Pharmacology, Biochemistry and Behavior 96 (2010) 160–165

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Stress-induced reinstatement of amphetamine-conditioned place preference and changes in tyrosine hydroxylase in the nucleus accumbens in adolescent rats

Fábio C. Cruz [a], Rodrigo M. Leão [a,b], Marcelo T. Marin [a], Cleopatra S. Planeta [a,b,*]

[a] Laboratory of Pharmacology, School of Pharmaceutical Sciences, Univ. Estadual Paulista-UNESP, Rod. Araraquara-Jaú Km 1, 14801-902, Araraquara, São Paulo, Brazil
[b] Interinstitutional Graduate Program in Physiological Sciences, UFSCar/UNESP, Rod. Washington Luiz, Km 235, São Carlos, São Paulo, Brazil

ARTICLE INFO

Article history:
Received 4 November 2009
Received in revised form 16 April 2010
Accepted 2 May 2010
Available online 10 May 2010

Keywords:
Amphetamine
Stress
Conditioned place preference
Reinstatement
Adolescence
Rat

ABSTRACT

Drug abuse among humans often begins during adolescence. Exposure to psychostimulants during this age period may have long-term consequences which can render the organism more susceptible to drug abuse and relapse later in life. It has been demonstrated that exposure to stress can promote relapse to drug use even after long periods of withdrawal. The reinstatement of conditioned place preference (CPP) is a useful animal model for studying relapse. In humans and animals, changes in tyrosine hydroxylase (TH) have been related to drug addiction. Our study examined whether amphetamine-induced CPP during adolescence could be reinstated by exposure to stress 1 (adolescence) and 30 (adulthood) days after the extinction test. We also investigated TH levels following the reinstatement of CPP. Our results showed that amphetamine-induced CPP during adolescence can be reinstated by stress exposure 1 day (P42, end of adolescence) but not 30 days after extinction (P71, adulthood). Moreover the reinstatement of AMPH-induced CPP by stress exposure occurred in the presence of decreased TH in the nucleus accumbens. In conclusion, our data add new evidence that neuroadaptations on TH may mediate relapse to drug-seeking behavior induced by stress within adolescence.

1. Introduction

Stress exposure has been related to initiation, maintenance and relapse to drug abuse (Gawin, 1991; Sinha, 2001; Gordon, 2002; Goeders, 2003; Weiss, 2005). For example, clinical studies have demonstrated that exposure to stress or simply the presentation of stress-related imagery can induce relapse to drug seeking in humans (Lamon and Alonzo, 1997; Brady and Sonne, 1999; Sinha et al., 1999; Sinha, 2001).

Two animal models have proven especially useful for studying relapse, the reinstatement of self-administration (Carroll, 1985; Lê and Shaham, 2002; Lu et al., 2003) and the reinstatement of conditioned place preference (CPP) (Mueller and Stewart, 2000; Itzhak and Martin, 2002; Lu et al., 2005; Biala and Budzynska, 2006). It has been observed that the same stimuli that reinstate self-administration are capable of inducing the reinstatement of CPP (Aguilar et al., 2009). In this sense, pre-clinical studies have shown that stress can reinstate cocaine, amphetamine, morphine, and heroin self-administration (Wit and Stewart, 1981; Shaham et al., 1997; Buczek et al., 1999; Lesage et al., 2004). Similarly, several studies have shown that stress exposure reinstate opioids-, cocaine- and nicotine-induced CPP (Will et al., 1998, 2004; Der-avakian et al., 2005, 2006; Leão et al., 2009).

It has been demonstrated that exposure to stress can promote relapse to drug use even after long periods of withdrawal (Lu et al., 2004). In rats, intermittent footshock reinstates nicotine self-administration up to 15 days after extinction (Buczek et al., 1999). Recently, we showed that the exposure to acute restraint stress caused the reinstatement of nicotine-induced CPP 15 days after the extinction of this behavior (Leão et al., 2009).

Recently, some studies have investigated the neurobiology of stress-induced reinstatement of drug seeking. Studies pointed to the involvement of dopamine, corticotropin-release factor and noradrenaline in brain areas such as bed nucleus of the stria terminalis (BNST), central nucleus of amygdala and nucleus accumbens in this phenomenom (Shaham et al., 2000). The increase of dopamine transmission in the nucleus accumbens has a critical role in the reinstatement of drug-seeking behavior (Khroyan et al., 2000; Schmidt et al., 2006). For instance, intra-accumbal infusion of dopamine antagonists attenuates reinstatement of drug seeking (Shaham and Stewart, 1996; Anderson et al., 2006). Moreover, drug-induced reinstatement has been associated with enhanced dopamine release in nucleus accumbens (De Vries et al., 1998; Di Ciano et al., 2001; Vezina et al., 2002).

