

Neuronal NOS expression in rat's Cuneate Nuclei following passive forelimb movements and median nerve stimulations

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ABSTRACT

Nitric oxide (NO) synthase (NOS) has been observed in the Cuneate Nuclei (CN), suggesting a role for NO in the modulation of their neurons' activity. The present study was undertaken to evaluate whether passive movement of forelimb as well as electric stimulation of medial nerve modulate the expression of neuronal isoform of NOS (nNOS) within CN.

The experiments were carried out on 21 male Wistar rats, by using two different protocols. In the first group of rats the median nerve was stimulated with high frequency trains (phasic stimulation) or at constant frequency (tonic stimulation); as a control, in the third group, no stimulus was delivered. Moreover, in the second group of rats, we imposed to the animal's left forepaw circular paths at a roughly constant speed (continuous movement), or rapid flexions and extensions of the wrist (sudden movement); as a control, in the third group, no movement was imposed. After the experimental session, free-floating frontal sections of medulla oblongata were processed for nNOS or glutamate (GLU) immunohistochemistry.

Phasic stimulation of the median nerve or sudden movements of the forelimb determines a significant decrement of the nNOS-positive neurons within the ipsilateral CN, whereas no effects were observed on GLU positive cells. We have also found a peculiar topographical distribution within IN of nNOS-positive neurons: positive cells were clustered at periphery of some "niches" having circular or elliptical form, with GLU positive cells at center.

Key words

Proprioception • Passive movement • Nerve stimulation • Nitric oxide • Glutamate • Immunohistochemistry

Introduction

It is well known that Cuneate Nuclei (CN), i.e. Main Cuneate Nucleus (MCN) and External Cuneate Nucleus (ECN), represent important relay structure involved in the transmission of sensory information directed to the thalamus and the cerebellum, respectively (for a review see Paxinos, 1995). Principal afferents are esteroceptive and proprioceptive messages coming from the neck, the anterior part of the

trunk and the ipsilateral forelimb (Mackie et al., 1998).

Several studies on synaptic communication have shown that nitric oxide (NO) acts in the CNS as a neuromodulator that, for its gaseous nature, cross nervous terminal by simple diffusion rather than for exocytosis (Prast and Philippu, 2001). NO, named "gasotransmitter" by Rui Wang (2002), is synthesized in the cytosol by calcium-dependent activation of nitric oxide synthase (NOS; Bredt et al., 1990).

So far, three NOS isoforms have been described: the inducible isoform (iNOS), whose activity is calcium-independent, the epithelial (eNOS) and the neuronal isoforms (nNOS), both of which depend on calcium (Dawson et al., 1991).

Immunohistochemical studies have showed the presence of nNOS in the cuneate nuclei (Valtschanoff et al., 1995; Wang et al., 2002; Baizer, 2009), suggesting a role for NO in the modulation of cuneate neuron activity. In fact, we observed that both spontaneous and NMDA-evoked activities of cuneate neurons were decreased by NO and that the inhibition of NO synthase, by application of L-NAME, instead, abolished the depressant effect induced by L-Arginine (Garifoli et al., 2006). We suggested a NO modulation of cuneate neurons and provide support for a physiologic role, not only in increasing the signal-to-noise ratio in the excited cells, but also in a form of surround inhibition.

The objective of the present study was to evaluate, in anesthetized adult rats, the effect of somatosensory inputs on the expression nNOS within CN. For this purpose, we have examined whether passive movements of forelimb as well as electric stimulation of medial nerve is capable of influencing the immunohistochemical expression of nNOS within MCN and/or ECN. In particular, we have verified whether the nNOS expression is modulated by continuous inputs from forelimb or only by sudden motor events. For this purpose, we compared the effects induced by continuous limb movements or by tonic electric stimulation of the median nerve with those elicited by rapid limb movements or phasic stimulation of the nerve. We also investigated the effects of forelimb activation on distribution within CN of glutamate (GLU) immunopositive neurons.

Methods

Animal preparation

The experiments were carried out on 21 male Wistar rats, weighing between 230 and 280 g, housed under a 12 hr light - 12 hr dark cycle and given food and water ad libitum. The animals were deeply anesthetized with urethane (1.2 g/kg i.p.) and mounted in a stereotaxic device; their body temperature was maintained at 37°C with a feed-back-controlled heating pad.

Median nerve stimulation

In 12 rats, after administration of local anesthetic (lidocaine), an incision was carried out along the proximal portion of the left forelimb, with the purpose to place in the median nerve bipolar stimulation electrodes (FHC inc., USA) connected to with a Grass stimulator (model SD9E) using square wave pulses. The median nerve was exposed at the volar side of the left forepaw, just proximal to the carpal ligament. Stimulus intensity ranged from 0.7 to 1.3 mA (mean value: 0.97 mA \pm 0.13); the stimulation strength was identified on the basis of capability of eliciting a slight noticeable twitch of the thumb and/or digits two and three (cfr. Jellema et al., 2004).

Although the median nerve was always stimulated with an average of 32,400 cathodal pulses (having a duration of 0.4 ms and intensity of 200 μ A) delivered in 90 min, two different stimulation protocols were used: in a first group of 4 rats, the nerve was activated with 30 ms trains (frequency: 400 Hz) delivered at intervals of 2 s for 90 min (phasic stimulation; Wan et al., 1992), whereas in a second group of 4 rats, the median nerve was stimulated with single cathodal pulses delivered every 167 ms for 90 min (tonic stimulation; Bosco et al., 1996). As a control, in a third group of 4 rats the stimulating electrode was inserted in the nerve but no stimulus was delivered.

