Pharmacology, Biochemistry and Behavior 91 (2008) 109–114

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Cocaine-induced behavioral sensitization in adolescent rats endures until adulthood: Lack of association with GluR1 and NR1 glutamate receptor subunits and tyrosine hydroxylase

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ARTICLE INFO

Article history:
Received 27 March 2008
Received in revised form 2 June 2008
Accepted 24 June 2008
Available online 29 June 2008

Keywords:
Behavioral sensitization
Cocaine
Adolescence
Glutamate receptors
Tyrosine hydroxylase

ABSTRACT

Exposure to repeated cocaine induces enduring behavioral sensitization, which has been implicated in the psychostimulant-induced craving and psychosis. Adaptations in dopamine and glutamate neurotransmission in the nucleus accumbens (NAc) and medial prefrontal cortex (mPFC) seem to mediate psychostimulant-induced behavioral sensitization. The abuse of drugs often begins during adolescence; however few studies have been devoted to study the effects of drugs of abuse at this age. The aim of our study was to examine whether repeated cocaine during adolescence could induce behavioral sensitization that endures into adulthood. Moreover, the protein levels of Tyrosine Hydroxylase (TH) and the glutamate receptor subunits GluR1 and NR1 in the NAc and mPFC were measured following the behavioral tests. Adolescent rats were treated with cocaine from postnatal day (PND) 30 to PND34 and behavioral sensitization was verified recording locomotor activity after cocaine challenge injection to adolescent (PND37) or adult (PND64 or 94) rats in separate groups at each time point. TH, GluR1, and NR1 protein levels were measured by Western blotting. Rats exposed to cocaine during adolescence expressed behavioral sensitization when tested on PND37 and PND64. In cocaine sensitized rats GluR1 protein was increased in the mPFC on PND37 but not in other ages. Thus, cocaine-induced behavioral sensitization during adolescence endures into early adulthood. However, cocaine pretreatment during adolescence induced a transient increase of GluR1 in the mPFC only when animals were challenged in the same age.

1. Introduction

Drug addiction can be defined as a loss of control over drug use, or the compulsive seeking and taking of drugs despite adverse consequence. This behavioral alteration requires repeated drug exposure and can be a life long condition in which individuals show intense drug craving and increased risk for relapse even after several years of abstinence (Nestler, 2001; Ujike and Sato, 2004).

Repeated administration of cocaine or other drugs of abuse can induce a progressive and enduring enhancement of the motor stimulant effect of these drugs, termed behavioral sensitization. This phenomenon has been implicated in the development of psychostimulant-induced craving and psychosis (Robinson and Becker, 1986; Robinson and Berridge, 1993; Covington and Miczek, 2001). In adult rats, it is well known that the enhanced motor stimulant effect of cocaine can persist for up to 6 months as a result of neuroadaptations in the mesocorticolimbic dopamine pathway engendered by intermittent drug administration (Henry and White, 1995; Vanderschuren and Kalivas, 2000; Vezina, 2004; Hope et al., 2006). Acute cocaine administration increases synaptic dopamine (DA) in the nucleus accumbens (NAc) and behavioral sensitization has been related to potentiated DA release in this brain region (Kalivas and Stewart, 1991; Cadoni et al., 2000). Moreover, repeated cocaine administration can alter tyrosine hydroxylase (TH; the rate-limiting enzyme for DA synthesis) level in the NAc of adult rats 1 or 2 weeks after the repeated treatment (Todtenkopf et al., 2000; Schmidt et al., 2001).