Changes in tyrosine hydroxylase levels (TH; the rate-limiting enzyme for dopamine synthesis) in the mesolimbic pathway have been related to repeated psychostimulant administration and drug addiction (Todtenkopf et al., 2000). For instance, Trulson et al. (1987) found decreased TH immunolabeling in the nucleus accumbens

* Corresponding author. Laboratory of Pharmacology, School of Pharmaceutical Sciences, Univ. Estadual Paulista-UNESP, Rod. Araraquara-Jaú Km 1, 14801-902, Araraquara, São Paulo, Brazil. Tel.: +55 16 3301 6981; fax: +55 16 3301 6980.
E-mail address: cplaneta@fcfar.unesp.br (C.S. Planeta).

0091-3057/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2010.05.001

following repeated cocaine administration. However, some studies have demonstrated that repeated psychostimulant administration produces increases or do not change TH levels (Beitner-Johnson and Nestler, 1991; Sorg et al., 1993; Vrana et al., 1993; Lu et al., 2003; Todtenkopf et al., 2000, Marin et al., 2008). Moreover, it was demonstrated that TH levels in the nucleus accumbens may be influenced by extinction training of a conditioned behavior (Schmidt et al., 2001). These authors demonstrated that 12 days of cocaine self-administration in rats reduced TH immunoreactivity by 29% in the nucleus accumbens after a 1 week withdrawal period. In contrast, TH immunoreactivity in the nucleus accumbens was completely restored in animals that experienced extinction training during the same withdrawal period. Thus, considering that dopamine release in the nucleus accumbens is involved in stress-induced reinstatement and TH levels is influenced by extinction training it would be interesting evaluated if neuroadaptations on TH in this brain are is involved in stress-induced reinstatement of amphetamine-induced CPP.

Drug abuse among humans often begins during adolescence, a period of ontogeny in which individuals exhibit age-specific behavioral characteristics, such as risk taking and novelty seeking, which could predispose them to initiate drug use (Spear, 2000; Casey et al., 2008). Exposure to psychostimulants during adolescence can have long-term consequences, because early drug exposure may cause enduring adaptations (Guerriero et al., 2006; McPherson and Lawrence, 2006), which can render the organism more susceptible to drug abuse and relapse later in life. Consequently, there is a need to model drug relapse during this developmental period and assess vulnerability to relapse and neuroadaptations through adulthood.

Our study examined whether amphetamine-induced CPP during adolescence could be reinstated by exposure to stress 1 (adolescence) and 30 (adulthood) days after the extinction test. We also investigated the TH levels in the nucleus accumbens of rats immediately after the reinstatement test.

2. Experimental procedures

2.1. Subjects

Subjects were male Wistar rats obtained from the animal breeding facility of the São Paulo State University-UNESP at postnatal day (P) 21. Groups of 3–4 animals were housed in plastic cages 32 (width) × 40 (length) × 16 (height) cm in a room maintained at 23 ± 2 °C. Rats were kept in a 12:12 h light/dark cycle (lights on at 07:00) and were allowed free access to food and water. Each animal was used only in one experimental procedure. The experimental procedure started on adolescence (P28). All experiments were performed during the light phase between 8:00 a.m. and 5:00 p.m. Each experimental group consisted of 7–8 animals.

The experimental protocol was approved by the Ethical Committee for use of Human or Animal Subjects of the School of Pharmaceutical Science-UNESP (CEP-13/2004) and the experiments were conducted according to ethics principles of the Brazilian College of Animals' Experimentation-(COBEA), based on NIH Guidelines for the Care and Use of Laboratory Animals.

2.2. Drug

D,L-Amphetamine (Sigma, St. Louis, MO, USA).

2.3. Reinstatement of amphetamine-induced CPP

The testing apparatus for the conditioned place preference paradigm consisted of Plexiglas boxes with two compartments of equal size (30.0 cm length × 21.0 cm width × 30.0 cm height) separated by removable guillotine doors from a small central gray area (15.0 cm length × 30.0 cm width × 30.0 cm height). One compartment had white walls and a thin parallel grid floor and the other had black and white stripes on the walls and a grid with small holes on the floor. The central gray area constituted a "neutral" chamber. The testing boxes were kept in a soundproof room with dim 40 −l× illumination.

The CPP-reinstatement procedure consisted of the following phases: pre-conditioning, conditioning, post-conditioning, extinction and reinstatement. It was used an unbiased place conditioning paradigm similar to method that described by Mueller and Stewart (2000). The procedure started on adolescence (P28) and the CPP-reinstatement test was performed in the end of adolescence (P42) or during adulthood (P71). These ages were selected according to Spear (2000).

2.3.1. Pre-conditioning (PRE-COND)

During this phase each rat was placed in the neutral compartment with the guillotine doors removed to allow access to the entire apparatus for 15 min for 3 days. On day 3, rats were placed in the apparatus and videotaped for 15 min and the time spent in each compartment was recorded and analyzed. Approximately 20% of the animals displayed strong unconditioned aversion (<15% of session time) or preference (>85%) for one of the compartments and were excluded from the study.