Forelimb passive movement

Two different protocols were used. In 3 rats, the animal's left forepaw was attached to a computer controlled robot arm programmed to execute 2,700 circular paths at a roughly constant speed of one turn in 2 s for 90 min (continuous movement; Fig. 1A). In a second group of 3 animals, the left forepaw was attached to a computer controlled robot arm programmed to execute 2,700 sequences of fast flexions and extension of wrist, having an amplitude of 25°; a total duration of 250 ms and repeated at intervals of 2 s for 90 min (sudden movement; Fig. 1B). As a control, in a third group of 3 rats, the forelimb was connected for 90 min to the robot arm but no movement was imposed.

Histology and immunohistochemistry

After one hour from the movement or stimulation offset, the rats were perfused sequentially with 500 ml of 0.9% saline at 42°C, 500 ml of a 1.25% glutar-

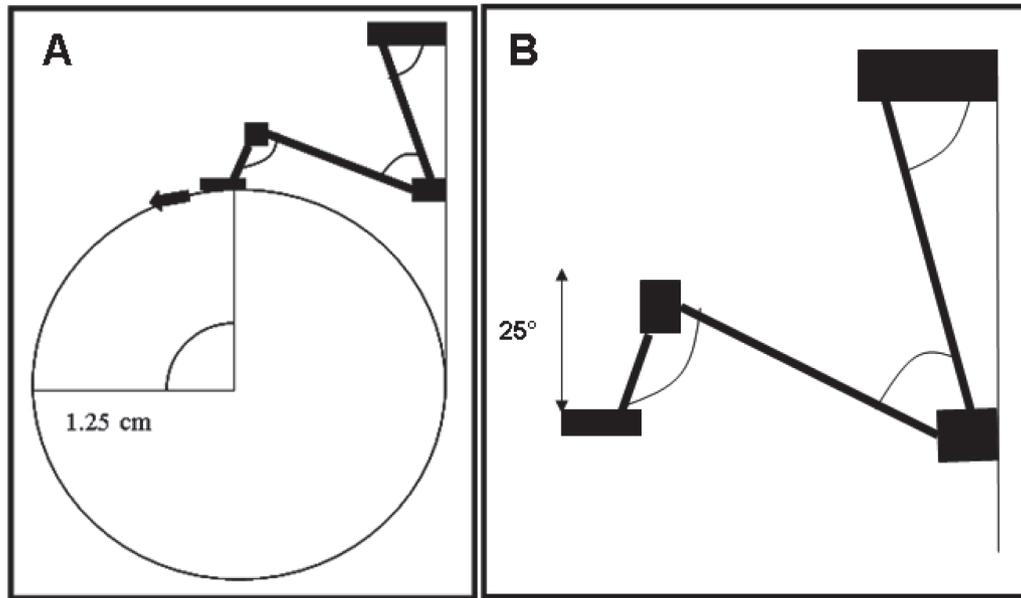


Fig. 1. - Rat forelimb kinematics during passive movements. (A) Stick diagram of the rat's forelimb with definition of the joint angles and circular trajectory of the rat's forepaw imposed during continuous movement (constant speed of one turn in 2 s for 90 min). (B) Stick diagram of the rat's forelimb with plots of shoulder, elbow and wrist angular trajectories obtained during experiments which imposed sequences of fast flexions and extension of wrist, having an amplitude of 25°; a total duration of 250 ms and repeated at intervals of 2 s for 90 min (sudden movements).

aldehyde-1% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) solution at room temperature and 500 ml of an ice-cold 10% sucrose solution in PB. The brains were removed and placed in the same 10% sucrose in PB solution at 4°C until sectioned. Free-floating frontal sections of brainstem cut at 30 µm on a freezing microtome were collected in 0.1 M pH 7.4 phosphate buffer. Out of three consecutive sections, one was used for nNOS immunohistochemistry, the second for GLU immunohistochemistry, whereas the third was counterstained with 1% neutral red (pH 4.8) for identification of cytoarchitectonic details.

Immunohistochemical procedures were carried out by the avidin-biotin complex (ABC) method, as previously described (Berretta et al., 1991). The immunohistochemical steps include: 1) 30 min preincubation at room temperature in normal goat serum (NGS) diluted 1:10 in phosphate-buffered saline at pH 7.4 (PBS); 2) two 5 min washes in PBS with 0.2% gelatine and 0.25% Triton X-100 (PBS + G + T); 3) 10 min rinse in PBS + G + T with 0.1% sodium azide (PBS + G + T + A); 4) overnight incubation at 4°C with a) polyclonal rabbit primary antibody for nNOS (Chemicon, Temecula, CA, USA) diluted 1:2500 in PBS + G + T + A or

b) polyclonal anti-GLU antiserum raised in rabbit (Sigma, St. Louis, MO, USA) diluted 1:1000 in PBS + G + T + A; 5) three 10 min washes in a solution with 0.05% triton X-100 in PBS (PBS + T); 6) 10 min rinse in PBS + G + T; 7) 2 h incubation at 4°C in biotinylated goat anti-rabbit IgG (Vector Laboratory Inc., Burlingame, CA) diluted in PBS + G + T + A (1:200); 8) three 10 min washes in PBS + T; 9) 10 min rinse in PBS + G + T; 10) 60 min incubation at room temperature in avidin-biotinylated horseradish peroxidase complex (Vecstain ABC kit PK-4000; Vector Laboratory Inc., Burlingame, CA); 11) three 10 min washes in PBS + T; 12) 10 min rinse in 0.05 M Tris Buffer, pH 7.6 (TB); 13) 5-25 min incubation at room temperature with 0.05% DAB in TB containing 0.002% H₂O₂; 14) two 5 min washes in TB; 15) 5 min rinse in PBS + G + T + A. The immunostained sections were mounted onto gelatin-coated slides and coverslipped with Entellan (Merk AG, Germany) to be examined by microscopy. Some of immunostained sections were stained with neutral red for study of cytoarchitecture, by using for reference the atlas of Paxinos and Watson (1998). Technical controls included the use of NGS instead of the first specific antiserum.

