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Fos immunoreactivity in the intermediolateral nucleus induced by tendon vibration of the *m. triceps surae* in rats pretreated with a nitric oxide blocker or precursor

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We investigated neuronal activation of the rat intermediolateral (IML) nucleus of the thoracolumbar spinal cord, initiated by Achilles tendon vibration, after intramuscular (*m. triceps surae*) administration of 7-nitroindazole (7-NI) or L-arginine (LA). The spindle afferent response to vibratory stimuli induced a distinct bilateral increase in the activation of c-Fos immunoreactivity in the spinal neurons in three groups of rats (tendon-vibrated, tendon-vibrated + 7-NI and tendon-vibrated + LA). The T5/T13 segments in tendon-vibrated +7-NI animals showed the highest increase of Fos-immunoreactive neurons. This increase was two times higher than that in tendon only-vibrated control rats and tendon-vibrated + LA animals. The highest mean number of labelled neurons were observed in the IML nucleus and in layers 4 and 7 of the T5-L3 segments in tendon-vibrated and tendon-vibrated + 7-NI animals, and in the IML nucleus and layer 4 in tendon-vibrated + LA rats. The highest mean number of activated neurons was found ipsilaterally in the IML nucleus of the T5/T13 segment. These results indicate that decreased nitric oxide release after injection of 7-NI was accompanied by a potentiation of the early c-fos gene expression induced by muscle proprioceptive activity within the thoracolumbar region of the rat spinal cord. Thus, enhanced c-Fos immunoreactivity in the IML nucleus indicated that the sympathetic nervous system can exert a direct influence on the muscle spindles.

Key words: intermediolateral nucleus, tendon vibration, c-Fos expression, 7-nitroindazole, L-arginine, rat

INTRODUCTION

Proprioceptors of skeletal muscles, particularly the receptors of intrafusal spindles, are important sources of signals to the segmental systems of motor control. Such signals initiate the generation of intraspinal and suprasegmental commands directed towards postural and locomotion regulation (Cordo et al. 2011, Proske et al. 2000, Windhorst 2007). Muscle spindles play important roles in various motor behaviours, and modulations of the sympathetic outflow to muscle spindles can change rhythmic movement patterns (Roatta et al. 2005). It is known that a direct sympathetic regulation of the muscle spindle occurs in parallel with the fusimotor regulation of the muscle spindle. Previous studies (Grassi et al. 1993, Passatore et al. 1985, Roatta et al. 2002) have shown that sympathetic stimulation during a muscle stretch can reduce the dynamic and static components of the stretch reflex response in rabbit and rat jaw muscles, indicating that an increase in sympathetic outflow may depress the feedback control of the muscle reflex system. Consequently, the motor reflex functions, coordination, and proprioception, could be influenced by

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sympathetic modulation (Roatta et al. 2002). The sympathetic preganglionic neurons (SPNs) have specific shape and size of the somas and nuclei, which differ from other interneurons within lateral horns of thoracic and lumbar spinal cord. SPN somas are situated in distinct clusters within thoracic and upper lumbar spinal segments (Petras and Cummings 1972, Zimmerman and Hochman 2010). The dendrites of SPNs are mainly oriented rostrocaudally within the lateral funiculus and to a lesser extent medially within the grey matter towards the area surrounding the central canal in lamina X, thus forming a ladder-like, symmetric distribution around the central canal (Anderson et al. 1989, Sah and McLachlan 1995). Spinal cord SPNs integrate activity from descending and sensory systems to determine the final central output of the sympathetic nervous system, and the intermediolateral (IML) in the grey matter of the spinal cord has the highest number and density of SPNs (Rando et al. 1981, Zimmerman and Hochman 2010).

The nitrergic system, providing synthesis of nitric oxide (NO), is an important modulatory system in the spinal cord. NO synthase (NOS) is localized in the sarcolemma of both the extrafusal and intrafusal fibres, particularly in the bag intrafusal fibres of the fast-twitch muscle fibres (Grozdanovic and Baumgarten 1999), the proprioceptive group Ia afferents (Maršala et al. 2006), and the postsynaptic region of neuromuscular junctions (Rothe et al. 2005). Endogenous NO could be involved in an increase or decrease in the strength of skeletal muscle contractility (Andrade et al. 1998, Eu et al. 2003). Inhibition of NOS in limb muscle was accompanied with a decrease in the force of twitch contractions, demonstrating NO-dependent muscle force production (King-Vanvlack et al. 1995, Maréchal et al. 1998). The location of neurons containing neuronal NO synthase (nNOS) in spinal structures is one of the most important characteristics of spinal centres involved in the control of proprioceptive and nociceptive activities (Man'kovskaya et al. 2014, Pilyavskii et al. 2001, Saito et al. 1994).

