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Activity-dependent reduction of dopamine D2 receptors during a postnatal critical period of plasticity in rat striatum is not affected by prenatal haloperidol treatment

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ABSTRACT

Motor activity induced in the Circling Training test (CT) during a postnatal (PN) critical period of plasticity (PN30–37) produces a long-lasting decrease in the number of binding sites and mRNA expression levels of the dopamine D2 receptor (D2R) in rat striatum. Prenatal exposure to the antipsychotic haloperidol also decreases postnatal levels of the striatal D2R in the offspring. We examined whether such fetal exposure to haloperidol could affect the activity-dependent reduction of the D2R system during the critical period. Half of the male offspring exposed to either haloperidol (2.5 mg/kg/day), i.p.) or saline during gestational days 5–18 were subjected to the CT during the critical period, while the remaining represented CT control animals. The adult number of binding sites and mRNA expression levels of the striatal D2R at PN90 were not changed by prenatal haloperidol treatment alone. On the other hand, only pups subjected to the CT during the critical period showed decreases in both studied parameters, regardless the prenatal treatment. These findings indicated that the postnatal reduction of the striatal D2R binding induced prenatally by haloperidol does not affect long-lasting activity-dependent plastic changes on the same receptor system elicited by motor activity in an ontogenetic critical period of plasticity in rat striatum.

1. Introduction

The striatum (caudate-putamen in humans) is a main component of basal ganglia involved in the extra-pyramidal regulation of motor control. It plays an important role in procedural learning and in the formation of behavioral habits, and represents a major target for drugs of abuse (Gerdeman et al., 2003; O'Doherty et al., 2004; Williams and Eskandar, 2006; Atallah et al., 2007; Balleine et al., 2007). The striatal dysfunction is implicated in the pathophysiology of neurological and neuropsychiatric disorders such as Parkinson's disease, Huntington's disease, Schizophrenia and Attention-Deficit Hyperactivity Disorder (Albin et al., 1989; Andersen, 2005).

During the postnatal (PN) development of the rat striatum, we have described a motor critical period of activity-dependent plasticity (PN30–37). In that period exclusively, the motor activity of running in a circular path induced in the operant conditioning-based Circling Training test (CT), permanently decreases the number of muscarinic–cholinergic and dopaminergic D2 (D2R) receptors in the striatum as determined by binding assays (Ibarra et al., 1995, 1996; Soiza-Reilly et al., 2004). Consistently, we have also shown a decrease of a similar magnitude in mRNA expression levels of the striatal D2R (Soiza-Reilly et al., 2004). In addition, we have described that the ontogenetic onset of the striatal critical period can be modulated by the neurotrophin nerve growth factor (Wolansky et al., 1999a,b) as has been shown for other sensory-cortical critical periods (Berardi and Maffei, 1999; Hensch, 2005). Together, our previous work has demonstrated the existence of a motor critical period of activity-dependent plasticity in rat striatum during the fifth week of life, when long-lasting changes on the D2R system take place.

The prenatal exposure to the prototypical antipsychotic drug and D2R blocker haloperidol also induces enduring postnatal changes on the number of binding sites of D2R in the striatum. Specifically, fetal haloperidol treatment during gestational days (GD) 5–18 decreases the number of binding sites of the striatal D2R in the offspring in the fifth week of postnatal life, when the striatal critical period takes place (Rosengarten and Friedhoff, 1979; Scalzo et al., 1989b). In this study, we examined the hypothesis that

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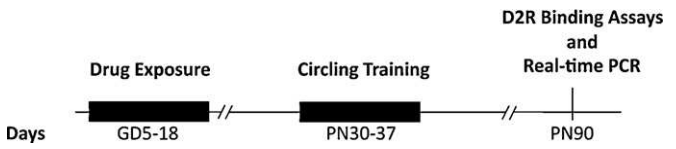

Fig. 1. Schematic timeline indicating the prenatal exposure to haloperidol (from GD5 to 18), the motor activity testing in the Circling Training test (CT) during the striatal critical period (PN30–37), and tissue processing for binding assays and real-time PCR (PN90).

