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Short communication

Delayed and bilateral changes of GAP-43/B-50 phosphorylation after circling training during a critical period in rat striatum

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Abstract

During the critical period of activity-dependent plasticity in rat striatum (30–37 days after birth) physiological circling behavior induces delayed modifications in GAP-43/B-50 phosphorylation by PKC. Postexercise, ipsi- and contralateral striatum to the circling direction show a similar temporal pattern of GAP-43/B-50 phosphorylation, with an initial decrease followed by a subsequent increase. However, there is a lag between initiation of the phosphorylation response in this asymmetrical task which does not occur when animals are subjected to exercise under conditions of symmetrical motor activity.

Theme: Motor systems and sensorimotor integration
Topic: Basal ganglia

Keywords: Striatum; Development; Plasticity; GAP-43/B-50; Circling training; Motor activity

The caudate–putamen (striatum in rodents) exerts a key role in extrapyramidal motor behavior control [12], and its function impairment is associated with motor and cognitive disturbances [5,18,23]. A striatal critical period of activity-dependent synaptic plasticity during rat postnatal development was described between postnatal days (PND) 30–37. During this period, but not before or after, motor activity on a circular path (circling behavior) induces a bilateral reduction in the striatal expression of muscarinic cholinergic (mAChR) and dopaminergic subtype D2 (D2R) receptors in adult life [15,16]. As in the case of the visual model of activity-dependent plasticity, the length of this critical period can be modified by local perfusion of nerve growth factor (NGF) [28,29]. Although cellular and molecular events underlying the permanent reduction of the expression in the receptors of the striatum are still unknown, we found endogenous GAP-43/B-50 phosphorylation by protein kinase C (PKC) in synaptic plasma membranes only when exercise took place during the critical period [22]. GAP-43 (also called B-50, pp46, F1, neuromodulin) is found in growth cones, axons and presynaptic nerve endings [1], and has been linked to functional and morphological neuroplasticity as well as to plasticity mechanisms of learning and memory [2,3,13,24]. At 30 min of post-circling behavior (but not immediately), the contralateral striatum to the circling direction shows a decrease in PKC activity and GAP-43 phosphorylation level. Moreover, a correlation between GAP-43 changes and the running speed developed during CT was found, in the presence [22] or absence [21] of associative learning inducements. These results suggest a possible intervening role of GAP-43 phosphorylation in the plasticity events which lead to adult reduction in the expression of the striatal receptors. However, the GAP-43 and PKC Postexercise modifications are exclusive to the contralateral striatum; conversely, adult neuroreceptor reduction is bilateral. To clarify this point, we carried out an extended evaluation of the GAP-43 phosphorylation delayed temporal pattern after circling behavior developed during the critical period in rat striatum.

Male Sprague–Dawley rats at PND30 were used in all cases. Circling Training (CT) tests was performed as previously described [4]. The exercise in a circular gyratory platform (GP) turning at 15 rpm was performed as men-

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tioned previously [21]. Each rat was required to complete 150 turns (150 m) per session for two sessions, and the circling direction for each animal was randomly selected in both paradigms. The symmetrical exercise was performed on the 1-m-circumference free running wheel (RW) apparatus. Each rat was required to complete 150 turns (150 m) per session for two sessions. Rats were terminated by decapitation at 0, 30, 60, and 90 min postexercise at the end of the second day of training. The brain was quickly removed, and left and right striata were dissected separately at 4°C and weighed. In animals subjected to asymmetrical motor activity (CT and GP), each striatum was pooled according to the circling direction during exercise according to whether it was ipsilateral or contralateral. For animals subjected to symmetrical motor activity (RW), each striatum was pooled as left or right. The tissue was then processed to obtain synaptic plasma membranes (SPM) preparations as previously detailed [21,22]. Protein concentration was assessed by the method of Lowry et al. [19], using bovine serum albumin as standard. In vitro GAP-43/B-50 endogenous phosphorylation was evaluated as previously described in detail [21,22]. PKC activity was determined by an in vitro phosphorylation assay using as substrate histone III-S [21,22]. Differences between groups were assessed by Student's *t*-test for unpaired samples or by Dunnett's test for unpaired or paired samples according to what was appropriate in each case.

