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Moderate and Severe Perinatal Asphyxia Induces Differential Effects on Cocaine Sensitization in Adult Rats

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ABSTRACT Perinatal asphyxia (PA) increases the likelihood of suffering from dopamine-related disorders, such as ADHD and schizophrenia. Since dopaminergic transmission plays a major role in cocaine sensitization, the purpose of this study was to determine whether PA could be associated with altered behavioral sensitization to cocaine. To this end, adult rats born vaginally (CTL), by caesarean section (C+), or by C+ with 15 min (PA15, moderate PA) or 19 min (PA19, severe PA) of global anoxia were repeatedly administered with cocaine (i.p., 15 mg/kg) and then challenged with cocaine (i.p., 15 mg/kg) after a 5-day withdrawal period. In addition, c-Fos, FosB/ΔFosB, DAT, and TH expression were assessed in dorsal (CPu) and ventral (NAcc) striatum. Results indicated that PA15 rats exhibited an increased locomotor sensitization to cocaine, while PA19 rats displayed an abnormal acquisition of locomotor sensitization and did not express a sensitized response to cocaine. c-Fos expression in NAcc, but not in CPu, was associated with these alterations in cocaine sensitization. FosB/ΔFosB expression was increased in all groups and regions after repeated cocaine administration, although it reached lower expression levels in PA19 rats. In CTL, C+, and PA15, but not in PA19 rats, the expression of TH in NAcc was reduced in groups repeatedly treated with cocaine, independently of the challenge test. Furthermore, this reduction was more pronounced in PA15 rats. DAT expression remained unaltered in all groups and regions studied. These results suggest that moderate PA may increase the vulnerability to drug abuse and in particular to cocaine addiction.
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INTRODUCTION

Despite substantial improvements in neonatal care practices, perinatal asphyxia (PA) remains one of the leading causes of neonatal morbimortality (Filippi et al., 2012). This situation is exacerbated in developing countries where the incidence of PA is five- to ten-fold higher than in developed countries, probably due to inadequate neonatal care and limited access to

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new technologies (Atasay and Arsan, 2003; McGuire, 2006).

PA is associated not only with short-term morbimortality and the development of neurological disorders, but also increases the likelihood of suffering from attention deficit-hyperactivity disorder (ADHD) and schizophrenia, in which altered dopaminergic transmission has been proposed (Cannon et al., 2002; Lewis and Murray, 1987; van Handel et al., 2007). Long-lasting dysregulation of dopaminergic function has also been extensively observed in the murine model of PA developed by Bjelke et al. (1991) (for review, see Boksa and El-Khodor, 2003). In this model, rat pups are subjected to a period of intra-uterine anoxia following caesarean section birth (Brake et al., 1997a). Interestingly, the duration of intra-uterine anoxia seems to modulate the dopaminergic system in different ways. For instance, adolescent and adult rats subjected to intermediate durations of PA (13–16 min of intra-uterine anoxia) showed increased number of TH-IR nerve cell bodies in the VTA (Bjelke et al., 1991), increased D_1 and D_2 dopamine receptor mRNA levels in NAcc and tuberculum olfactorium (Gross et al., 2000), and exacerbated dopamine release in the NAcc after repeated stress or acute methamphetamine administration (Brake et al., 1997b; Wakuda et al., 2008). At a behavioral level, an increase of apomorphine-, amphetamine- and methamphetamine-induced locomotion (Bjelke et al., 1991; Chen et al., 1995; El-Khodor and Boksa, 1998; Wakuda et al., 2008) and a stress-induced behavioral sensitization to amphetamine (Brake et al., 1997a) were observed. On the other hand, rats which had undergone longer durations of intra-uterine anoxia (19–22 min) showed a reduction in mesencephalic dopaminergic neurons (Chen et al., 1997a), reduced levels of DA, DA metabolites (DOPAC and HVA) and D_2 agonist affinity in CPu and NAcc (Bustamante et al., 2003; Chen et al., 1997b; Ungethüm et al., 1996), and a decreased striatal dopamine release under basal, D-amphetamine and K+-depolarising conditions (Bustamante et al., 2007; Loidl et al., 1994). In addition, severe PA was associated with a significant decrement of locomotor activity and stereotyped behaviors under basal conditions (Chen et al., 1995; Galeano et al., 2011) or after the administration of D_1 receptor agonist SKF 82958 (Venerosi et al., 2004).

Cocaine is a psychostimulant that increases locomotor activity and induces rewarding effects (Johanson and Fischman, 1989). Although the molecular actions of cocaine include the potentiation of monoamine transmission by blocking the three monoamine transporters (i.e., DAT, NET, and SERT), cocaine stimulant effects are mainly mediated by its actions on the mesocorticolimbic dopamine system (Koob and Nestler, 1997; Nestler et al., 2005a). Repeated exposure to cocaine produces a progressive increase of cocaine-induced behavioral responses which persists after long periods of withdrawal (Everitt and Wolf, 2002). This phenomenon, known as behavioral sensitization, is thought to underlie drug craving and relapse, and therefore it is one of the possible mechanisms implicated in cocaine abuse and dependence (Robinson and Berridge, 1993, 2003; Steketee, 2005). In rodents, behavioral sensitization is frequently studied using a challenge injection of cocaine in animals withdrawn from repeated cocaine administration (Blanco et al., 2012a,b). This challenge injection elicits a higher locomotor activity than that observed in animals injected with an acute dose of cocaine, indicating a sensitized response to the drug (Kalivas and Stewart, 1991). The expression of this behavioral sensitization requires several neuroadaptations in dopamine transmission that involve the nigrostriatal and mesoaccumbens dopamine pathways (Vanderschuren and Kalivas, 2000). This raises the question whether the dysregulation of dopaminergic transmission found in animals subjected to PA could alter cocaine-induced locomotor sensitization.

Abbreviations

ADHD	attention deficit hyperactivity disorder
aca	anterior commissure
C+	rats born by cesarean section
Coc	cocaine
Coc-Veh	rats pretreated with cocaine and challenged with vehicle
Coc-Coc	rats pretreated with cocaine and challenged with cocaine
CPu	caudate-putamen
CTL	rats born by vaginal delivery
DA	dopamine
DAT	dopamine transporter
DOPAC	3,4-dihydroxyphenylacetic acid
G3PDH	glyceraldehyde-3-phosphate dehydrogenase
HVA	homovanillic acid
IEGs	immediate-early genes
NAcc	nucleus accumbens
NET	norepinephrine transporter
OF	open field
PA	perinatal asphyxia
PA15	rats born by C-section plus 15 min of intra-uterine anoxia
PA19	rats born by C-section plus 19 min of intra-uterine anoxia
mPFC	medial prefrontal cortex
SERT	serotonin transporter
TH	tyrosine hydroxylase
TH-IR	tyrosine hydroxylase immunoreactive
Veh	vehicle
Veh-Veh	rats pretreated with vehicle and challenged with vehicle
Veh-Coc	rats pretreated with vehicle and challenged with cocaine
VTA	ventral tegmental area