Quantitative analysis was carried out with bright-field microscopy (Axioplan; Carl Zeiss MicroImaging GmbH, Germany); the only cells that had significant levels of DAB reaction product tissue background levels were considered immunopositive and, therefore, counted. Thresholding procedure was based on an average gray level cut-off of 130 units (out of 255) when the labeled neurons were analyzed with a digital image analysis system (Image Biological System 200, IBS, Italy). The images acquired with a CCD video camera were transferred to a video monitor for computerized pseudocolor processing. The immunopositive cells were counted in all sections of each animal for both ipsi- and contralateral CN with respect to the stimulated forelimb, and the two highest counts per nucleus for each side were recorded and summed, so giving a single value per nucleus.

Statistical analysis

Statistical analysis was carried out by using the software package SYSTAT, version 11 (Systat Inc., Evaston, IL, USA). All data in text and figures are reported as mean \pm standard deviation; significance was set at $P < 0.05$. Data were collected and averaged, and then compared by using unpaired Student's t-test or one-way repeated measures ANOVA (Friedman

test) followed by the Dunn's Multiple Comparison Test. Statistical analysis was carried out according to guidelines for reporting statistics in journals published by the American Physiological Society (Curran-Everett and Benos, 2004).

Results

Morphological aspects

In sections of medulla oblongata immunostained for nNOS and GLU, we observed the presence of immunopositive cells in MCN as well as ECN, both in controls and in stimulated rats. In controls, a similar number of nNOS and GLU immunopositive neurons was present in both ECN and MCN (Fig. 2). By comparing the topographical distribution of nNOS and GLU immunopositive neurons, we observed that both immunopositive cells were present within multiple areas of both MCN and ECN, roughly oval in shape (niches). These areas displayed an uneven distribution of immunostained cells, with a relative lack of nNOS-positive cells at the center and an increased presence at the periphery (Fig. 3). On the contrary, GLU-positive neurons were concentrated at the center of the niche and decreased at the periphery (Fig. 4).

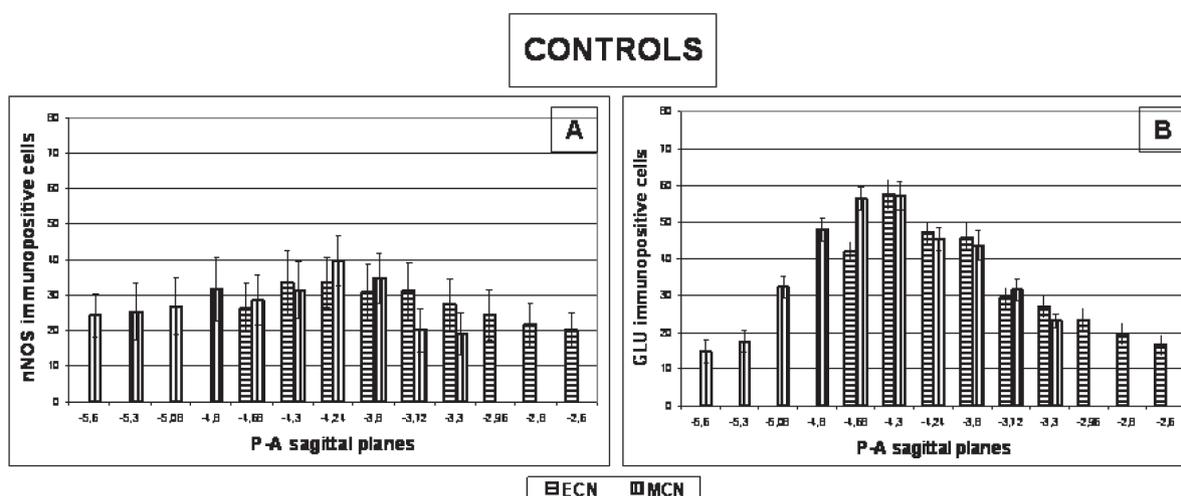


Fig. 2. - Mean number (\pm standard deviation) of neuronal NOS (nNOS) and glutamate (GLU) immunopositive neurons present in controls in the different postero-anterior (P-A) planes of Main Cuneate Nucleus (MCN) and External Cuneate Nucleus (ECN), according to the atlas of Paxinos and Watson (1998).