Emerging data indicate that changes in glutamate transmission are critical for cocaine-induced behavioral sensitization. For example, Zhang et al. (2001) demonstrated enhanced cocaine-induced glutamate release in the NAc after repeated treatment with this drug. Moreover, the increase in glutamate release seems to occur only in sensitized rats (Pierce et al., 1996). Glutamate projections from medial prefrontal cortex (mPFC) appear to be essential to the expression of behavioral sensitization (Pierce et al., 1998). Besides alterations of glutamate release, long-term adaptations of glutamatergic receptors have been demonstrated following repeated cocaine. For example, repeated cocaine administration increased the expression of GluR1, a subunit of AMPA ionotropic glutamatergic receptor, in the NAc of adult rats. Indeed, this increase was observed only in the animals that

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doi:10.1016/j.pbb.2008.06.018

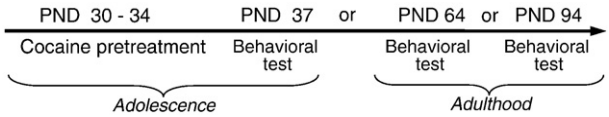

Fig. 1. Schematic representation of the experimental design.

developed behavioral sensitization (Churchill et al., 1999). In addition, cocaine self-administration not only induces long-term elevation of GluR1 but also increases the protein levels of NR1 (an essential subunit of NMDA ionotropic glutamatergic receptors) in the NAc of adult rats up to 90 days following withdrawal (Ozawa et al., 1998; Lu et al., 2003). In the mPFC, increases of GluR1 and NR1 immunolabeling were also found after psychostimulant administration (Lu and Wolf, 1999; Hemby et al., 2005).

Drug abuse often begins during adolescence, a period of ontogeny that individuals exhibit age specific behavioral characteristics, such as risk taking and novelty seeking, which could predispose them to initiate drug use (Spear, 2000). Brain pathways that play a key role in reward and motor effects of psychostimulant drugs undergo maturational changes during this transitional period and can engender different responsivity to drugs of abuse such as cocaine and amphetamine (Laviola et al., 1995; Bolanos et al., 1998; Kelley et al., 2004; Marin and Planeta, 2004).

Given that long-term behavioral and molecular effects of repeated cocaine exposure during adolescence are poorly investigated, the aim of our study was to examine whether repeated cocaine administration during adolescence could induce behavioral sensitization that endures to adulthood. Moreover, after the behavioral tests, animals were sacrificed to measure the protein levels of TH, GluR1 and NR1 in the NAc and mPFC.

2. Materials and methods

2.1. Subjects

Male Wistar rats were obtained from the animal breeding facility of the São Paulo State University (Botucatu-SP, Brazil) just after weaning, on postnatal day (PND) 21. They were housed in groups of 3–5 animals in a room maintained at 23±2 °C and a 12:12 hour light/dark cycle (lights on at 7:00) with free access to food and water. All experiments were performed during the light phase (between 9:00 and 17:00).

The experimental procedures were approved by the Ethical Committee for Use of Human or Animal Subjects of the School of Pharmaceutical Science-UNESP (CEP-11/2004) and the experiments were conducted according to ethics principles of the Brazilian College of Animals' Experimentation — (COBEA), in compliance with NIH Guide for Care and Use of Laboratory Animals.

2.2. Behavior apparatus

Behavioral testing was conducted in a commercially available (Columbus Instruments, CA) activity monitoring chambers, consisting of Plexiglas cages. The chambers, measuring 44 (width)×44 (length)×20 (height) cm, have included 10 pairs of infrared photocells, which were used to measure the horizontal locomotor activity. The consecutive interruption of two beams was recorded as one unit of locomotion count.

2.3. Cocaine-induced behavioral sensitization

The cocaine treatment to induce behavioral sensitization was performed as described previously (Planeta and Marin, 2002). Adolescence was defined as the age period between PND 28–42, during which behavior discontinuities from younger to older (PND 60 forward) rats are evident and a time when growth spurt and neuronal changes mainly occur (Spear, 2000).