postnatal reductions of the striatal D2R levels induced by haloperidol could affect long-lasting plastic changes on the same receptor system elicited by motor activity during the striatal critical period of plasticity (PN30–37). Thus, prenatally induced changes on the striatal D2R system might affect the postnatal brain developmental trajectory and contribute to long-standing behavioral outcomes. For this purpose, we applied haloperidol or saline systemically to pregnant rats from GD5 to GD18. After that, half of the male offspring from saline and haloperidol treated dams was subjected to the CT during the critical period (PN30–37) to induce the motor activity-dependent plastic changes on the striatal D2R system, while the remaining animals represented CT controls. Finally, we assessed the adult number of binding sites and mRNA expression levels of the striatal D2R at PN90 in all animals.

2. Materials and methods

2.1. Subjects

Primiparous Sprague–Dawley female rats obtained from the School of Veterinary Sciences of the University of Buenos Aires (Buenos Aires, Argentine) were used for breeding. Cages containing three female rats (about 200 g) and one male (about 380 g) per each were placed in a temperature-controlled environment (22–24 °C) under a normal 12 h dark/light cycle (lights on at 7 a.m.) with free access to food and water. The date of conception was determined by the presence of a copulating plug in the vagina and was considered gestational day (GD) 0. All procedures followed the National Institutes of Health guidelines for the care and use of laboratory animals and all efforts were made to reduce the number of animals used.

2.2. Drugs and drug administration

Sixteen pregnant rats were treated intraperitoneally once daily between 2 and 4 p.m. from GD5 to 18 with 2.5 mg/kg/day haloperidol (Janssen-Cilag, Buenos Aires, Argentine) ($N = 8$) or saline solution (sterile NaCl 0.9%, w/v) ($N = 8$) in a final volume of 1 ml/kg (Fig. 1). Haloperidol dose was individually adjusted depending on daily weighing and selected based on our previous studies (Brusés et al., 1991; Wolansky et al., 2004).

2.3. Litters and experimental groups

The day of birth was considered PN1 and the total number of pups per dam was recorded. All pups were sexed and litters with more than twelve pups were reduced to twelve. Litters with less than six live progeny were discarded. Body weights were recorded daily, measuring total weight of the litter during the nursing period, and the mean pups weight was calculated by dividing the litter weight by the total number of pups.

After weaning at PN21, up to four pups of the same sex and litter were housed per cage, and only one or two male pups were randomly used per cage for Circling Training test (CT) experiments starting at PN30 (Fig. 1). The male offspring from saline and haloperidol treated groups were assigned randomly to trained (CT) or non-trained (CT control) subgroups. All behavioral testing was conducted during the light phase of the cycle. Rats had free access to high-protein food and water except when indicated.

2.4. Behavioral testing

2.4.1. Surface righting and negative geotaxis

All the pups were tested by the appearance of the surface righting reflex and by the negative geotaxis test during the nursing period as previously described (Vorhees et al., 1979; Brusés et al., 1991). Briefly, for surface righting reflex, each pup was placed in a supine position and the time required for placing all four feet in contact with the surface was recorded. Rats were tested once daily in an all-or-none evaluation procedure beginning at PN8 until all pups from the same litter could right themselves in less than 2 s.

For the negative geotaxis test, the pups were timed for completing a 180° turn when placed in a head-down position on a 25° inclined plywood surface. Pups were given one daily trial from PN7 to PN16 and allowed a maximum time of 60 s per trial. The mean of the litter in each day was scored.