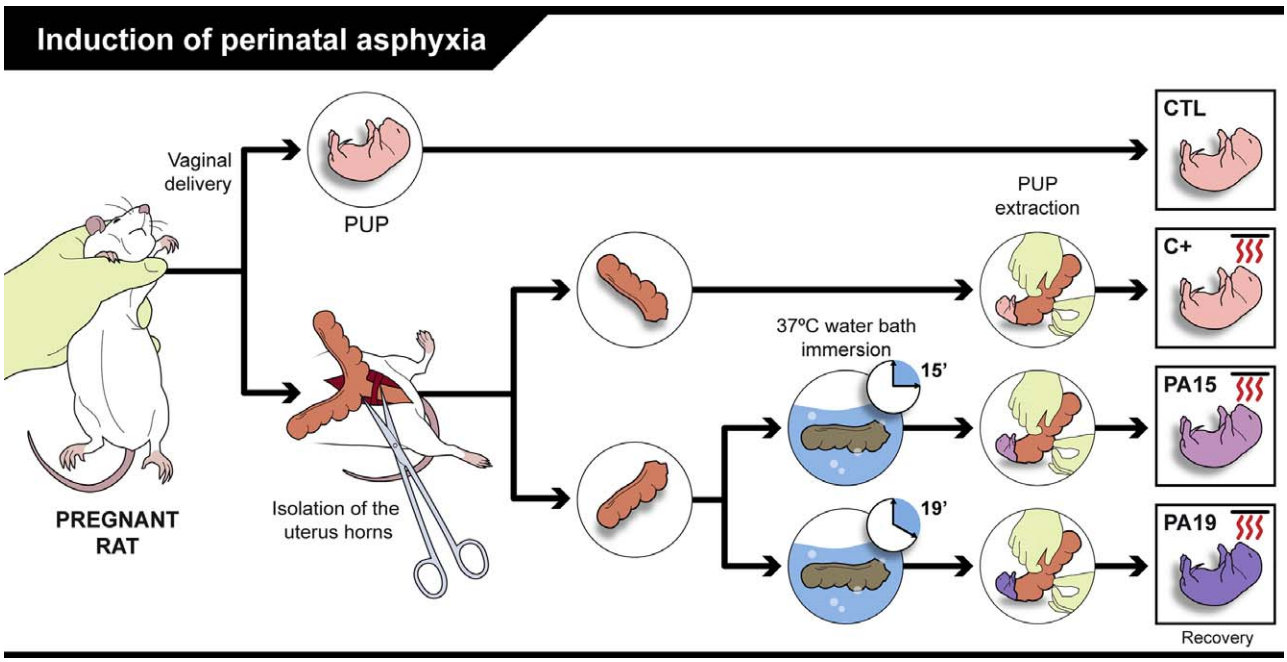

Fig. 1. Schematic illustration of the procedures performed for the induction of perinatal asphyxia. At embryonic day 22 (E22), dam rats that vaginally delivered no more than two pups were euthanized and the uterus horns were rapidly isolated. Next, one of the uterus horns was opened and pups removed (pups born by cesarean section, C+). The other uterus horn was immersed in a water bath at 37°C for 15 min (moderate PA) or 19 min (severe PA) (pups born by cesarean section followed by intra-uterine anoxia, PA15 and PA19). Then, rat pups were left to recover under a heating lamp and given to surrogate mothers. Vaginally delivery controls (CTL) were obtained from another group of pregnant rats that were left to deliver spontaneously.

To study the neural circuits involved in behavioral sensitization to cocaine, the expression of the immediate early gene (IEG) products c-Fos, FosB and ΔFosB in different brain regions were also examined in various previous studies (Brenhouse and Stellar, 2006; Crombag et al., 2002; Hope et al., 2006; Mattson et al., 2008). IEGs are rapidly induced in response to a wide variety of stimuli, including drugs of abuse (Nestler et al., 2001). This induction could persist for short- or long-term periods of time. For example, the c-Fos expression in NAcc after acute cocaine administration reaches its peak at 2 h, returning to baseline after 6 h (Nestler, 2001). On the other hand, isoforms of ΔFosB are highly stable and accumulate after repeated cocaine exposure in NAcc and dorsal striatum for weeks (Nestler et al., 2001).

Against this background, the main aim of this study was to assess whether PA may produce changes in the acquisition and expression of locomotor sensitization to cocaine and, if so, whether these behavioral alterations could be associated with differential IEGs (c-Fos and FosB/ΔFosB) and dopamine-related protein (TH and DAT) expression in ventral and dorsal striatum (NAcc and CPu).

MATERIAL AND METHODS

Animals and housing conditions

Ninety-five pregnant Sprague Dawley rats were obtained from the School of Veterinary Sciences' central vivarium at the Universidad de Buenos Aires. Pregnant rats arrived to our local vivarium 1 week prior to delivery in order to acclimate to the new environment. All animals were housed in individual cages and maintained in a temperature- (21 ± 2°C) and humidity- (65 ± 5%) controlled environment on a 12-h light/dark cycle. Animals had ad libitum access to food (Purina chow) and tap water.

Perinatal asphyxia procedure

We employed a murine model of PA originally developed by Bjelke et al. (1991) and extensively described by our group (Capani et al., 2003, 2009; Galeano et al., 2011; Saraceno et al., 2010, 2012) (Fig. 1). At the expected delivery date (E22), pregnant rats ($n = 53$) were individually observed and when no more than two pups were delivered, the dams were immediately euthanized by decapitation and the uterus horns were rapidly isolated through an abdominal incision. Next, one of the uterus horns was rapidly opened, pups were removed, the amniotic fluid was cleaned, and the umbilical cord was ligated (cesarean section or C-section procedure). The other uterus horn was submerged in a water bath at 37°C for 15 min (moderate PA) or 19 min (severe PA). Immediately after the time of asphyxia elapsed, the same procedures applied for the C-section were followed, but before ligation of the umbilical cord took place, pups were stimulated to breathe by performing

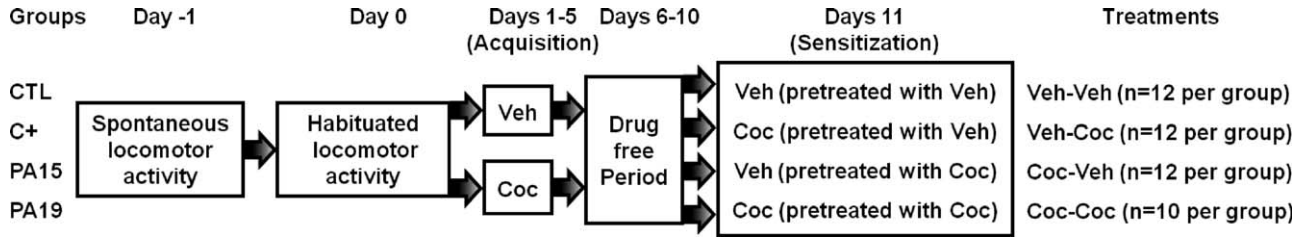

Fig. 2. Experimental design and treatments schedule. Schematic representation of administered treatments and the behavioral protocol consisting of the assessment of the spontaneous and habituated locomotor activity (day −1 and 0), an acquisition of cocaine sensitization phase (days 1–5), a drug free period (days 6–10) and a cocaine sensitization test (day 11). Abbreviations: CTL, vaginally delivery controls ($n = 48$); C+, rats born by cesarean section ($n = 48$); PA15 and PA19, rats born by cesarean section followed by 15 ($n = 48$) or 19 ($n = 40$) min of intra-uterine anoxia. Veh, vehicle; Coc, cocaine.

tactile intermittent stimulation with pieces of medical wipes for a few minutes until regular breathing was established. This was unnecessary for pups born by C-section since they started breathing spontaneously. Pups born by C-section (C+ group, $n = 48$) or by C-section plus 15 min (PA15 group, $n = 48$) or 19 min of asphyxia (PA19 group, $n = 40$) were left approximately for 1 h under a heating lamp in order to allow the asphyxiated pups improve their physiological conditions. Next, all pups were given to surrogate mothers ($n = 30$) which had delivered normally within the last 24 h. Another group of pregnant rats ($n = 12$) were left to deliver spontaneously (vaginal delivery or control group, CTL; $n = 48$). All pups were cross-fostered and each surrogate dam received an approximately equal number of pups from each experimental group. In every case, we maintained litters of no more than 10 pups with each surrogate dam. Rats were weaned at 21 days of age and housed in groups of four rats per cage throughout the experiment. Only male pups were retained for this study.

All procedures involving animals were approved by the Institutional Animal Care and Use Committee at the Universidad de Buenos Aires (School of Medicine, Resolution No. 4081/04) and conducted according to the principles of the Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996). All efforts were made to reduce the number of animals used and to minimize their suffering.

Drug

Cocaine–HCl was obtained from Sigma-Aldrich (St Louis, MO) and dissolved in sterile 0.9% NaCl solution just before experimentation. Rats were given repeated injections (15 mg/kg) and/or an acute challenge injection (15 mg/kg) of cocaine. The drug was injected intraperitoneally (i.p.) in a final volume of 1 ml/kg.

Apparatus

The mazes used were four open field (OF) black melamine handmade boxes (L60 × W60 × H40 cm). Arenas were uniformly and indirectly illuminated by four spiral compact fluorescent lamps in each corner facing the walls. Light intensity in the center of the arenas was 60–75 lux. White noise was provided throughout testing. Behavioral procedures were carried out between 7:00 a.m. and 5:00 p.m. Animals were placed individually in the center of each arena after treatments and its behavior was analyzed using a computerized video-tracking system (Ethovision XT, version 5, Noldus Information Technology, Wageningen, The Netherlands). The apparatus was cleaned between sessions with 70% ethanol and then dried. Locomotor activity was assessed by measuring the total distance travelled in centimeters (cm) by each rat.

Behavioral protocols

When animals reached an adult age of 3 months, the procedures listed below were performed. In all behavioral procedures performed, the testing order of groups and treatments were counterbalanced to avoid the confounding effect of the time of the day at which animals were tested.

Handling and acclimatization

Throughout 5 days, animals were weighed, handled for 10 min and habituated to injection procedures (holding and pseudo-injection) to minimize stress effects. For the pseudo-injection, the lower abdomen of the rat was pressed with a capped syringe. Before starting the experiment every day, animals were acclimated to the testing room for 30 min.