Rats received intraperitoneal (i.p.) injections of cocaine hydrochloride (10 mg/kg, Sigma®) or saline (1 ml/kg) twice a day (9:00 and 18:00) during 5 consecutive days in their home cages. Animals were treated between PND 30–34. Subsets of animals were tested for behavioral sensitization on PND 37, PND 64 or PND 94, respectively 3, 30 or 60 days after the last injection, as shown in Fig. 1. In the behavioral test the animals from saline pretreated group were given a challenge dose of saline (1 ml/kg, i.p., $n=7$ per age, SAL–SAL group) or cocaine (10 mg/kg, i.p., $n=7$ per age, SAL–COC group) and the cocaine pretreated group were given a challenge dose of cocaine (10 mg/kg, i.p., $n=8$ per age, COC–COC group). Following the challenge injections, locomotion counts accumulated in 5-min intervals were recorded during a 40-min session. The animals were allowed a 20-min adaptation period to the photocell apparatus immediately prior the challenge injections. The dose of cocaine used in these experiments is known to induce locomotor activity in the absence of focused stereotypy (Ushijima et al., 1995; Planeta and Marin, 2002).

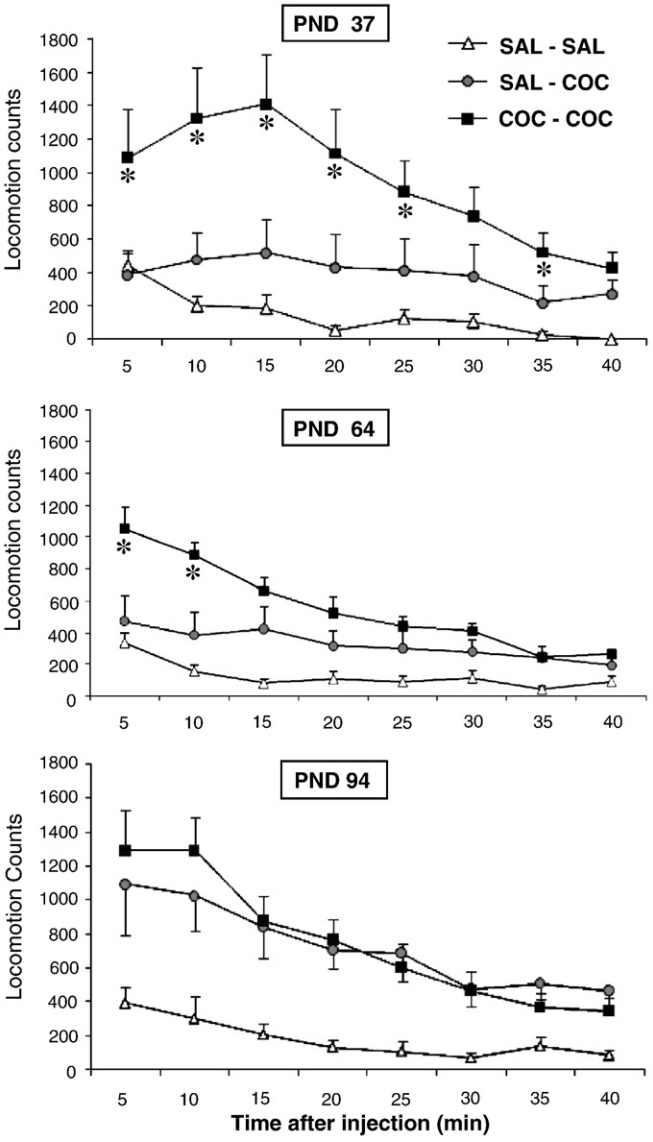

Fig. 2. Locomotor activity following saline or cocaine (10 mg/kg) challenge injection on postnatal day (PND) 37, 64 or 94 of rats pretreated with saline or cocaine during adolescence (PND30–34). Data represent mean±SEM ($N=7$–8 animals per group) of cumulative locomotion counts in 5-min intervals recorded immediately after the animals received the challenge injection. SAL–SAL, saline pretreatment and saline challenge; SAL–COC, saline pretreatment and cocaine challenge; COC–COC, cocaine pretreatment and cocaine challenge. *$p<0.05$: SAL–COC vs COC–COC (Newman–Keuls post-hoc test).