In rats, descending projections from the sensorimotor cortex and various brainstem centers are known to spread down to layers 4–6 of the grey matter and also to dorsal and ventromedial parts of the intermediate zone (lamina 7) (Kuypers 1982). Such descending projections can modulate the activity of spinal interneurons, which is a key factor in the control of motor activity in animals. Elimination or modulation of the influence different descending pathway results in changes in the balance between excitatory and inhibitory influences of peripheral afferents in spinal neuronal networks and in disorders of interaction between various muscle groups; this is manifested in disturbances of posture and movements (Musienko et al. 2011). It has been suggested that modulation of the level of NO production by its blocker or precursor should, in some way, affect the activity of the neuronal networks that control motor phenomena. Recently, we observed (Pilyavskii et al. 2012) that the preliminary systemic application of 7-nitroindazole (7-NI) in rats subjected to vibrational stimulation of the tendon of the *gastrocnemius-soleus* muscle results increases the number of Fos-immunoreactive (Fos-ir) interneurons and motoneurons within the lower lumbar spinal segments related with afferents projection of these muscles. Injection of 7-NI did not induce significant changes in the level of c-Fos immunoreactivity in the rat spinal cord. However, after 7-NI application, the intensified expression of c-Fos protein (induced by influences from low-threshold muscle afferents) was further increased. The preliminary systemic application of reserpine (an irreversible suppressor of vesicular monoamine transporter) subjected to vibrational stimulation of the Achilles tendon of the *m. triceps surae* in rats also increased the level of c-Fos immunoreactivity in interneurons and motoneurons in rats within the lumbar spinal segments (Maznychenko 2014). Remarkably, in the present study, a high level of c-Fos expression (after reserpine injection) was revealed in the IML nucleus of the lumbar spinal cord. This data on increase in the number of Fos-ir neurons in SPNs in the IML spinal nuclei of reserpinized rats show that preganglionic sympathetic activity was intensified under conditions of our experiments. Although these changes in the intensification of *c-fos* gene expression in the activity of spinal neuronal populations induced by the intense stimulation of segmental peripheral afferents were examined, the neuronal processing of proprioceptive information, particularly from the muscle spindle afferents is less clear.

The purpose of the present study was to examine the spatial and quantitative distribution of Fos-ir neuron in the IML nucleus as well as inter- and motoneurons within the thoracic and upper lumbar spinal cord, initiated by tendon vibration of the *m. triceps surae* in control rats and animals preliminarily injected with a selective blocker of nNOS – 7-NI or precursor for the synthesis of NO – L-arginine (LA).

METHODS

Experimental groups and stimulation protocol

Male Wistar rats, weighing 280–320 g, were used in the present study. The animals were purchased from a state-controlled experimental animal supplier through the common animal facility of Bogomoletz Institute of Physiology (Kyiv). The use of the animals was approved

by the Ethics Committee of the Institute and performed in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

All animals were divided into the following 8 groups: 1st group – intact animals (n=4); 2nd group – sham-vibrated (animals in which the Achilles tendon contacted the head of a vibrator, but no vibrational stimulation was applied, n=4); 3rd group – vehicle-injected (rats with intramuscular (i.m.) injections of dimethylsulfoxide (DMSO) solution, n=4); 4th group – 7-NI-injected (n=4); 5th group – LA-injected (n=4); 6th group – tendon-vibrated (n=4); 7th group – 7-NI-injected + tendon-vibrated (n=4); and 8th group – LA-injected + tendon-vibrated (n=4). The animals in all groups were anaesthetized with chloral hydrate (400 mg/kg, i.p., Sigma, USA). Thereafter, the rats in groups 6, 7 and 8 were gently fixed to the platform by gauze tie-strings. The left hindlimb remained horizontal with the natural ankle angle. The head of the vibrator was placed on the Achilles tendon, and the skin above this area was anaesthetized with lidocaine (intracutaneous injection of 0.1 ml, 2%). A high frequency (100 s^{-1}) sinusoidal vibration was used (series of 1 mm peak-to-peak amplitude, 1 min application and 1 min intervals for 30 min of exposure) (De-Doncker at al. 2003). The applied vibration parameters did not induce any visible movements of the hindlimb. DMSO aqueous solution and 7-NI (5 mg/kg, Sigma, USA) or LA (20 mg/kg, Sigma, USA) dissolved in DMSO (15% final DMSO concentration in 0.5 ml of distilled water) were injected into the left *m. triceps surae* of the animals in groups 3, 4 and 5. DMSO, 7-NI or LA was injected into 4 sites along the muscle. After injection, the needle remained in place for 1 min to prevent the agent from leaking into subcutaneous tissues. The animals in groups 7 and 8 were subjected to tendon vibration 30 min after the preliminary i.m. injection of 7-NI or LA, respectively. It should be noted, although unilateral application of these agents was used, a systemic effect of such injections take place in the present experimental model, because the post injection time was sufficient for bilateral changes in the level of c-Fos expression in the grey matter of spinal cord. For monitoring the blood pressure, a catheter was inserted into the common carotid artery; arterial blood pressure was measured with a pressure transducer. Notably, during the experiments, the mean arterial pressure did not change.

