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Cerebellar control of cortico-striatal LTD

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Abstract. *Purpose:* Recent anatomical studies showed the presence of cerebellar and basal ganglia connections. It is thus conceivable that the cerebellum may influence the striatal synaptic transmission in general, and synaptic plasticity in particular.
Methods: In the present neurophysiological investigation in brain slices, we studied striatal long-term depression (LTD), a crucial form of synaptic plasticity involved in motor learning after cerebellar lesions in rats.
Results: Striatal LTD was fully abolished in the left striatum of rats with right hemicerebellectomy recorded 3 and 7 days following surgery, when the motor deficits were at their peak. Fifteen days after the hemicerebellectomy, rats had partially compensated their motor deficits and high-frequency stimulation of excitatory synapses in the left striatum was able to induce a stable LTD. Striatal plasticity was conversely normal ipsilaterally to cerebellar lesions, as well as in the right and left striatum of sham-operated animals.
Conclusions: These data show that the cerebellum controls striatal synaptic plasticity, supporting the notion that the two structures operate in conjunction during motor learning.

Keywords: EPSP, long-term depression, motor recovery, rat, synaptic plasticity

Abbreviations: ACSF: artificial cerebrospinal fluid; HCb: hemicerebellectomy; HCbed: hemicerebellectomized; HFS: High frequency stimulation; LTD: long-term depression; EPSP: excitatory postsynaptic potential

1. Introduction

The cerebellum and the striatum are key components of two cortico-subcortical circuits differentially involved in motor learning and motor control (Sanes et al., 1990; Pascual-Leone et al., 1993; Grafton, 1994; Jenkins et al., 1994; Jueptner et al., 1997; Laforce and Doyon, 2001; 2002).

Despite the fact that the cerebellum and the striatum have long been considered as scarcely connected, cerebellar lesions have been shown to increase monoamine levels in the caudate nucleus (Cano et al., 1980), and evoked responses in the cerebellar cortex have been identified after basal ganglia stimulation (Fox and Williams, 1968; Bratus and Moroz, 1978). Furthermore, recent anatomical studies proposed a cerebellar and basal ganglia interaction, based on the identification of a di-synaptic pathway originating from the cerebellum and projecting to the striatum *via* the thalamus (Ichinohe et al., 2000; Hoshi et al., 2005). These findings raise the idea that the cerebellum may influence striatal synaptic transmission, including synaptic plasticity. Thus, in the present neurophysiological investigation, we studied striatal long-term depression (LTD), a form of synaptic plasticity believed to represent a synaptic correlate of motor learning (Calabresi et al., 2007), in the striatum of rats with cerebellar damage.

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2. Experimental procedures

2.1. Animals and surgery

The experiments were conducted in conformity with the European Communities Council Directive of November 1986 (86/609/EEC). Rats (250–270 g; Harlan, Italy) were randomly assigned to receive either a right hemicerebellectomy (HCb) ($n = 21$) or sham surgery ($n = 18$). Rats were anaesthetised with an i.p. solution of ketamine (90 mg/kg) and xylazine (15 mg/kg). A craniotomy was performed over the right hemicerebellum. The dura was excised and the right cerebellar hemisphere and hemivermis as well as the fastigial, interpositus and dentate cerebellar nuclei were ablated by suction. Care was taken not to lesion extra-cerebellar structures. The cavity was filled with sterile gel foam, the wound edges were sutured and the animals were then allowed to recover from anaesthesia and surgical stress. The animals belonging to the sham surgery group (controls) were anaesthetized to perform the craniotomy over the cerebellar structures. Neither excision of meningeal membranes nor cerebellar ablation were made. The wound edges were then sutured and the animals were allowed to recover from anaesthesia and surgical stress (Federico et al., 2006).

2.2. Behavioral test

Behavioral testing was performed at variable time intervals starting from 24 hours to 15 days after cerebellar lesion or sham-surgery. In all instances, last behavioral evaluation was obtained 3–6 hours before the electrophysiological experiments. Testing procedures were similar to those described elsewhere (Federico et al., 2006). Postural asymmetries and motor behavior were assessed by means of a behavioral rating scale. Presence or absence of head and body tilt, hyperflexion or hyperextension of fore- and hind-limbs in relation to trunk, ankle extra-rotation, hypotonia, eye nystagmus, head oscillations (bobbing) and tremor were evaluated. Furthermore, features of locomotion, such as wide-base, collapses on the belly, steering, circling, pivoting and side falls were analyzed. Specific postural and motor abilities, such as vestibular drop reaction and rearing behavior, were examined. Finally, complex motor skills, such as ascending a ladder and suspension on a wire were assessed. Video-records were taken throughout the entire cycle of testing and were used to supplement the direct behavioral observations. A score ranging from 0 to 2 was assigned to each symptom according to its degree of severity. Since 21 behaviors were taken into account, the total score ranged from 0 (absence of any postural and motor deficit) to 42 (presence of all postural and motor symptoms to the highest degree).

