UDC 612.83:612.884:612.014.42

I.V. Melnick

Electrically silent neurons in substantia gelatinosa of the rat spinal cord

Substantia gelatinosa (SG) neurons are usually categorized on three main types: tonic, adapting and delayed firing (DFNs), based on characteristic firing response evoked by sustained stimulation. Here, the existence of electrically silent neurons (ESNs, 9.3 %) is reported by using patch-clamp recording and confocal microscopy in spinal cord slices from 3-5 weeks-old rats. Those neurons does not generate spikes at their resting membrane potential (~ -69 mV) but only at preliminary depolarization to > -60 mV. In the latter case, spikes appeared starting from the end of stimulation, which is characteristic feature of DFNs. With the exception of APs block, all other passive and active electrophysiological properties of ESNs and DFNs were similar. Their main morphological properties (vertical orientation of dendritic tree and axonal trajectory) were close too. A distinctive feature of ESNs was larger amplitude of outward A-type K^+ current (K_A). The results suggest that the latter might cause a block of APs in ESNs, while these cells likely are a functional (i.e., non-firing) subtype of DFNs. The role of DFNs in descending control of pain transmission via modulation of its K_A is hypothesized.

Key words: spinal cord, substantia gelatinosa, firing pattern, action potentials, A-type current, pain.

INTRODUCTION

Substantia gelatinosa (SG) or lamina II of the spinal cord plays an important role in the processing of nociceptive and thermoreceptive information. SG cells receive monosynaptic input from afferent fibers and relay it further to projection neurons located in laminas I and IV-V. Therefore, SG cells operate as interneurons in sensory dorsal horn networks. Their structural and physiological characteristics are highly heterogeneous, however. Morphologically, there have been described a variety of neuronal cell types [4, 5, 7, 12–14, 20, 21]. According to physiological classification, three major groups have been shown: neurons with tonic, adapting and delayed firing (DFNs) [1, 5, 11–14, 18, 19, 22]. The latter has distinct electrophysiological and morphological properties. Among the first are a high threshold of action potentials (APs) in response to stimulation, appearance of APs starting from the end of stimulus (i.e., with delay) and possession of high amplitude subthreshold K^+ current of A-type (K_A), which activates before inward Na^+ current and thus is believed to be a cause of APs delay [5, 10, 13, 17, 18]. Morphologically, DFNs have vertical orientation of their dendritic and axonal trees [13]. Here, I report the existence of electrically silent neurons (ESNs), which do not respond by APs to stimulation at their resting membrane potential (RMP). Despite this distinctive feature, all other physiological and morphological properties of ESNs are similar with those of DFNs, except of the larger amplitude of K_A in silent neurons. The results suggest that the larger K_A might cause not only a delay but also a full block of APs in ESNs, while the latter likely is a functional subgroup of DFNs in substantia gelatinosa. It is proposed that DFNs might have a role in

descending control of pain transmission via modulation of its K_A.

METHODS

Patch-clamp recordings were performed on 300-mm parasagittal and coronal slices prepared from the lumbar enlargement of the spinal cord of 3-5 week-old Sprague-Dawley rats. The animals were anesthetized by Na-pentobarbital (30 mg/kg ip). Slices were incubated in artificial cerebrospinal fluid (ACSF) for >30 min at 32-33°C before experiments (carried at similar temperature). Lamina II was identified as a translucent band in the dorsal horn. The neurons were visualized by infrared-differential interference contrast (IR-DIC) optics of a Zeiss FS2 microscope (Carl Zeiss, Germany). ACSF contained (in mM): NaCl, 124; KCl, 3; $CaCl_2$, 2.5; $MgSO_4$, 1.3; NaH_2PO_4, 1.4; $NaHCO_3$, 26; glucose, 10 (pH 7.4 when bubbled with 95% O_2 - 5% CO_2). Pipettes had resistances of 5-7 MW when filled with an internal solution containing (in mM): K-gluconate, 125; KCl, 4; MgATP, 5; NaGTP, 0.3; EGTA, 5; HEPES, 5; neurobiotin, 0.2 % (pH adjusted to 7.25 with KOH, osmolarity adjusted to 295 mOsm). Pipette tips were filled separately, with internal solution without neurobiotin. The MultiClamp 700B amplifier was used for recordings. Signals were filtered at 3 kHz and digitized at 10 kHz using a computer interface (Digidata 1322) and pCLAMP 9.2 software (Axon Instruments, Burlingame, CA). Recordings were not corrected for the liquid junction potential. Series resistance was <25 MW and was not compensated. Firing of APs was evoked by 500 ms depolarizing current steps of varying intensities (range 10-650 pA). Passive electrical properties of cells were measured as described earlier [14]. Following recordings, slices were fixed in 4 % paraformaldehyde in 0.1 M phosphate-buffered saline (PBS) at 4°C for 48 h. After washing overnight in 20 % sucrose solution, unsectioned slices were incubated with neutravidin-conjugated Alexa 594 (1:300) for 1 h, washed with PBS and mounted in Prolong® anti-fading medium (Molecular Probes). Images (1-2 mm interval) were taken and processed with a Zeiss LSM510 confocal microscope. Data are presented as mean ± S.E.M. They were statistically analyzed by Student t-test, and probability level $P < 0.05$ was taken as significant.