Fos immunohistochemistry

All studied animals were deeply anaesthetized with sodium pentobarbital (75 mg/kg, i.p., Nembutal, USA) and perfused (2 h after the end of the Achilles tendon vibration (groups 6–8) or injections (groups 3–5)) through the ascending aorta with 0.9% physiological saline (500 ml), followed by fixative solution (500 ml) containing 4% paraformaldehyde in 0.1 M of phosphate buffer (PB) (pH 7.4). Blocks of the thoracic and lumbar spinal cord were quickly removed, post-fixed overnight in the same fixative and cryoprotected in phosphate-buffered sucrose at 4°C for 48 h. Frozen sections of the frontal spinal cord (40 µm thick) were cut. Ten serial sections from each thoracic and lumbar segment of spinal cord were collected in wells containing cold phosphate-buffered saline (PBS) (0.1 M of PB containing 0.9% NaCl, pH 7.4), for subsequent immunohistochemical processing. Fos-immunoreactive (Fos-ir) spinal neurons were detected according to a standard avidin-biotin-peroxidase technique (Hsu at al. 1981) using a rabbit polyclonal antibody directed against c-Fos protein (1:2000, Ab-5, PC38, Oncogene Research, USA) and a commercial kit (ABC Kit, PK 4001, Vector Laboratories, USA). Briefly, following several rinses in PBS, the sections were placed in PBS containing 3% normal goat serum (Sigma, USA) and 0.3% Triton X-100 (Sigma, USA) for 30 min at room temperature. Free-floating sections were subsequently incubated for 48 h at 4°C in primary antiserum against c-Fos protein diluted (1:4000) in PBS containing 3% normal goat serum and 0.4% Triton X-100. The sections were then incubated in biotinylated goat anti-rabbit immunoglobulin G (1:200) and avidin-biotin complex (ABC) using a standard protocol. Fos-ir nuclei were visualized with nickel-intensified 3,3′- diamino-benzidine tetrahydrochloride (DAB, Sigma, USA). Sections were incubated in 0.05 M of Tris-buffer (pH 7.4) containing 0.035% DAB, 0.2% nickel ammonium sulphate and 0.005% hydrogen peroxide for 10 min at room temperature to produce a purple-black reaction product, washed in PBS and subsequently mounted into gelatine-subbed slides, air-dried, cleared in xylene and finally cover-slipped.

Data analysis

The Fos-ir labelled neurons in the layers of the spinal grey matter were detected as black nuclei and calculated using an optic microscope at magnifications of ×250 and ×400; their localization was identified according to a rat brain atlas (Paxinos and Watson 1997). The mean number ± S.E.M. of Fos-ir neurons per section was calculated in layers 1–10 of the spinal grey matter on both sides of the thoracic (T5–T13) and lumbar (L1–L3) spinal segments in the rats of all groups. The potential double-count errors of the same cell in the neighbouring section were corrected using the Abercrombie equation (Abercrombie 1946). Patterns of the

quantitative distribution of Fos-ir neurons in the grey matter under the same conditions were similar in some segments of the thoracic (from T5 to T13) and lumbar (L2 and L3) spinal cords. To optimize presentation of the data, the values of these segments were merged. The data were subjected to three-way statistical analysis of variance (ANOVA) and displayed graphically. The factors of variation included three conditions: groups of animals, spinal segments (T5/T13, L1 and L2/L3) and contra- and ipsilateral sides. To estimate integrated data (activated Fos-ir neurons in different layers within T5–L3 on ipsi- and contralateral sides together) obtained from rats groups 6–8, the one-way ANOVA was additionally used. Values with $P<0.05$ were considered to be significant. Newman-Keuls *post hoc* analysis was used when a significant difference was detected.

RESULTS

Immunohistochemical detection of the activated Fos-ir neurons within thoracic and lumbar spinal cord slices was performed in eight groups of animals. In the intact and sham-vibrated rats, the basal level of c-Fos expression on both sides of the thoracic and upper lumbar spinal cord was low (approximately 4–6 Fos-ir neurons per 40-µm-thick section). The mean numbers of Fos-ir neurons in the above spinal segments of the rats in groups 1 and 2 were practically similar, suggesting that chloral hydrate anaesthesia does not considerably influence the expression of the early gene *c-fos* in the absence of some special afferent and/or modulatory influences on neuronal networks of the above mentioned spinal levels. In the next three groups of studied animals