2.3. Electrophysiology

Rats were killed by cervical dislocation 3, 7 and 15 days after the HCb or of sham surgery for *in vitro* electrophysiological experiments. Corticostriatal slices were prepared from the left and right striatum according to previous reports (Centonze et al., 2003). Vibratome-cut coronal slices (200–300 μm) were transferred to a recording chamber and submerged in a continuously flowing artificial cerebrospinal fluid (ACSF) (34°C, 2–3 ml/min) gassed with 95% O_2- 5% CO_2. The solution composition was (in mM): 126 NaCl, 2.5 KCl, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 2.4 $CaCl_2$, 11 Glucose, 25 $NaHCO_3$. Intracellular recording electrodes were filled with 2M KCl (30–60 MΩ). Bicuculline (10 μM) was constantly applied to block depolarizing GABAA-mediated synaptic potentials.

Signals were recorded with the use of an Axoclamp 2A amplifier, displayed on a separate oscilloscope and stored and analysed on a digital system (pClamp 8, Axon Instruments, USA). For synaptic stimulation, bipolar electrodes were used, located in the white matter between the cortex and the striatum to try to activate corticostriatal fibers. High frequency stimulation (HFS) of excitatory synapses (3 trains, 3 s duration, 100 Hz frequency, 20 s interval) was used as a LTD-inducing protocol (Centonze et al., 2003). Quantitative data on modifications of EPSPs are expressed as percentage of the controls, the latter representing the mean of responses recorded during a stable period (around 10 min) before the HFS. EPSP values presented in the figures represent means $\pm$ SEM. Only one cell per slice and less than four neurons per animal were recorded. Student's t test for unpaired and paired observations was used to compare two groups of means and ANOVA was used for multiple comparisons. Drugs were applied by dissolving them to the desired final concentration in saline and by switching the perfusion from control saline to drug-containing saline. Drugs were: bicuculline (Sigma-RBI, St. Louis, USA), CNQX and MK-801 (Tocris, Bristol, UK).