The phosphorylation level of GAP-43 in striatal SPM was assessed up to 90 min after the end of the running activity performed during the critical period. As GAP-43 phosphorylation has been reported to be associated with plastic processes of learning [2,26], we employed two paradigms with an identical circular path but including (CT) or not (GP) an associative learning task to discriminate motor from purely cognitive components. The results obtained in both paradigms used were notably similar (Fig. 1A and B). Fig. 1 shows the phosphorylation level of GAP-43 in striatal SPM up to 90 min postexercise with respect to non-running controls, differentiating the contralateral (Fig. 1A) and ipsilateral (Fig. 1B) striatum according to the circling direction, independently of which had been selected (clockwise or anticlockwise). Control animals from both paradigms did not show significant differences either between both striata at each tested time or between each striatum at different experimental times ($p > 0.6$). For the contralateral striatum, the endogenous GAP-43 phosphorylation levels did not show significant differences immediately following exercise (0 min) with respect to control values. At 30 min, we detected a significant decrease of 30% for CT and GP exercised animals, as previously reported [21,22]. In contrast, 60 min after running, a significant increase of GAP-43 phosphorylation (67% and 70% CT and GP, respectively) was observed, which decreased at 90 min postexercise approaching control values.

For the ipsilateral striatum (Fig. 1B), a significant difference was not found in the GAP-43 phosphorylation levels at

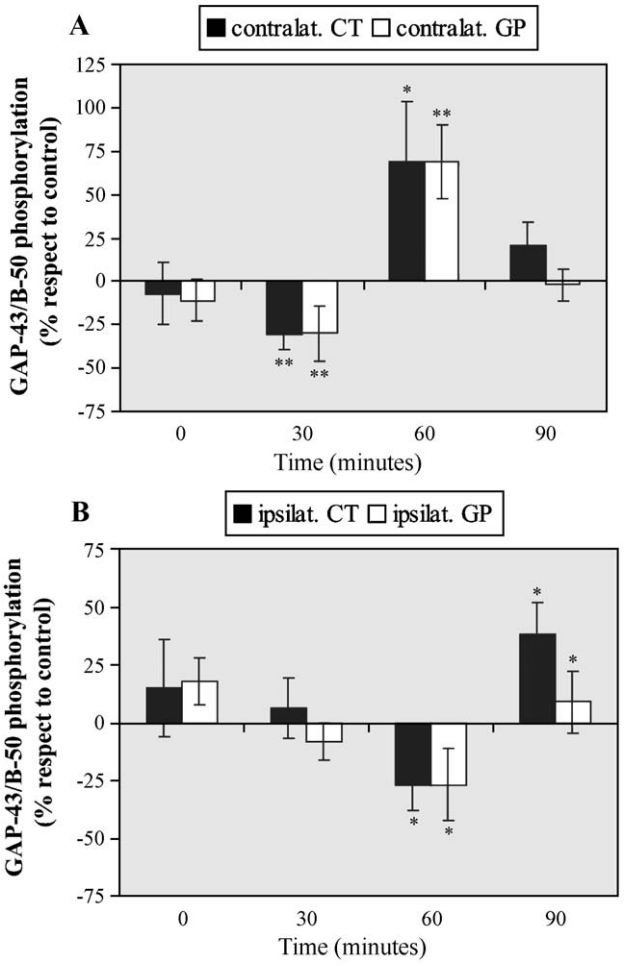

Fig. 1. Temporal pattern of endogenous GAP-43/B-50 phosphorylation level (0, 30, 60, and 90 min) after asymmetrical motor activity performed during the striatal critical period. Animals were subjected to the CT or GP paradigm and then were grouped all striata as contra- or ipsilateral striata. GAP-43/B-50 phosphorylation levels from contralateral (A) or ipsilateral (B) striata are shown. Data are expressed as percentages of the control value and represent the mean ± S.D. of three independent determinations ($n = 6$) measured by triplicate. $^{**}p < 0.01$ and $^{*}p < 0.05$ vs. its respective control values by Student's *t*-test for unpaired samples.

