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Short communication

Different dopamine D1 and D2 receptors expression after motor activity in the striatal critical period

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Abstract

Circling training during rat striatum postnatal critical period (PN30 to 37 days) induces a life-lasting co-reduction of muscarinic acetylcholine receptors (mAChR) and dopamine D2 receptors (D2R) binding. Here, we evaluated the expression of D1R and D2R under similar experimental conditions. Trained rats showed a decrease of 40% in D2R binding sites ($p < 0.01$) and of 45% in the D2R mRNA expression which involve short ($p < 0.05$) and long ($p < 0.01$) isoforms. In contrast, D1R binding sites nor its mRNA expression levels were affected by training, indicating a differential synaptic refinement during this ontogenetically fixed period.

Theme: Motor systems and sensorimotor integration
Topic: Basal ganglia

Keywords: Motor plasticity; Dopamine; Rat striatum; Critical period; D1 receptor; D2 receptor

The rat striatum (caudate-putamen in humans), a main subcortical brain component related on extrapyramidal regulation of motor activity as well as for the formation of behavioral habits, is a major target for drugs of abuse [9]. In addition, striatal dysfunction has been implicated in several pathologies such as Parkinson's disease and Huntington's chorea [1]. During the ontogenetic development of striatum, there is a critical period of activity-dependent plasticity between postnatal days (PN) 30–37. Exclusively during this period circling motor activity performed under physiological conditions induces a life-lasting reduction of 35% in the B_{max} of muscarinic acetylcholine receptors (mAChRs) [14] with co-reduction of 40% in dopamine D2 receptors (D2R) [15]. Currently, two main output pathways of the striatal circuitry are known: the direct (striatonigral) and indirect (striatopallidal) pathways, whose projective gabaergic medium spiny neurons express dopamine D1 receptor subtype (D1R) and D2R, respectively [11]. A single mRNA isoform of D1R has been described [20] while for D2R mRNA a long and short isoforms, generated by alternative splicing [12] have been shown. Recently, it has been proposed a postsynaptic role for the long D2R subtype and presynaptic one, as autoreceptor, for the short isoform [18].

In the present work, we further evaluate the effects elicited by circling motor activity during the postnatal critical period on the adult expression of D1R and D2R in striatal synaptic membranes as well as their respective mRNAs.

Male Sprague–Dawley rats were subjected to the circling training test (CT) at 30 days old, as described previously [3]. Briefly, animals deprived of water for 24 h were trained to turn in an 8 cm wide and 100 cm long circular path for a water reward (50 μl drop per turn) in eight consecutive sessions. In the first training session, 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 in the last 10 min 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 2–4 and 150 turns (= 150 m) in the last four sessions. The left or right-hand turning direction was

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randomly selected for each animal in the first session and kept until the last session. Untrained control animals were also deprived of water and placed in the CT apparatus for 30 min each day but not training was performed. In all the cases, the body weight was daily recorded and animals with more than a 10% decrease in weight were discarded. After training all rats have performed 900 turns (900 m). All rats (including untrained control animals) were killed by decapitation at 90 days old. The brain was quickly removed, left and right striata from each animal were dissected and pooled together or as ipsi- and contralateral striatum to the circling direction according to the respective study (total sample). A portion of the total sample was processed as previously described [14] for synaptic membranes isolation. D1R was measured by saturating ligand binding assay as described elsewhere [5] using [^{3}H]-SCH23390 (New England Nuclear) and D2R was measured by saturating ligand binding assay as described elsewhere [22] using [^{3}H]-Raclopride (New England Nuclear).