2.4.2. Circling Training test (CT)

At PN30, both CT subgroups of saline and haloperidol treated pups were trained in the CT as previously described (Brusés and Azcurra, 1993), while the two remaining subgroups represented CT control animals. Briefly, rats deprived of water for 24 h were trained to turn in a black circular maze (path: 8 cm wide × 100 cm long × 21 cm high) for a 10% (w/v) sucrose solution reward (60 μl drop per turn) in eight consecutive sessions. In the first training session (S_0), each rat was rewarded for a successive approximation to a full turn in the prescribed direction (selected randomly) over a 30 min period: in the first 10 min, a quarter turn in the appropriate direction was rewarded, in the next 10 min a half turn and during the last 10 min only a full turn in the appropriate direction was rewarded. Training was then conducted daily for 7 days according to a continuous reinforcement schedule. Each rat was required to perform 100 complete turns (=100 m) on days 1–3 (S_1, S_2, S_3) and 150 complete turns (=150 m) on days 4–7 (S_4, S_5, S_6, S_7). The left or right-hand turning direction was randomly selected for each animal in the first session and kept until the last session. After the complete training all rats performed approximately 950 rewarded turns (~950 m). The total correct sense turns (one turn = one reinforcement) in each session divided by the required time (min) represent the circling velocity (V). The number of incorrect sense turns (associative spatial errors) was also monitored during sessions in order to avoid a possible bias in the estimation of circling velocities. Turn registration, reinforcement delivery and time recording were automatically executed by a computer following a sequence of interruption of four photo beams equidistantly distributed in the maze walls (one per each quarter turn) during rat ambulation. After completing the behavioral task, rats were returned to the general housing facilities and maintained with free access to food and water until sacrifice.

2.5. Binding assays

All rats were sacrificed by decapitation at PN90 (Fig. 1). The brain was quickly removed and both striata from each animal were dissected (total sample). A fraction of the total sample was added to ice cold 20 mM Tris–HCl buffer, pH 7.4 (5%, w/v solution), containing 0.32 M sucrose, 1 mM EDTA and 0.5 mM PMSF. Homogenization was performed at 1300 rpm in a Potter-Elvehjem glass homogenizer fitted with a Teflon pestle for 10 min. Following centrifugation at 1090 × g for 10 min, the pellets were washed twice and then discarded. Supernatants were centrifuged again at 13,000 × g for 30 min. Following two washes, the pellets were re-suspended in the same buffer and stored at −70 °C for receptor binding assays. The number of D2R binding sites was measured by saturating ligand binding assay using [^{3}H]-raclopride as previously described (Young et al., 1991). Briefly, 100 μg protein were incubated for 90 min in 1 ml of 50 mM Tris–HCl, pH 7.4, 120 mM NaCl, 5 mM $CaCl_2$, 1 mM EDTA, 5 mM $MgCl_2$ at 25 °C in the presence of saturating concentration of [^{3}H]-raclopride (5 nM) (New England Nuclear Corp.). Each determination was performed in triplicate and the non-specifically bound [^{3}H]-raclopride was determined in duplicate in the presence of 300 μM sulpiride. After incubation, 3 ml ice-cold incubation buffer was added and the content was filtered under vacuum through a Whatman GF/B filter. Following three washes with 5 ml of the same buffer, the filters were placed in glass vials and dried overnight at 42 °C. 10 ml toluene–POPOP–PPO solution (0.25% POPOP, 4% PPO w/v) was added to each vial and after 30 min of incubation at room temperature under darkness, radioactivity was measured by liquid scintillation spectrometry. Protein was determined by the method of Lowry (Lowry et al., 1951), using bovine serum albumin as the standard.