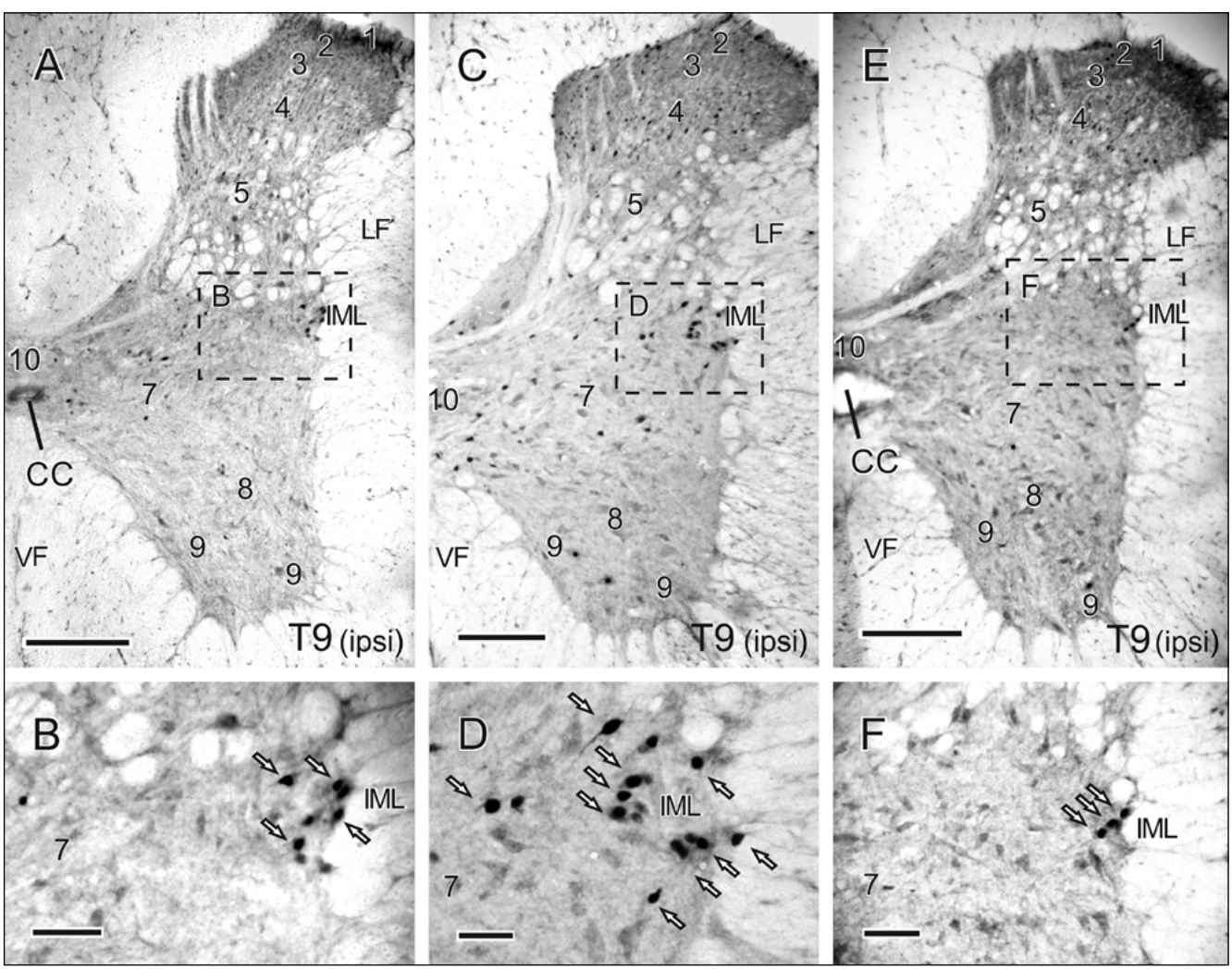

Fig. 1. Photomicrographs of frontal sections of the thoracic (T9) segment of rat spinal cord (ipsilateral side) after vibrational stimulation of the Achilles tendon (A, B), i.m. injection of 7-nitroindazole + tendon vibration (C, D) and i.m. injection of L-arginine + tendon vibration (E, F). Rectangle regions limited by dashed lines in (A, C and D) are shown in (B, D and F) at greater magnification. Structures: (1–10) layers of grey matter; (CC) central canal; (IML) intermediolateral nucleus; (LF and VF) lateral and ventral funiculi, respectively. Labelled nuclei of Fos-ir neurons are shown by arrows. Scale bar= 200 µm for A, C, E and 50 µm for B, D, F.

(vehicle-injected, 7-NI-injected and LA-injected) the level of the Fos immunoreactivity in the spinal segments was slightly higher than that in the spinal segments in group 1 and 2 animals. In these rats, the number of Fos-ir neurons bilaterally increased in the thoracic and lumbar segments in response to injection-related pain. For example, in the T5/T13, L1 and L2/L3 segments of the vehicle-injected animals, the mean number of labelled neurons unilaterally increased to 5.3±0.7, 5.1±0.4 and 4.5±0.6 cells, respectively. The distribution patterns of the activated cells in the spinal cords of these rats were also similar. It should be noted that there was no significant difference in mean numbers of Fos-ir neurons for T5/T13, L1 and L2/L3 levels of the spinal cord within the same animal group, as well as between groups (vehicle-injected, 7-NI-injected and LA-injected).

In the rats of group 6, vibrational stimulation of the Achilles tendon induced a clear increase in the activation of spinal Fos-ir neurons in different layers of the grey matter on both ipsi- and contralateral slice halves with respect to the side of stimulation. The greatest number of labelled cells was detected in layers 4 and 7 and in the IML nucleus of the studied segments (Figs 1A, B, 2A, B). For example, in these sites (ipsilaterally) was detected 5.5±0.4, 2.3±0.2 and 3.5±0.4 units in T5/T13, 8.2±1.0, 3.1±0.4 and 3.3±0.3 labelled cells in L1, 8.1±1.1, 3.2±0.5 and 2.1±0.4 activated neurons in L2/L3, respectively (Figs 3A–C). A notable number of labelled motoneurons were observed in layer 9 of these segments (0.5±0.2, 1.8±0.4 and 1.6±0.3 units in T5/T13, L1 and L2/L3 segments, respectively). In the other layers, a small number of Fos-ir neurons was also found (Figs 3A–C).