Spontaneous locomotion

All animals received a pseudo-injection and were immediately placed in the OF for 30 min to measure spontaneous locomotor activity (day −1) (Fig. 2). Twenty-four hours later, animals were re-exposed to the OF for 30 min to assess the habituated locomotor activity (day 0) (Fig. 2).

Acquisition of cocaine locomotor sensitization

From day 1 to 5, half of the animals in each group were injected (i.p.) once-daily with vehicle (saline)

and the other half with cocaine (15 mg/kg). Immediately after the injection, rats were placed in the OF for 30 min. Thereafter, rats were left undisturbed in their home cages from day 6 to 10 (Fig. 2).

Expression of cocaine locomotor sensitization

On the 11th day, vehicle and cocaine pretreated animals received a challenge injection (i.p.) of either vehicle or cocaine (15 mg/kg) and locomotor activity in the OF was assessed during 60 min. In this way, CTL, C+, PA15, and PA19 animals were randomly assigned to the following treatments: animals pretreated with vehicle and challenged with either vehicle (Veh-Veh treatment) or cocaine (acute cocaine administration; Veh-Coc treatment), and animals pretreated with cocaine and challenged with either vehicle (cocaine-conditioned locomotion; Coc-Veh treatment) or cocaine (cocaine sensitization; Coc-Coc treatment). In summary, we obtained 16 experimental groups that are shown in Fig. 2.

Although, it is well known that locomotor activity in rodents reaches its peak during the dark phase of the light/dark cycle (Benstaali et al., 2001), we carried out the cocaine sensitization protocol during the light phase since we previously found robust cocaine-sensitized locomotor responses during this phase of the cycle (Bilbao et al., 2013; Blanco et al., 2012a,b; Luque-Rojas et al., 2013). In addition, melatonin seems to have suppressive effects on cocaine sensitization (Uz et al., 2002). However, many studies have showed a strong sensitization response during the dark phase of the light/dark cycle (Kupferschmidt et al., 2011; Sondheimer and Knackstedt, 2011; Winstanley et al., 2009; to cite only a few). Since sensitization to cocaine has proved to be influenced by circadian genes and rhythm (Abarca et al., 2002), it would be interesting to test, in further experiments, if the relative differences between groups in cocaine sensitization observed in this work would change as a consequence of testing the animals during the dark phase of the light/dark cycle.

Immunohistochemistry for c-Fos and FosB/ΔFosB

Expression of IEGs (c-Fos and FosB/ΔFosB) was assessed by immunohistochemistry in striatal region (ventral, NAcc; dorsal, CPu). The expression of the IEGs was quantified in the same rats that were used for behavioral testing. One hour after finishing the expression of cocaine sensitization, half of the rats from each group ($n = 6$ for CTL, C+, and PA15, and $n = 5$ for PA19) were deeply anesthetized with sodium pentobarbital (50 mg/kg i.p.) and transcardially perfused with 100 ml of sterile saline solution (0.9% NaCl) followed by approximately 350 ml of 4% paraformaldehyde (Sigma; St. Louis, MO) in 0.1 M phosphate buffer (pH 7.3). Brains were removed from the skull, post-fixed for 2 h in 4% paraformaldehyde at 4°C, equilibrated in PBS containing 30% sucrose, cut into coronal sections (30 μm thick) by using a sliding microtome (Leica, Germany) and stored at 4°C in phosphate buffer with 0.002% (w/v) of sodium azide until they were later used for inmunostaining. Coronal sections containing the CPu and NAcc (~1.6 mm from bregma) were obtained according to Paxinos and Watson (2007). For immunohistochemistry, free-floating sections were washed several times with 0.2% Triton-X100 in PBS (PBS-Tx) and incubated in 3% hydrogen peroxide in PBS-Tx for 20 min in darkness at room temperature to inactivate endogenous peroxidase. After several washes in PBS-Tx, sections were preincubated in 10% normal goat serum in PBS-Tx 1 h and then incubated overnight in diluted primary antibody at 4°C. The primary antibodies used were: rabbit polyclonal anti-c-Fos antibody (Calbiochem, PC38; 1:10,000) and rabbit polyclonal anti-Fos B antibody (Santa Cruz Biotechnology, sc-48; 1:500) that recognizes both FosB and isoforms of ΔFosB. The second day and after several washes in PBS-Tx, sections were incubated with biotinylated goat anti-rabbit IgG (Vector) for 2 h at room temperature, washed again in PBS-Tx, and incubated in ExtrAvidin peroxidase (Sigma, St Louis, MO) diluted 1:1000 in darkness at room temperature for 1 h. After several washes in PBS-Tx, the immunolabeling was revealed with 0.05% diaminobenzidine (Sigma), 0.05% nickel ammonium sulfate, and 0.03% H_2O_2 in PBS. Finally, following several washes in PBS, sections were mounted on slides treated with poly-L-lysine solution (Sigma), air dried, dehydrated in ethanol, cleared with xylene, and coverslipped with Eukitt mounting medium (Kindler GmBH, Freiburg, Germany). Immunostaining was observed under a Nikon Eclipse 800 microscope, and images were acquired with a Nikon DXM1200, high resolution digital camera.

The number of c-Fos and FosB/ΔFosB-immunoreactive nuclei in CPu and NAcc were counted in both hemispheres and averaged. Six coronal sections from each brain region and animal were quantified and the means calculated for each region and animal were used for subsequent statistical analysis. Two delineated areas of 2×2 mm^2 (for CPu) and 2×1.5 mm^2 (for NAcc) were used to determine the number of immunoreactive nuclei in each region. Quantification was done by a blind observer using the ImageJ software (NIH, Bethesda, MD).

Western Blot for TH and DAT

Western blotting was performed as we previously described in Blanco et al. (2012a,b) with some modifications. Half of the animals from each group ($n = 6$ for CTL, C+, and PA15, and $n = 5$ for PA19) were euthanized by decapitation, brains were removed, dorsal (CPu) and ventral (NAcc) striatum from each

hemisphere were dissected, snap frozen in liquid nitrogen and stored at −80°C until use. Lysates were homogenised and incubated in RIPA buffer 1× (Thermo Scientific, Rockford, IL) containing a proteinase and phosphatase inhibitor cocktail (sodium fluoride 50 mM, sodium orthovanadate 1 mM, sodium pyrophosphate 10 mM, β-glycerophosphate 10 mM, NaF 5 mM, NaOV4 100 μM, NaH2PO4 1 mM, aprotinin 80 μM, pepstatin A 2 mM, trypsin inhibitor 1 μM, phenylmethylsulfonyl fluoride 50 μM; Merck) for 2 h at 4°C and then centrifuged at 10,000×g for 15 min at 4°C. Equivalent amounts of protein extract (35 μg) were separated by 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electroblotted onto nitrocellulose membranes. Blots were pre-incubated with a blocking buffer containing PBS, 0.1% Tween 20, and 2% albumin fraction V from bovine serum (Merck) at room temperature for 1 h. Membranes were then incubated with their corresponding primary antibodies overnight at room temperature: monoclonal anti-tyrosine hydroxylase antibody (1:10,000; cat. no. T1299, Sigma, USA) and polyclonal anti-dopamine transporter (1:1000; cat. no. AB1591P, Millipore, Billerica, MA). After extensive washing in PBS containing 1% Tween 20 (PBS-T), a peroxidase-conjugated goat anti-rabbit antibody or goat anti-mouse antibody (Promega, Madison, WI) was added, both diluted at 1:10,000, for 1 h at room temperature. Membranes were then subjected to repeated washing in PBS-T and the specific protein bands visualised using the enhanced chemiluminescence technique (Western Blotting Luminol Reagent; cat. no. sc-2048, Santa Cruz Biotechnology, Santa Cruz, CA) and Auto-Biochemi Imaging System (LTF Labortechnik GmbH, Wasserburg/Bodensee, Germany). The same reference sample from a CTL animal (Veh-Veh treatment) was loaded in every polyacrylamide gel and used for standardization. Every sample was then expressed as a percentage of that reference sample. In addition, the G3PDH loading control was used as a way to determine the same amount of protein was loaded for each sample. Images were analysed and compiled using Adobe Photoshop 11.0 CS4. No other manipulation other than contrast and brightness was performed.

Statistical analysis

All data were expressed as the mean ± SEM. Data were analyzed by one-way, two-way or three-way between-subjects or mixed ANOVA followed by Tukey's HSD post-hoc tests or paired t-test with the Bonferroni correction. The Greenhouse-Geisser nonsphericity correction was employed in mixed ANOVA when appropriate. The significance level was set up at $P \leq 0.05$. All statistical analyses were performed using the SPSS software (version 15.0.1, SPSS, Chicago, IL).