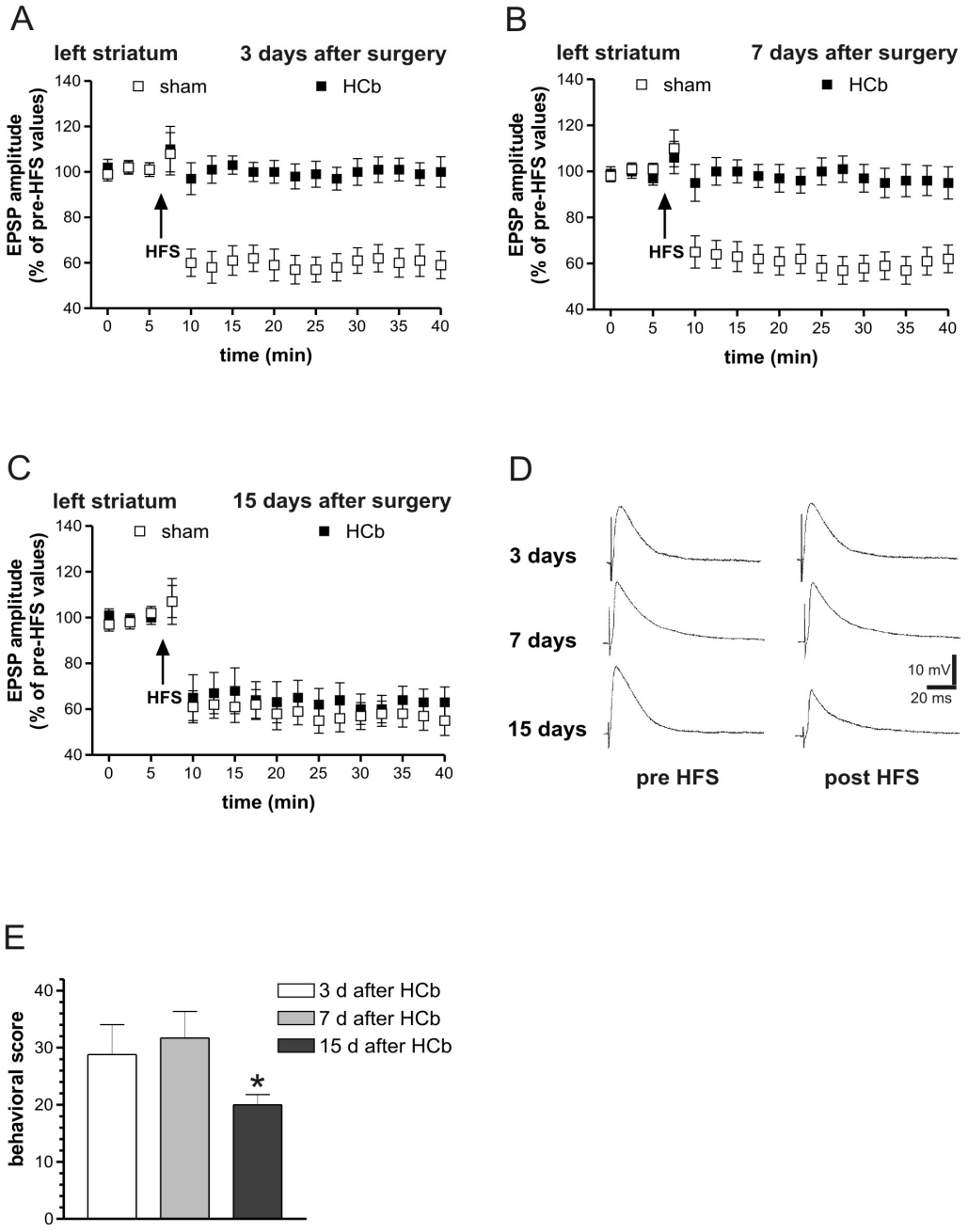

Fig. 1. HCb alters contralateral corticostriatal synaptic plasticity. A,B. HFS of corticostriatal fibres failed to induce LTD of EPSP amplitude in the left striatum (contralateral to HCb) 3 days (A) and 7 days (B) after surgery. C. HFS of corticostriatal synapses induced a stable LTD in the left striatum of HCbed rats 15 days after surgery. D. Examples of EPSPs recorded before and 10 min after HFS. E. The graph shows the behavioral scores of HCbed rats and indicates the clinical amelioration at 15 days after surgery. 15 days vs 3 days or 7 days after HCb: $p < 0.01$ (*).

3. Results

Recordings from striatal principal neurons were performed from control and HCbed rats. HFS was able to produce LTD of EPSP amplitude in the left (Fig. 1) and right striatum (Fig. 2) of control rats, 3 ($n = 7$ for both hemispheres), 7 ($n = 7$ for both hemispheres) and 15 days ($n = 8$ for both hemispheres) after the sham surgery ($p < 0.01$ and $t > 3.7$ respect to pre-drug values for each hemisphere and time point after surgery).

