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Afferent facilitation of corticomotor responses is increased by IgGs of patients with NMDA-receptor antibodies

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Abstract

A severe subacute encephalitis associated with auto-antibodies to the NMDA receptor (NMDA-R) has been reported in humans. These antibodies are directed to NR1/NR2 heteromers of the NMDA receptor. We studied the effects of patients' cerebrospinal fluid (CSF) injected in rFr2 (the prefrontal area) on the afferent facilitation in a conditioning paradigm for corticomotor responses. The afferent facilitation was assessed in forelimbs and hind-limbs of rats, before and after application of trains of high-frequency stimulation (HFS) which are known to modulate the excitability of M1. Before HFS, patients' CSF did not modify afferent facilitation. After HFS, the amplitudes of corticomotor responses before conditioning were significantly larger in forelimbs and hindlimbs. There was an increase of the afferent facilitation in forelimbs. The same effect was observed after injection of purified IgGs from patients' sera. Our results highlight that IgGs of patients with NMDA-R antibodies induce a state of corticomotor hyperexcitability following application of HFS over the prefrontal area.

Keywords

Antibodies; NMDA receptor; Encephalitis; Afferent facilitation; Conditioning

Introduction

Up to one-third of patients presenting with limbic encephalitis have antibodies (Ab) against neuronal cell surface antigens [1]. One of these antigens corresponds to extracellular epitopes of NR1/NR2 heteromers of the NMDA (*N*-methyl-D-aspartate) receptors (NMDA-R). In most cases, patients are young women with teratoma of the ovary and presenting with acute psychiatric manifestations, seizures, dyskinesias, hypoventilation and autonomic instability [2]. Early removal of the ovarian teratoma followed by plasma exchange, intravenous immunoglobulins, and corticosteroids administration frequently result in neurological improvement and even full recovery [2].

One key question is whether the NMDA-R Ab are involved in the pathogenesis of the encephalitis. Recent *in vitro* and *in vivo* studies have shown that NMDA-R Ab decrease the number of clusters of NMDA-R in postsynaptic dendrites in the hippocampus and also cause a decrease of NMDA-R mediated currents [3]. This decrease is reversed by antibody removal [4]. The *in vivo* effects of NMDA-R Ab in the cerebral cortex are unknown. The demonstration of an effect on intensities of corticomotor responses would argue in favor of a direct effect of NMDA-R Ab on cerebral cortex. We tested this hypothesis using a conditioning paradigm with application of trains of high-frequency stimulation (HFS) over premotor cortex. These trains enhance afferent facilitation [5].

Methods

Cerebrospinal fluid (CSF) and IgG purification

Controls' CSF—Controls' CSF ($n = 3$) were obtained from one patient with herpes simplex encephalitis, one patient with neurodegenerative disorders and one with paraneoplastic sensory neuropathy associated with anti-Hu antibodies and small cell lung carcinoma [6].

Patients' CSF positive for NMDA-R Ab—CSF was obtained from three patients with encephalitis and NMDA-R-Ab (one from University of Pennsylvania-USA, and two from University of Lyon-France: 9049 and 9052). These CSF samples are referred to in the text as *patients' CSF*. In all cases the CSF was collected at symptom presentation, before any treatment. In addition, we also used purified IgGs to confirm the results obtained with patients' CSF: IgGs were purified from the serum of one patient with NMDA-R-Ab (patient 9052) as previously reported [7]. The presence of NR1/NR2 antibodies was demonstrated in all patients as reported earlier [2].

Infusion of antibodies

Experiments were approved by the Animal Care Committee of ULB. We made all efforts to reduce animal suffering as much as possible and to reduce the number of animals used for the study. On the basis of a previous study demonstrating that rFr2 (the equivalent of the premotor/supplementary motor area in primates; see [8, 9]) is an area of the brain modulating the excitability of M1 [5], we decided to perform injections in this zone. Adult Wistar rats (weight: 240–430 g) were anaesthetized with chloral hydrate (400 mg/kg administered *ip*) prior to surgery. We selected the procedure of continuous anaesthesia because of the absence of interference of voluntary motor activity. Guides (CMA12, CMA, Sweden) were inserted in the right premotor area rFr2 according to the atlas of Paxinos-Watson (Fig. 1, 1). Coordinates for rFr2 were (relative to bregma): A/P + 2 mm, Lat 1 mm, D/V –1 mm, respectively. Dental cement was used to fix the guide on the skull. A needle (Hamilton point style 4, Hamilton) was inserted in the guide. The extremity of the needle was located between –1 and –2 mm. Infusion of antibodies was performed using a