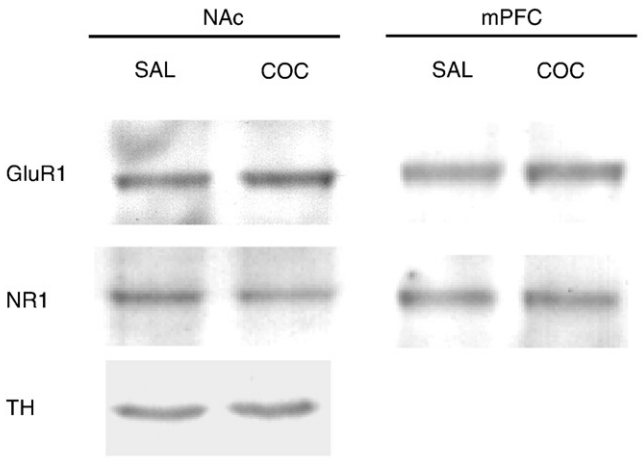

Fig. 3. Representative Immunoblots of GluR1, NR1 and TH in the nucleus accumbens (NAc) and medial prefrontal cortex (mPFC) of adolescent (PND 37) rats. SAL, saline pretreated rats; COC, cocaine pretreated rats.

2.4. Tissue preparation and Western blot analyses

Immediately after the behavioral analysis, the animals were decapitated and their brains were removed and sectioned coronally in slices of 1.5 mm using a brain matrix (Insight®, Ribeirão Preto-SP/Brazil). The appropriate brain slices (approximately +2.5 to +4.0 mm and from +1.0 to +2.5 mm relative to bregma, respectively for mPFC and NAc; Paxinos and Watson, 2005) was ice-cooled in a plate and bilateral brain areas were dissected using a 14-gauge tissue punch and stored at −80 °C until Western blot analysis. The punches of mPFC comprised both dorsal and ventral portions of this area. Tissues samples were then sonicated in 250 mM Tris–HCl, 1% SDS, 5 μg/ml Leupeptin; 5 μg/ml Pepstatin-A; 1 mM PMSF and 10 mM EDTA; pH 8. Protein content determination was made using the method of Lowry (Bio-Rad Laboratories). Samples of 30 μg of protein were subjected to SDS-polyacrylamide gel electrophoresis and transferred onto polyvinylidene fluoride (PVDF) membrane for immunoblotting. PVDF membranes were blocked with 5% nonfat dry milk and 0.1% Tween 20 in Tris buffer (TTBS, pH 7.5) for 1 h at room temperature. The primary antibodies (Santa Cruz Biotechnology®) were incubated overnight at 4 °C in TTBS (1:2000, GluR1; 1:3500, NR1; 1:6000, TH). Next, the blots were washed and incubated for 1 h with horseradish peroxidase-conjugated IgG (1:2000; Amersham Pharmacia Biotech). Protein bands were visualized on a Kodak Biomax Light film with enhanced chemiluminescence (ECL) procedure of Amersham Pharmacia Biotech. Equal protein loading was confirmed by stripping the blots and re-probing them with a monoclonal actin antibody (1:500, Santa Cruz Biotechnology®), followed by incubation with secondary antibody and visualization as described above. The films were scanned in transparence mode and the volume of the bands was quantified using Image-Master® software (Amersham Pharmacia Biotech) with subtraction of background. Each gel was loaded with at least three samples from each group under analysis (saline and cocaine) and the data were normalized as percentage of the saline values in the same blot. Western blot assays aimed to analyze the effect of cocaine pretreatments on TH, GluR1 and NR1. Thus, samples from rats pretreated with saline and challenged with cocaine (SAL+COC) were compared to samples from animals pretreated with cocaine and challenged with the same drug (COC+COC). The technique and antibodies employed allow measuring the total level of each protein, in spite of intracellular localization, phosphorylation level or activity. Moreover, the time interval between the challenge injections and sacrifice of animals (40 min) is not supposed to be long enough to induce alterations in the total level of such complex proteins.