2.3.2. Conditioning

Animals were randomly paired to drug or saline administration. Conditioning was performed using a protocol consisting of 8 alternate injections of 5.0 mg/kg i.p. of amphetamine or saline (2/day) over 4 consecutive days. (i.e. saline in the first session and amphetamine in the second). Injections were administrated immediately before confinement in one of the two large compartments for 30 min before returning to the home cage. In each group half of the animals injected with amphetamine were confined to the preferred compartment and the other half were confined to the initially non-preferred compartment. Conditioning sessions were conducted twice a day with an interval of 4 h. The control group received saline everyday in both compartments. The neutral chamber was never used during conditioning and was blocked by guillotine doors.

2.3.3. Conditioning test (COND)

The test was conducted 24 h after the last conditioning session. Each rat was placed in the neutral compartment with the guillotine doors removed to allow access to the entire apparatus. The time spent in each compartment was recorded for 15 min as described for the pre-conditioning phase. Amphetamine or saline was not injected before tests.

2.3.4. Extinction (EXT)

Beginning the day after the test for CPP, rats underwent 12 sessions (6-day cycles of 2 sessions in each day) of extinction training that consisted of exposure to the saline- and amphetamine-paired compartment immediately after a saline injection (i.e. six exposures to each compartment). Twenty-four hours after the last extinction session the extinction test was carried out recording the time spent in each compartment as described in the pre-conditioning phase.

2.3.5. Reinstatement (REINST)

One (P42) or 30 (P71) days after the last extinction session, stress-induced reinstatement of amphetamine CPP was evaluated. Rats were exposed to restraint stress during 30 min to this end they were removed from their home cage and were restrained for 30 min in plastic cylinders [20.0 cm (length) × 5.5 cm (internal diameter) for adult rats; 17.0 cm (length) × 4.5 cm (internal diameter) for adolescent rats] in a separated room then immediately tested for reinstatement of CPP. During this reinstatement test each rat was placed in the neutral compartment with the guillotine doors removed to allow access to the entire apparatus for 15 min, the time spent in each compartment was measured as described above.

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 slice containing shell and core of the nucleus accumbens (approximately from +1.0 to +2.5 mm relative to bregma, Paxinos and Watson, 2005) was placed in an ice-cooled plate and bilateral brain areas were dissected using a 14-gauge tissue punch and stored at −80 °C until Western blot analysis. 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. The homogenate was used for the western blotting analysis. 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 blots were incubated with TH antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight at 4 °C in TTBS (1:6000). Next, the blots were washed and incubated for 1 h with horseradish peroxidase-conjugated IgG (1:2000; Amersham). Protein bands were visualized on a Kodak Biomax Light film with enhanced chemiluminescence procedure (ECL-Amersham). 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 of both groups (saline or amphetamine) and the data were normalized as percentage of the saline values in the same blot. All assays were conduct under conditions in which densitometric signal intensity was linear with protein concentration as determined by preliminary experiments.

2.5. Statistical analysis

The behavioral data are expressed as means ± SEM of CPP score. The conditioned score is expressed by the ratio between the time spent in the drug-paired and the time spent in both compartments (drug and saline paired), (i.e. total time minus time spent in the neutral chamber) multiplied by 100. Levene tests for homogeneity of variance were performed to the behavioral and protein levels data. Levene did not show statistically significant differences, indicating the homogeneity of variance. Thus the reinstatement of CPP was analyzed by two-way ANOVA for repeated-measured [treatment factor (saline and amphetamine) *versus* phases (PRE-COND, COND, EXT and REINST)]. The phase was used as repeated-measured. When a significant ($p<0.05$) main effect was observed *F*-tests for contrast analysis were applied. The Western blotting data were analyzed using Student's *t*-tests between amphetamine and saline groups.

3. Results

3.1. Reinstatement of amphetamine-induced CPP

At adolescence (P42) (1 day after extinction) two-way ANOVA for repeated measures did not reveal significant differences for treatment factor [$F(1,14)=0.25$; $p>0.05$]. However, it showed significant differences for phase factor [$F(3,42)=5.19$; $p<0.05$]. This analysis also detected the interaction between factors [$F(3,42)=3.49$; $p<0.05$] (Fig. 1A).

Further analysis (*F*-test) revealed an increase in the time spent in amphetamine-paired compartment in the COND when compared to PRE-COND [$F(1,14)=15.57$; $p<0.01$], indicating that amphetamine-induced CPP. In addition, no difference was observed comparing PRE-COND with EXT phases [$F(1,14)=1.26$; $p>0.05$], indicating the extinction of CPP. Significant differences in the time spent in amphetamine-paired compartment were detected comparing REINST to PRE-COND [$F(1,14)=9.96$; $p<0.01$]. In addition, time spent in REINST was significantly higher than EXT [$F(1,14)=4.22$; $p<0.05$], indicating the reinstatement of CPP.

At early adulthood (P71; 30 days after EXT) two-way ANOVA for repeated-measured did not revealed differences for treatment factor [$F(1,16)=0.15$; $p>0.05$]. However, it detected significant differences for phase factor [$F(3,48)=3.62$; $p<0.05$]. No significant interaction between factors was observed [$F(3,48)=1.04$; $p<0.38$] (Fig. 1B).