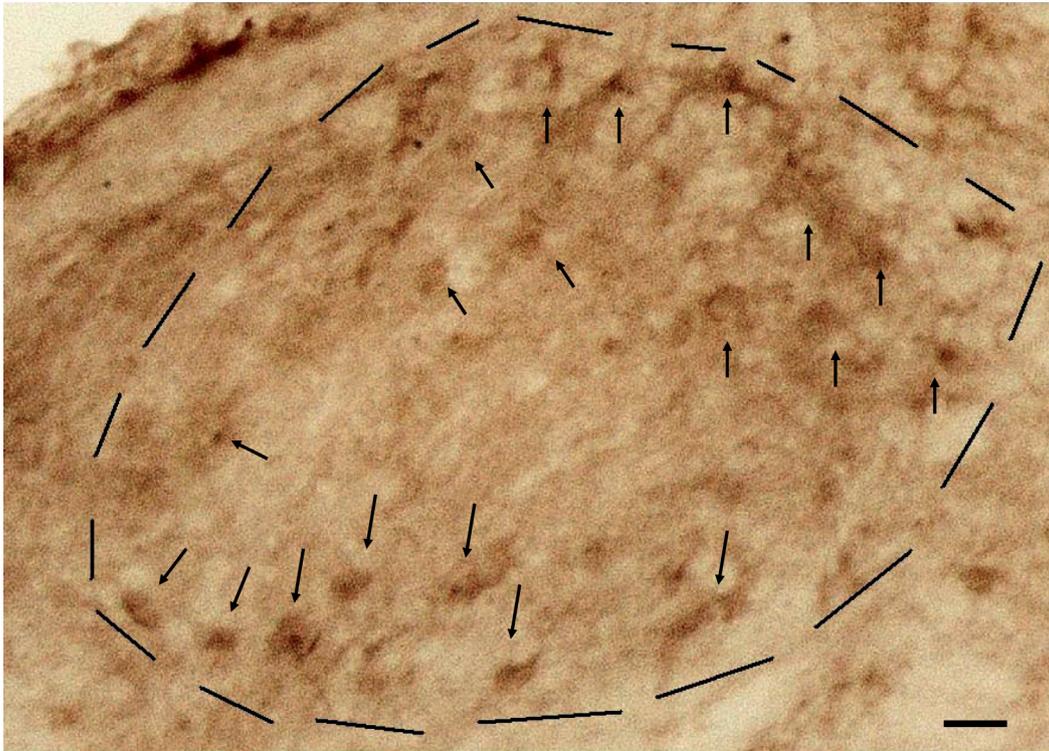


Fig. 3. - Microphotography showing topographical distribution of nNOS immunopositive neurons within the Cuneate Nuclei in a frontal section of a control rat. Immunopositive cells were observed within multiple areas of both MCN and ECN, roughly oval in shape (broken lines). These areas (niches) displayed an uneven distribution of nNOS immunostained cells (arrows) which were absent at the center of the niches. Calibration bar (bottom-right corner): 25 μ m. Abbreviations as in Fig. 2.

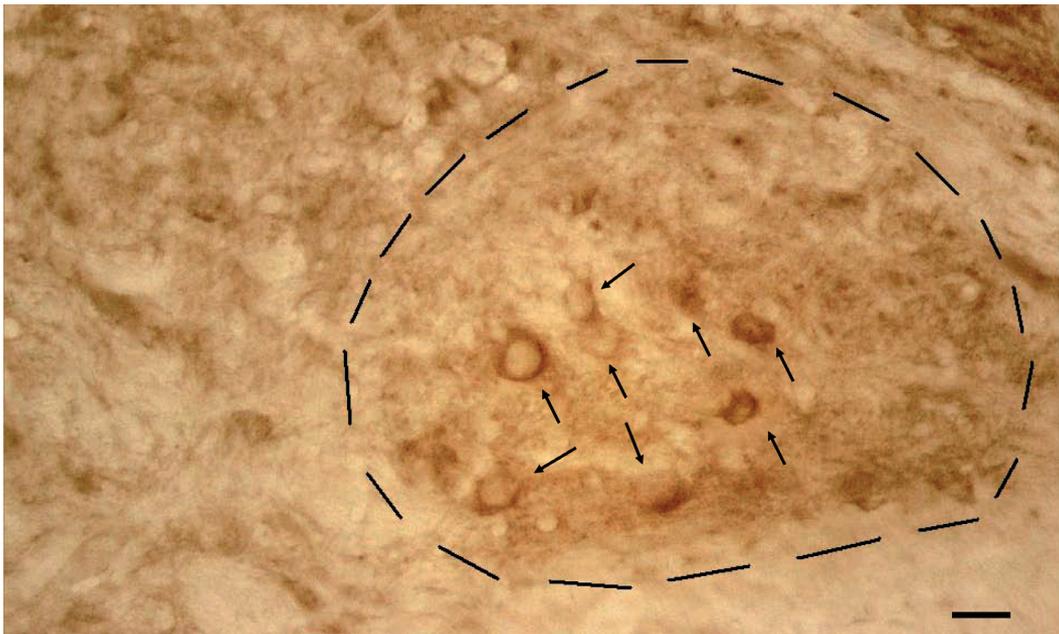


Fig. 4. - Microphotography showing topographical distribution of GLU immunopositive neurons within the Cuneate Nuclei in a frontal section of a control rat. GLU-positive neurons (arrows) were concentrated at the center of the niche (broken lines) and decreased at the periphery. Calibration bar (bottom-right corner): 25 μ m. Abbreviations as in Fig. 2.

Electric stimulation of median nerve

In rats submitted to the electric activation of median nerve, we observed that the phasic stimulation was capable of inducing within CN a significant decrement of nNOS immunopositive neurons, mainly ipsilaterally to the stimulated side (Fig. 5A).