2.6. Real-time quantitative PCR

On the remaining fraction of the total sample, the striatal RNA was isolated following the single step procedure described previously (Chomczynski and Sacchi, 1987) using commercially available Trizol reagent (Invitrogen, Carlsbad, CA). RNA integrity was verified on agarose gels by ethidium bromide staining. The quantity and purity of extracted RNA were determined by spectrophotometric readings at absorbances of 260 and 280 nm. The ratios of 260/280 nm absorbances were between 1.7 and 1.9, indicating low protein contamination of the samples. The striatal RNA (2 μg) was denatured in a sterile tube at 70 °C for 10 min. The cDNA synthesis was then performed by adding (final concentration): 1 mM of each dNTP, 2 U/μl RNAsin, 2.5 μM random primers, 0.1 U/μl Reverse Transcriptase (RT) AMV (Promega; Madison, WI) and DEPC-treated water in a final volume of 20 μl. The reaction was carried out at 48 °C for 45 min and stopped at 95 °C for 5 min (RT inactivation). After optimal PCR conditions were established, we used 1 μl of cDNA per well in a final reaction volume of 20 μl including (final concentration): 1× free-Mg^{2+} buffer, 0.1 mM dNTPs, 2 mM $MgCl_2$, 1.25 μM primers, 3.4% Dimethyl Sulfoxide, 0.0016% SYBR Green, 0.0625 U/μl Taq DNA Polymerase (Invitrogen, Carlsbad, CA) and Milli-Q water. The relative expression of D2R mRNA was estimated using as standard the expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA. The following PCR primers were used: forward 5′-AGCCAACCTGAAGACACCACTC-3′ and reverse 5′-AGCATCCATTCTCCGCCTGTTC-3′ for D2R (118 bp product); forward 5′-GGCAAGTTCAACGGCACAGTC-3′ and reverse 5′-TCGCTCCTGGAAGATGGTGATG-

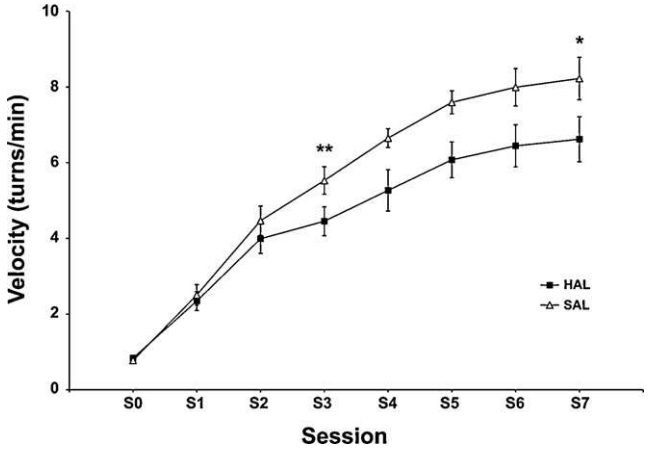

Fig. 2. Motor activity developed in the Circling Training test (CT) during the striatal critical period (PN30–37) after prenatal exposure to haloperidol. SAL (saline) and HAL (haloperidol) treated male pups were subjected to the CT ($N = 11$ per treatment group). Data represent the mean velocity (turns/min) ± SEM. $^{**}p < 0.05$ and $^{*}p < 0.004$ vs. saline for haloperidol treatment in S_3 and S_7, respectively.

3′ for GAPDH (81 bp product). Real-time PCR was performed in a DNA Engine Opticon (MJ Research, Waltham, MA). After a initial 3 min denaturalization at 94 °C, the PCR cycle consisted of a 45 s denaturalization incubation at 94 °C, then a 30 s annealing incubation at 64 °C (D2R) or 70 °C (GAPDH), and a 1 min extension incubation at 72 °C. Final extension of the products was conducted for 10 min at 72 °C, and a melting curve was performed to discard undesirable products. Parallel reactions were performed using as a template total RNA, in the absence of the cDNA synthesis step (negative controls). Cycle thresholds were selected 2 cycles past baseline amplification within the linear range on log scaling. Relative mRNA expression was calculated by using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

2.7. Statistical analysis

The CT data from saline and haloperidol treated groups ($N = 11$ per group) were evaluated by two-way ANOVA where prenatal treatment was the between-subject factor (2 levels) and CT session (3 levels) was the repeated measure factor. In order to satisfy the corresponding validation requirements (e.g., Mauchley's Sphericity test) we analyzed CT data of three representative sessions of the behavioral test (S1, S3 and S7). Significant interactions were analyzed using simple-effect slice ANOVA at each level of the repeated measures and treatment factors. Once ANOVA results showed significance, we compared data by Tukey test as a multiple comparison method. The number of D2R binding sites and real-time PCR data (after $2^{-\Delta Ct}$ transformation) from all the four experimental subgroups were analyzed by using two-way ANOVA, after data satisfy the corresponding requirements (e.g., normality and homogeneity of variances). The prenatal treatment and training were the between-subject factors. Significant differences were considered when $p < 0.05$.