Further analysis (*F*-test) revealed an increase in the time spent in drug-paired compartment in the COND when compared to PRE-COND [$F(1,16)=17.76$; $p<0.001$], indicating that amphetamine-induced CPP. In addition, no difference was observed comparing PRE-COND with EXT phases [$F(1,16)=0.45$; $p>0.05$], indicating the extinction of CPP. No differences in the time spent in amphetamine-paired compartment were detected comparing REINST to PRE-COND phase [$F(1,16)=0.08$; $p>0.05$], indicating that exposure to restraint stress failed in reinstating CPP 30 days after extinction. In all experiments, no significant differences were observed in the time spent for saline group across phases.

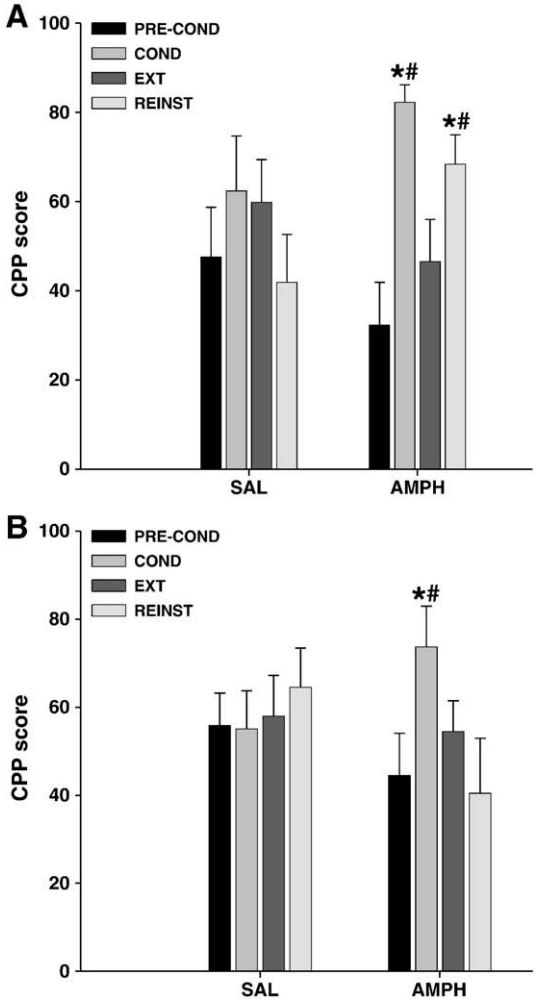

Fig. 1. Reinstatement of amphetamine-conditioned place preference in rats caused by exposure to 30-min restraint stress 1 (A) or 30 days (B) after the extinction test in saline (SAL) and amphetamine (AMPH) groups. Bars represent means ± SEM of CPP score ($N=7$–8 animals per group). *$p<0.05$, compared to PRE-COND; #$p<0.05$ compared to EXT.

No differences were observed neither in locomotor activity nor freezing behavior between adolescent and adult rats following stress exposure.

In summary, our results showed that exposure to restraint stress was able to reinstate CPP 1, but not 30, days after extinction in rats the developed amphetamine CPP during adolescence.

No differences on time spent in the "neutral" compartment during any of the phases were observed between treatments.

3.2. Alterations in TH in the nucleus accumbens

When the reinstatement test was performed 1 day (P42) after the extinction test *t*-test revealed a significant decrease in expression of TH in amphetamine-conditioned animals compared to saline conditioned animals [$t(1,12) = 2.97$; $p = 0.01$]. However, when the reinstatement test was performed 30 days (P71) after the extinction test no significant changes were observed in expression of this enzyme in amphetamine-conditioned animals compared to saline conditioned animals [$t(1,12) = 0.46$; $p = 0.65$] (Fig. 2).

4. Discussion

Our results showed that amphetamine-induced CPP during adolescence can be reinstated by the exposure to stress 1 day (P42, end of adolescence) but not 30 days after extinction (P71, adulthood). Moreover the reinstatement of AMPH-induced CPP by stress exposure occurred in the presence of decreased TH in the nucleus accumbens.

Adolescent rats developed a clear-cut amphetamine-induced CPP. This finding agrees with previous studies from our laboratory (Cruz et al., 2008) and also corroborates findings reported by Adriani and Laviola (2003).