0 and 30 min postexercise, when compared to its respective control values. However, at 60 min, a significant decrease of 28% was observed in both paradigms. As in the case of contralateral striatum, a significant increase of GAP-43 phosphorylation was equally present in the ipsilateral striatum, but at 90 min post-running (40% and 10% CT and GP, respectively). However, the percentage of increase is less, when compared to contralateral striatum. At this point in time, we cannot determine whether this difference is due to experimental conditions, as we are not sure if times selected for the measurements are concurrent with the peak of the phosphorylation response for each of the two striata.

The comparison between Fig. 1A and B indicates that both striata follow a similar two-phase pattern in the delayed GAP-43 phosphorylation response after asymmetrical motor activity during the critical period. However, the lag in the

Table 1
The PKC activity temporal pattern after circling motor activity performed in the gyratory platform during the striatal critical period

	Ipsilateral striatum (pmol min^{-1} mg_{prot}^{-1})	Contralateral striatum (pmol min^{-1} mg_{prot}^{-1})
Control	66.95 ± 8.07	62.04 ± 7.90
0 min	70.41 ± 9.02	63.51 ± 10.62
30 min	76.59 ± 5.77	50.07 ± 5.68*
60 min	59.37 ± 7.84	83.78 ± 10.66**,#
90 min	79.55 ± 6.34#	65.28 ± 8.53*

Data were obtained from controls and trained animals sacrificed at 0, 30, 60, or 90 min postexercise and expressed as pmol min^{-1} mg $^{-1}_{protein}$ of SPM ± S.D. of three independent determinations measured by quadruplicate ($n = 5–6$).

*$p < 0.05$ vs. ipsilateral by Student's t-test for paired samples.

**$p < 0.01$ vs. ipsilateral by Student's t-test for paired samples.

#$p < 0.05$ vs. control by Dunnett's test for unpaired samples.

initiation of that response is more extended in the ipsilateral striatum.

To test whether changes in GAP-43 phosphorylation levels in the ipsi- and contralateral striatum were also associated with changes in PKC activity, we measured the enzyme activity in SPM from animals exercised in CT (see Table 1). Significant differences were not found on striatal PKC activity levels at 0 min after exercise between ipsi- and contralateral striatum with respect to their control values. On the other hand, contralateral striatal values showed a significant decrease (35%) in PKC activity at 30 min postexercise compared to ipsilateral ones, but PKC activity in ipsilateral striatum was unaffected as previously shown [21,22]. At 60 min after motor activity, a significant increase (41%) in PKC activity was found in the contralateral striatum. Ipsilateral striatum showed the lowest level of PKC activity at this time, although this was not statistically significant with respect to control values. At 90 min, a significant increase (24.5%) in PKC activity was shown in the ipsilateral striatum, while enzyme activity in the contralateral striatum went down to control values.

The onset of different GAP-43 phosphorylation events between ipsi- and contralateral striatum may be related to the asymmetrical motor activity developed while running in a circular path. To test this possibility, the striatal GAP-43 phosphorylation level was assessed in animals subjected to spontaneous symmetrical motor activity on a running wheel (Fig. 2). As in the case of circling motor activity, significant differences were not found in striatal GAP-43 phosphorylation levels between right and left striatum and their respective control values immediately after exercise (0 min). However, animals measured 30 min postexercise showed a simultaneous decrease of GAP-43 phosphorylation level in both striata. Thus, these results and those shown in Fig. 1A and B indicate that the difference in the initiation of GAP-43 phosphorylation events between both striata depends on the asymmetric posture conditions during the running activity.

Several studies report delayed changes in GAP-43 phosphorylation by PKC after physiologic stimulation with molecular events associated with plasticity mechanisms in synaptic function [13,14,24], and we were tempted to follow this interpretation in the adult bilateral reduction in receptor number in striatum after motor activity during the critical period [21].