On the remaining portion of the total sample, RNA was isolated by the single step procedure described elsewhere [4] using commercially available Trizol reagent (Invitrogen; Carlsbad, CA). RNA integrity was verified on agarose gels with 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 were consistently better than 1.8, 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). The optimal PCR amplification conditions and cycle number were determined experimentally to ensure specific and exponential signal generation. Expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA was used as internal standard to quantify the relative expression of D1R and D2R mRNAs. The cDNA samples (1 μl) were amplified by PCR reactions in a 0.5-ml sterile tube by adding (final concentration): reaction mix containing 1 × buffer free-Mg^{2+}, 0.4 mM of each dNTP, 0.5 mM (D2R) or 0.3 mM (D1R) $MgCl_2$, 0.5 μM primers, 0.05 U/μl Taq DNA Polymerase (Promega, Madison, WI) and autoclaved distilled water in a final volume of 25 μl. The following PCR primers were used: (D1R) forward 5′-CTTGGTGGCTGTCCTGGTCAT-3′ and reverse 5′-GGTCATCTTCCTCTCATACTG-3′, which generated a 196 bp PCR product; (D2R) forward 5′-GCAGTCGAGCTTTCAGAGCC-3′ and reverse 5′-TCTGCGGCTCATCGTCTTAAG-3′, which generated two 317 bp (short isoform) and 404 bp (long isoform) PCR products; (GAPDH) forward 5′-TGATGACATCAAGAAGGTGGTGAAG-3′ and reverse 5′-TCCTTGGAGGCCATGTAGGCCAT-3′, which generated a 240 bp PCR product. After a initial 2 min denaturalization at −94 °C, the PCR cycle consisted of a 40 s denaturalization incubation at −94 °C, then a 30 s annealing incubation at 54 °C (D1R), 64 °C (D2R) or 70 °C (GAPDH), and a 30-s extension incubation at 72 °C was performed. Final extension of the products was conducted by 10 min at 72 °C. PCR was carried out through 25 (D1R and D2R) and 20 (GAPDH) cycles. Parallel reactions were performed in the absence of RT to control for the presence of contaminant DNA and or replacing the cDNA template by distilled autoclaved water (negative controls). The PCR products were simultaneously resolved on a 3% agarose gel with ethidium bromide staining to verify the expected size of the amplified fragments, then photographed and quantitated by scanning densitometry using the Image Gauge 3.12 (Fuji Photo Film, Japan). Statistical analysis was performed by ANOVA or Student's *t*-test according with the number of variables evaluated. When significant differences appears in ANOVA's studies, a post hoc Scheffé test was performed.

Table 1 shows the number of D1R and D2R binding sites in rat striatum 53 days after the end of the training. These data confirms a reduction of 40% in D2R B_{max} of [^{3}H]-Raclopride in trained rats in respect to control ones ($p < 0.01$) as reported previously using [^{3}H]-Spiperone [15]. In contrast, the striatal D1R binding sites (B_{max} of [^{3}H]-SCH23390) were unaffected by training (Table 1). Measurements of Raclopride binding discriminating ipsi- and contralateral striatum to the circling direction have shown a reduction of 38% and 46% respectively ($p < 0.01$ vs. control, $n = 7$, data not shown).

Dopamine receptor subtypes mRNA expression in the striatum of animals under identical experimental conditions of Table 1 is presented in Fig. 1. A 45% decrease in the expression of both D2R's isoforms mRNAs in trained animals is shown ($p < 0.01$ vs. control, long D2R and $p < 0.05$ vs. control, short D2R), while D1R mRNA expression was unaltered by training. In addition, the constitutive expression of the enzyme GAPDH mRNA, used as internal standard, was unmodified. This results indicate the specificity of the changes triggered by circling motor activity on D2Rs. Fig. 2 shows that reduction of both isoforms of D2R mRNA expression are present in ipsi- and contralateral striatum to the circling direction. Specifically, a decrease

Table 1
D1R and D2R binding in rat striatum after circling training during a postnatal critical period

	[^{3}H]-SCH23390 binding (fmol/mg protein)	[^{3}H]-Raclopride binding (fmol/mg protein)
Control	456 ± 85	355 ± 66
Trained	448 ± 93	221 ± 53*

Comparison between trained and control rats. [^{3}H]-SCH23390 binding reflects specific binding to D1R. [^{3}H]-Raclopride binding reflects specific binding to D2R. Values are expressed as means ± S.E.M. of three independent experiments.

* $p < 0.01$ vs. control by Student's *t*-test for unpaired samples, nine rats per group.