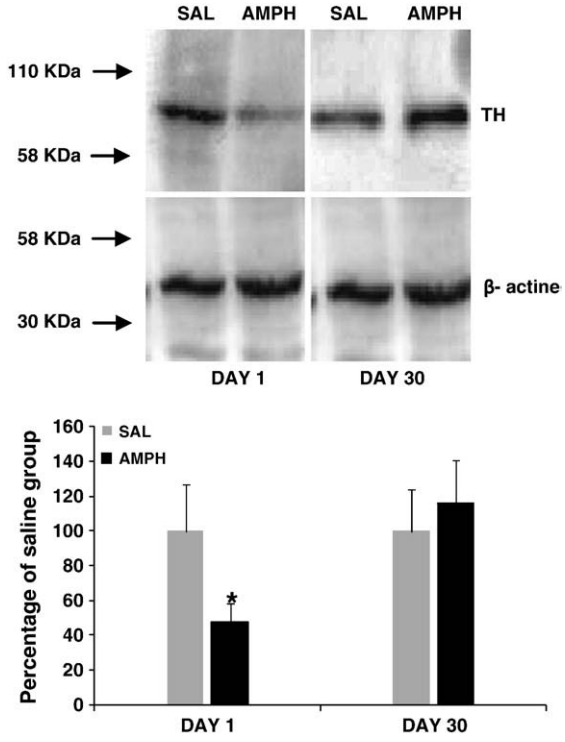

Fig. 2. On the top: Representative immunoblots of tyrosine hydroxylase (TH) in the nucleus accumbens obtained immediately following the reinstatement test in saline (SAL) and amphetamine (AMPH) groups. On the bottom: Protein levels of tyrosine hydroxylase in the NAc obtained immediately following the reinstatement test that was carried-out 1 and 30 days after the extinction test in saline (SAL) and amphetamine (AMPH) groups. Data represent the mean ± SEM of percentage change respective to saline values of saline-treated rats for each immunoblot. ($N = 7$–8 animals per group). *$p < 0.05$, compared to SAL group.

Exposure to stressful events is considered one of the major factors responsible for drug relapse (Sinha et al., 1999, 2006). Our results showed that acute exposure to restraint stress reinstated amphetamine-induced CPP when tests were performed during adolescence (1 day following extinction), but not when animals were tested 30 days after extinction (i.e., on P71). There are some studies showing that exposure to acute stress reinstates psychostimulant-induced CPP in adult rats and mice when both acquisition of CPP and exposure to stress occurred in adulthood (Lu et al., 2002; Ribeiro do Couto et al., 2006). This is the first study to demonstrate the effect of stress on psychostimulant reinstatement during adolescence.

The lack of the reinstatement in adulthood could be related to the fact that the period of 30 days after extinction is quite long to induce CPP reinstatement. However, previously data from our laboratory show that amphetamine priming injection was capable to induce CPP reinstatement following 30 days after extinction in rats that acquired the CPP in adolescence (Cruz et al., 2008). In adult rats it has been shown that a similar exposure to stress reinstated nicotine-CPP 15 days after the extinction (Leão et al., 2009). Moreover, studies have demonstrated that stress exposure is able to reinstate psychostimulant and heroin self-administration and CPP even after long periods of withdraw (Shaham and Stewart, 1995). For instance, it has been observed in adult rats, that morphine and cocaine-induced CPP was reinstated by the exposure to footshock stress 37 days after CPP extinction (Lu et al., 2000, 2001; Wang et al., 2000). Then, we can hypothesize that the absence of reinstatement in adulthood could be related to the fact that drug-induced neuroadaptations in adolescence did not persist in the transition to adulthood. In fact, it has been observed that repeated cocaine treatment during adolescence of rats promoted increase in GluR1 subunit of a glutamate receptor at the prefrontal cortex that was not observed when they become adults (P60) (Marin et al., 2008).

Although a 30 min restraint stress is able to fully activate the HPA axis in adolescent and adult rats (Doremus-Fitzwater et al., 2009; Gray et al., 2010), it may be considered that the time of restraint (30 min) which is able to induce reinstatement in adolescent perhaps is different to that effective in the adulthood. Some studies have shown that the CPP acquired and extinguished during adolescence can be reinstated in the adulthood by a priming injection of the drug (Balda et al., 2006; Cruz et al., 2008). Thus, drug exposure appears to be a stronger cue to promote relapse to drug-seeking than stress in adult rats that had early experience with the drug. Adolescent take longer than adult rats to extinguish cocaine-induced CPP and they exhibit a stronger reinstatement upon priming, suggesting that the cocaine cue may have greater salience during adolescence (Brenhouse and Andersen, 2008).

The lack of the reinstatement in adulthood could also be related to kind of stress. Studies have demonstrated that some kind of stress cannot be able to reinstatement a conditioned behavior for a specific drug (Ribeiro do Couto et al., 2006). For example, it has been demonstrated that acute food deprivation, but neither restraint nor footshock stress reinstated heroin-seeking in rats (Shalev et al., 2000).

We investigated also whether differences in TH levels in the nucleus accumbens could be related to stress-induced reinstatement of amphetamine CPP. Our results showed that TH protein levels were reduced in amphetamine-treated animals compared to saline 1 day after CPP extinction, i.e., in adolescent rats. However no change in TH levels was observed in the animals following 30 days of CPP extinction. The time course of these protein alterations were similar to the behavioral data, in which CPP reinstatement was demonstrated following 1 but not 30 days after extinction. These results suggest that changes in TH in the nucleus accumbens may at least partially, be related to the reinstatement of amphetamine-induced CPP by the exposure to acute stress. However these changes on TH levels in the nucleus accumbens in our study may be related to development of rats. There are evidence showing that TH expression varies during

ontogeny. For example, it was demonstrated that tyrosine hydroxylase immunoreactivity in control rats (without any kind of treatment) is higher on P90 compared to P30, P40 and P50 (Mathews et al., 2009). Then, in our experiment, the absence of amphetamine-induced changes on P71 might be related to the animal development.

