, Fig. 8F).

The levels of IL-6, IL-1β, TNFα, and COX-2 provide information about the inflammatory status in the hippocampus. Expression levels of IL-6, IL-1β, and TNFα were significantly higher in dHP and vHP of cocaine-treated rats than that of saline-treated controls.
BDNF, CREB, and ΔFOSB proteins are known to be closely associated with drug dependence and addiction (Nestler, 2001; Corominas et al., 2007; McGinty et al., 2010). Compared with their levels in the saline group, the levels of BDNF and ΔFOSB were significantly increased ($p < 0.05, n = 4$ rats/group, Fig. 10A–C), and the level of p-CREB-1 ($p < 0.05, n = 4$ rats/group, Fig. 10D) was significantly decreased, in dHP and vHP of adult rats that exposed to cocaine during adolescence.

**DISCUSSION**

Adolescence represents a critical developmental period for the brain, with a heightened vulnerability to cocaine abuse. It has been proposed that psychostimulant exposure during adolescence may disturb the developmental proceed and consequently result in maladaptive modifications in the brain (Cao et al., 2007), which might contribute to later increased susceptibility for psychiatric problems. Yet, few animal studies have been performed to investigate the long-term effects of adolescent cocaine exposure on the changes in structure, neurochemistry and function in adult hippocampus, an important region that involved in cocaine addiction and toxicity. In this study, spatial cognitive behavior and anxiety behavior, in which the hippocampus involved a lot, were examined in adult rats with an adolescent cocaine use history. As expected, adult rats with cocaine exposure history throughout adolescence showed greater anxiety-like behavior than controls, as indicated by EPM test. There are no differences in distances traveled between groups, suggesting this increased anxiety behavior was not due to changes in their locomotive function. Until now, different results about the effects of adolescent cocaine exposure on anxiety behaviors in rats have been reported. Valzachi et al. (2013) find that repeated exposure to cocaine on P30–P37 significantly increases anxiety behaviors in rats on P47. While, another recent work reports that a binge cocaine protocol (15 mg/kg cocaine injection, three times daily) on P35–P50 fails to influence anxiety-like behaviors in early adulthood (Alves et al., 2014). Differently, Sillivan and his colleagues (2011) found that a similar cocaine treatment on P35–P46 significantly decreases anxiety-like
Fig. 5. Oxidative stress in the hippocampus of adult rats. (A) Activity of superoxide dismutase (SOD). (B) Total antioxidative capacity (T-AOC). (C) Levels of glutathione (GSH). (D) Levels of malondialdehyde (MDA). \( n = 6 \) rats/group. *\( p < 0.05 \), **\( p < 0.01 \) vs. saline control. C, cocaine group; S, saline group; dHP, dorsal hippocampus; vHP, ventral hippocampus.

Fig. 6. Expressions of neuron and glial cell markers in the hippocampus of adult rats. (A) Representative western blot images of the protein bands. (B) Fold change of Tuj1. (C) Fold change of GFAP. (D) Fold change of OX42. (E) Fold change of S-100β. \( n = 4 \) rats/group. *\( p < 0.05 \), **\( p < 0.01 \) vs. saline-treated group. C, cocaine group; S, saline group; dHP, dorsal hippocampus; vHP, ventral hippocampus.

Fig. 7. Expressions of synaptic proteins in the hippocampus of adult rats. (A) Representative western blot images of the protein bands. (B) Fold change of synapsin I. (C) Fold change of synaptophysin (SYP). \( n = 4 \) rats/group. *\( p < 0.05 \), **\( p < 0.01 \) vs. saline-treated group. C, cocaine group; S, saline group; dHP, dorsal hippocampus; vHP, ventral hippocampus.

Fig. 8. Expressions of apoptosis proteins in the hippocampus of adult rats. (A) Representative western blot images of the protein bands. (B) Fold change of caspase-3 (CAS3). (C) Fold change of caspase-8 (CAS8). (D) Fold change of Bcl-2. (E) Fold change of Bax. (F) The ratio of Bcl-2 to Bax. \( n = 4 \) rats/group. *\( p < 0.05 \), **\( p < 0.01 \) vs. saline-treated group. C, cocaine group; S, saline group; dHP, dorsal hippocampus; vHP, ventral hippocampus.
**Fig. 9.** Expressions of inflammatory mediators in the hippocampus of adult rats. (A) Representative western blot images of the protein bands. (B) Fold change of IL-6. (C) Fold change of IL-1β. (D) Fold change of TNFα. (E) Fold change of COX-2. n = 4 rats/group. *p < 0.05, **p < 0.01 vs. saline-treated group. C, cocaine group; S, saline group; dHP, dorsal hippocampus; vHP, ventral hippocampus.