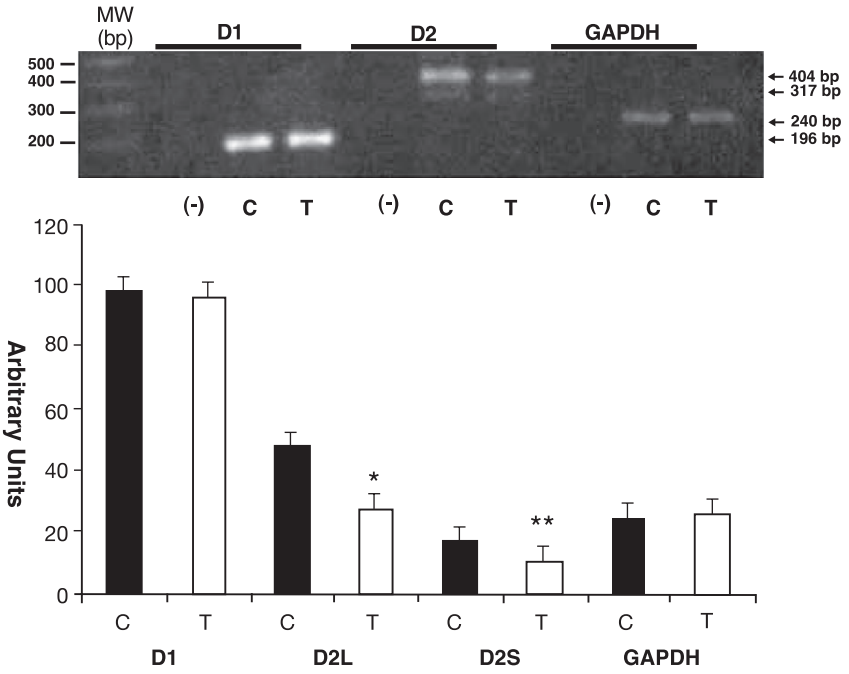

Fig. 1. The circling training during the postnatal critical period alters the striatal D2R mRNA expression but not D1R in rat. Upper panel: A representative agarose gel stained with ethidium bromide of the RT-PCR products subjected to electrophoresis running for D1R, D2R and GAPDH from trained (T) and control (C) rats. The expected products (D1R: 196 bp; D2RShort: 317 bp; D2RLong: 404 bp; GAPDH: 240 bp) were detected by comparison with a loaded molecular weight marker (MW) in an adjacent lane. (−): negative control. Lower panel: Quantitative densitometric analysis of RT-PCR products shown in upper panel. Values are expressed as means ± S.E.M. of three independent experiments. *$p < 0.01$ and **$p < 0.05$ vs. control by Student's t-test for unpaired samples, nine rats per group.

of 45% for ipsilateral striatum and of 44% for contralateral striatum in trained rats were observed ($p < 0.01$ vs. control, long D2R and $p < 0.05$ vs. control, short D2R).

Previously, it has been shown that circling motor activity triggers during a postnatal critical period (PN30–37) but not before (PN20–27) or after (PN60–67), a permanent co-alteration on B_{max} of mAChRs and D2Rs in adult rats [15]. Data of Table 1 using the more specific ligand [^{3}H]-Raclopride confirm a D2R reduction in the B_{max} of the same extent than that previously reported using [^{3}H]-Spiperone [15]. In addition, synaptic receptor protein modification is accompanied with a reduction of the D2R mRNA in the tissue as shown by RT-PCR (Fig. 1) indicating a permanent cellular adjustment of regulatory process upstream the translation step. Although precise quantification is limited due to the employed methodology, both short and long D2R mRNA isoforms show a similar reduction when compared to control values. Moreover, the adults ratio between short and long D2R isoforms mRNA in favor of the latter is conserved in trained animals (Figs. 1 and 2). As short D2R localizes presynaptically and could act as autoreceptor while long D2R are present in postsynaptic membranes [18], motor activity induced D2R adjustment probably involve axonal endings of nigrostriatal neurons and postsynaptic sites of striatal neurons.

In contrast to D2R, D1R is not modified in its membrane expression as measure by specific antagonist binding using [^{3}H]-SCH23390 (Table 1) or its mRNA expression evaluated by RT-PCR (Fig. 1). These results obtained by two independent techniques coincidentally shown that D1R expression is excluded of the persistent activity-dependent adjustment of mAChRs and D2Rs induced in the critical period. D1R and D2R are the main expressed dopaminergic receptors in striatal projection gabaergic neurons, and they are mainly circuitry segregated as well as mAChR subtypes. D2R and mAChR M1 are expressed on the indirect striatal gabaergic projection neurons, while D1R and mAChR M4 are expressed on the direct striatal gabaergic projection neurons [6]. This pathways segregation has "in vivo" expression in microdialysis experiments on adult freely moving rats, where phenylethylamine-induced reduction in striatal acetylcholine release was blocked by co-perfusion with Raclopride but not with SCH23390 [17].