The alteration in TH was observed in adolescent rats that reinstated amphetamine - induced CPP, these results add relevant findings on the ontogeny of amphetamine effects. However, lack of data on adolescent rats makes it difficult to further discuss these data.

Changes in TH levels seem to be also dependent on the time interval after drug repeated administration. It has been shown that repeated cocaine administration decreased TH immunoreactivity in the nucleus accumbens core 2 days after withdrawal, but increased TH immunoreactivity in the nucleus accumbens shell 14 days following withdrawal (Todtenkopf et al., 2000). Moreover, Schmidt et al. (2001) found decreased TH levels in the accumbens following 7 days of withdrawal of repeated cocaine self-administration. This decrease in TH levels returned to basal levels within 15 days of withdrawal. Alternatively, the different results may be due to differences in the accumbens' dissection, drug administration method, treatment duration and age of rats that the drug was administrated.

Experiments on adult have shown that increased dopamine transmission in the nucleus accumbens has a critical role in the reinstatement of drug seeking behavior by a priming injection. (Khroyan et al., 2000; Schmidt et al., 2006). However the role of dopamine on stress-induced reinstatement of drug seeking is not clear yet. Some evidences show that the administration of a mixed dopamine receptor antagonist, flupenthixol, attenuated relapse induced by footshock (Shaham and Stewart, 1996).On the other hand, selective D1- or D2-like receptor antagonists (SCH 23390 or raclopride) have no effect on footshock-induced reinstatement. Moreover it was demonstrated that heroin priming induces a greater release of DA in the nucleus accumbens than footshock under the conditions of the reinstatement (Shaham and Stewart, 1996). Thus these authors have suggested that dopaminergic systems play only an indirect role in this effect.

Conversely, we found that TH, a rate-limiting enzyme to dopamine synthesis, TH protein levels were reduced in amphetamine-treated animals compared to saline only when amphetamine CPP was reinstated by exposure to stress (i.e. 1 day after CPP extinction). Thus we could suppose that the levels of dopamine would also be reduced in these animals. However we only evaluated total levels of TH but not its activity (i.e. its activity is regulated by its phosphorylation state.

Another hypothesis is that the alteration observed in TH levels in our results could be related to extinction of CPP. In fact, extinction of conditioned behavior has been related to decreased of nucleus accumbens levels of TH, since animals that did not show reduced levels of TH did not display extinction (Schmidt et al., 2001).

In conclusion, our data add new evidence that neuroadaptations on TH can be related to relapse to drug seeking induced by stress within adolescence. These results also show the relevance of considering stress as a factor in strategies for drug abuse intervention in particular during the adolescence.

Acknowledgements

The authors appreciate the excellent technical assistance by Elisabete Zocal Paro Lepera and Rosana Finoti Pupim Silva. We also thank Celso Luís Borsato for animal care. This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP — 04/01606-0) and a CAPES PhD fellowship to F.C.C.

References

Adriani W, Laviola G. Elevated levels of impulsivity and reduced place conditioning with d-amphetamine: two behavioral features of adolescence in mice. Behav Neurosci 2003;117:695–703.

Aguilar MA, Rodríguez-Arias M, Miñarro J. Neurobiological mechanisms of the reinstatement of drug-conditioned place preference. Brain Res Rev 2009;59:253–77.

Anderson SM, Schmidt HD, Pierce RC. Administration of the D2 dopamine receptor antagonist sulpiride into the shell, but not the core, of the nucleus accumbens attenuates cocaine priming-induced reinstatement of drug seeking. Neuropsychopharmacology 2006;31:1452–61.

Balda MA, Anderson KL, Itzhak Y. Adolescent and adult responsiveness to the incentive value of cocaine reward in mice: role of neuronal nitric oxide synthase (nNOS) gene. Neuropharmacology 2006;51:341–9.

Beitner-Johnson D, Nestler EJ. Morphine and cocaine exert common chronic actions on tyrosine hydroxylase in dopaminergic brain reward regions. J Neurochem 1991;57:344–7.

Biala G, Budzynska B. Reinstatement of nicotine-conditioned place preference by drug priming: effects of calcium channel antagonists. Eur J Pharmacol 2006;537:85–93.

Brady KT, Sonne SC. The role of stress in alcohol use, alcoholism treatment, and relapse. Alcohol Res Health 1999;23:263–71.

Brenhouse HC, Andersen SL. Delayed extinction and stronger reinstatement of cocaine conditioned place preference in adolescent rats, compared to adults. Behav Neurosci 2008;122:460–5.

Buczek Y, Lê AD, Wang A, Stewart J. Stress reinstates nicotine seeking but not sucrose solution seeking in rats. Psychopharmacology 1999;144:183–8.

Carroll ME. The role of food deprivation in the maintenance and reinstatement of cocaine-seeking behavior in rats. Drug Alcohol Depend 1985;16:95-109.

Casey BJ, Jones RM, Hare TA. The adolescent brain. Ann NY Acad Sci 2008;1124:111–26.