The vibration of the *m. triceps surae* tendon in the rats of group 7 performed at 30 min after preliminary injections of 7-NI provided higher intensity of Fos immunoreactivity in the thoracic and upper lumbar spinal segments (see Figs 1C, D, 2C, D). The values of the mean

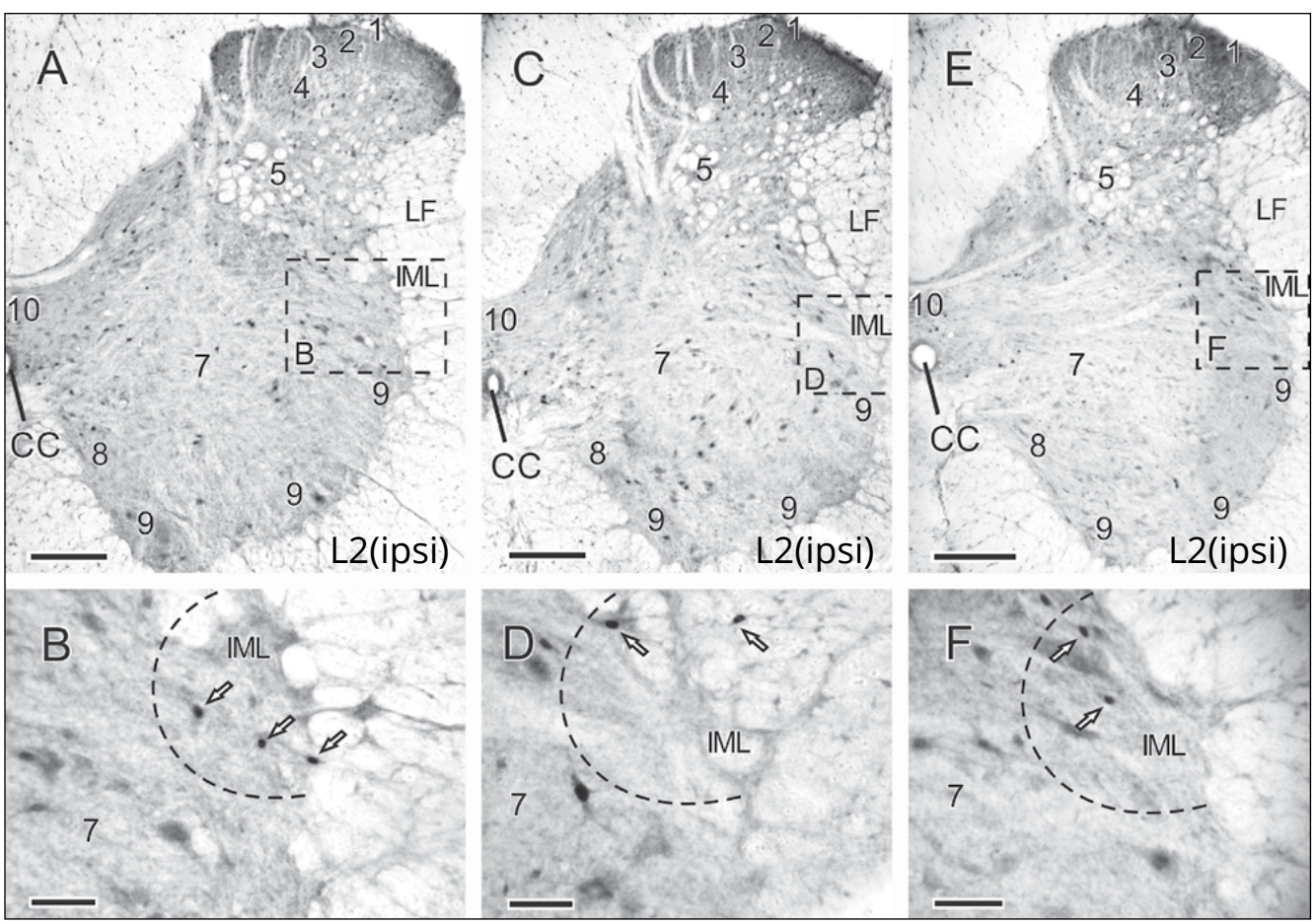

Fig. 2. Photomicrographs of frontal sections of the lumbar (L2) segment of the rat's spinal cord (ipsilateral side) after vibrational stimulation of the Achilles tendon (A, B), i.m. injection of 7-nitroindazole + tendon vibration (C, D) and i.m. injection of L-arginine + tendon vibration (E, F). Rectangle regions limited by dashed lines in (A, C and D) are shown in (B, D and F) at greater magnification. Structures: (1–10) layers of grey matter; (CC) central canal; (IML) intermediolateral nucleus; (LF and VF) lateral and ventral funiculi, respectively. Labelled nuclei of Fos-ir neurons are shown by arrows. Scale bar=150 μm for A, C, E and 50 μm for B, D, F.

number of activated spinal neurons exceeded those observed in the same segments in the animals of group 6. The total number of activated neurons in the sections of the studied segments was 202.2±18.4 units (animals of