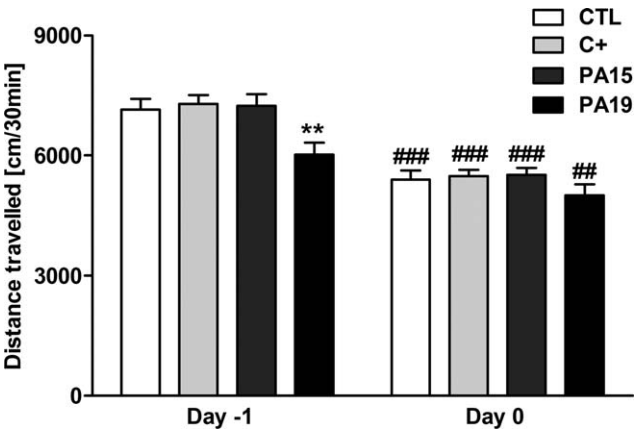

Fig. 3. Levels of spontaneous locomotion during two OF sessions separated by 24 h. $n = 48$ for CTL, C+ and PA15 groups and $n = 40$ for PA19 group. For abbreviations, see list. Data represent mean distance travelled + SEM. $^{**}P < 0.01$ vs. other groups in day −1; $^{\#\#}P < 0.01$ day 0 vs. day −1; $^{\#\#\#}P < 0.001$ day 0 vs. day −1.

RESULTS

Spontaneous locomotion

The two-way ANOVA test showed that the main effects of group and day were significant ($F_{(3,\ 180)} = 4.03$, $P = 0.008$; $F_{(1,\ 180)} = 128.63$, $P < 0.001$, respectively). Although the interaction group × day did not reach statistical significance ($F_{(3,\ 180)} = 1.67$, P = n.s.), post-hoc analysis indicated that PA19 rats displayed a significantly reduced locomotor activity compared with other groups during the first exposure to the OF (Fig. 3). Further post-hoc comparisons revealed that all groups significantly reduced their locomotor activity twenty-four hours after the first exposure to the OF, but in this second exposure no differences in locomotion were observed between groups (Fig. 3). These results indicate that all groups habituated their locomotor activity reaching the same level of locomotion during the second exposure to the OF despite the fact that not all groups displayed the same levels of basal locomotion.

Acquisition of cocaine locomotor sensitization

No differences were found between groups administered with vehicle in the distances travelled and therefore we expressed the distances travelled by groups treated with cocaine as the percent change with respect to distances travelled by vehicle-treated groups. The four (group) × two (pretreatment) × five (day) mixed ANOVA indicated that the main effects of group ($F_{(3,\ 176)} = 2.51$, $P = 0.05$), pretreatment ($F_{(3,\ 176)} = 411.28$, $P < 0.001$), day ($F_{(3.07,\ 540.39)} = 27.45$, $P < 0.001$) and the interactions group × pretreatment ($F_{(3,\ 176)} = 2.51$, $p = 0.05$), day × pretreatment ($F_{(3.07,\ 540.39)} = 27.45$, $P < 0.001$), group × day ($F_{(9.21,\ 540.39)} = 2.26$, $P < 0.05$) and group × pretreatment × day ($F_{(9.21,\ 540.39)} = 2.26$, $P < 0.05$) were all significant. Post-hoc pairwise comparisons indicated that the four

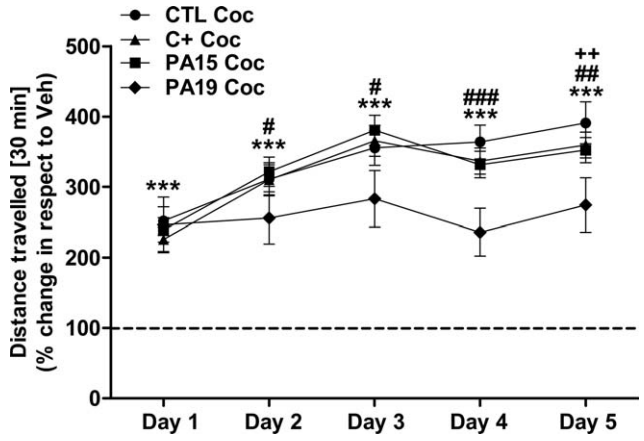

Fig. 4. Acquisition of cocaine sensitization during 5 consecutive days. CTL Coc = vaginally delivered controls treated with cocaine ($n = 24$); C+ Coc = rats born by cesarean section treated with cocaine ($n = 24$); PA15 Coc = rats born by cesarean section plus 15 min of global asphyxia treated with cocaine ($n = 24$); PA19 Coc = rats born by cesarean section plus 19 min of global asphyxia treated with cocaine ($n = 20$). Data represent mean distance travelled normalized to percent change with respect to vehicle-treated groups (dashed line) ± SEM. $^{***}P < 0.001$ vs. vehicle-treated groups (dashed line); $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ PA19 Coc group vs. CTL, C+, and PA15 Coc groups; $^{++}P < 0.01$ day 5 vs. day 1 for CTL, C+ and PA15 Coc groups.

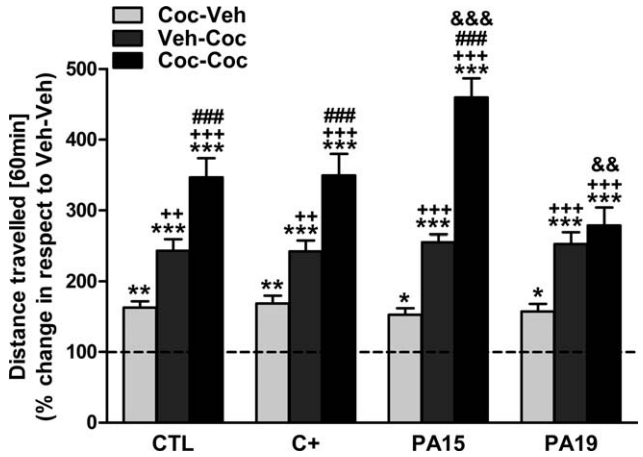

Fig. 5. Expression of locomotor sensitization to cocaine in controls and perinatally asphyxiated rats. Rats were pretreated with vehicle or cocaine (15 mg/kg) and challenged with vehicle or cocaine (15 mg/kg) after 5 days of withdrawal. For abbreviations, see list. Each bar represents the mean distance travelled normalized to percent change with respect to Veh-Veh treatment (dashed line) + SEM of 12 (CTL, C+, PA15) or 10 (PA19) rats. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. Veh-Veh treatment (dashed line); $^{++}P < 0.01$, $^{+++}P < 0.001$ vs. Coc-Veh treatment; $^{\#\#\#}P < 0.001$ vs. Veh-Coc treatment; $^{\&\&}P < 0.01$, $^{\&\&\&}P < 0.001$ PA19 or PA15 rats pretreated with cocaine and challenged with cocaine vs. CTL or C+ rats pretreated with cocaine and challenged with cocaine.

groups that were administered with cocaine (CTL Coc, C+ Coc, PA15 Coc and PA19 Coc groups) displayed a significantly increased locomotor activity during the 5 days of the acquisition of cocaine sensitization phase compared with vehicle-treated groups (Fig. 4). Nevertheless, from day 2 to day 5 PA19 rats treated with cocaine (PA19 Coc group) showed a significantly reduced locomotion in comparison to the other groups treated with cocaine (CTL Coc, C+ Coc, and PA15 Coc groups) (Fig. 4). Finally, CTL, C+, and PA15 rats administered with cocaine (CTL Coc, C+ Coc, and PA15 Coc groups) showed a significantly increased locomotor activity during the last day in comparison to the first day ($t = -3.66$, $d.f. = 23$, $P = 0.001$; $t = -5.00$, $d.f. = 23$, $P < 0.001$; $t = -4.16$, $d.f. = 23$, $P = 0.001$, respectively), while the PA19 rats administered with cocaine (PA19 group) did not show this increase ($t = -1.75$, $d.f. = 19$, $P =$ n.s.). These results mean that all groups showed a similar locomotor response to an acute administration of cocaine (day 1), while the repeated cocaine administration progressively increased locomotor activity in CTL, C+, and PA15 rats but not in PA19 rats (day 2 to 5).