Cruz FC, Marin MT, Planeta CS. The reinstatement of amphetamine-induced place preference is long-lasting and related to decreased expression of AMPA receptors in the nucleus accumbens. Neuroscience 2008;151:313–9.

De Vries TJ, Cools AR, Shippenberg TS. Infusion of a D-1 receptor agonist into the nucleus accumbens enhances cocaine-induced behavioural sensitization. Neuroreport 1998;9:1763–8.

Der-Avakian A, Will MJ, Bland ST, Deak T, Nguyen KT, Schmid MJ, et al. Surgical and pharmacological suppression of glucocorticoids prevents the enhancement of morphine conditioned place preference by uncontrollable stress in rats. Psychopharmacology 2005;179:409–17.

Der-Avakian A, Bland ST, Schmid MJ, Watkins LR, Spencer RL, Maier SF. The role of glucocorticoids in the uncontrollable stress-induced potentiation of nucleus accumbens shell dopamone and conditioned place preference responses to morphine. Psychoneuroendocrinology 2006;31:653–63.

Di Ciano P, Blaha CD, Phillips AG. Changes in dopamine efflux associated with extinction, CS-induced and D-amphetamine-induced reinstatement of drug-seeking behavior by rats. Behav Brain Res 2001;120:147–58.

Doremus-Fitzwater TL, Varlinskaya EI, Spear LP. Social and non-social anxiety in adolescent and adult rats after repeated restraint. Physiol Behav 2009;97:484–94.

Gawin FH. Cocaine addiction: psychology and neurophysiology. Science 1991;251:1580–6.

Goeders NE. The impact of stress on addiction. Eur Neuropsychopharmacol 2003;13:435–41.

Gordon HW. Early environmental stress and biological vulnerability to drug abuse. Psychoneuroendocrinology 2002;27:115–26.

Gray M, Bingham B, Viau V. A comparison of two repeated restraint stress paradigms on hypothalamic-pituitary-adrenal (HPA) axis habituation, gonadal status, and central neuropeptide expression in adult male rats. J Neuroendocrinol 2010;22:92-101.

Guerriero RM, Hayes MM, Dhaliwal SK, Ren JQ, Kosofsky BE. Preadolescent methylphenidate versus cocaine treatment differ in the expression of cocaine-induced locomotor sensitization during adolescence and adulthood. Biol Psychiatry 2006;60:1171–80.

Itzhak Y, Martin JL. Cocaine-induced condicioned place perference in mice: Induction, extinction and reinstatement by related psycostimulants. Neuropsychopharmacology 2002;26:130–4.

Khroyan TV, Barrett-Larimore RL, Rowlett JK, Spealman RD. Dopamine D1- and D2-like receptor mechanisms in relapse to cocaine-seeking behavior: effects of selective antagonists and agonists. J Pharmacol Exp Ther 2000;294:680–7.

Lamon BC, Alonzo A. Stress among males recovering from substance abuse. Addict Behav 1997;22:195–205.

Lê A, Shaham Y. Neurobiology of relapse to alcohol in rats. Pharmacol Ther 2002;94:137–56.

Leão RM, Cruz FC, Planeta CS. Exposure to acute restraint stress reinstates nicotine-induced place preference in rats. Behav Pharmacol 2009;20:109–13.

Lesage MG, Burroughs D, Dufek M, Keyler DE, Pentel PR. Reinstatement of nicotine self-administration in rats by presentation of nicotine-paired stimuli, but not nicotine priming. Pharmacol Biochem Behav 2004;79:507–13.

Lu L, Ceng X, Huang M. Corticotropin-releasing factor receptor type I mediates stress-induced relapse to opiate dependence in rats. Neuroreport 2000;11:2373–8.

Lu L, Liu D, Ceng X. Corticotropin-releasing factor receptor type 1 mediates stress-induced relapse to cocaine-conditioned place preference in rats. Eur J Pharmacol 2001;415:203–8.

Lu L, Zhang B, Liu Z, Zhamg Z. Reactivation of cocaine conditioned place preference induced by stress is reverse by cholecystokinin-B receptor antagonist in rats. Brain Res 2002;954:132–40.

Lu L, Grimm JW, Shaham Y, Hope B. Molecular neuroadaptations in the accumbens and ventral tegmental area during the first 90 days of forced abstinence from cocaine self-administration. J Neurochem 2003;85:1604–13.

Lu L, Grimm JW, Hope BT, Shaham Y. Incubation of cocaine craving after withdrawal: a review of preclinical data. Neuropharmacology 2004;47:214–26.