3. Results

3.1. Offspring early physical development

We did not observe differences between saline and haloperidol treated groups in the gestation time, sex ratio, malformation rate, neonatal mortality, and mother cannibalism. However, haloperidol-treated litters showed a more delayed pattern of body mass gain during the first three postnatal weeks in comparison to control pups. Additionally, haloperidol-exposed pups showed a trend to a more delayed response in the negative geotaxis test at early ages (until PN8). On the other hand, we did not find differences between both treated groups in the appearance of the surface righting reflex (data not shown).

3.2. Motor response in the Circling Training test

After fetal exposure to haloperidol or saline, motor performances of male pups were evaluated in the operant conditioning-based CT starting at PN30 (Fig. 2). The ANOVA results showed a significant interaction between session and treatment factors ($F_{2,60} = 3.64$, $p < 0.04$). The simple-effect ANOVA for the treatment factor was significant for saline ($F_{2,60} = 134.72$, $p < 10^{-16}$) and haloperidol ($F_{2,60} = 74.31$, $p < 10^{-11}$) levels. Post hoc comparisons for all three sessions within each of these levels showed significant differences among all of them ($p < 0.01$ for S_1, S_3 and S_7 vs. each other by Tukey test). These results evidenced the effect of training throughout consecutive sessions in the CT.

The simple-effect ANOVA results for session factor were significant for S_3 ($F_{1,60} = 4.35$, $p < 0.05$) and S_7 ($F_{1,60} = 9.27$, $p < 0.004$) but not for S_1 ($F_{1,60} = 0.17$, $p = 0.69$) levels. These results indicated that the sensitivity of the CT to detect haloperidol-induced motor alterations depends on the accumulation of motor activity developed throughout sessions.

3.3. Striatal D2R binding sites and mRNA expression

We first corroborated that prenatal exposure to haloperidol induces a postnatal decrease of the striatal D2R binding persistent into the fifth week of life. To do that, we used [^{3}H]-raclopride to measure the number of D2R binding sites at PN30, when the striatal critical period begins. These results showed a significant decrease of about 20% in haloperidol-exposed pups in comparison to control animals (saline: 397 ± 15 fmol/mg of protein vs. haloperidol: 323 ± 13 fmol/mg of protein; $F_{1,6} = 13.91$, $p < 0.01$).

After that, we assessed the number of striatal D2R binding sites and mRNA expression levels at PN90 to analyze whether postnatal changes on D2R system induced prenatally by haloperidol could affect long-lasting activity-dependent plastic changes on the same receptor system elicited by motor activity during the critical period of plasticity (PN30–37).

The ANOVA did not show significant differences either for treatment ($F_{1,20} = 3.91$, $p = 0.06$) or for the interaction ($F_{1,20} = 0.99$, $p = 0.33$), indicating that gestational exposure to haloperidol alone does not produce long-lasting changes on the adult number of D2R binding sites in the striatum. On the other hand, only the effect of CT training was significant ($F_{1,20} = 139.86$, $p < 10^{-9}$), revealing the long-lasting activity-dependent reduction of about 40% in the number of binding sites of the striatal D2R (Fig. 3A). Consistent results were obtained when analyzing D2R mRNA levels by quantitative PCR using GAPDH expression as reference (Fig. 3B and C). In this case, ANOVA results did not show significant effects either for treatment ($F_{1,8} = 1.23$, $p = 0.30$) or for the interaction ($F_{1,8} = 1.48$, $p = 0.26$). Only the effect of CT training was significant ($F_{1,8} = 482.07$, $p < 10^{-7}$), showing a long-lasting reduction of about 50% in the striatal D2R mRNA expression.