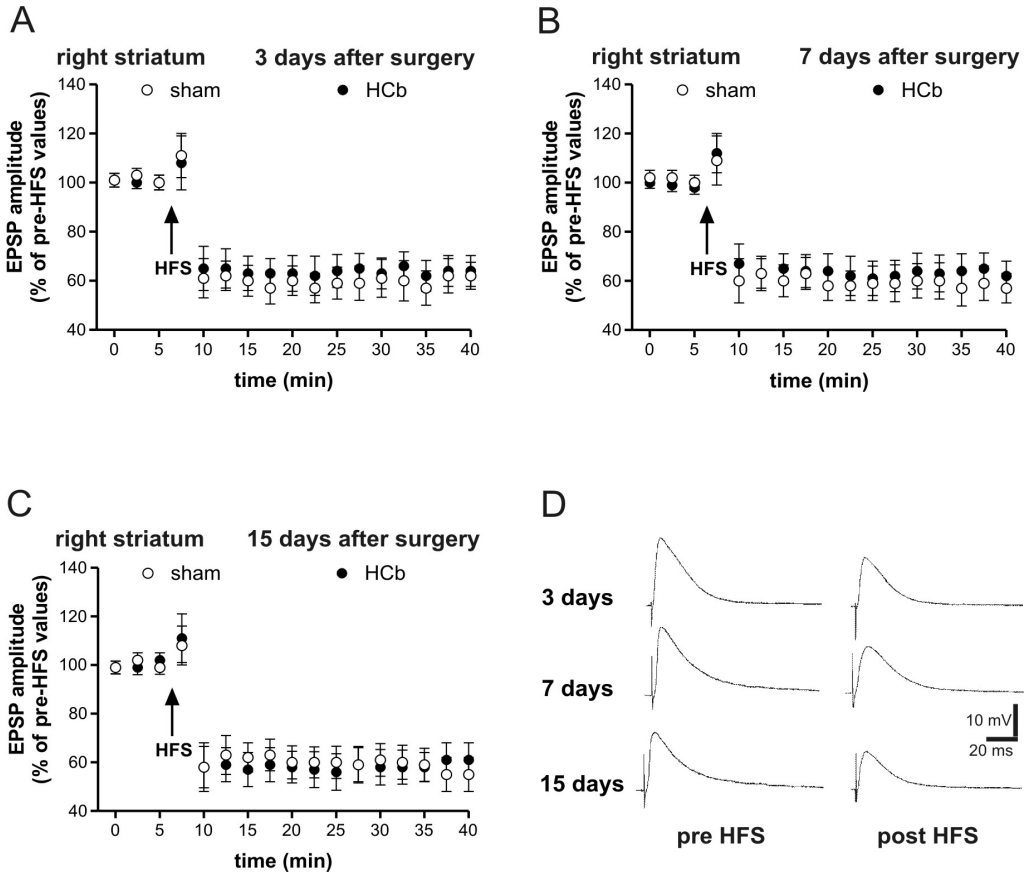

Fig. 2. HCb does not alter synaptic plasticity in the ipsilateral striatum. A,B HFS of corticostriatal synapses induced a stable LTD in the right striatum (ipsilateral to HCb) 3 days after surgery (A) and 7 days (B) after surgery. C. HFS induced a stable LTD in the right striatum of HCbed rats recorded 15 days after surgery. D. Examples of EPSPs recorded before and 10 min after HFS.

In contrast, LTD was fully abolished in the left striatum of HCbed rats recorded 3 and 7 days following surgery ($n = 7$, p>0.05 and t=1.3 respect to pre-drug values for both time points; $F = 13.6$ and $p < 0.0001$ comparing the post-HFS values of sham and HCbed rats), when the postural and motor symptoms of the animals were maximal. Conversely, fifteen days after right HCb, rats had partially compensated their motor deficits ($p < 0.01$, $F = 21.16$) and HFS of contralateral striatal excitatory synapses was able to induce a stable LTD ($n = 7$, $p < 0.01$ and $t = 3.8$ respect to pre-drug values) (Fig.1).

We also studied striatal plasticity in the right striatum of HCbed rats, ipsilaterally to the lesioned side. Three ($n = 7$), 7 ($n = 8$) and 15 days ($n = 8$) after HCb, striatal LTD was normally expressed following HFS of excitatory fibers ($t > 3.8$ and $p < 0.01$ compared to pre-drug values for each time point; $F = 0.25$ and $p > 0.1$ comparing the post-HFS values of sham and HCbed rats) (Fig. 2).

We wondered whether the loss of LTD in the first week after HCb could be caused by hyperactivity of NMDA receptors at corticostriatal synapses. The pharmacology of cortically evoked EPSPs was however similar in the left striatum of both control and HCbed rats, irrespective of the post-operative time point analyzed. MK-801 (30 μM), antagonist of glutamate NMDA receptors, in fact, failed to affect EPSP amplitude and duration in all the recorded neurons 3, 7, and 15 days after surgery ($n =$ at least 5 for each experimental group and time point). In contrast, subsequent application of CNQX (10 μM) fully suppressed striatal EPSPs in all instances (Fig. 3).

We also measured the amplitude of the membrane depolarization induced by HFS in the left striatum of control and HCbed rats 3 days after surgery, to investigate whether differential amplitudes to HFS responses could account for the different expression of synaptic plasticity in the two groups. HFS-induced depolarizations, however, did not significantly differ in the two