The similar temporal pattern obtained in both paradigms led us to think that as in the case of the initial decrease, the subsequent increase in GAP-43 phosphorylation also depends on motor activity. Previously, we have shown that in our experimental conditions, GAP-43 phosphorylation level at 30 min post-running is not due to other GAP-43 phosphorylase but to PKC [21]. Although in the above experiments, we have not excluded the possibility of other phosphorylase participation at longer time lapses, the PKC activity increase is coincidental with the increase of the protein phosphorylation (Table 1), suggesting that PKC participates in the rebound of GAP-43 phosphorylation after running. If GAP-43 phosphorylation reflects presynaptic

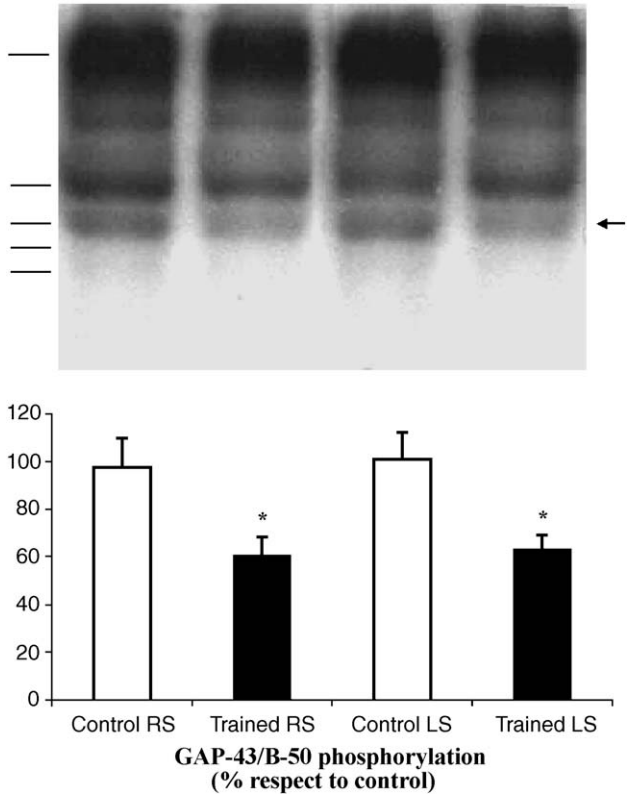

Fig. 2. Phosphorylation of the endogenous GAP-43/B-50, 30 min after symmetrical motor activity performed during the striatal critical period. Animals were subjected to the RW paradigm and then were grouped all striata as left (LS) or right (RS) striata. Upper panel shows representative autoradiogram of the GAP-43 phosphorylation in synaptic plasma membrane for control and exercised animals sacrificed 30 min after symmetrical motor activity. Lateral lines indicate the position of molecular weight markers: 95.5, 55, 43, 36, and 29 kDa. The arrow indicates the band corresponding to GAP-43/B-50. Lower panel shows a quantification of GAP-43/B-50 phosphorylation level on the upper panel. Data are expressed as percentages of the control value and represent the mean + S.D. of four independent experiments ($n = 5–6$) measured by triplicate. *$p < 0.01$ vs. its respective control values by Student's t-test for unpaired samples.

activity related to vesicle release [9], the patterns of Fig. 1A and B indicate at least two phases of neuronal activity in the striatum. This pattern may be originated by activity of the same or different neuronal groups. As it is known that distinct PKC isoenzymes are expressed by different neuronal types in striatum [27], the total PKC activity measured (Table 1) preclude us from clarifying this point.

GAP-43 phosphorylation events have been linked to LTP in vivo and in vitro [13,20]. In addition, for cortical plasticity, maximum susceptibility to LTP and LTD are coincidental with critical periods [8,10,17]. Therefore, it seems likely that reported changes in striatal GAP-43 phosphorylation may be related to LTD or LTP induced by motor activity. On the other hand, in the striatum, at least two different types of interneurons are present in the processing of information flow after corticostriatal fiber stimulation leading to LTP or LTD [6].