4. Discussion

The aim of this study was to examine whether postnatal changes on D2R system induced prenatally by haloperidol (GD5–18) could affect long-lasting activity-dependent plastic changes on the same receptor system elicited by motor activity during an ontogenetic critical period of plasticity (PN30–37).

The prenatal administration of haloperidol alone did not modify substantially the gross trajectory of the gestational development in agreement with previous studies (Brusés et al., 1991; Williams et al., 1992; Wolansky et al., 2004). However, we found a delayed postnatal increase in body mass gain in haloperidol-exposed pups as suggested by previous studies (Scalzo et al., 1989b; Holson et al., 1994; Singh and Singh, 2002).

The therapeutic actions of haloperidol are mediated principally by its antagonism of the striatal D2R signaling, although it has numerous other activities as well (Creese et al., 1976). Previous studies have shown that fetal exposure to haloperidol decreases postnatal levels of [^{3}H]-spiroperidol binding sites in striatal plasma

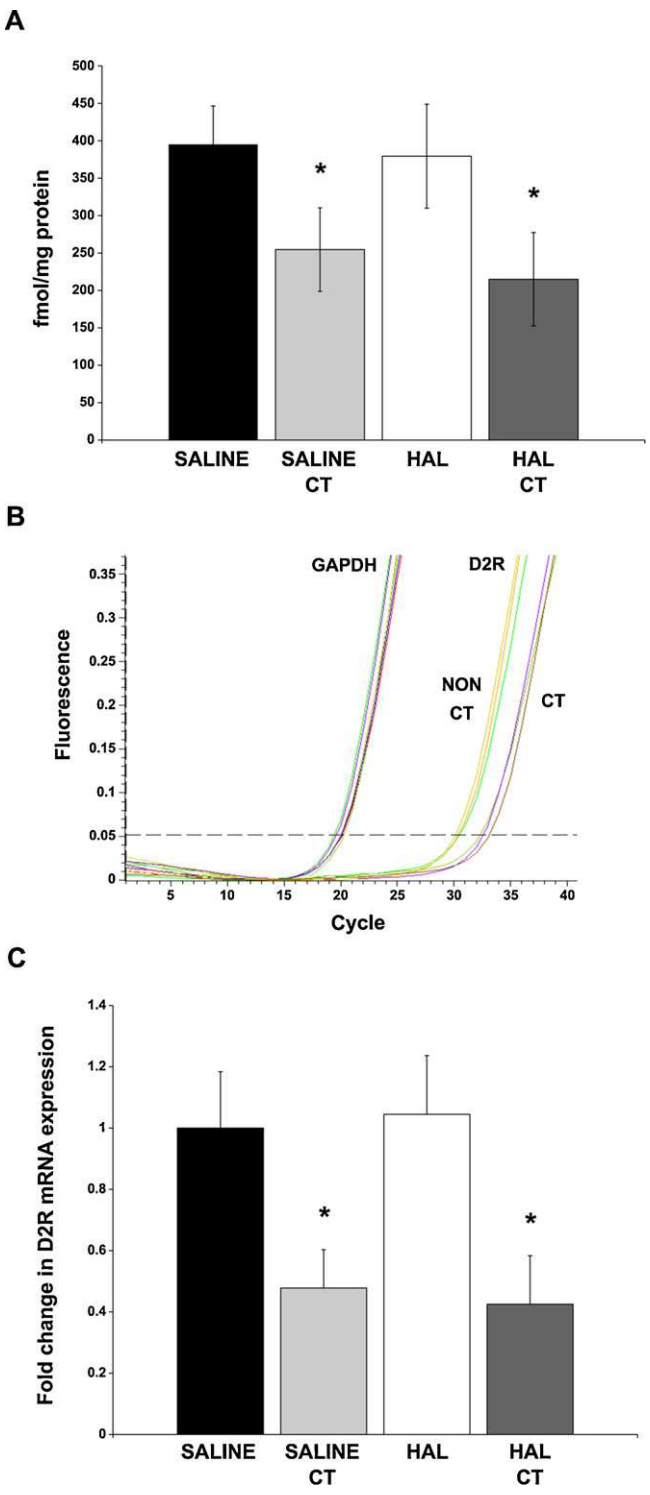

Fig. 3. (A) Adult number of binding sites of the D2R in the striatum at PN90, after the prenatal exposure to haloperidol and the Circling Training test (CT) during the striatal critical period (PN30–37). SAL (saline) and HAL (haloperidol) treated male pups subjected or not to the CT were sacrificed at PN90 and their striatal synaptic membranes obtained for binding assays using [^{3}H]-raclopride ($N = 6$ replicates per experimental subgroup). Data represent the mean ± SEM. $^*p < 10^{-9}$ vs. non-trained animals for both CT subgroups. (B and C) Adult mRNA expression levels of the striatal D2R at PN90, after the prenatal exposure to haloperidol and the CT during the striatal critical period (PN30–37). (B) Representative examples of real-time PCRs for D2R (right traces) using GAPDH as reference (left traces) on samples of HAL (haloperidol) treated male pups subjected or not to the CT assayed by triplicate (individual traces). The dashed line indicates the cycle threshold used for quantitative analysis. (C) Quantitative results of real-time PCR measurements of all experimental groups ($N = 3$ replicates per experimental group). Data represent the mean ± SEM. $^*p < 10^{-7}$ vs. non-trained animals for both CT groups.