The differential activity-dependent plasticity response found between D1R and D2Rs in our experimental conditions open the possibility to the existence of a different critical period for the striatal D1R synapses. The development of appropriate neuronal circuits involves activity-dependent processes [19], and the existence of multiple sensitive periods during development in primates have been shown in visual cortex [13]. An alternative explanation is that circling training may be inappropriate or insufficient to trigger D1R plasticity response. However, recently we have found that mAChR activity-dependent adjustment during the striatal critical period involves mRNA expression of mAChR M1 and M4 subtypes [8], showing that gabaergic

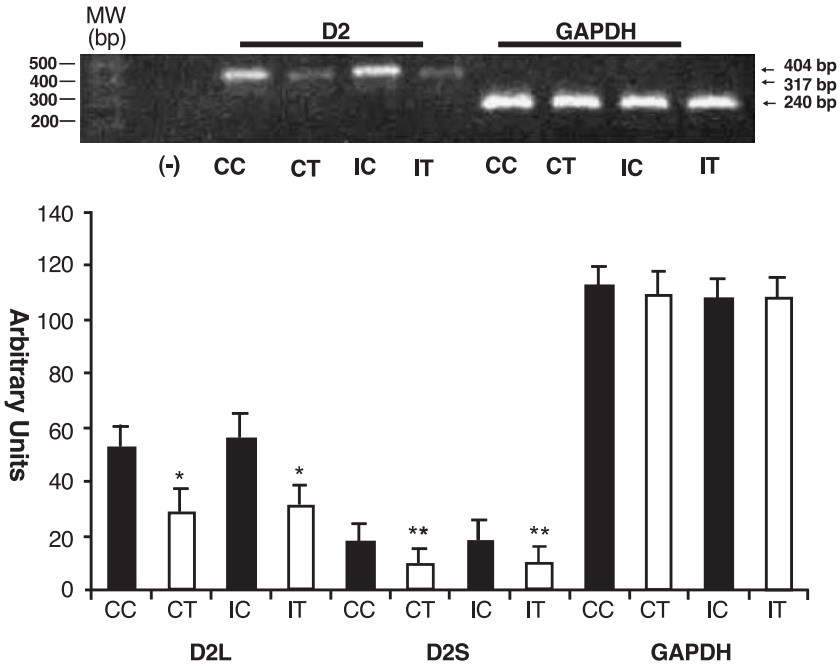

Fig. 2. Bilateral effects of circling training carried out during the postnatal critical period on D2R mRNAs expression in rat striatum. Upper panel: A representative agarose gel stained with ethidium bromide of the RT-PCR products subjected to electrophoresis running for D2R and GAPDH from Trained (T) and Control (C) rats, indicating Ipsilateral (I) and Contralateral (C) striata to the circling direction. The expected products (D2RShort: 317 bp; D2RLong: 404 bp; GAPDH: 240 bp) were detected by comparison with a loaded molecular weight marker (MW) in an adjacent lane. (–): Negative control. Lower panel: Quantitative densitometric analysis of RT-PCR products shown in upper panel. Values are expressed as means ± S.E.M. of three independent experiments. *$p < 0.01$ and **$p < 0.05$ vs. respective controls by post hoc Scheffé test, six rats per group.

projective neurons from both striatal pathways are involved in the activity-dependent reshaping during the critical period. Therefore, above presented results support that ontogenetic period (PN30–37) correspond to a critical period of activity-dependent synaptic refinement [19]. The complexity of circuital regulation that triggers post-stimulation plasticity events [2] and receptor signaling pathways individualities underlie the different plasticity response of individual neuronal synapses.

Pharmacological experiments have shown a differential D1R-D2R plasticity induced in striatum. Early postnatal estradiol administration affects adult expression of D2R binding sites but not D1R [7]. In adult animals, ablation of striatal cholinergic interneurons has been shown to exclusively [23] or preferentially [16] decrease D2R expression. A differential regulation of D1R plasticity in dopamine-depleted animals has been shown [10]. A specific increase in D2R as secondary response to dopamine low levels in an epileptic strain of mice has been reported [21]. Above presented results confirm that differential plasticity of D1R and D2R is present during late postnatal maturation under physiological stimulation.

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