**Fig. 10.** Expressions of addiction-related proteins in the hippocampus of adult rats. (A) Representative western blot images of the protein bands. (B) Fold change of BDNF. (C) Fold change of p-CREB-1. (D) Fold change of ΔFOSB. n = 4 rats/group. *p < 0.05, **p < 0.01 vs. saline-treated group. C, cocaine group; S, saline group; dHP, dorsal hippocampus; vHP, ventral hippocampus.

Behavior in rats on P70. The differences among these studies may be due to different cocaine treatment paradigms or different ages to perform EPM test in rats. Both in animal studies and in humans, elevated anxiety levels or stress is involved in the etiology of cocaine addiction, such as affecting risk of cocaine relapse (Salas-Ramirez et al., 2010; Buffalari et al., 2012). In contrast, no changes in spatial learning and memory were found in adult rats that were subjected to cocaine exposure during adolescence, as indicated by MWM test. As mentioned above, dHP is a key region for cognition, especially in the acquisition and retrieval of spatial information (Florian and Roullet, 2004; Bahar et al., 2011), whereas vHP is mainly implicated in emotional processing, such as anxiety and fear (Strange et al., 2014). Accordingly, both dHP and vHP were examined to explore the molecular mechanisms that may underlie the effects of adolescent cocaine exposure on changed or unchanged behaviors in adult rats.

Previous studies find that substance abuse is able to reduce the volume of the hippocampus in adolescents (De et al., 2000; Ozsoy et al., 2013). Here, we found that exposure to cocaine during adolescent period also resulted in obviously damaged neurons in the hippocampus in adult rats. These morphologic deficits occurred both in vHP and dHP. Yet, we observed no notable difference in spatial learning and memory properties in adult rats between saline and cocaine groups, even though the CA3 of dHP in cocaine-treated rats showed signs of neuronal damage. Similar to this finding, a recent study reported that CA3 atrophy in adult dHP induced by nutrient deficiency during the fetal period had no effect on learning and memory in adulthood (Lopes et al., 2013). We speculated that these mostly intact spatial cognitive abilities in rats with toxic exposure at early ages may be due to compensatory changes during a later developmental period. Compared with rats that receive acute CA3 lesions, rats treated with cocaine in adolescence may have a much longer time for recovery or adaptation before reaching adulthood. During this period, compensatory changes may occur in the hippocampus or other neural circuitry for learning and memory, such as the prefrontal cortex. Accordingly, we examined expressions of two synaptic proteins in the hippocampus. Synapsin I is highly expressed in presynaptic terminals (Tao-Cheng, 2006), whereas SYP is mainly anchored to synaptic vesicles (Vallortiga et al., 2004). To some extent, their expression levels are able to reflect the activities of synaptic transmission in the local brain area (Boido et al., 2010; Pereno and Beltramino, 2010). We found that levels of synapsin I and SYP were increased in both vHP and dHP of adult rats that had received cocaine during adolescence. The two proteins are essential for neuroplasticity and involved in cognitive functions (Thiel, 1993; Vawter et al., 2002). Although the physiologic implications of these changes in synaptic proteins remain unclear, these results may imply potential compensatory effects and neuronal plasticity in adult hippocampus after adolescent cocaine exposure.

The impairment of pyramidal neurons in adult hippocampus following adolescent cocaine exposure might be due to a disorder in local environments. Glia cells, including microglia and astrocytes, play an
important role in maintaining a homeostatic environment, which is critical for proper development and functioning of neurons (Vernadakis, 1988; Reichenbach and Pannicke, 2008; Harry and Kraft, 2012). Both clinical and animal studies have shown that repeated exposure to psychostimulant drugs, such as cocaine, may alter the activities of glia cells (Narita et al., 2006; Miguel-Hidalgo, 2009; Cooper et al., 2012; Beardsley and Hauser, 2014). Indeed, we found an increase in astrocyte activation in dHP and vHP of adult rats after adolescent cocaine exposure, as evidenced by increased expression of GFAP and S-100β. In contrast, microglia seemed to be unchanged, as showed by unchanged OX42 in the hippocampus. Dysfunctions in astrocyte are associated with many neurodegenerative diseases and mood disturbances, including anxiety (Mitterauer, 2004; Mrak and Griffin, 2005; Sloan and Barres, 2014; Tong et al., 2014; Verkhratsky et al., 2014). Glia cells are responsible for regulating the immune response, inflammatory process, apoptotic process and oxidative status in the brain (Andersen, 2004; Sugama et al., 2009; Bajramovic, 2011). Thus, we further observe neurochemical environment in adult hippocampus.