All assays were conducted under conditions in which densitometric signal intensity was linear with protein concentration, as determined by preliminary experiments.

2.5. Statistical analyses

Behavioral data were analyzed by two-way ANOVA (group and time after injection factors) in each age period. Time was a repeated measure and Newman–Keuls' test was employed for individual post-hoc comparisons. The Western blot data were analyzed using Student's *t* tests between cocaine and saline pretreated groups within the same age. Significant differences are reported for $p<0.05$.

3. Results

3.1. Cocaine-induced behavioral sensitization

Fig. 2 depicts the locomotor activity following a cocaine challenge on PND 37, 64 or 94 in rats treated with repeated cocaine during adolescence (PND 30–34).

In adolescent animals (PND 37) two-way ANOVA revealed significant differences in locomotor activity considering both group [$F_{(2,19)}=7.16$; $p<0.005$] and time [$F_{(7,133)}=13.16$; $p<0.001$] factors. In addition, significant interaction between factors was detected [$F_{(14,133)}=4.17$; $p<0.001$]. Pair wise comparisons were then performed by the Neuman–Keuls test for each time interval. This comparison detected significant differences between SAL–COC and COC–COC groups on time intervals 5, 10, 15, 20, 25 and 35 ($p<0.05$), revealing that repeated cocaine treatment induced behavioral sensitization.

In young adult rats (PND 64) there was a main effect of group [$F_{(2,18)}=12.47$; $p<0.001$] and time [$F_{(7,126)}=21.93$; $p<0.01$] factors. Furthermore, a significant interaction between the factors [$F_{(14,126)}=5.36$; $p<0.001$] was detected. Newman–Keuls comparisons for each time interval revealed significant differences between SAL–COC and COC–COC groups at 5 and 10 min after cocaine injections ($p<0.05$), revealing the expression of behavioral sensitization to cocaine.

At PND 94, ANOVA revealed a main effect of group [$F_{(2,19)}=11.66$; $p<0.001$] and time [$F_{(7,133)}=14.87$; $p<0.01$], but no interaction between the factors [$F_{(14,133)}=1.69$; $p>0.05$]. The significant effect of group was due to differences between SAL–SAL and SAL–COC group ($p<0.001$; Newman–Keuls) but not between SAL–COC and COC–COC groups ($p>0.05$). Thus, there was an acute effect of cocaine but at this age animals did not exhibit behavioral sensitization.

3.2. Protein alterations induced by cocaine pretreatment

Representative Western blots of proteins TH, GluR1 and NR1 are shown in the Fig. 3.

Table 1
Results of Western blotting assays of animals from saline or cocaine pretreatment

Age	Protein	NAc		mPFC	
		Saline pretreat.	Cocaine pretreat.	Saline pretreat.	Cocaine pretreat.
PND 37	GluR1	100.0±5.6	110.9±7.5	100.0±3.7	124.5±8.0 *
	NR1	100.0±21.5	91.9±12.6	100.0±6.0	101.4±4.6
	TH	100.0±12.2	112.5±9.3	ND	ND
PND 64	GluR1	100.0±4.9	110.9±14.6	100.0±8.3	100.9±6.2
	NR1	100.0±11.9	127.5±17.1	100.0±9.2	100.6±10.8
	TH	100.0±16.0	108.6±12.1	ND	ND
PND 94	GluR1	100.0±2.6	118.1±8.6	100.0±15.2	92.6±12.2
	NR1	100.0±8.9	88.9±4.6	100.0±18.4	98.5±16.9
	TH	100.0±7.3	95.9±11.8	ND	ND

Values are expressed as percentage of the mean values from the animal group pretreated with saline and challenged with cocaine (mean±SEM; $n=7$–8 per group). ND=Non-detectable; *$p<0.05$ compared to Saline pretreated group (Student's *t* test).