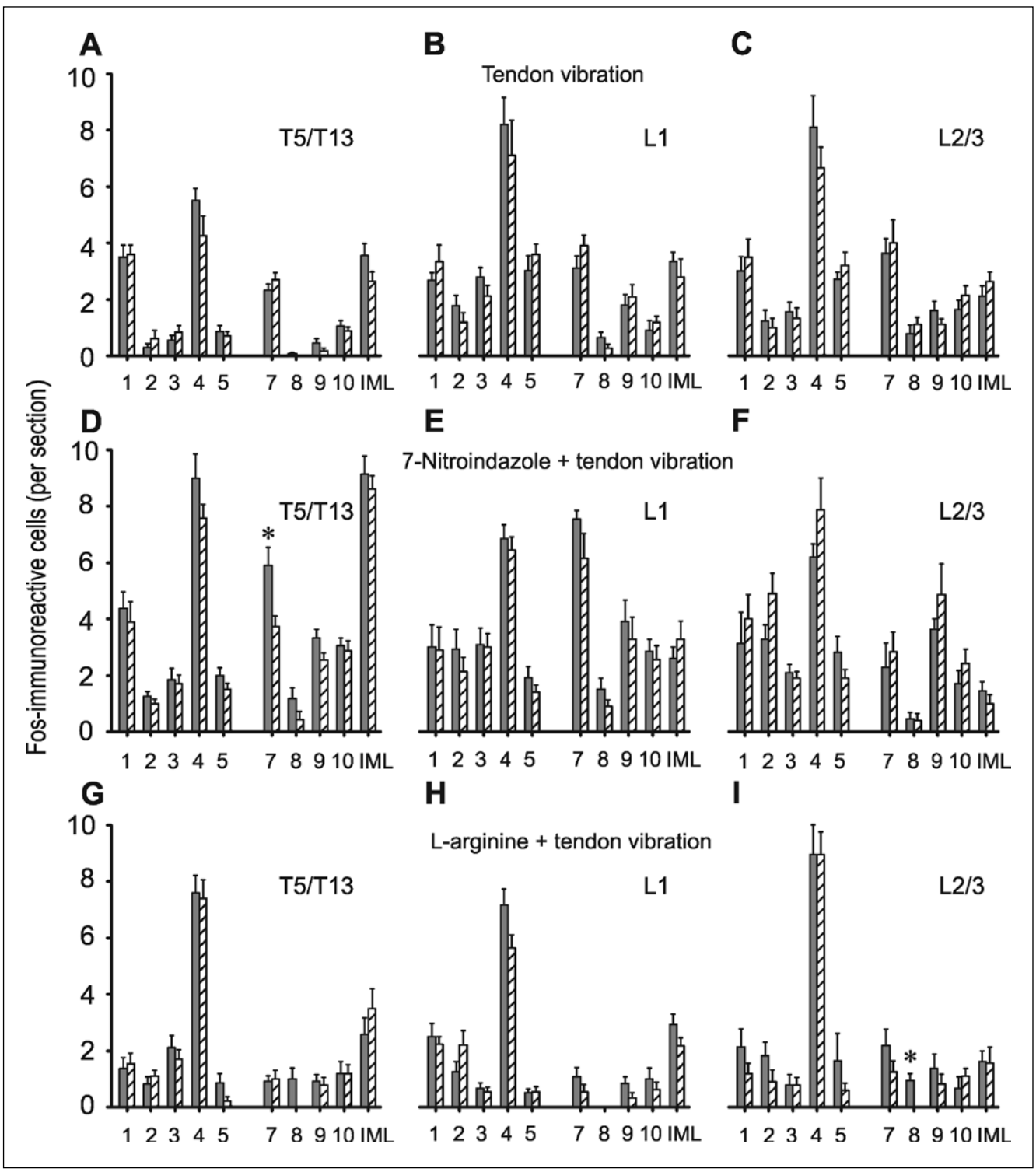

Fig. 3. The mean number ± S.E.M. of Fos-ir neurons localized at the ipsilateral (filled columns) and contralateral (open dashed columns) section halves in layers 1–10 and intermediolateral nucleus (IML) in the T5/T13, L1 and L2/L3 segments. After Achilles tendon vibration (A–C), i.m. injection of 7-nitroindazole + tendon vibration (D–F) and after i.m. injection of L-arginine + tendon vibration (G–I). Asterisks above the columns show cases of significant differences between the numbers of Fos-ir neurons within layers in the ipsi- and contralateral sides of the slice ($P<0.05$).

group 7) *vs.* 143.5±11.5 cells (rats of group 6). In the 7-NI pretreated and vibrated rats, the greatest total number of Fos-ir neurons was observed in the thoracic region with 74.2±5.8 labelled cells, a value two-fold higher compared with only vibrated animals (34.7±2.6 neurons). Notably, the identified Fos-ir neurons were primarily localized in the IML nucleus and layers 4, 7 in T5/T13 (9.1±0.6, 9.0±0.8 and 5.9±0.6 labelled units ipsilaterally), and layers 4 and 7 in L1, L2/L3 (6.8±0.5, 7.5±0.3 and 6.6±0.4 Fos-ir cells, respectively). Moreover, an increase in the number of Fos-ir motoneurons (layer 9) was also recorded in the rats of group 7, and bilaterally in all studied segments (for example, 3.3±0.3 and 2.7±0.3 units in T5/T13 ipsi- and contralaterally, respectively). In other layers, the level of c-Fos immunoreactivity was also higher than that in the studied spinal segments in animal group 6 (Figs 3D–F).