The mean number of neurons present in each plane of CN was of 38.76 ± 3.55 in the ipsilateral side, and of 41.85 ± 3.84 in the contralateral sides, with respect to a mean value, in the controls, of 60.08 ± 3.90 and 60.59 ± 3.90 , respectively (Table I). The decrement of the nNOS-positive neurons following the phasic stimulation was slightly more evident in level of the ipsilateral MCN, with a reduction of 38.1% (mean number of neurons for plane: 19.3 ± 7.1) in comparison to a decrease of 32.5% in the ipsilateral ECN (26 ± 6.4). In the contralateral MCN we had a reduction of 27.6% (mean number of neurons for plane: 22.5 ± 6.9) versus a decrease of 11.9% in the contralateral ECN (32.5 ± 5.8). As can be seen from the figures, the reduction was present to a quite homogeneous extent in all planes.

The tonic stimulation of median nerve was not capable to induce significant reductions in amount of nNOS immunopositive neurons in bot ipsilateral and contralateral CN (Fig. 5B). Also in this case the reduction was evenly present in all planes, with

a mean number of neurons present in each plane of CN of 53.07 ± 3.68 in the ipsilateral side and of 60.96 ± 3.58 in the contralateral sides, without significant differences with respect to the controls (Table II).

Concerning the distribution of immunostained cells within the niches, with a relative lack of nNOS positive cells at the center and an increased presence at the periphery, easily visible in controls, it was significantly modified by electric stimulation of the median nerve. In fact, whereas the concentration of GLU-positive cells remained unmodified, that of nNOS-positive neurons decreased strongly after the forelimb activation, mainly on the ipsilateral side.

Forelimb passive movements

In rats submitted to fast flexo-extension movements of wrist (having an amplitude of 25° ; a total duration of 250) we observed that this sudden movement was able to induce a significant decrement of nNOS immunopositive neurons, mainly in the same side of the activated forelimb. In fact, we observed a significant reduction of nNOS immunopositive neurons ($P < 0.05$) in the ipsilateral CN and non significant changes in the contralateral nuclei, with respect to the controls (Fig. 6A). As can be seen, also in this experimental condition the reduction was evenly dis-

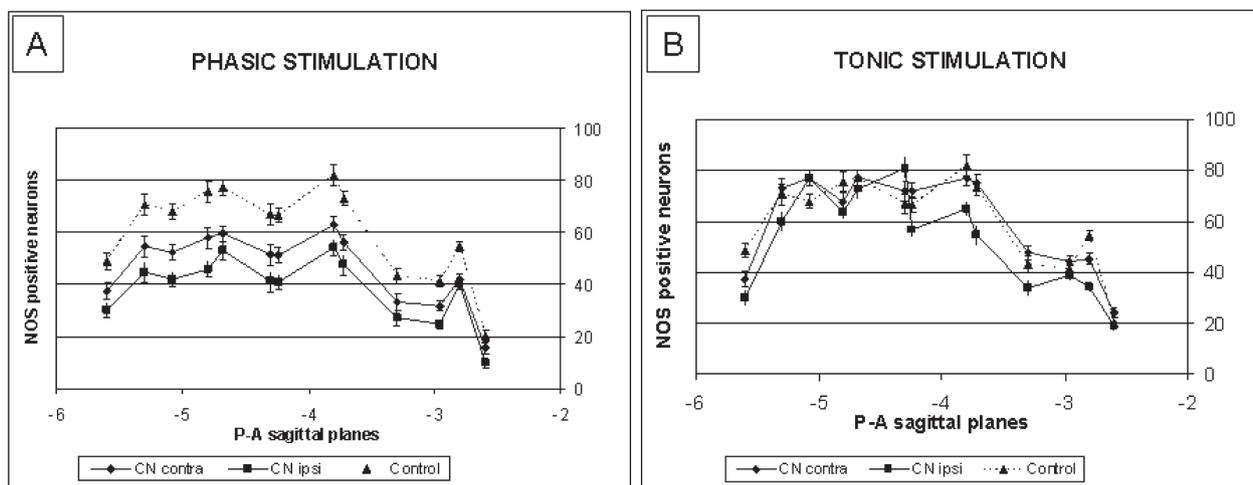


Fig. 5. - Effects induced by electric stimulation of median nerve. (A) Phasic stimulation: it was observed a significant decrement of nNOS immunopositive neurons within the Cuneate Nuclei (CN), with respect to the controls, mainly ipsilaterally to the stimulated forelimb. (B) Tonic stimulation: it was detected that a significant reduction of nNOS immunopositive neurons, with respect to the controls, mainly ipsilaterally to the stimulated forelimb. Abbreviations as in Fig. 2.

Table I. - Mean number of nNOS- and GLU immunopositive neurons (\pm standard deviation, S.D.) counted in right and left Cuneate Nuclei of rats submitted to PHASIC STIMULATION of median nerve in the left forelimb and in controls. In the table, there are also reported the results of One-way analysis of variance (ANOVA) of data, followed by Bonferroni's Multiple Comparison Test.

nNOS neurons	Right stimulated	Left stimulated	Right control	Left control
Mean \pm S.D.	41.85 \pm 3.84	38.76 \pm 3.55	60.59 \pm 3.90	60.08 \pm 3.90
ANOVA				
dx phasic vs. sx phasic	t = 1.151	P > 0.05		
dx phasic vs. dx control	t = 6.976	P < 0.001		
dx phasic vs. sx control	t = 6.785	P < 0.001		
sx phasic vs. dx control	t = 8.127	P < 0.001		
sx phasic vs. sx control	t = 7.936	P < 0.001		
dx control vs. sx control	t = 0.1910	P > 0.05		