micropump (CMA100, CMA, Sweden). The volume injected was 5 μL , with a flow rate of 1 $\mu\text{L}/\text{min}$. Rats were lying over a temperature regulator (Heating Controller 872/1, Harvard apparatus). Indeed, it has been shown that the temperature is a key-factor for NMDA assessments (the channel kinetics play an important role in determining amplitude and time course of NMDA receptor-mediated postsynaptic currents, see [10]).

Three groups of rats were thus considered:

- Group 1: no infusion ($n = 8$ rats).
- Group 2: infusion of controls' CSF (each control CSF was infused in three rats; $n = 9$ rats).
- Group 3: infusion of NMDA-R Ab-positive CSF (each patient CSF was infused in five rats; $n = 15$ rats).

After these first experiments, a confirmatory study in a fourth group of rats was performed. The purified IgG were infused in 4 rats in order to verify the results found with patient's CSF.

In order to exclude a local bleeding following the experimental procedures, we monitored the local blood flow at the beginning and at the end of the experiments using laser flowmetry (Oxylab, Oxford Optronix). A laser flow probe was inserted near the tip of the guide in order to monitor the blood flow locally in the brain (microvascular perfusion). This technique allows the early detection of bleeding (immediate drop in blood flow). The method had been validated first in 12 rats. We determined the blood flow (expressed in arbitrary units BPU—blood per units—allowing evaluation of *relative* changes in perfusion, see [11]) at the beginning and at the end of each experiment. Rats with impaired blood flow were excluded from the analysis (one rat with local bleeding and blood flow decreasing below 45% of basal values, whereas the blood flow values remained above 65% of baseline values in all the other rats for all the experiments performed).

Analysis of the corticomotor excitability, conditioned corticomotor responses, trains of stimulation over rFr2 and spinal cord excitability

Analysis of corticomotor excitability—The methodology of muscle recordings is similar to the method of Hosoido et al. [12]. For forelimbs and hind-limbs, we analysed the corticomotor responses evoked in interosseus muscles on the left side following stimulation of the right motor cortex [13]. We used subcutaneous electrodes (Technomed 017K025) implanted in interosseus muscles. The impedance was kept below 5 kOhms.

In previous studies, we determined the 'hot spot' of the gastrocnemius muscle using a matrix of 6×9 sites of stimulation [5]. This allowed us to identify the precise location corresponding to the largest motor evoked potential (MEP; confirmed by epidural stimulation with tungsten microelectrodes TM33A05, World Precision Instruments, UK), which was found to be located between 2 and 4 mm laterally, and between 1 mm anterior and 2 mm posterior (coordinates relative to bregma). A similar methodology for mapping of MEPs (identification of the hot spot for each muscle) was applied here. The duration of stimuli was 1 ms (square waves; NeuroMax 4, Xltek, Canada). Recruitment curves (detection of motor threshold MT defined as the lowest intensity eliciting at least 5 out of 10 evoked responses with an amplitude $>20 \mu\text{V}$, followed by increases of the intensity of stimulation with steps of 0.1 mA until a plateau) of corticomotor responses were analyzed to confirm the classical sigmoid course using a sigmoid fitting with three parameters: $y = a/(1 + \exp(-(x - x_0)/b))$ [13]. Subsequently, the motor cortex was stimulated at an intensity of 130% of MT to assess corticomotor potentials. Peak-to-peak amplitudes in motor responses

of the left interosseus muscles were studied for groups of 10 successive corticomotor responses (Filter settings were 30 Hz to 1.5 kHz; NeuroMax 4, Xltek, Canada).

Conditioned corticomotor responses—At a short latency between a peripheral nerve (conditioning) stimulus and a second stimulus (called test stimulus) applied contralaterally on motor cortex, the corticomotor response is increased due to facilitation [5]. To evaluate conditioned corticomotor responses, test stimuli on right motor cortex (NeuroMax 4, Xltek, Canada) were preceded by a conditioning stimulus (DS70 stimulator, Digitimer, UK) in contralateral medialis nerve (for forelimbs) at an inter-stimulus interval (ISI) of 4 ms and in contralateral sciatic nerve (for hindlimbs) at an ISI of 6 ms [5].