Expression of cocaine locomotor sensitization

As in the acquision phase, the mean distances travelled were not statistically different between groups when the Veh-Veh treatments were applied and data were normalized to percent change with respect to Veh-Veh treatment. Next, a two-way ANOVA test with group (CTL, C+, PA15, and PA19) and treatment (Veh-Veh, Veh-Coc, Coc-Veh, and Coc-Coc) as between-subject factors was performed. The main effects of group and treatment and the interaction group x treatment were all significant ($F_{(3,\ 168)} = 4{,}94$, $P < 0.01$; $F_{(3,168)} = 184.99$, $P < 0.001$; $F_{(9,\ 168)} = 5.21$, $P < 0.001$, respectively). Post-hoc pairwise comparisons indicated that all groups showed a cocaine-conditioned locomotion, since rats pretreated with cocaine and challenged with vehicle (i.e., Coc-Veh treatment) displayed a significantly increased locomotor activity compared with rats pretreated with vehicle and challenged with vehicle (i.e., Veh-Veh treatment) (Fig. 5). On the other hand, CTL, C+, and PA15 rats pretreated with cocaine and challenged with cocaine (i.e Coc-Coc treatment) showed a significantly increased locomotor activity compared with rats that received a acute injection of cocaine (i.e., Veh-Coc treatment) (Fig. 5). Moreover, PA15 rats displayed an increased sensitized response to cocaine compared to CTL and C+ rats. On the contrary, PA19 rats pretreated with cocaine and challenged with cocaine did not increase their locomotor activity compared with that observed after an acute injection of cocaine (Fig. 5).

Overall, these results mean that CTL and C+ groups displayed a normal locomotor sensitization to cocaine, while moderate and severe PA induced differential effects on cocaine sensitization. The PA15 group showed an increased sensitization to the motor effect of cocaine, while the PA19 group did not express locomotor sensitization to cocaine.

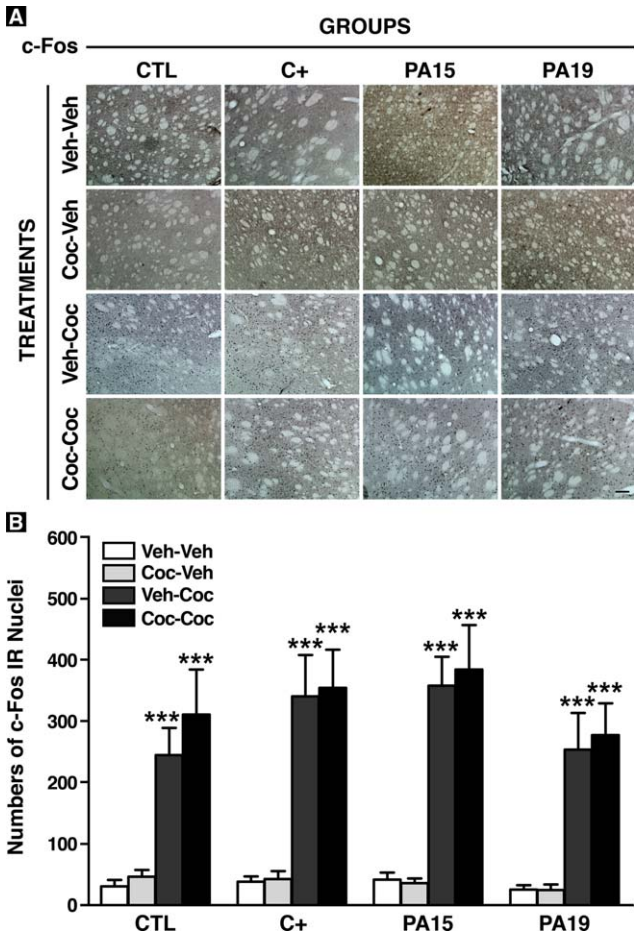

Fig. 6. Expression of c-Fos in the caudate-putamen (CPu). (**A**) Representative photomicrographs illustrating c-Fos immunopositive nuclei in different experimental groups (CTL, C+, PA15, and PA19) after treatments (Veh-Veh, Coc-Veh, Veh-Coc, and Coc-Coc). aca, anterior commissure; for the rest abbreviations, see list. Scale bar = 200 μm. (**B**) Number of c-Fos IR nuclei in the CPu induced by treatments. Data represent mean + SEM. $^{***}P < 0.001$ vs. Veh-Veh and Coc-Veh treatments.

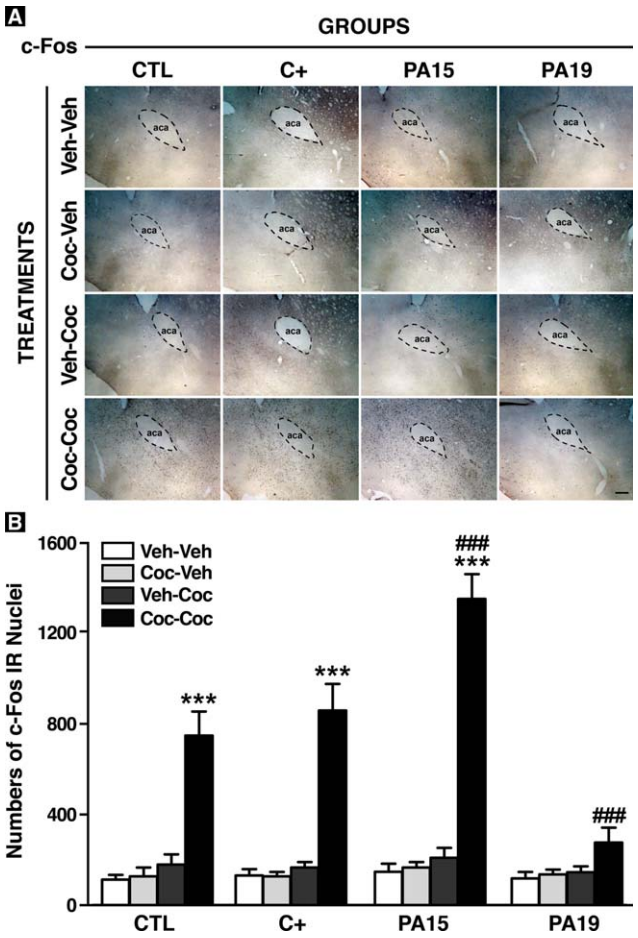

Fig. 7. Expression of c-Fos in the nucleus accumbens (NAcc). (**A**) Representative photomicrographs illustrating c-Fos immunopositive nuclei in different experimental groups (CTL, C+, PA15 and PA19) after treatments (Veh-Veh, Coc-Veh, Veh-Coc and Coc-Coc). aca, anterior commissure; for the rest of abbreviations, see list. Scale bar = 200 μm. (**B**) Number of c-Fos IR nuclei in the NAcc induced by treatments. Data represent mean + SEM. $^{***}P < 0.001$ vs. Veh-Veh, Coc-Veh, and Veh-Coc treatments; $^{\#\#\#}P < 0.001$ PA15 or PA19 rats pretreated with cocaine and challenged with cocaine vs. CTL or C+ rats pretreated with cocaine and challenged with cocaine.

Number of c-Fos-immunoreactive nuclei in CPu and NAcc

In the CPu, the two-way ANOVA test showed that the main effect of treatment was significant ($F_{(3,\ 76)} = 54.05$, $P < 0.001$), but neither the main effect of group nor the interaction group × treatment was significant ($F_{(3,\ 76)} = 1.63$, $P =$ n.s.; $F_{(9,\ 76)} < 1$, respectively). In all groups, post-hoc comparisons revealed a significant increase in c-Fos-IR nuclei in those animals challenged with cocaine regardless of the pretreatment condition (i.e., Veh-Coc and Coc-Coc treatments) (Fig. 6). In the NAcc, the two-way ANOVA test showed that the main effects of group and treatment and the interaction group × treatment were all significant ($F_{(3,\ 76)} = 16.63$, $P < 0.001$; $F_{(3,\ 76)} = 124.35$, $P < 0.001$; $F_{(9,\ 76)} = 12.25$, $P < 0.001$, respectively). Post-hoc analysis indicated that CTL, C+, and PA15 animals pretreated with cocaine and challenged with cocaine (i.e., Coc-Coc treatment) showed a significant increase in c-Fos-IR nuclei compared with other treatments (Fig. 7), and this increase was even more pronounced in PA15 rats. On the contrary, the increase in c-Fos-IR nuclei was not observed in PA19 rats (Fig. 7).