membranes (Rosengarten and Friedhoff, 1979; Scalzo et al., 1989b). These changes were more robust around the fifth postnatal week and were attenuated by PN60. Consistent with that, in this study we found that prenatal haloperidol treatment induced a decrease of about 20% in the number of [^{3}H]-raclopride binding sites at PN30, when the striatal critical period begins.

The CT has been shown to be sensitive for the detection of motor alterations induced by developmental exposures to haloperidol (Brusés and Azcurra, 1993; Wolansky et al., 2004; Soiza-Reilly and Azcurra, 2009). Consistent with our previous work, results showed a decrease in the CT activity of haloperidol-exposed pups in comparison to saline treated animals. Additionally, fetal haloperidol treatment has a similar effect on apomorphine-induced stereotypy (Rosengarten and Friedhoff, 1979; Scalzo et al., 1989a). The neurobiological substrate underlying these behavioral effects might be the reduction in the number of spontaneously active dopamine neurons in the substantia nigra and ventral tegmental area (Zhang et al., 1996). However, this effect was obtained in two-week-old pups and it is unknown whether such reduction in the activity of dopamine neurons could persist into adolescence or at later ages.

The adult levels of D2R binding or its mRNA expression were not modified by prenatal exposure to haloperidol, supporting a close association between transcription and translation of this receptor in the striatum as previously suggested (Angulo et al., 1991; Fishburn et al., 1994). Indeed, postnatal silencing of D2R mRNA expression using antisense RNAs produces long-term decreases in the number of D2R binding sites and D2R-mediated behaviors (Davidkova et al., 1998).

This study also indicates an ontogenetic compensation of postnatal changes on the striatal D2R binding induced prenatally by haloperidol. A recent study using a prenatal immunological activation supports these findings (Kirsten et al., 2010). Specifically, they showed that prenatal exposure to a single injection of lipopolysaccharide (LPS) produces a marked reduction in the adult levels of striatal dopamine and its metabolites, without affecting motor behavior. Thus, prenatal manipulations of the striatal dopaminergic system could be compensated by ontogenetic events during postnatal development. In fact, we have shown that alterations on motor behavior induced by prenatal exposure to haloperidol can be reverted by postnatal exposure to the same drug (Wolansky et al., 2004).