Trains of high-frequency stimulation (HFS) over rFr2—Trains of stimulation were applied in right prefrontal region rFr2. Location of the rFr2 area was based on the Atlas of Paxinos and Watson (anode: +1.5 mm lateral, +4.5 mm anterior; cathode around the guide: +2 mm anterior, +1 mm lateral; coordinates relative to bregma). Stimulus intensity was set at 40% of motor thresholds (current supplied by a constant current stimulator A310–A365; a battery charger A362 was used to charge the stimulator between the experiments—World Precision Instruments, UK). *Trains of HFS* consisted of 15 successive rectangular pulses, with a pulse duration of 600 μ s and an intra-train interval between two successive pulses of 1 ms (1,000 Hz). The selection of pulse duration and timing between successive pulses within trains is based upon previous studies [14, 15]. Trains were repeated every 50 ms for a period of 10 min. We studied here the effects of premotor HFS on the conditioned corticomotor responses.

For group 1, corticomotor responses were analysed (a) without and (b) with conditioning (baseline state). Trains of HFS were applied subsequently for 10 min and the corticomotor responses were re-assessed both without and with conditioning immediately after (*HFS effect*). For groups 2 and 3, corticomotor responses were first assessed without and with conditioning (baseline state). Following infusion of CSF in rFr2 (controls' CSF in group 2 and patients' CSF in group 3), corticomotor responses were re-assessed between 30 and 45 min after infusion without and with conditioning (to evaluate the *infusion effect*; in a preliminary study in three rats, we assessed the time-course of the effects of infusion of NMDA-R Ab on corticomotor responses in forelimbs and hindlimbs and found no change in unconditioned and conditioned responses at the following times: 30–45, 60–75 and 90–105 min). Trains of HFS over rFr2 were subsequently applied for 10 min and the corticomotor responses were re-assessed immediately after (at a timing of about 60–75 min after infusion), both without and with conditioning (*HFS effect*). The same procedure was applied for the confirmatory study in group 4 with purified IgG.

Spinal cord excitability—For groups 1–3, we also investigated the H-reflex in fore-limbs and hindlimbs on the left side to evaluate spinal cord excitability at the beginning and at the end of experiments. H-reflex is an electrical analogue of the monosynaptic stretch reflex [12]. The direct motor responses (M responses) were studied in the left interosseus muscles using a method adapted from Gozariu et al. [16]. Electrical stimulation was performed using needle electrodes inserted subcutaneously. Electrical stimuli consisted of single square-wave shocks of 0.5 ms duration, delivered every 6 s (NeuroMax4, Xltek, Canada). Integrals of H- and M-responses were plotted against stimulus intensity to analyze the recruitment curves for the H/M ratios and to obtain the Hmax/Mmax ratios [14].

Histological verification—At the end of the experiments, brains were extracted from the skull to exclude traumatic lesion. We assessed brain sections in a way very similar to a method previously published [17]. Only data with correct location of the guide (the extremity of the guide located in the target region) were analysed.

Statistical analysis—Data were exported to Microsoft Excel. Statistical analysis was performed using SigmaStat (Jandel Scientific, Germany). For both forelimbs and hindlimbs, we applied to analysis of variance to compare the amplitudes of MEPs in the groups 1–3 at baseline before conditioning (in order to exclude differences between the three groups at the beginning of the experiments). For MEPs in both forelimbs and hindlimbs, we assessed the effects of the conditioning procedure in two states for group 1 (basal state and post-HFS), and in three states for groups 2 and 3 (basal state, post-injection and post-HFS) using the analysis of variance with post-hoc multiple comparison procedures (Bonferonni test). The interaction conditioning by state was analysed. We also compared the MEPs in forelimbs and the MEPs in hindlimbs in the three groups of rats without/with conditioning using multiple comparison procedures (analysis of variance with post-hoc Bonferonni test). For group 4, the same procedure was applied. For Hmax/Mmax ratios, the limb effect, the state effect and the limb by state interaction were also analysed using the analysis of variance followed by the Bonferonni test.

Results

In the four groups, latencies of corticomotor responses in interosseus muscles were between 5.2 and 6.8 ms in forelimbs and between 8.4 and 9.6 ms in hindlimbs. The range of peak-to-peak intensities of M responses were between 3.7 and 6.2 mV.