Number of FosB/ΔFosB-immunoreactive nuclei in CPu and NAcc

In the CPu and NAcc, the two-way ANOVA tests revealed that the main effects of group and treatment and the interaction group x treatment were all significant (CPu: $F_{(3,\ 76)} = 5.79$, $P = 0.001$; $F_{(3,\ 76)} = 80.49$, $P < 0.001$; $F_{(9,\ 76)} = 2.22$, $P = 0.029$, respectively. NAcc: $F_{(3,\ 76)} = 11.54$, $P < 0.001$; $F_{(3,\ 76)} = 104.69$, $P < 0.001$; $F_{(9,\ 76)} = 3.77$, $P = 0.001$, respectively). In both regions, the post-hoc comparisons showed a

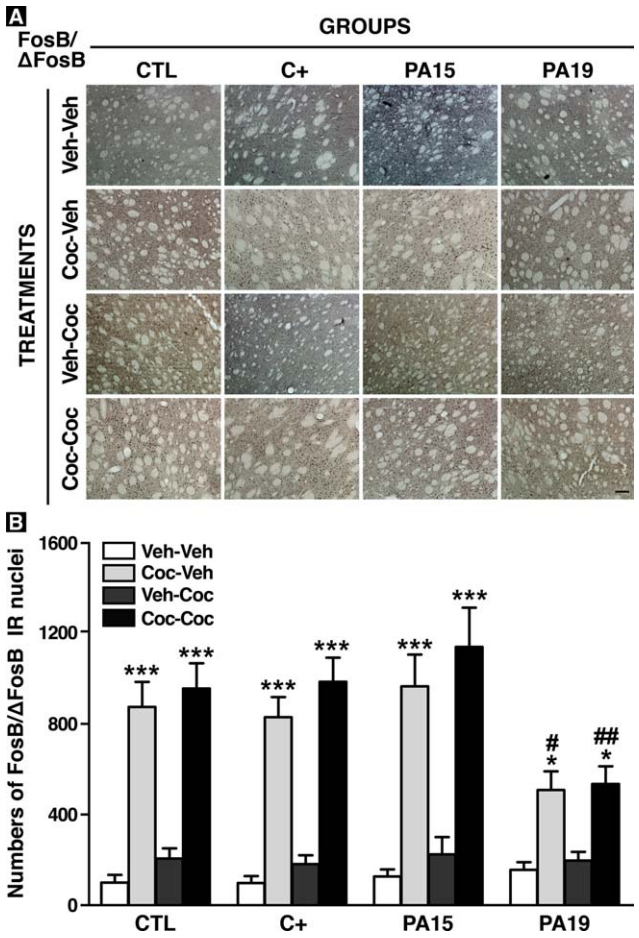

Fig. 8. Expression of FosB/ΔFosB in the caudate-putamen (CPu). (**A**) Representative photomicrographs illustrating FosB/ΔFosB immunopositive nuclei in different experimental groups (CTL, C+, PA15, and PA19) after treatments (Veh-Veh, Coc-Veh, Veh-Coc, and Coc-Coc). aca, anterior commissure; for the rest of abbreviations, see list. Scale bar = 200 μm. (**B**) Number of FosB/ΔFosB IR nuclei in the CPu induced by treatments. Data represent mean + SEM. $^{*}P < 0.05$, $^{***}P < 0.001$ vs. Veh-Veh and Veh-Coc treatments. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. Coc-Veh and Coc-Coc treatments in CTL, C+ and PA15 groups.

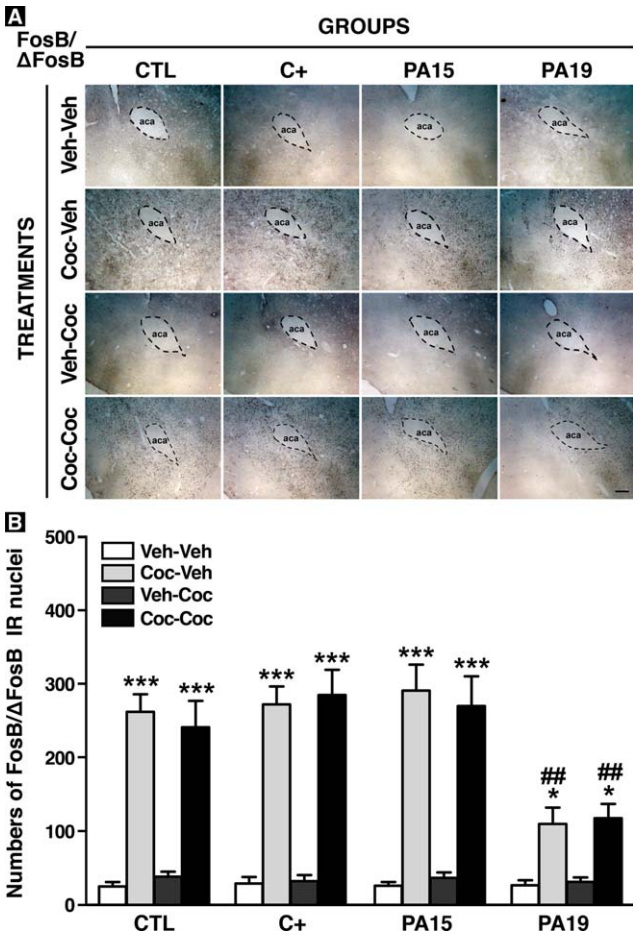

Fig. 9. Expression of FosB/ΔFosB in the nucleus accumbens (NAcc). (**A**) Representative photomicrographs illustrating FosB/ΔFosB immunopositive nuclei in different experimental groups (CTL, C+, PA15, and PA19) after treatments (Veh-Veh, Coc-Veh, Veh-Coc, and Coc-Coc). aca, anterior commissure; for the rest of abbreviations, see list. Scale bar = 200 μm. (**B**) Number of FosB/ΔFosB IR nuclei in the NAcc induced by treatments. Data represent mean + SEM. $^{*}P < 0.05$, $^{***}P < 0.001$ vs. Veh-Veh and Veh-Coc treatments. $^{\#\#}P < 0.01$ vs. Coc-Veh and Coc-Coc treatments in CTL, C+ and PA15 groups.

significant increase of FosB/ΔFosB-IR nuclei in those animals that were pretreated with cocaine regardless of the challenge used (i.e., Coc-Veh and Coc-Coc treatment), although this increase was less pronounced in PA19 rats (Figs. 8 and 9).

Expression of TH in CPu and NAcc

In the CPu, the two-way ANOVA test showed that neither the main effect of group nor the main effect of treatment nor the interaction group × treatment were significant ($F_{(3,\ 76)} = 1.19$, P = n.s.; $F_{(3,\ 76)} < 1$; $F_{(9,\ 76)} < 1$, respectively) (Fig. 10a). On the contrary, in the NAcc the two-way ANOVA test indicated that the main effect of group and treatment, and the interaction group x treatment were all significant ($F_{(3,\ 76)} = 12.95$, $P < 0.001$; $F_{(3,\ 76)} = 38.36$, $P < 0.001$; $F_{(9,\ 76)} = 4.04$, $P < 0.001$, respectively). Post-hoc comparisons indicated that CTL, C+, and PA15 rats pretreated with cocaine, regardless of the challenge injection (i.e., Coc-Veh and Coc-Coc treatments), showed a significantly reduced expression of TH compared with rats pretreated with vehicle and then challenged with vehicle or cocaine (i.e., Veh-Veh and Veh-Coc treatments) (Figs. 10b and 10e). This reduction of TH expression was significantly higher in PA15 rats (Figs. 10b and 10e). No changes of TH expression were observed in PA19 rats (Figs. 10b and 10e).

Expression of DAT in CPu and NAcc

The expression of DAT did not change, neither in the CPu nor in the Nacc, regardless of group or treatment (Figs. 10c and 10d) (CPu: group: $F_{(3,\ 76)} = 1.69$, P = n.s.; treatment: $F_{(3,\ 76)} = 1.20$, P = n.s.; group ×