Three days after cocaine treatment during adolescence (PND 37) a significant increase of GluR1 protein was detected in the mPFC ($p<0.05$, Student's t test). This increase was not enduring and vanished in adulthood (PND 64 and 94). No significant change of GluR1 receptor subunit was detected in the NAc ($p>0.05$). NR1 or TH proteins in the NAc and mPFC were not altered in response to cocaine pretreatment in any age, as shown in Table 1. The bands of TH protein in the mPFC were not detectable in our assay and probably reveal small amount of this protein in the mPFC.

4. Discussion

We investigated the long-lasting behavioral and molecular effects of cocaine exposure during adolescence. Our results show that repeated cocaine administration to adolescent rats induces cocaine behavioral sensitization in this age period, which endures until early adulthood, while changes in GluR1, NR1 and TH in the NAc or mPFC were not associated with long-lasting behavioral sensitization.

Repeated cocaine administration during adolescence produced behavioral sensitization when the challenge injection of cocaine was administrated during the same age period (PND 37). This result is in line with previous results from our and others' laboratories (Laviola et al., 1995; Planeta and Marin, 2002; Frantz et al., 2007). The major finding of our study is the observation that cocaine-induced sensitization during adolescence endures to early adulthood (PND 64). This is particularly relevant considering the lack of investigations on the long-lasting sensitization to cocaine when animals were exposed to the drug during adolescence. Only one early study (Ujike et al., 1995) showed that cocaine administration from PND 28 to 32 induced behavioral sensitization that lasted until nearly adulthood (PND 53). Our study extended the withdrawal period and showed the sensitized cocaine response beyond the adulthood boundary of PND 60. Recently, McPherson and Lawrence (2006) have shown similar enduring behavioral sensitization after amphetamine administration to adolescent rats.

Behavioral sensitization has been implicated in the development of drug addiction and relapse to drug-seeking behavior (Robinson and Becker, 1986; Robinson and Berridge, 1993; De Vries et al., 1998). The neuroadaptations related to behavioral sensitization are involved in craving and relapse to drug taking (Kalivas et al., 1998; Nestler, 2001). Considering the great risk of initiation of drug abuse and addiction during adolescence (Spear, 2000), our results suggest that exposure to cocaine during adolescence could predispose individuals to cocaine addiction and relapse later in life. Moreover, these results highlight the importance of investigating psychostimulant effects throughout ontogeny.

On PND 94 (i.e. when the test was performed 60 days after the last cocaine injection) behavioral sensitization was not detected following the cocaine challenge. The absence of sensitized behavioral response might be related to the fact that on PND 94 acute cocaine (SAL–COC group) caused more pronounced increase of locomotion as compared to younger rats. It could be pointed out that this enhanced acute locomotion could impair the observation of sensitization measured only by locomotor activity, since on PND 94 the occurrence of stereotyped behavior could emerge in sensitized rats. However, the dose of cocaine used (10 mg/kg) is known to induce locomotor activity in the absence of focused stereotypy (Ushijima et al., 1995) and we have previously demonstrated locomotor sensitization with the same dose of cocaine in adult rats (Araujo et al., 2003). Thus, we can argue that 60 days after withdrawal, neuronal alterations induced by cocaine pretreatment in adolescent rats are no longer present with the same intensity. In adult animals, Henry and White (1995) have reported a similar time course of cocaine-induced behavioral sensitization. They have shown that behavioral sensitization is evident up to 1 month, but it is no longer present following 2 months of withdrawal from repeated (10 mg/kg) cocaine administration. Nevertheless, most studies on cocaine-induced behavioral sensitization were performed in adult rats with drug challenge after withdrawal periods ranging from 1 to 30 days of repeated drug treatment (Ujike et al., 1995; De Vries et al., 1998; Cadoni et al., 2000; Scheggi et al., 2002; Hope et al., 2005). Differently from our experiments, in studies using the environment-paired sensitization protocol, the sensitized behavior can be seen for up to 6 months following repeated cocaine administration (Hope et al., 2006). Then, procedures of cocaine treatment in the testing apparatus could induce a more enduring sensitized behavior in adolescents and is a good target for future studies.