At baseline, amplitudes of MEPs were similar in groups 1–3 both for forelimbs (range of intensities: 641–889 μ V) and hindlimbs (range of intensities: 634–907 μ V). There was no inter-group difference ($p = 0.356$ and $p = 0.429$, respectively for forelimbs and hindlimbs). Typical examples of afferent facilitation are illustrated in Fig. 1(2), before (baseline), after injection of CSF, and after application of trains of stimuli (HFS) over the premotor cortex for an illustrative rat of group 2 and an illustrative rat of group 3. For both forelimbs and hindlimbs, a conditioning effect was found in the three groups of rats in all the states ($p < 0.001$; Bonferonni test: $p < 0.05$; see Table 1).

In group 1 (no infusion), trains of HFS were associated with an increase in the intensities of MEPs for both fore-limbs and hindlimbs (state effect; $p < 0.001$; Bonferonni test: $p < 0.05$). Moreover, we found an enhancement of the afferent facilitation following application of HFS in fore-limbs (interaction conditioning $\times$ state: $p = 0.013$). The enhancement of the afferent facilitation was not statistically significant in hindlimbs ($p = 0.108$).

In group 2, infusion of controls' CSF did not modify the intensities of MEP for either forelimbs or hindlimbs (no infusion effect; Bonferonni test: $p > 0.05$). Moreover, the afferent facilitation remained unchanged as compared to baseline state, for both forelimbs and hindlimbs (no interaction conditioning $\times$ state; Bonferonni test: $p > 0.05$). Following application of HFS, the intensities of MEPs were increased before conditioning as compared to basal state and post-injection ($p < 0.001$). However, for both forelimbs and hindlimbs, there was no statistically significant change in the afferent facilitation following HFS, although a slight increase was observed in forelimbs (no interaction conditioning $\times$ state; forelimbs: $p = 0.072$, hindlimbs: $p = 0.541$).

In group 3, infusion of patients' CSF did not modify the intensities of MEP as compared to baseline for either forelimbs or hindlimbs (no infusion effect; Bonferonni test: $p > 0.05$). In addition, the afferent facilitation remained unchanged, for both forelimbs and hindlimbs. Following application of HFS, the intensities of MEPs were markedly increased before conditioning ($p < 0.001$) and we observed a strong enhancement of the afferent facilitation in fore-limbs. Indeed, a significant conditioning by limb effect was found, with an afferent facilitation larger in forelimbs as compared to hindlimbs ($p < 0.001$). After HFS, this

asymmetry forelimbs/hindlimbs was significantly higher in group 3 as compared to groups 1 and 2 ($p < 0.001$).

These findings were confirmed with the use of purified IgG. For forelimbs, responses were as follows (respectively, without conditioning and with conditioning; values in the basal state without conditioning were set at 100% and served as a reference for subsequent measurements): at baseline 1.000 ± 0.118 and 1.222 ± 0.130 (conditioning effect: 122.15%), following injection: 1.016 ± 0.095 and 1.220 ± 0.112 ($\times 120.06\%$), following HFS: 1.564 ± 0.077 and 2.275 ± 0.082 ($\times 145.42\%$). For hindlimbs, responses were (respectively, without conditioning and with conditioning): at baseline 1.000 ± 0.064 and 1.213 ± 0.033 ($\times 121.31\%$), following injection: 1.020 ± 0.073 and 1.237 ± 0.034 ($\times 121.29\%$), following HFS: 1.637 ± 0.074 and 1.932 ± 0.045 ($\times 118.01\%$). For both forelimbs and hindlimbs, responses were significantly higher following HFS as compared to baseline and post-injection (HFS effect; $p < 0.001$). In the HFS state, the conditioning effect was significantly higher in forelimbs as compared to hindlimbs (limb effect; $p < 0.001$).