Intramuscular pretreatment with LA and tendon vibration induced Fos immunoreactivity within the T5–L3 segments in the animals of group 8 (Figs 1E, F, 2E, F). Patterns of the quantitative and spatial distribution of activated neurons were similar to those observed in the spinal segments in the animals of group 6, but the total number of Fos-ir neurons within T5–L3 was approximately 20% smaller (115.2±7.3 units). Compared with group 7, the total number of Fos-ir neurons within the T5/T13 and L1 segments of LA + tendon-vibrated animals was two times less (37.5±2.3 *vs.* 74.2±5.8 in T5/T13 and 32.5±1.6 *vs.* 68.4±5.9 in L1, respectively). The identified Fos-ir neurons were primarily localized in layer 4 and in the IML nucleus within T5/T13 and L1 (7.9±0.6 and 2.6±0.6 in T5/T13 and 7.1±0.5, 2.9±0.4 labelled units in L1, respectively, ipsilaterally) and in layer 4 in the L2/L3 segments (9.0±1.0 Fos-ir cells). A small number of Fos-ir motoneurons in the T5/T13, L1 and L2/L3 segments were also detected (Figs 3G–I). Notably, ipsi- or contralateral prevalence in mean number of Fos-ir neurons was not detected within the studied segments in all groups of animals.

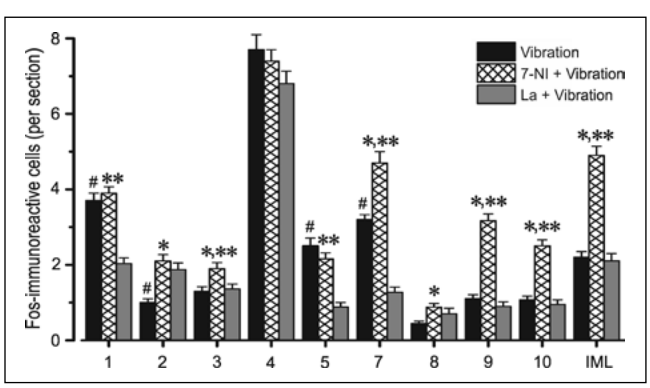

Fig. 4. The mean number ± S.E.M. of Fos-ir neurons (bilaterally) localized within layers 1–10 and IML nucleus of thoracolumbar region (T5–L3) of the spinal cord after Achilles tendon vibration (black columns), i.m. injection of 7-NI + tendon vibration (open dashed columns) and after i.m. injection of LA + tendon vibration (grey columns). Asterisk above the columns show cases of significant differences between the numbers of Fos-ir neurons within layers of the segments of the tendon-vibrated and 7-NI + tendon-vibrated animals (P<0.05); two asterisks – between 7-NI + tendon-vibrated and LA + tendon-vibrated rats; # – between tendon-vibrated and LA + tendon-vibrated animals (P<0.05).

To evaluate the integrated values, the mean number of activated Fos-ir neurons (per section) was calculated within different layers of thoracolumbar region (T5–L3) of the spinal cord on ipsi- and contralateral sides together (Fig. 4). One-way ANOVA revealed a significant effect of application of 7-NI with subsequent tendon vibration on neuronal activation compared with vibrated and LA + tendon-vibrated rats within IML ($F_{2,292}$=63.46, P<0.001) and layers 3 ($F_{2,292}$=5.96, P<0.01), 7 ($F_{2,292}$=65.56, P<0.001), 9 ($F_{2,292}$=66.19, P<0.001) and 10 ($F_{2,292}$=42.49, P<0.001).

DISCUSSION

The functioning of the spinal centres involved in the regulation of skeletal muscle activity is significantly dependent on proprioceptive muscle signals coming to these neuronal systems. Signals from primary and secondary muscle afferents form a significant component of such signals. Intramuscular factors mostly determine the intensity of the generation of proprioceptive signals, while the central action of the last one is also controlled by intrasegmental mechanisms. The detection of the interneurons and motoneurons with c-Fos protein, a product of the early *c-fos* gene expression in different layers of the spinal cord may be a potential marker of those neurons, which are components of the pathways related to the realization of specific functions (Herdegen and Leah 1998). Immunohistochemical analysis revealed a high level of c-Fos immunoreactivity in segmental neuron populations in the thoracic and lumbar spinal segments. These changes were induced by vibration of the Achilles tendon, demonstrating an additional increase in the number of Fos-ir neurons compared with control. The highest changes were primarily revealed in premotor neurons (layers 4 and 7), which are the direct recipients of signals of group I and II afferents from muscle stretch receptors. The Fos-ir neurons detected in these layers were localized within regions of the spinal grey associated with the transmission of proprioceptive effects from intrafusal primary and secondary afferents (groups Ia and II), which are sensitive to vibration (Bove et al. 2003, Fallon and Macefield 2007). A small number of Fos-ir cells were also detected within motor nuclei. Ia muscle afferents contact monosynaptically with motoneurons in layer 9, but in the present study, only a few activated Fos-ir motoneurons (unilaterally) were revealed within