As striatal LTD and LTP respectively require PKG and PKA activation [7], the changes we have shown in GAP-43 phosphorylation activity in synaptic membranes may not be located in the direct chain of molecular signaling events that lead to permanent reduction in the receptor expression, but do participate in complex and necessary cellular and/or circuitry processes.

Fig. 2 shows that the difference for the onset of delayed changes in phosphorylation of GAP-43, between ipsilateral and contralateral striatum in our experimental conditions, is not apparent when motor training is performed under symmetrical postural conditions (running wheel). Physiological asymmetries in biochemical markers of striatum have been reported in adult rats after circling behavior [11], while in other reports, these findings have failed to be confirmed [25]. Although the initial post-running fall of GAP-43 phosphorylation occurs under both symmetrical and asymmetrical running conditions, the follow-up dynamics of GAP-43 phosphorylation events in both striata after symmetrical running is an open question, since differential neuronal involvement can be presumed in both postural conditions. Taken together, above results show that the response of GAP-43 phosphorylation after motor stimulation in the critical period is present in each striatum, but independently regulated. The independence of each striatum in the permanent modification in the number of receptors after motor stimulation in the critical period has previously been shown [29].

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References

[1] K.A. Alexander, B.T. Wakim, G.S. Doyle, K.A. Walsh, D.R. Storm, Identification and characterization of the calmodulin-binding domain of neuromodulin, a neurospecific calmodulin-binding protein, J. Biol. Chem. 263 (1988) 7544–7549.
[2] S.M. Ali, S. Bullock, S. Rose, Phosphorylation of synaptic proteins in chick forebrain: changes with development and passive avoidance training, J. Neurochem. 50 (1988) 1579–1587.
[3] L.I. Benowitz, A. Routtenberg, A membrane phosphoprotein associated with neural development, axonal regeneration, phospholipid metabolism and synaptic plasticity, Trends Neurosci. 10 (1987) 527–531.
[4] J.L. Brusés, J.M. Azcurra, The circling training: a behavioral paradigm for functional teratology testing, in: P.M. Conn (Ed.), Paradigms for the Study of Behavior, Methods in Neuroscience, vol. 14, Academic Press, New York, 1993, pp. 166–179.
[5] M. Carlson, A. Carlson, Interactions between glutamatergic and monoaminergic systems within the basal ganglia, implication for schizophrenia and Parkinson's disease, Trends Neurosci. 13 (1990) 272–276.
[6] D. Centonze, P. Gubellini, G. Bernardi, Permissive role of interneurons in corticostriatal synaptic plasticity, Brain Res. Rev. 31 (1999) 1–5.
[7] D. Centonze, B. Picconi, P. Gubellini, G. Bernardi, P. Calabresi, Dopaminergic control of synaptic plasticity in the dorsal striatum, Eur. J. Neurosci. 13 (2001) 1071–1077.
[8] M.C. Crair, R.C. Malenka, A critical period for long-term potentiation at thalamocortical synapses, Nature 375 (1995) 325–328.
[9] L.V. Dekker, P.N.E. De Graan, W.H. Gispen, Transmitter release: target of regulation by protein kinase C, Prog. Brain Res. 46 (1991) 209–233.
[10] S.M. Dudek, M.F. Bear, A biochemical correlate of the critical period for synaptic modifications in the visual cortex, Science 240 (1989) 673–675.
[11] C.R. Freed, B.K. Yamamoto, Regional brain dopamine metabolism: a marker for the speed, direction, and posture of moving animals, Science 229 (1985) 62–65.
[12] C.R. Gerfen, The neostriatal mosaic: multiple levels of compartmental organization, Trends Neurosci. 15 (1992) 133–139.
[13] C. Gianotti, M.G. Nunzi, W.H. Gispen, R. Corradetti, Phosphorylation of the presynaptic protein B-50 (GAP-43) is increased during electrically induced long term potentiation, Neuron 8 (1992) 843–848.
[14] W.H. Gispen, H.B. Nielander, P.N.E. De Graan, A.B. Oestreicher, L.H. Schrama, P. Schotman, Role of the growth-associated protein B-50/GAP-43 in neuronal plasticity, Mol. Neurobiol. 5 (1991) 61–85.
[15] G.R. Ibarra, J.A. Rodríguez, G.Ch. Paratcha, J.M. Azcurra, Permanent alteration of muscarinic acetylcholine receptor binding in rat striatum following circling training during a limited period of striatal development, Brain Res. 705 (1995) 39–44.
[16] G.R. Ibarra, G.Ch. Paratcha, M.J. Wolansky, J.M. Azcurra, Co-alteration of dopamine D2 receptor and muscarinic acetylcholine receptor binding in rat striatum after circling training, NeuroReport 7 (1996) 2491–2494.
[17] A. Kirkwood, L. Hey-Kyoung, M.F. Bear, Co-regulation of long-term potentiation and experience-dependent synaptic plasticity in visual cortex by age and experientia, Nature 35 (1995) 328–331.
[18] H.C. Lou, L. Henriksen, P. Bruhn, H. Borner, J.B. Nielsen, Striatal dysfunction in attention deficit and hyperkinetic disorder, Arch. Neurol. 46 (1989) 48–52.
[19] O.H. Lowry, N.J. Rosebrough, A. Farr, R.J. Randall, Protein measurement with follin reagent, J. Biol. Chem. 193 (1951) 265–272.
[20] R.B. Nelson, D.J. Linden, A. Routtenberg, Phosphoproteins localized to presynaptic terminal linked to persistence of long-term potentiation (LTP): quantitative analysis of two dimensional gels, Brain Res. 497 (1989) 30–42.
[21] G.Ch. Paratcha, G.R. Ibarra, R. Cabrera, J.M. Azcurra, Decreased phosphorylation of GAP-43/B-50 in striatal synaptic plasma mem-