In the basal condition, the latency of the H-reflex ranged from 5.2 to 6.1 ms in forelimbs and from 9.0 to 10.2 ms in hindlimbs for the three groups of rats. Table 2 shows the ratios Hmax/Mmax in the groups 1–3 for the different states. In the three groups of rats, a significant limb effect was found (ratios of Hmax/Mmax were significantly higher in hindlimbs; $p < 0.001$). In group 1 in the basal state, the range of Hmax/Mmax ratios was 21.4–27.3% in forelimbs and 43.2–56.4% for hindlimbs. At the end of the experiments, the Hmax/Mmax ratios remained unchanged in forelimbs ($p = 0.987$) and hindlimbs ($p = 0.857$). Similar values were found for groups 2 and 3 in the basal state. In group 2, infusion of controls' CSF followed by application of HFS did not modify the Hmax/Mmax ratios (state effect: $p = 0.838$) and there was no significant limb by state interaction ($p = 0.408$). A similar observation was made for group 3 (state effect: $p = 0.389$; no limb by state interaction: $p = 0.833$).

Discussion

This study of the effects of trains of HFS on the premotor area on conditioned corticomotor responses show that IgGs of patients with NMDA-R Ab markedly enhance the excitability of the motor cortex. By contrast, the excitability of the spinal cord is unaffected by patients' CSF infused at a supra-spinal level. Our data also provide an indirect confirmation that the control of forelimbs and hindlimbs muscles by central commands differ. Indeed, (1) we confirm that the Hmax/Mmax ratios are higher in hindlimbs [12], and (2) we show that blockade of NMDA-R sensitizes the MEPs in interosseus muscles of forelimbs to the HFS procedure to a greater extent as compared to the hindlimbs.

It has been shown that rFr2 is a key modulator of the activity of the motor cortex and that application of trains of stimuli over rFr2 influence the corticomotoneuronal output in conditioning paradigms using peripheral stimulation [5]. From the experimental standpoint, we show here that infusion of patients' CSF represents an experimental method for increasing the excitatory effects of rFr2 upon M1 area in conditioning paradigms. This represents the first demonstration that the projection from rFr2 to M1 is functionally dependent on NR1/NR2 heteromers of NMDA receptors and confirms that rFr2 plays a key role in shaping motor commands in a context of afferent sensory information.

An imbalance between AMPA (alpha-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate) and NMDA pathways is deleterious for the brain circuitry, as indicated by recent studies on AMPA/NMDA ratios [18]. In particular, it has been demonstrated that blockade of NMDA-R might stop AMPA-R endocytosis, and thus deregulate fast and slow glutamatergic

signalling [19]. We show here that blocking the extracellular epitopes of NR1/NR2 heteromers of the NMDA-R is associated with a sensitization to hyperexcitability. The current findings might provide some explanation for patients' symptoms such as episodes of anxiety, agitation and seizures that may occur at early stages or during the phase of recovery from anti-NMDA-R encephalitis. We speculate that abnormal activities of AMPA pathways might result from blockade of NMDA-R and contribute to these symptoms, which are suggestive of an overactivity of glutamatergic pathways. This glutamatergic hyperactivity could also be related to an inhibition of NMDA-R expressed on GABAergic interneurons [20]. Our data provide additional support for an antibody-mediated pathogenesis of anti-NMDA-R encephalitis [21].

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Fig. 1.

1 Experimental procedure for infusion of antibodies and application of trains of high-frequency stimulation (HFS) over rFr2. **A** the guide (*arrow*) is implanted in the skull. **B** illustration of the localization of the anode and cathode on the skull. *a* hot spot corresponding to the interosseus muscle in left forelimb; *b* hot spot corresponding to the interosseus muscle in left hindlimb. **C** brain extracted from the skull at the end of the experiment. The site of entry of the guide is visible in right premotor cortex (*arrow*). **D** cryosection with superimposition of the guide (*arrow*). *Inj* site of injection, *R* right side. **2** Superimposition of averaged motor evoked potentials (MEPs) obtained with no preceding peripheral stimulation (unconditioned responses, thin traces) and with a conditioning stimulus (thick trace). Duos of corticomotor reponses are shown for a control rat (injected with a control CSF; rat from group 2) and a rat injected with CSF positive for NMDA-R antibodies (rat from group 3). Averaged MEPS in left interosseus muscles (forelimbs and hindlimbs) are illustrated. **A** baseline state; **B** post-injection state; **C** post-HFS (high-frequency stimulation) state. Note the marked increase in the intensity of conditioned responses in forelimb after HFS for the rat injected with the NMDA-R antibodies (*arrow*)

Table 1

Amplitudes of corticomotor responses in left interosseus muscles (mean $\pm$ SD)