GLU neurons	Right stimulated	Left stimulated	Right control	Left control
Mean \pm S.D.	87.44 \pm 7.35	91.45 \pm 8.45	89.34 \pm 6.89	90.81 \pm 8.02
ANOVA				
dx phasic vs. sx phasic	t = 0.764	P > 0.05		
dx phasic vs. dx control	t = 0.3489	P > 0.05		
dx phasic vs. sx control	t = 0.6189	P > 0.05		
sx phasic vs. dx control	t = 0.3875	P > 0.05		
sx phasic vs. sx control	t = 0.1175	P > 0.05		
dx control vs. sx control	t = 0.2700	P > 0.05		

Table II. - Mean number of nNOS- and GLU immunopositive neurons (\pm standard deviation, S.D.) counted in right and left Cuneate Nuclei of rats submitted to TONIC STIMULATION of median nerve in the left forelimb and in controls. In the table, there are also reported the results of One-way analysis of variance (ANOVA) of data, followed by Bonferroni's Multiple Comparison Test.

nNOS neurons	Right stimulated	Left stimulated	Right control	Left control
Mean \pm S.D.	60.96 \pm 3.58	53.07 \pm 3.68	60.59 \pm 3.90	60.08 \pm 3.90
ANOVA				
dx tonic vs. sx tonic	t = 2.962	P > 0.05		
dx tonic vs. dx control	t = 0.1395	P > 0.05		
dx tonic vs. sx control	t = 0.3320	P > 0.05		
sx tonic vs. dx control	t = 2.823	P > 0.05		
sx tonic vs. sx control	t = 2.630	P > 0.05		
dx control vs. sx control	t = 0.1926	P > 0.05		

GLU neurons	Right stimulated	Left stimulated	Right control	Left control
Mean \pm S.D.	91.25 \pm 8.98	90.64 \pm 8.33	89.34 \pm 6.89	90.81 \pm 8.02
ANOVA				
dx tonic vs. sx tonic	t = 0.1066	P > 0.05		
dx tonic vs. dx control	t = 0.3339	P > 0.05		
dx tonic vs. sx control	t = 0.0769	P > 0.05		
sx tonic vs. dx control	t = 0.0072	P > 0.05		
sx tonic vs. sx control	t = 0.0297	P > 0.05		
dx control vs. sx control	t = 0.2570	P > 0.05		

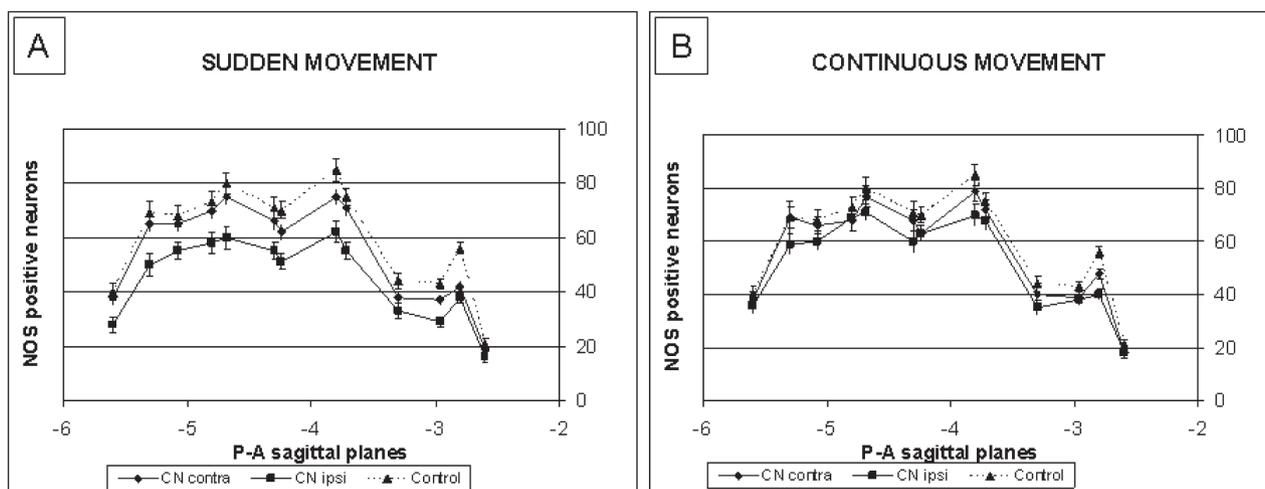


Fig. 6. - Effects induced by passive movements of the forelimb. (A) Sudden movements: it was observed that a significant decrement of nNOS immunopositive neurons, with respect to the controls, only ipsilaterally to the stimulated forelimb. (B) Continuous movements: it was detected that a decrement of nNOS immunopositive neurons, with respect to the controls, only ipsilaterally to the stimulated forelimb. Abbreviations as in Fig. 2.

tributed in all planes, with a mean number of neurons present in each plane of CN was of 45.83 ± 3.67 in the ipsilateral side and of 56.17 ± 3.74 in the contralateral sides, with respect to the controls (Table III). Continuous movements of the wrist caused non significant decreases of nNOS immunopositive neurons

both in ipsilateral and contralateral CN with respect to the stimulated forelimb (Fig. 6B). The mean number of neurons present in each plane of CN of 53.23 ± 3.51 in the ipsilateral side and of 57.80 ± 4.03 in the contralateral sides, values similar to those of the controls (Table IV).