40 μm thick sections. These results are consistent with data showing that c-Fos expression is increased in motoneurons after fictive locomotion on a treadmill (Ahn et al. 2006). Notably, unilateral tendon vibration and subsequent muscle spindles activity induced the activation of ipsi- and contralateral Fos-ir neurons in the grey matter of studied spinal segments. The transmission of afferent influences to the contralateral side of the spinal cord can be achieved with the involvement of cross-linkage interneurons (Bannatyne at al. 2006). Such interneurons, which are components of excitatory and inhibitory neuronal pathways, provide interlimb interactions and coordination during locomotion; these interneurons are components of the neuronal networks related to the manifestation of contralateral effects in the case of unilateral training (Carroll at al. 2006). The present results also showed that the unilateral vibrational stimulation of even relatively small muscle groups of one hindlimb results in the considerable involvement of crossed interneurons in the activity. In the present study, a high level of Fos immunoreactivity was also found in the sympathetic preganglionic neurons within the IML nuclei of the spinal cord. Thus, SPNs integrate the activity of the descending and sensory systems of autonomic control; such cells function as important centres of the efferent signal formation in the sympathetic nervous system and are primarily localized in the IML nuclei of the thoracolumbar region of the spinal cord (Anderson at al. 1989). These cells receive the main inputs from spinal interneurons and suprasegmental populations of sympathetic premotor neurons. However, a certain number of segmental sympathetic interneurons involved in the control of SPN activity were observed in layers 5 and 7 of the spinal cord, within zones of the projection of muscle afferents (Cabot at al. 1994, Joshi at al. 1995). Previous studies have shown that the intact spinal cord transsegmental connections with SPNs are mostly inactive (Schramm 2006).

7-NI is widely used as a selective inhibitor of nNOS and used to study the role of the neuronal NO pathway in the nervous system (Matsumura et al. 2008, Pilyavskii et al. 2012, Vlasenko et al. 2013). NOS is expressed in large quantities in the spinal cord, and NO plays an important role in central mechanisms of nociception and in the regulation of autonomic function (Chapman et al. 1995, Krukoff 1999). Our study demonstrates that preliminary i.m. administration of 7-NI markedly potentiates Fos immunoreactivity within the spinal cord in rats induced by high frequency vibration of the Achilles tendon. The highest effect of 7-NI application on *c-fos* expression was observed bilaterally in thoracic segments. Compared with the vibrated group of rats, the mean numbers of Fos-ir neurons (in the case of 7-NI injection) were significantly higher in the IML nucleus and layers 7–10 in the studied segments. These data suggest that the activity of the spinal neurons, as targets for muscle proprioceptive afferents, can be under background inhibitory control of central NOS-containing neurons. Such types of neurons are found in different spinal and suprasegmental regions, and most of these neurons are γ-aminobutyric acid (GABA) or glycine-immunoreactive cells (Johnson and Ma 1993, Spike et al. 1993). Studies have shown that nNOS is present in primary Ia afferents as a component of monosynaptic stretch reflex in neurons of lamina 7 and within laminae 8 and 9 (Maršala et al. 2004). An inhibitory influence of NO on fictive swimming activity in tadpoles, which is realized in part through the facilitation of glycinergic and GABAergic inhibition, was also shown (McLean and Sillar 2002, 2004). Thus, we proposed that the disinhibition of proprioceptive neurons after 7-NI administration might contribute to the facilitation of their responsiveness and consequent increase in c-Fos expression in the distinctive regions of the spinal grey matter in rats.

The present study also showed that i.m. administration of LA and subsequent tendon vibration in rats decreased the level of Fos immunoreactivity in the T5–L3 spinal segments compared with tendon-vibrated and 7-NI + tendon-vibrated animals (by 20% and 44%, respectively). LA is an amino acid essential for rapid growth, severe catabolic stress or injuries. LA is also a precursor used in the synthesis of many biologically active components, such as NO, creatine phosphate, agmatine, polyamines, and ornithine (Wu and Morris 1998). Notably, NO plays a dual role in the brain, producing beneficial or deleterious biological effects. A low concentration of NO via the activation of nNOS in the spinal cord exerts antinociceptive effects via kyotorphin-Met-enkephalin system (Kawabata et al. 1993). However, at high concentrations, NO mediates the maintenance of neuropathic pain following peripheral nerve injury through both the NO-cGMP-PKG and the NO-peroxynitrite pathways in the spinal cord (Tanabe et al. 2009). A recent study revealed the dose-dependent action of L-arginine on Fos immunoreactivity within the lumbar cord in rats (Kuan and Wen-Xiu 2010). These data are consistent with the decreased level of c-Fos expression within the spinal cord in rats after the administration of LA together with Achilles tendon vibration of the animals found in the present study.

Notably, NO can activate and promote the expression of the immediate early gene *c-fos*, as a marker of neuronal activation. The present study has shown that changes in NO content (after application of NO blocker or precursor together with tendon vibration in rats) can distinctly affect neuronal activation within the thoracolumbar region and the sequential enhancement or inhibition of the neuronal responsiveness to muscle proprio-

ceptive inflow. A high concentration of Fos-ir neurons in the IML nucleus indicates that the sympathetic nervous system can also exert a direct influence on muscle spindles under conditions of tendon vibration in rats.

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