Impairment in local environment, including neurochemistry, contributes a lot to brain disorders and impaired behaviors. In humans, drugs of abuse affect behaviors in part by enhancing the inflammatory process (Clark et al., 2013). In line with this notion, we found that some pro-inflammatory cytokines (e.g. IL-6, IL-1, and TNFα), and pro-apoptotic proteins (e.g. CAS3, CAS8, and Bax) were significantly increased, indicating a heightened inflammatory status and apoptotic status in adult hippocampus following adolescent cocaine exposure. These findings are in consistent with the increased activities of astrocyte found in this study. Under inflammatory conditions, neurons in the brain become susceptible to toxic effects of cocaine abuse (Downey and Loftis, 2014). Thus, the damaged neurons and increased apoptosis found in the hippocampus might partially due to the heightened inflammatory status. Accordingly, the prolonged inflammation caused by cocaine use during adolescence may contribute to pathologic conditions in adult brains.

Cocaine exposure in adult animals causes an increase in hippocampal oxidative stress, which might contribute to impaired behaviors (Bashkatova et al., 2005; Aksenov et al., 2006; Pomierny-Chamioo et al., 2013; Jang et al., 2014). However, we found no significant oxidative damage in adult hippocampus following adolescent cocaine exposure, as suggested by an unchanged level of MDA. Moreover, although the decrease in SOD activity indicated a slight effect on antioxidant capacity, we observed no change in the level of GSH activity or T-AOC in adult rats after adolescent cocaine exposure. These findings suggest that different time windows exposed to drugs (e.g. adolescence vs. adult) result in different influence on oxidative status in the brain.

Changes in the expression of BDNF, CREB, and ΔFOSB in the brain reward circuitry, including the hippocampus, are important to cocaine abuse and addiction-related behaviors (Nestler, 2001; Corominas et al., 2007; McGinty et al., 2010; Rinaldi et al., 2012). BDNF mediates cell development and synaptic connections in the hippocampus, and modulates mood, learning, and reward (Nair and Vaidya, 2006; Nestler and Carlezon, 2006; Martinowich et al., 2007; Cohen-Cory et al., 2010). CREB and ΔFOSB are transcription factors that are involved in reward, memory, and stress (Nestler, 2001; Tully et al., 2003; Carlezon et al., 2005). Changes in levels of BDNF and CREB in the hippocampus are involved impaired emotion that results from exposure to and withdrawal from psychostimulant drugs (Filip et al., 2006; Yang and Pu, 2009; Cao et al., 2013). ΔFOSB has been shown to mediate long-term changes in brain function after chronic stimuli, such as drugs of abuse (Nestler, 2001; Nikulina et al., 2008; Cao et al., 2010). Previous studies have emphasized the effects of ΔFOSB in the striatum and cortex on drug addiction (Nestler, 2001; Hostetter and Bales, 2012; Lobo et al., 2013). ΔFOSB is also important to hippocampal plasticity and neurogenesis (Bing et al., 1997; Chen et al., 2000). Here, we found that BDNF level was increased, but that the phosphorylated CREB level (activated CREB form) was decreased, and ΔFOSB protein expression was increased in dHP and vHP of adult rats after adolescent cocaine exposure. These changes in addiction-related proteins in the hippocampus in adult rats may alter emotional state and result in an increased risk of psychiatric problems and drug addiction.

An interesting finding of this study is that changes in morphology and neurochemistry by adolescent cocaine exposure occur both in vHP and dHP of adult rats, both of which are of a similar trend. However, only vHP-involved anxiety-like behavior, but not dHP-dependent spatial memory are affected by adolescent cocaine use in adult rats. As speculated above, the inconsistence between local environment and behavior results might due to compensatory mechanisms occur in the hippocampus or in other neural circuitry for spatial memory, such as the prefrontal cortex, during later development period. This study is limited to observe changes in behaviors of adult rats and in morphology and neurochemistry of adult hippocampus by adolescent cocaine treatment. In future studies, the correlation between changes in behaviors and in adult hippocampus by adolescent substances use need to be further investigated.

CONCLUSION

The current study shows repeated cocaine exposure to rats during their adolescent period results in increased anxiety-like behaviors in their adulthood. Changes in morphologic and neurochemical properties in adult hippocampus induced by adolescent cocaine exposure might contribute to this behavioral impairment. These results may help to better understand the molecular mechanisms underlying the susceptibility for psychiatric disorders and addiction behaviors in adults following adolescent drugs of abuse.

CONFLICT OF INTEREST

The authors declare no conflict of interest relating to this study.
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