branes after circling motor activity, Neurochem. Res. 23 (1998) 1241–1249.
[22] G.Ch. Paratcha, G.R. Ibarra, M.J. Wolansky, J.A. Rodríguez, J.M. Azcurra, Decreased GAP-43/B-50 phosphorylation in striatal synaptic plasma membranes after circling motor behavior during development, Mol. Brain Res. 65 (1999) 34–43.
[23] J.B. Penney, A.B. Young, Speculations on the functional anatomy of basal ganglia disorders, Annu. Rev. Neurosci. 6 (1983) 73–94.
[24] G.M.J. Ramakers, P.N.E. De Graan, I.J.A. Urban, D. Kraay, T. Tang, P. Pasinelli, A.B. Oestreicher, W.H. Gispen, Temporal differences in the phosphorylation state of pre- and post synaptic protein kinase C substrates B-50/GAP-43 and neurogranin during LTP, J. Biol. Chem. 270 (1995) 13892–13898.
[25] K.E. Sabol, J.B. Richards, C.R. Freed, In vivo dialysis measurements of dopamine and DOPAC in rats trained to turn on a circular treadmill, Pharmacol. Biochem. Behav. 36 (1990) 21–28.
[26] F.S. Sheu, B.J. Mc Cabe, G. Horn, A. Routtenberg, Learning selectively increases protein kinase C substrate phosphorylation in specific regions of the chick brain, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 2705–2709.
[27] C. Tanaka, Y. Nishizuka, The protein kinase C family for neuronal signaling, Annu. Rev. Neurosci. 17 (1994) 551–567.
[28] M.J. Wolansky, R.J. Cabrera, G.R. Ibarra, L.A. Mongiat, J.M. Azcurra, Exogenous NGF alters a critical motor period in rat striatum, NeuroReport 10 (1999) 1–5.
[29] M.J. Wolansky, G.Ch. Paratcha, G.R. Ibarra, J.M. Azcurra, Nerve growth factor preserves a critical motor period in rat striatum, J. Neurobiol. 38 (1999) 129–136.