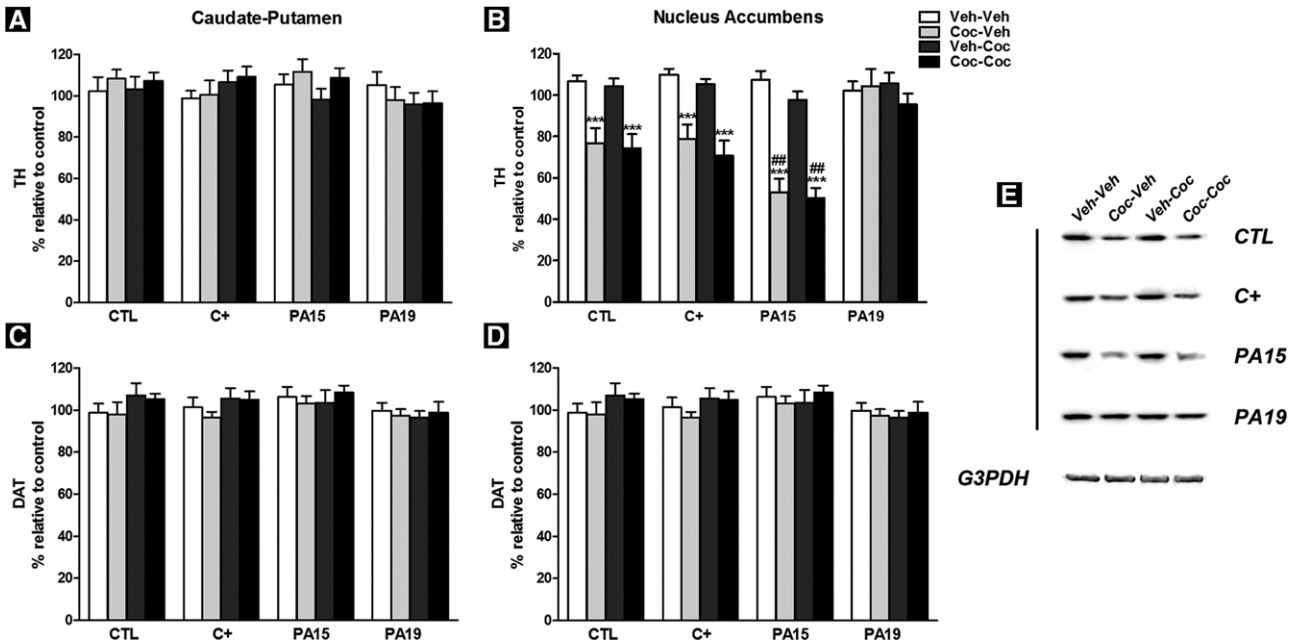

Fig. 10. Quantitative analysis of TH and DAT protein expression levels in the CPu and NAcc induced by different treatments (Veh-Veh, Coc-Veh, Veh-Coc, and Coc-Coc) in experimental groups (CTL, C+, PA15, and PA19). Analysis of protein levels of: TH in (**A**) CPu, (**B**) NAcc, and DAT in (**C**) CPu, (**D**) NAcc using Western blot. (**E**) Representative immunoblots of TH and G3PDH in experimental groups under different treatments. Data represent mean + SEM. G3PDH, glyceraldehyde-3-phosphate dehydrogenase; for abbreviations, see list. $^{***}P < 0.001$ vs. Veh-Veh and Veh-Coc treatments in CTL, C+, PA15 and PA19 groups; $^{\#\#}P < 0.01$ vs. Coc-Veh and Coc-Coc treatments in CTL, C+, and PA19 groups.

TABLE I. Summary of behavioral results

Groups	Spontaneous locomotion		Acquisition of cocaine locomotor sensitization	Expression of cocaine locomotor sensitization
	Day −1	Day 0		
CTL	Normal	Normal	Normal	Normal
C+	Normal	Normal	Normal	Normal
PA15	Normal	Normal	Normal	Increased
PA19	Decreased	Normal	Impaired	Impaired

treatment: $F_{(9,\ 76)} < 1$. NAcc: group: $F_{(3,\ 76)} < 1$; treatment: $F_{(3,\ 76)} < 1$; group × treatment: $F_{(9,\ 76)} < 1$).

DISCUSSION

The main finding of this study is that both the locomotor response to repeated cocaine administration and the sensitized locomotor response to cocaine are differentially modulated by PA duration. In addition, the expressions of the IEGs and TH in the striatum were altered by the administration of the different treatments in control (CTL and C+) and asphyxiated groups (PA15 and PA19) (see Tables I and II for a summary of the behavioral, immunohistochemical and biochemical results).

Moderate PA induced an increased locomotor sensitization to cocaine

In this work, adult rats which had undergone 15 min of intra-uterine anoxia (moderate PA) showed an increased sensitized response to a challenge injection of cocaine following withdrawal from repeated cocaine administration (Fig. 5). Although moderate PA has been associated with an exacerbated locomotor response to acute administration of psychostimulants and stress-induced sensitization to amphetamine (Brake et al., 1997a; Chen et al., 1995; El-Khodor and Boksa, 1998; Wakuda et al., 2008), this is the first study to demonstrate that moderate PA induces an increased locomotor sensitization to cocaine. We have also found a significant increment in the number of c-Fos-IR nuclei in the NAcc in all of the groups that showed cocaine sensitization. However, this enhancement was more pronounced in the PA15 group (PA15) (Fig. 7). Therefore, higher levels of c-Fos expression observed in this group suggest an augmented neural activity in the NAcc that could underlie the increased locomotor sensitization to cocaine. Regarding c-Fos expression in the CPu, we

have found a similar significant increase in the number of c-Fos-IR nuclei, both in acute cocaine administration (i.e., Veh-Coc treatment) and after a challenge injection of cocaine (i.e., Coc-Coc treatment) in all of the groups. Hence, this increase in c-Fos expression might underlie the augmented neural activity in the CPu that follows acute cocaine administration but not the neural activity that underlies the sensitized response. The enhancement of c-Fos expression in the NAcc, but not in the CPu, after cocaine sensitization is in agreement with previous reports by Crombag et al. (2002) and Hope et al. (2006). It is important to note that although both the NAcc and the CPu have been implicated in behavioral sensitization to cocaine (Pierce and Kalivas, 1997), the mesoaccumbens dopamine pathway plays a key role in behavioral sensitization to drugs of abuse (Henry and White, 1995; Koob, 1992). Furthermore, Hope et al. (2006) demonstrated that the neuronal activity in the NAcc, determined by c-Fos labeling, mediates the sensitized locomotor response to cocaine in a protocol of behavioral sensitization similar to the one used in this study.

In both striatal regions, the FosB/ΔFosB expression was significantly increased in all of the groups that were submitted to a repeated administration of cocaine (i.e., Coc-Veh and Coc-Coc treatment), but not after an acute treatment (i.e., Veh-Coc treatment). This result was expected since the expression of FosB, and its isoform ΔFosB, accumulates during repeated cocaine administration and it persists up to several weeks after the administration of the drug has ceased (Nestler et al., 2001). Therefore, this increment in FosB/ΔFosB expression might reflect the striatal neural activity related to the acquisition of cocaine sensitization but not its expression. In fact, PA19 rats showed lower levels of FosB/ΔFosB expression which could be related to the impairment in the acquisition of cocaine sensitization observed for this group (see next subsection).

TH expression in NAcc was significantly reduced after repeated cocaine administration (i.e., Coc-Veh and Coc-Coc treatments) in CTL, C+, and PA15 groups. This reduction could be associated to neuroadaptations occurring during the acquisition of behavioral sensitization and the withdrawal period, since TH expression was not altered after acute cocaine injection (i.e., Veh-Coc treatment). Although previous findings are somewhat contradictory, other studies have also found a reduction of TH levels or activity in the NAcc of animals withdrawn from repeated cocaine administration (Beitner-Johnson and Nestler, 1991; Brock et al., 1990; Todtenkopf et al., 2000; Trulson et al., 1987; but see Licata and Pierce, 2004). Moreover, in the PA15 group the reduction of TH expression in those rats that had received the Coc-Veh and Coc-Coc treatments was more pronounced compared to that seen in CTL and C+ group. Therefore, it seems that the higher reduction of TH expression in the PA15 group could be associated with the increased locomotor sensitization to cocaine. It could be hypothesized that the reduction of TH expression in the NAcc might be due to a down-regulation of the enzyme after repeated cocaine administration. Repeated cocaine administration increases dopamine release in NAcc (Weiss et al., 1992). This enhancement of dopamine signaling has shown to increase D_1 dopamine receptor sensitivity for up to several weeks in NAcc (Henry and White, 1991, 1995), which in turn could trigger compensatory mechanisms, such as a decreased synthesis of DA (Brock et al., 1990) by reducing TH activity and/or expression. The disruption of axonal transport of TH from the VTA to the NAcc could also account for the reduced TH expression observed after repeated cocaine administration (Beitner-Johnson et al., 1992). However, this hypothesis seems unlikely because TH levels remained unaltered in PA19 group regardless of the administered treatment, and we previously found that severe PA induces striatal cytoskeleton alterations (Cebral et al., 2006; Saraceno et al., 2012). In spite of the mechanisms by which repeated cocaine administration decreased TH levels, it seems that this reduction underlies the cocaine sensitization displayed by CTL and C+ groups and the increased locomotor sensitization observed in the PA15 group.

Although one of the mechanisms of action of cocaine is the blockade of DAT, we were unable to find changes in their expression levels neither in CPu nor in NAcc. The lack of association between changes in striatal DAT expression and behavioral sensitization to cocaine is in agreement with many previous reports (Blanco et al., 2012a; Kula and Baldessarini, 1991; Letchworth et al., 1997; Samuvel et al., 2008). Hence, it seems that changes in striatal DAT levels are not critical neuroadaptations contributing to cocaine sensitization.