Table III. - Mean number of nNOS- and GLU immunopositive neurons (\pm standard deviation, S.D.) in right and left Cuneate Nuclei of rats submitted to SUDDEN MOVEMENTS of left forelimb and in controls. In the table, there are also reported the results of One-way analysis of variance (ANOVA) of data, followed by Bonferroni's Multiple Comparison Test.

nNOS neurons	Right stimulated	Left stimulated	Right control	Left control
Mean \pm S.D.	56.17 ± 3.74	45.83 ± 3.67	62.75 ± 3.38	62.04 ± 3.38
ANOVA				
dx sudden vs. sx sudden	$t = 3.566$	$P < 0.05$		
dx sudden vs. dx control	$t = 2.271$	$P > 0.05$		
dx sudden vs. sx control	$t = 2.024$	$P > 0.05$		
sx sudden vs. dx control	$t = 5.837$	$P < 0.01$		
sx sudden vs. sx control	$t = 5.591$	$P < 0.01$		
dx control vs. sx control	$t = 0.2468$	$P > 0.05$		

GLU neurons	Right stimulated	Left stimulated	Right control	Left control
Mean \pm S.D.	90.33 ± 8.87	88.89 ± 8.83	91.44 ± 8.28	91.16 ± 3.43
ANOVA				
dx sudden vs. sx sudden	$t = 0.2049$	$P > 0.05$		
dx sudden vs. dx control	$t = 0.1580$	$P > 0.05$		
dx sudden vs. sx control	$t = 0.1181$	$P > 0.05$		
sx sudden vs. dx control	$t = 0.3629$	$P > 0.05$		
sx sudden vs. sx control	$t = 0.3230$	$P > 0.05$		
dx control vs. sx control	$t = 0.0399$	$P > 0.05$		

Table IV. - Mean number of nNOS- and GLU immunopositive neurons (\pm standard deviation, S.D.) in right and left Cuneate Nuclei of rats submitted to CONTINUOUS MOVEMENTS of left forelimb and in controls. In the table, there are also reported the results of One-way analysis of variance (ANOVA) of data, followed by Bonferroni's Multiple Comparison Test.

nNOS neurons	Right stimulated	Left stimulated	Right control	Left control
Mean \pm S.D.	57.80 \pm 4.03	53.23 \pm 3.51	62.75 \pm 3.38	62.04 \pm 3.38
ANOVA				
dx cont vs. sx cont	$t = 1.560$	$P > 0.05$		
dx cont vs. dx control	$t = 1.691$	$P > 0.05$		
dx cont vs. sx control	$t = 1.447$	$P > 0.05$		
sx cont vs. dx control	$t = 3.251$	$P > 0.05$		
sx cont vs. sx control	$t = 3.007$	$P > 0.05$		
dx control vs. sx control	$t = 0.2442$	$P > 0.05$		

GLU neurons	Right stimulated	Left stimulated	Right control	Left control
Mean \pm S.D.	90.98 \pm 8.73	92.77 \pm 8.57	91.44 \pm 8.28	91.16 \pm 8.43
ANOVA				
dx cont vs. sx cont	$t = 0.2578$	$P > 0.05$		
dx cont vs. dx control	$t = 0.0663$	$P > 0.05$		
dx cont vs. sx control	$t = 0.0259$	$P > 0.05$		
sx cont vs. dx control	$t = 0.1915$	$P > 0.05$		
sx cont vs. sx control	$t = 0.2319$	$P > 0.05$		
dx control vs. sx control	$t = 0.0403$	$P > 0.05$		

In relation to the distribution of immunostained cells in the niches, we observed reductions of nNOS-positive neurons following natural activation of the forelimb, on the ipsilateral side, whereas the number of GLU-positive cells remained unmodified.

Discussion

The main result of the present study is that the phasic stimulation of the median nerve or sudden passive movements of the forelimb determine a decrement of the nNOS-positive neurons at level of cuneate nuclear complex, and in particular within ECN; the reduction was statistically significant only ipsilaterally to the activated side (Fig. 7). Therefore, somatosensory inputs coming from the forelimb succeed to inhibit the expression of the nNOS at level of structures that give rise to the cuneocerebellar projections.

A peculiar topographical distribution of the nNOS-positive neurons was also founded: immuno-positive neurons were clustered to form some "niches" of circular or elliptical form along the CN, with the nNOS-positive cells at periphery and those

GLU-positive at the center (Figs. 3 and 4). This particular disposition of the nNOS-positive neurons appears to be coherent with the well known cells' distribution inside the CN (Sanchez et al., 1999). The median regions of the CN basically includes two types of neurons: a) glutamatergic projection cells that send their axons mainly to the thalamus, through the medial lemniscus, and to the cerebellum via the cuneocerebellar bundle; b) local inhibitory cells that regulate the projection cells. Both types of neurons receive somatosensory inputs and modulate the esteroceptive transmission towards more rostral centers (Barbaresi et al., 1986).

The projection cells are arranged in nests that receive monosynaptic contacts deriving almost exclusively from the fibers of a single nerve (Sanchez et al., 1999). The local interneurons, of smaller dimensions, are only partially intermixed to the projection cells, forming a sort of shell around this last ones. It has been shown that whereas the projection cells are mainly glutamatergic, local modulation neurons are instead GABAergic (Barbaresi et al., 1986); the nNOS seems represented mainly in these preceding cells (Wang et al., 2001). In fact, various researches confirm that in the cuneate cells the NOS has been found co-local-