Lu L, Chen H, Su W, Ge X, Yue W, Su F, et al. Role of withdrawal in reinstatement of morphine-conditioned place preference. Psychopharmacology 2005;181:90-100.
Marin MT, Cruz FC, Planeta CS. Cocaine-induced behavioral sensitization in adolescent rats endures until adulthood: lack of association with GluR1 and NR1 glutamate receptor subunits and tyrosine hydroxylase. Pharmacol Biochem Behav 2008;91: 109–14.
Mathews IZ, Waters P, Mccormick CM. Changes in hyporesponsiveness to acute amphetamine and age differences in tyrosine hydroxylase immunoreactivity in the brain over adolescence in male and female rats. Dev Psychobiol 2009;51: 417–28.
Mcpherson CS, Lawrence AJ. Exposure to amphetamine in rats during periadolescence establishes behavioural and extrastriatal neural sensitization in adulthood. Int J Neuropsychopharmacol 2006;9:377–92.
Mueller D, Stewart J. Cocaine-induced conditioned place preference: reinstatement by priming injections of cocaine after extinction. Behav Brain Res 2000;115:39–47.
Paxinos G, Watson C. The rat brain in stereotaxic coordinates, 5th ed. San Diego: Elsevier Academic Press; 2005.
Ribeiro do Couto B, Aguilar MA, Manzanedo C, Rodriguez-Arias M, Armario A, Minarro J. Social stress is as effective as physical stress in reinstating morphine-induced place preference in mice. Psychopharmacology 2006;185:459–70.
Schmidt EF, Sutton MA, Schad CA, Karanian DA, Brodkin ES, Self DW. Extinction training regulates tyrosine hydroxylase during withdrawal from cocaine self-administration. J Neurosci 2001;21:137.
Schmidt HD, Anderson SM, Pierce RC. Stimulation of D1-like or D2 dopamine receptors in the shell, but not the core, of the nucleus accumbens reinstates cocaine-seeking behaviour in the rat. Eur J Neurosci 2006;23:219–28.
Shaham Y, Stewart J. Stress reinstates heroin self-administration behavior in drug-free animals: an effect mimicking heroin, not withdrawal. Psychopharmacology 1995;119:334–41.
Shaham Y, Stewart J. Effects of opioid and dopamine receptor antagonists on relapse induced by stress and re-exposure to heroin in rats. Psychopharmacology (Berl) 1996;125:385–91.
Shaham Y, Adamson LK, Grocki S, Corrigall WA. Reinstatement and spontaneous recovery of nicotine seeking in rats. Psychopharmacology 1997;133:106.
Shaham Y, Erb S, Stewart J. Stress-induced relapse to heroin and cocaine seeking in rats: a review. Brain Res Rev 2000;33:13–33.
Shalev U, Highfield D, Yap J, Shaham Y. Stress and relapse to drug seeking in rats: studies on the generality of the effect. Psychopharmacology 2000;150:337–46.
Sinha R. How does stress increase risk of drug abuse and relapse? Psychopharmacology (Berl) 2001;158:343–59.
Sinha R, Catapano D, O'malley S. Stress-induced craving and stress response in cocaine dependent individuals. Psychopharmacology 1999;142:343–51.
Sinha R, Garcia M, Paliwal P, Kreek MJ, Rounsaville BJ. Stress-induced cocaine craving and hypothalamic-pituitary-adrenal responses are predictive of cocaine relapse outcomes. Arch Gen Psychiatry 2006;63:324–31.
Sorg BA, Chen SY, Kalivas PW. Time course of tyrosine hydroxylase expression after behavioral sensitization to cocaine. J Pharmacol Exp Ther 1993;266:424–30.
Spear LP. The adolescent brain and age-related behavioral manifestations. Neurosci Biobehav 2000;24:417–63.
Todtenkopf MS, De Leon KR, Stellar JR. Repeated cocaine treatment alters tyrosine hydroxylase in the rat nucleus accumbens. Brain Res Bull 2000;52:407–11.
Trulson ME, Joe JC, Babb S, Raese JD. Chronic cocaine administration depletes tyrosine hydroxylase immunoreactivity in the meso-limbic dopamine system in rat brain: quantitative light microscopic studies. Brain Res Bull 1987;19:39–45.
Vezina P, Lorrain DS, Arnold GM, Austin JD, Suto N. Sensitization of midbrain dopamine neuron reactivity promotes the pursuit of amphetamine. J Neurosci 2002;22: 4654–62.
Vrana SL, Vrana KE, Koves TR, Smith JE, Dworkin SI. Chronic cocaine administration increases CNS tyrosine hydroxylase enzyme activity and mRNA levels and tryptophan hydroxylase enzyme activity levels. J Neurochem 1993;61:2262–8.
Wang B, Luo F, Zhang WT, Han JS. Stress or drug priming induces reinstatement of extinguished conditioned place preference. NeuroReport 2000;21:2781–4.
Weiss F. Neurobiology of craving, conditioned reward and relapse. Curr Opin Pharmacol 2005;5:9-19.
Will MJ, Watkins LR, Maier SF. Uncontrollable stress potentiates morphine's rewarding properties. Pharmacol Biochem Behav 1998;60:655–64.
Will MJ, Der-Avakian A, Bland ST, Grahn RE, Hammack SE, Sparks PD, et al. Eletrolitic lesion and pharmacological inhibition of the dorsal raphe nucleus prevent stressor potentiation of morphine conditioned place preference in rats. Psychopharmacology 2004;171:191–8.
Wit H, Stewart J. Reinstatement of cocaine-reinforced responding in the rat. Psychopharmacology 1981;71:134–43.