Although in this study haloperidol treatment was restricted to GD5–GD18, we cannot exclude the possibility that the drug or its metabolites continue to act after the cessation of treatment due to their accumulation after binding to cellular constituents (Schmalzing, 1988). Most striatal neurons in the fetal brain are generated between GD13 and GD18 (Das and Altman, 1970; Bayer, 1984). In addition, specific binding to [^{3}H]-spiroperidol is detected as early as GD14 (Miller and Friedhoff, 1986), while D2R stereoselectivity reaches adult levels on PN5 (Miller and Friedhoff, 1986). On the other hand, haloperidol could also affect non-D2R-dependent signaling pathways by modifying the lipid composition of cellular membranes (Sánchez-Wandelmer et al., 2010).

Our previous work has shown that motor activity developed in the CT during a striatal critical period of plasticity (PN30–37) induces a long-lasting decrease of 40% in the number of binding sites of the D2R, accompanied by a similar reduction in its mRNA expression levels (Ibarra et al., 1996; Soiza-Reilly et al., 2004). In this study, we further validated our previous mRNA measurements using quantitative real-time PCR. We found that postnatal changes induced prenatally by haloperidol did not affect long-lasting activity-dependent reductions in the number of binding sites and mRNA expression levels of the striatal D2R, elicited by a fixed amount of motor activity (~950 m) developed during the critical period of plasticity (PN30–37). These findings suggest that

independent mechanisms of plasticity would be operating on the striatal D2R system during the fetal and postnatal periods. Interestingly, dopamine neurons of the nigrostriatal pathway can be regulated by dopamine through two different mechanisms (axon terminal vs. somatodendritic release), which have been suggested to present age-dependent variations during children development (Segawa, 2000). This raises the possibility that these variations may be contributing to developmental susceptibilities of the striatal D2R system.

Previous studies have shown that motor activity developed in the CT induces transient increases in dopamine levels and its metabolism in the striatum (Yamamoto and Freed, 1982; Yamamoto et al., 1982). This increase of dopamine may trigger events of desensitization/internalization of D2R and probably downregulation of its mRNA expression. However, we would not expect that these events would persist as far as 53 days after the end of the motor activity, supporting the hypothesis of a permanent activity-dependent synaptic adjustment of the striatal D2R system during the critical period. Additionally, we previously showed that rats trained a week before or after this critical period did not present the long-lasting biochemical changes induced by motor activity in the striatum (Ibarra et al., 1995, 1996). On the other hand, a substantial increase in the number of D2R binding sites and mRNA expression levels takes place in the striatum during the first four postnatal weeks (Pardo et al., 1977; Creese et al., 1992). After that, in the fifth week, receptor levels are pruned to finally reach its adult levels (Gelbard et al., 1989; Teicher et al., 1995; Tarazi and Baldessarini, 2000). At the same time, there is an overshoot of synapses followed by their competitive elimination (Andersen, 2003). Taken together, these data support our hypothesis of a permanent activity-dependent adjustment of certain synaptic sites within the striatum during the critical period of plasticity.

In the last decade, clinicians and pre-clinical researchers have put substantial efforts to understand whether drug treatments during developmental periods of sensitivity could affect the trajectory of the nervous system development, causing long-standing clinical outcomes later in life (Adams et al., 2000; Stanwood and Levitt, 2004; Thompson et al., 2009). The use of haloperidol in pregnant women was reported not to affect the gross fetal development (Diav-Citrin et al., 2005; Einarson and Boskovic, 2009). However, prospective studies will be necessary to determine whether haloperidol exposure during fetal development could represent a long-term risk for fetal brain maturation, with consequences on the adult life. These studies will also contribute to develop health policies that better define at-risk populations (Thompson et al., 2009).

In conclusion, we showed that postnatal changes on the striatal D2R induced prenatally by haloperidol do not affect the long-lasting activity-dependent adjustments on the same receptor system elicited by motor activity in an ontogenetic critical period of plasticity in rat striatum. Although we found that prenatal haloperidol alone did not modify the adult striatal D2R system, this work also emphasizes in the study of long-standing consequences after developmental drug exposure that could represent a prospective risk for individuals.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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