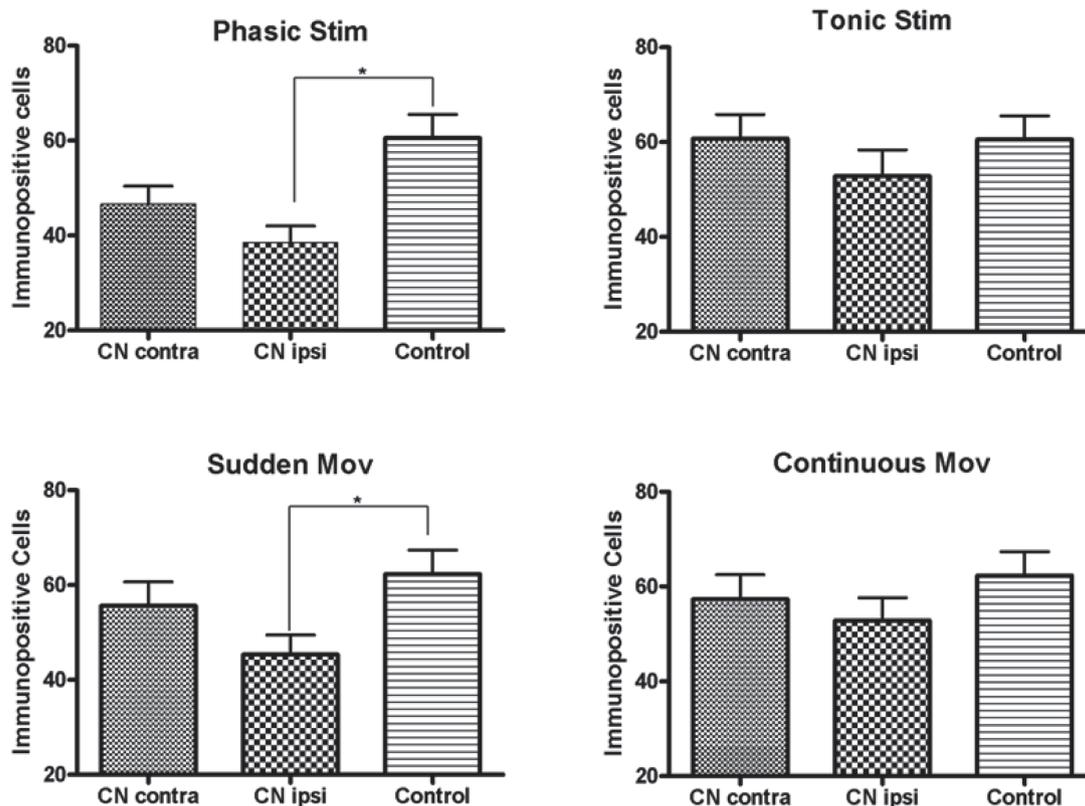


Fig. 7. - Summary of the results obtained in the present study. In the first row the effects induced by electric stimulation of median nerve, whereas on the second row those induced by passive movement of the forelimb are shown. It has been observed that only phasic stimulation of the median nerve or sudden movements of the forelimb were capable of inducing significant decreases of the number of nNOS immunopositive neurons within both divisions of the Cuneate Nuclei (CN). Furthermore, the reduction of nNOS immunopositive cells reached a significant amount only ipsilaterally to the activated forelimb. Abbreviations as in Fig. 2.

ized together with the GABA (Valtschanoff et al., 1995), suggesting a possible synergic inhibitory role, inside the cuneate local circuits, of neurotransmitter GABA and gaseous neuromodulator NO.

It can be suggested that the primary afferents can exert on the cuneate projection neurons a biphasic effect through the activation of both not-NMDA (AMPA and Kainate) and NMDA receptors. The not-NMDA component is faster by and large, but not exclusively, due to the AMPA receptors, whereas the NMDA component appears to be slower and more modulated (Soto et al., 2004). The same Authors suggest that GABAergic cells, which express nNOS, could be inhibited by high frequency afferent volleys through the involvement of glycinergic interneurons and of both NMDA and non-NMDA receptors (Soto et al., 2004).

It is, therefore, possible that an high frequency volley, as that generated by prompt stimulation of a receptive

field, excites monosynaptically the cuneate projection cells through NMDA and non-NMDA receptors and, at the same time, annuls the lateral inhibition induced by GABAergic interneurons expressing nNOS.

In this way, the activation of a receptive field would increase the activity of a cluster of cuneocerebellar cells either for direct excitatory synaptic action of afferent fibers and for a concomitant reduction of the activity in local inhibitory interneurons. Furthermore, the higher activity of nitrergic interneuron on cuneate inactive cells, around the subset of activated neurons, it could represent a form of "surround inhibition mechanism" at this level, useful to focus on neuronal activity and to select neuronal responses (Sohn & Hallet, 2004; Soto et al., 2004; Garifoli et al., 2006). Thus, during a forelimb movement, the proprioceptive signals could be parceled and processed into separate neuronal channels, in the second-order sensory neurons, and the nitrergic

system could contribute to progressively reduce the multiplicity of sensory information into a stereotyped representation of arm movements (Garifoli et al., 2002). In fact, in the rat, forelimb passive movements induced characteristic response patterns of neurons belonging to the ECN (Garifoli et al., 2002). This discharge pattern codified data related to the kinematics of limb, i.e. direction and speed, so furnishing the cerebellum with more integrated information.

We conclude that a significant elaboration of somatosensory afferent inputs happens already at a level of the first sensory station; in this way, cuneate projection neurons would furnish the more rostral centers, not a simple copy of the somatosensory input, but information elaborated by integrating more afferent inputs. In this contest, the reduction of the nNOS expression within specific "niches" of CN, decreases the formation of the inhibitory gasotransmitter NO, so activating specific clusters of CN neurons. This mechanism would contribute to select specific CN output channels, by maintaining reduced the activity of neurons non involved in transmission of somatosensory inputs.

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