Administration of cocaine (10 mg/kg) to adolescent rats that have never received cocaine (SAL–COC group at PND 37) did not significantly increase locomotor activity in nearly all time intervals after cocaine injection. On the other hand, the same dose of cocaine yielded a pronounced increase in locomotion of adult rats (PND 94). The finding that adolescent rats are less sensitive to the acute effects of cocaine is consistent with previous reports, which indicated that adolescent rats have a characteristic hyporesponsivity relative to adults to the effects of acute administration of psychostimulants on locomotor activity (Laviola et al., 1995; Bolanos et al., 1998; Adriani and Laviola, 2000). Maturational changes such as pruning of dopamine receptors and increase of monoamine transporters occur in the NAc and caudate putamen during adolescence (Teicher et al., 1995; Tarazi et al., 1998). These changes in brain pathways, which play key role in reward and motor effects of drugs of abuse, can engender different acute responsivity to psychostimulants.

A great deal of interest has been devoted characterizing cellular neuroadaptations associated with behavioral sensitization (Kalivas et al., 1998; Nestler, 2001). Our results showed that TH levels were not altered in the NAc when rats received cocaine during adolescence. Similar results in adult rats were described by Hope et al. (2005). These authors described no alteration on TH levels in the NAc of adult rats 1, 7 or 21 days after cocaine treatment, despite the development of behavioral sensitization to cocaine. Taken together these results indicate that behavioral sensitization to cocaine can develop without changes of TH in the NAc. However, contradictory results are reported in the literature. It has been shown that a cocaine treatment similar to ours in adult rats decreased TH immunoreactivity in the NAc core 2 days after withdrawal, but increased TH immunoreactivity in the NAc shell 14 days following withdrawal (Todtenkopf et al., 2000). On the other hand, Schmidt et al. (2001) have described decreased levels of TH in the NAc shell 7 days after cocaine self-administration. In addition, some studies have pointed out that cocaine-induced alterations on TH occur mainly in the cell bodies of dopaminergic neurons in the Ventral Tegmental Area (VTA), instead of dopaminergic terminal in the NAc (Sorg et al., 1993; Masserano et al., 1996). The absence of TH alteration in our study might be related to the fact that we did not dissect apart NAc core from shell or due to the age period of cocaine exposure.

Concerning GluR1 and NR1 glutamate receptor subunits in the NAc, our results showed no changes 3, 30 or 60 days following withdrawal from repeated cocaine administrations to adolescent rats. Churchill et al. (1999) showed no changes on GluR1 or NR1 in the NAc 1 day after treatment with cocaine during 7 days. Conversely, the same authors showed increased expression of GluR1 in the NAc of adult rats 3 weeks following cocaine treatment. Their GluR1 alteration was only shown in the animals that developed behavioral sensitization. Other study demonstrated that both GluR1 and NR1 subunits were increased 17 days following the cocaine treatment for 14 days (Scheggi et al., 2002). Thereby, it seems that the increase of GluR1 and NR1 in the NAc does not occur after short-term withdrawal from non-contingent cocaine administration. This observation could explain the absence of glutamate receptor alterations 3 days after cocaine treatment in this study. However, the lack of changes 30 and 60 days after cocaine treatment could be due to the innumerous neuroadaptations in the dopaminergic and glutamatergic systems that adolescent animals undergo until they reach adulthood (Spear, 2000; Crews et al., 2007).