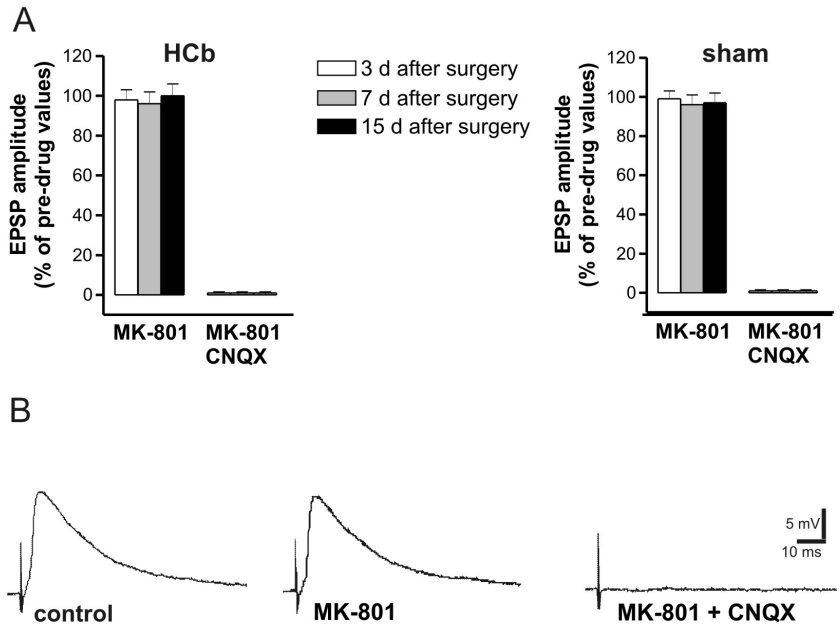

Fig. 3. HCb does not alter the pharmacology of corticostriatal EPSP. A. MK-801 failed to affect EPSP amplitude in all the recorded neurons 3, 7, and 15 days after HCb or sham surgery. Co-application of CNQX was required to fully suppress corticostriatal EPSPs. B. Examples of corticostriatal EPSP recorded 3 days after HCb before drugs, during MK-801 application and during MK-801 plus CNQX co-application.

classes of animals (HCbed rats: 48 ± 5 mV, $n = 7$; sham: 47 ± 8 mV, $n = 7$; $p > 0.05$) (not shown).

4. Discussion

The present results show that cerebellar lesion results in a transient blockade of LTD induction in the striatum. Although stressful events have been shown to disrupt LTD in other brain structures (Maroun, 2006), the effect here described is unlikely related to surgical stress, since striatal LTD was normally expressed in sham operated rats and, more importantly, in the ipsilateral striatum of HCbed rats. Furthermore, our data do not support the involvement of NMDA receptors in the loss of LTD seen in these mice. Accordingly, although potentiation of the NMDA receptor-mediated component of striatal EPSPs has been shown to block LTD induction, this effect is mediated, at least in part, by a greater depolarization of striatal neurons during HFS of glutamatergic fibers (Calabresi et al., 1997). In contrast, we observed: 1. no changes of EPSP amplitude and duration after blockade of NMDA receptors, and 2. no changes of the amplitude of HFS-induced membrane depolarization in HCbed rats. Both results are hardly compatible with the idea that striatal NMDA receptors are sensitized after cerebellar lesion.

Our data, therefore, support the notion that HCb does interrupt a neuronal pathway connecting the cerebellum and the contralateral striatum important for striatal synaptic plasticity. Notably, the recent description of a di-synaptic pathway connecting the cerebellar nuclei to the contralateral striatum allows some speculations on this topic. Accordingly, it has been found that fastigial, interpositus and dentate cerebellar nuclei do project to the striatum via intralaminar nuclei of the thalamus (Ichinohe et al., 2000; Hoshi et al., 2005). Within the striatum, axon terminals from neurons of the intralaminar nuclei of the thalamus mainly contact striatal interneurons, rather than spiny projection cells (Sidibe and Smith, 1999). Since the output of cerebellar nuclei is likely glutamatergic (Uusisaari et al., 2007), as it is that of the thalamus to the striatum (Sidibe and Smith, 1999), it might be conceivable that the interruption of cerebellar excitatory drive to thalamic nuclei would result in a decreased excitation of striatal interneurons. Since interneurons assist striatal synaptic plasticity, and namely LTD, via multiple mechanisms (Centonze et al., 1999; Wang et al., 2006), it is possible that reduced excitation of these neurons might play a role in the observed effects of cerebellar ablation on striatal synaptic plasticity. However, experiments with muscarinic receptor antagonists or direct recordings of the activity of cholinergic interneurons in HCbed are mandatory to substantiate this hypothesis.

Striatal LTD was rescued two weeks after HCB, indicating that adaptive processes take place in the brains of HCbed rats to compensate the loss of striatal synaptic plasticity. In this respect, it is relevant that the reappearance of striatal LTD parallels the spontaneous compensation of the motor deficits exhibited by HCbed rats, a finding which supports the notion that neuronal rearrangements mediate both clinical and synaptic recovery.

In summary, here we have described an unexpected cerebellar role in the control of striatal synaptic plasticity. In our opinion, this finding is particularly remarkable because the cerebellum and the striatum are segregated both anatomically and functionally. Further studies are required to clarify the functional meaning of the loss of striatal LTD following cerebellar damage, as well as of its reappearance during the recovery from the motor symptoms. As a final note of caution, it should be reminded that our results, rather than representing the synaptic correlate of cerebellar lesion, might reflect the effects of other factors caused by the experimental procedure, as endocrine or immunological responses or might be secondary to the behavioral changes induced by the lesion.

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