Gr.	Limb	Basal state		Post-injection		Post-HFS	
		No Cond.	Cond.	No Cond.	Cond.	No Cond.	Cond.
1	F1	1.000 $\pm$ 0.058	1.201 $\pm$ 0.066 ($\times$ 120.16%)	–	–	1.199 $\pm$ 0.066	1.561 $\pm$ 0.201 ($\times$ 130.23%)
	H1	1.000 $\pm$ 0.065	1.222 $\pm$ 0.160 ($\times$ 122.21%)	–	–	1.244 $\pm$ 0.193	1.644 $\pm$ 0.173 ($\times$ 132.10%)
2	F1	1.000 $\pm$ 0.092	1.201 $\pm$ 0.169 ($\times$ 120.09%)	1.004 $\pm$ 0.080	1.196 $\pm$ 0.169 ($\times$ 119.16%)	1.220 $\pm$ 0.165	1.589 $\pm$ 0.158 ($\times$ 130.18%)
	H1	1.000 $\pm$ 0.105	1.312 $\pm$ 0.235 ($\times$ 131.17%)	1.014 $\pm$ 0.075	1.266 $\pm$ 0.110 ($\times$ 124.78%)	1.271 $\pm$ 0.105	1.625 $\pm$ 0.160 ($\times$ 127.81%)
3	F1	1.000 $\pm$ 0.097	1.195 $\pm$ 0.114 ($\times$ 119.46%)	0.991 $\pm$ 0.077	1.233 $\pm$ 0.097 ($\times$ 124.42%)	1.418 $\pm$ 0.089	2.070 $\pm$ 0.135 ($\times$ 146.02%)*
	H1	1.000 $\pm$ 0.104	1.213 $\pm$ 0.108 ($\times$ 121.38%)	0.996 $\pm$ 0.081	1.235 $\pm$ 0.096 ($\times$ 123.96%)	1.444 $\pm$ 0.103	1.799 $\pm$ 0.139 ($\times$ 124.58%)*

Values in the basal state without conditioning were set at 100% in each group and served as a reference for subsequent measurements. Corticomotor responses were evoked by stimulation of the right motor cortex. Gr: Groups. 1: no injection, 2: injection of controls' CSF, 3: injection of NMDA-R antibodies-positive CSF. Limb: F1 corresponds to forelimb, H1 corresponds to hindlimbs. Post-injection: values refer to corticomotor responses after injection of CSF in rF2 on the right side. Post-HFS: values refer to corticomotor responses after application of trains of high-frequency stimulation (HFS) over rF2 on the right side. No Cond.: no conditioning stimulus; Cond.: a peripheral conditioning stimulus was applied. Values in brackets correspond to the mean ratio conditioned responses/unconditioned responses in each state (basal state, post-injection, post-HFS)

* Significant conditioning by limb effect in Group 3: values in forelimbs were significantly higher than values in hindlimbs after conditioning ($p < 0.001$)

Table 2Ratios Hmax/Mmax in left interosseus muscles (mean $\pm$ SD)

Gr.	Limb	Basal state *	Post-injection *	Post-HFS *
1	Fl	24.19 $\pm$ 2.00	–	24.16 $\pm$ 2.24
	HI	50.86 $\pm$ 5.63	–	50.59 $\pm$ 5.51
2	Fl	24.84 $\pm$ 1.92	24.63 $\pm$ 2.47	25.53 $\pm$ 2.08
	HI	51.81 $\pm$ 4.89	51.86 $\pm$ 5.13	49.82 $\pm$ 6.21
3	Fl	24.70 $\pm$ 2.53	25.94 $\pm$ 2.47	25.00 $\pm$ 2.88
	HI	48.37 $\pm$ 5.96	50.66 $\pm$ 5.92	50.00 $\pm$ 6.10

Values are expressed in % and correspond to the ratios Hmax/Mmax in left interosseus muscles in forelimb and hindlimb. Gr: Groups. (1) no injection, (2) injection of controls' CSF, (3) injection of NMDA-R antibodies-positive. CSF Limb: Fl corresponds to forelimb, HI corresponds to hindlimbs. Post-injection: values refer to corticomotor responses after injection of CSF in rFr2 on the right side. Post-HFS: Hmax/Mmax ratios were studied after application of high-frequency stimulation (HFS) over rFr2 on the right side

* Significant limb effect. In each group and in each state, values in hindlimbs were significantly higher than values in forelimbs