Severe PA impairs the acquisition of cocaine sensitization and blocks its expression

While in this study moderate PA was associated with an increased locomotor sensitization to cocaine, adult rats which had undergone severe PA (19 min of intra-uterine anoxia) showed a hyporesponsiveness to repeated cocaine administration (cocaine-induced locomotor response did not significantly increase from day 1 to 5 in the acquisition of cocaine locomotor sensitization phase, see Fig. 4). Furthermore, PA19 rats did not develop a sensitized response to cocaine since those pretreated with cocaine and challenged with cocaine (Coc-Coc treatment) did not increase their locomotor activity compared to the ones pretreated with vehicle and challenged with cocaine (acute cocaine administration, Veh-Coc treatment). (Fig. 5). Even

TABLE II. Summary of immunohistochemical and biochemical results

	c-Fos		FosB/ΔFosB	
Groups	CPu	NAcc	CPu	NAcc
CTL				
C+	$^{+++}$Veh-Coc	***Coc-Coc	$^{\uparrow\uparrow\uparrow}$Coc-Veh $^{\uparrow\uparrow\uparrow}$Coc-Coc	$^{\uparrow\uparrow\uparrow}$Coc-Veh $^{\uparrow\uparrow\uparrow}$Coc-Coc
PA15	$^{+++}$Coc-Coc	***Coc-Coc ▲▲▲Coc-Coc		
PA19		▼▼▼Coc-Coc	$^{\uparrow}$Coc-Veh $^{\uparrow}$Coc-Coc ▼Coc-Veh ▼Coc-Coc	$^{\uparrow}$Coc-Veh $^{\uparrow}$Coc-Coc ▼Coc-Veh ▼Coc-Coc

	TH		DAT	
Groups	CPu	NAcc	CPu	NAcc
CTL	=	$^{\downarrow\downarrow\downarrow}$Coc-Veh $^{\downarrow\downarrow\downarrow}$Coc-Coc	=	=
C+				
PA15		$^{\downarrow\downarrow\downarrow}$Coc-Veh $^{\downarrow\downarrow\downarrow}$Coc-Coc ▼▼Coc-Veh ▼▼Coc-Coc		
PA19		$^{\leftrightarrow}$		

$^{+++}P < 0.001$ significant increase compared to Veh-Veh or Coc-Veh; $^{***}P < 0.001$ significant increase compared to Veh-Veh, Coc-Veh or Coc-Coc; $^{\uparrow\uparrow\uparrow}P < 0.001$, $^{\downarrow\downarrow\downarrow}P < 0.001$, $^{\uparrow}P < 0.05$ significant increase/decrease compared to Veh-Veh or Veh-Coc; ▲▲▲$P < 0.001$, ▼▼▼$P < 0.001$, ▼▼$P < 0.01$, ▼$P < 0.05$ significant increase/decrease compared to the same treatment/s in the other groups; $^{=}$no differences between treatments and groups; $^{\leftrightarrow}$no differences between treatments.

though PA19 rats showed a reduced spontaneous locomotor activity during day −1, these results could not be explained by an impairment of the motor function, since during the second day the spontaneous locomotion no differences were observed between groups (Fig. 3). Moreover, these results could not be ascribed to an impaired locomotor response to acute cocaine administration since all the groups displayed similar levels of locomotor activity during the first day of the acquisition phase (Fig. 4), and after an acute challenge injection of cocaine (Fig. 5).

On the other hand, the repeated administration of cocaine (Coc-Veh and Coc-Coc treatments) was unable to increase FosB/ΔFosB expression, in both the CPu and NAcc, to the same levels observed in the other groups (CTL, C+ and PA15) (Figs. 8 and 9). ΔFosB is a transcription factor which plays an important role in drug addiction processes. Chronic administration of many drugs of abuse, including cocaine, induces the long-lasting expression of ΔFosB (Robison and Nestler, 2011). The lower increment of ΔFosB in the PA19 group (Figs. 8 and 9) may underlie the inability of cocaine to induce a sustained increase of locomotor activity over time (Fig. 4). In this sense, it has been reported that fosB knockout mice were unable to maintain an increase in locomotor activity throughout the repeated cocaine administration protocol, but showed a normal conditioned locomotor activity (Hiroi et al., 1997). Nevertheless, these mutant mice showed a significant increase in locomotor activity, compared with wild-type littermates, when an acute cocaine injection was administered (Hiroi et al., 1997). The mechanisms by which severe PA could affect the expression of FosB/ΔFosB induced by repeated cocaine administration, if this were the underlying cause of the reduced increment in the locomotor activity throughout the days of the acquisition phase, remains to be determined in further studies.

Regarding the lack of cocaine-induced locomotor sensitization (Fig. 5), this could be ascribed, in part, to the behavioral impairment shown by this group of animals during the acquisition phase. Moreover, the neural activity in NAcc showed to be reduced in PA19 rats pretreated with cocaine and challenged with cocaine (i.e., Coc-Coc treatment), as indicated by decreased c-Fos expression (Fig. 7). This fact could also explain the lack of behavioral sensitization observed in severe PA, since Hope et al. (2006) showed that the infusion of GABA agonists baclofen and muscimol into NAcc reduces c-Fos expression and attenuates the expression of the cocaine-sensitized response. In relation to the dopaminergic system, it was observed that neither TH nor DAT expression was altered in PA19 rats regardless of the administered treatment (Fig. 10).

The possible role of the mPFC in the differential effects induced by PA on cocaine sensitization

Another critical brain region, besides NAcc and CPu, involved in behavioral sensitization to cocaine is the medial prefrontal cortex (mPFC) (Steketee, 2003). mPFC dopaminergic system excerpts an inhibitory modulation of dopaminergic transmission in NAcc through its glutamatergic innervations to the VTA and NAcc (Steketee, 2003, 2005). Interestingly,

Brake et al. (2000) found that rats which had undergone moderate PA (15 min of intra-uterine anoxia) showed a persistent blunting of stress-induced DA release in the right PFC. This hyporesponsiveness of the PFC dopaminergic system was associated with an increased spontaneous locomotion. It could be hypothesized that repeated administration of cocaine to PA15 rats would also induce a blunting DA release in the mPFC, which would lead to a desinhibition of the dopaminergic transmission in the NAcc. This, in part, may explain the increased locomotor sensitization to cocaine showed by the PA15 group in this study. On the other hand, Li et al. (1999) demonstrated that when the mPFC is bilaterally lesioned, behavioral sensitization to cocaine is not expressed. It could also be hypothesized that the impairment in the acquisition and expression of cocaine sensitization in the PA19 group was due, in part, to mPFC damage induced by severe intra-uterine anoxia. Regrettably, as far as we know, no studies have been published regarding the consequences of severe PA on the mPFC in the model of global asphyxia used in this study. Given this evidence, it seems important to further study the role that the mPFC could play in the differential effects induced by PA on cocaine sensitization

Implications for vulnerability to drug addiction

It is well known that not all individuals that use drugs become addicts. A myriad of factors, ranging from genetic to cultural factors, can make an individual more prone to switch from occasional drug use to pathological abuse (Swendsen and Le Moal, 2011). In murine models, it has been demonstrated that prenatal and postnatal adverse experiences, such as maternal stress or maternal separation, alters cocaine and alcohol intake patterns or responsiveness to cocaine in the offspring (Kippin et al., 2008; Moffett et al., 2007). As it was mentioned in the introduction, PA is a known risk factor to develop attention deficit hyperactivity disorder (ADHD) and schizophrenia (Cannon et al., 2002; Lewis and Murray, 1987; van Handel et al., 2007). This could be due to the fact that PA produces a dopaminergic system dysregulation (Boksa and El-Khodor, 2003), which in turn has been proposed as one of the neurobiological abnormalities underlying both psychiatric disorders (Del Campo et al., 2011; Howes and Kapur, 2009; Landreau et al., 2012). Moreover, ADHD and schizophrenia are very frequently comorbid with substance abuse (Arias et al., 2008; Gudjonsson et al., 2012; Ringen et al., 2008) and all drugs of abuse cause common effects on the mesolimbic dopamine pathway (Nestler, 2005b). This raises the question whether PA may increase the vulnerability to drug addiction. Although no systematic studies are available, some isolated cases have been reported (Vecellio et al., 2003).

In this study, we demonstrated that PA induces differential effects on cocaine sensitization. Interestingly, moderate PA was associated with an increased locomotor sensitization to cocaine. Since behavioral sensitization to cocaine is a phenomenon that has been proposed as a relevant mechanism in cocaine abuse, further basic and epidemiological studies are required to determine if mild obstetric complications, such as moderate PA, may increase the vulnerability to drug addiction.

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