Furthermore, the studies that showed GluR1 or NR1 increase in the NAc used cocaine doses of 30 or 40 mg/kg, which is higher than the one used in our experiments (10 mg/kg, twice a day) (Churchill et al., 1999; Scheggi et al., 2002). Studies of cocaine self-administration have also shown glutamate receptor subunit alterations. Lu et al. (2003) revealed that GluR1 and NR1 protein levels were increased for up to 90 days in the NAc of adult rats after cocaine self-administration. In contrast, no significant alterations were observed on GluR1 or NR1 in the NAc 15–16 h following the end of the cocaine self-administration (Hemby et al., 2005). In other experiments, cocaine self-administration did not alter glutamate receptor subunits *per se*, but one week of extinction training during withdrawal increased GluR1 subunit in the NAc (Sutton et al., 2003).

Our results demonstrated increased GluR1 level in the mPFC 3 days after cocaine repeated injections in the adolescence. However, this alteration was small (only 24.5% increase) and did not remain until early adulthood as observed for sensitized behavior. Thus, we can argue that GluR1 increase in the mPFC can be related to development or short-term expression of cocaine-induced behavioral sensitization. Studies with cocaine sensitization in adult rats have shown no alterations of GluR1 in the mPFC (Fitzgerald et al., 1996; Churchill et al., 1999; Scheggi et al., 2002). Then, this alteration might be specific of adolescent animals. Besides glutamate receptor alterations in the NAc and mPFC, it has been reported that those proteins were altered in other brain regions, such as the VTA (Fitzgerald et al., 1996; Churchill et al., 1999; Lu et al., 2003; Hemby et al., 2005). Thus, alterations in other brain areas related to behavioral sensitization and drug addiction are good targets to further investigations using this animal model of adolescence.

The studies cited above were performed following withdrawal from repeated drug treatment but no drug challenge. In our study receptor subunit levels were measured shortly (40 min) after a cocaine challenge. Thus is also important to consider whether this procedure could affect our results. The literature about cocaine effect on total level of these proteins report alterations only after repeated cocaine administration (Churchill et al., 1999; Scheggi et al., 2002; Lu et al., 2003; Hemby et al., 2005). Moreover, a study of Fitzgerald et al. (1996) showed no alteration 16 to 18 h following acute cocaine (20 mg/kg) injection. Thus, we believe that the cocaine challenge of our experiment (10 mg/kg cocaine injected 40 min before sacrifice of the animals) do not change total levels of those proteins. Short term alterations, such as protein phosphorylation, receptor internalization, mRNA synthesis or enzyme activity could happen in this period of time. However, synthesis of such complex proteins takes a longer time. In spite of this, we performed Western blots of animal groups with same challenge injection and differing from each other only in the pretreatment procedure (SAL–COC group vs COC–COC group).

Boudreau and Wolf (2005) evaluated the contribution of glutamate receptor trafficking associated with behavioral sensitization. They showed that behavioral sensitization to cocaine is associated with increased GluR1 and GluR2/3 AMPA receptor subunits specifically in the cell surface into NAc. This redistribution occurs in the absence of changes in the total levels of these receptor subunits. Then, our results do not exclude the participation of glutamate receptors in the behavioral sensitization, because other alterations besides total protein, such as subunit composition, localization or function; can occur in glutamatergic signaling.

Therefore, our results showed that repeated cocaine administration during adolescence could have long-term consequences, producing enduring behavioral sensitization that lasts into early adulthood. Considering that behavioral sensitization is induced by neuroadaptations, which may render the organism more susceptible to drug craving and relapse, this study highlights the importance of drug exposure during adolescence and its consequence on drug abuse and relapse latter in life. Moreover, investigations into neuroadaptations underlying long-term behavioral sensitization induced by cocaine in the adolescence need to be extended.

Acknowledgements

The authors appreciate the excellent technical assistance by Elisabete Z. P. Lepera and Rosana F. P. Silva. This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) PhD fellowship 04/01894-6 to MTM and grant 04/01606-0 to CSP. FCC is a CAPES fellowship recipient. CSP is a CNPq Research Fellow.

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