RESULTS

The recordings were obtained from 108 neurons, which were categorized based on their typical firing response (i.e., firing pattern) evoked by sustained depolarization of increasing amplitude [14]. A small proportion of nerve cells (9.3 %) at their natural RMP did not respond by APs to stimulation of any intensity (Fig. 1, Aa); such cells were called "electrically silent neurons" (ESNs). This non-responsiveness did not result from the cell' damage or some other technical reasons, judging on their negative RMP (-69 ± 1.4 mV) and high values of input resistance (701 ± 122 MW). The overall shape of depolarizing membrane response in ESNs (notably, a notch on depolarizing phase and its gradually increasing amplitude to the end of stimulation) was similar to that of subthreshold responses in DFNs (Fig. 1, traces 2-3 in panel Aa vs. trace 1 in panel Ba). With sufficient membrane depolarization to > -60 mV, ESNs were able to fire usual APs with a delay starting from the end of depolarization (Fig. 1, Ab), which was again similar to the behavior of DFNs (Fig. 1, Ba, traces 2-3). The delay in DFNs decreased with membrane depolarization (Fig. 1, traces 2^s in panels Ba vs. Bb). This voltage sensitivity was quantified for both DFNs and ESNs (Fig. 1, C). From the graph, it was evident that, qualitatively, the delay behaved similarly in both cell types, in ESNs however the voltage dependence was shifted to more positive potentials. All other passive electrical properties of two cell types were close too (RMP and input resistance of DFNs, –68 ± 1.2 mV and 675 MW respectively, were not different

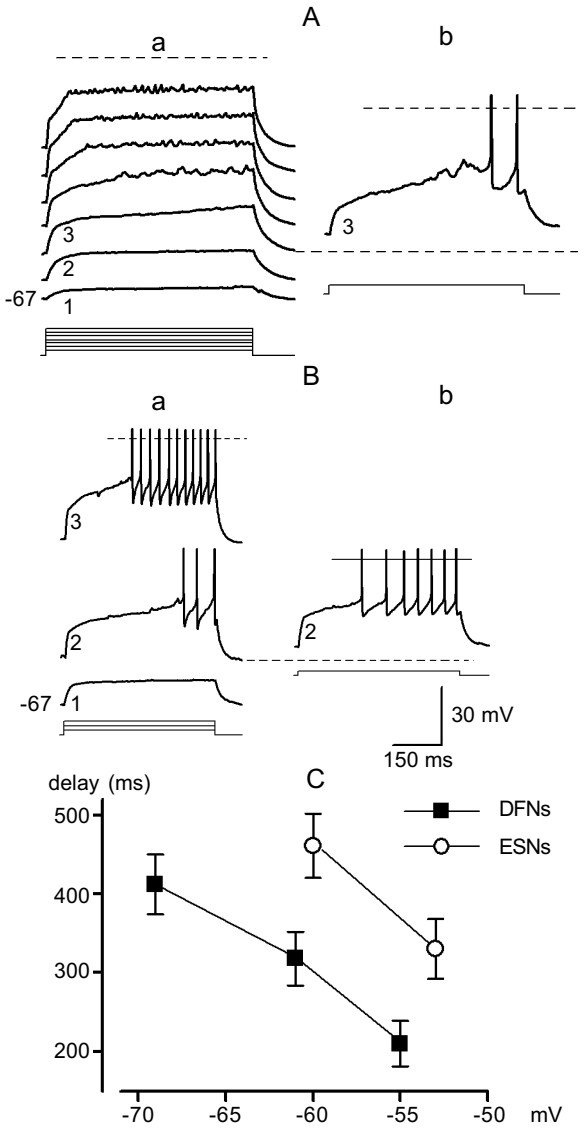

Fig. 1. Firing behavior of electrically silent (ESNs) and delayed firing neurons (DFNs). (A-B) Voltage responses of ESN (Aa) and DFN (Ba) to depolarizing currents of increasing intensity (protocol is shown below traces). ESN did not generate action potentials (APs) to stimulations. Upper dashed line in all panels show 0 mV level for the last (upper) trace. RMP is indicated near the first response. (Ab), stimulation of previously depolarized ESN by ~9 mV evoked APs. The intensity of stimulation is the one used for trace 3 from the panel a, RMP is the same for traces 3^s in both panels (indicated by dotted line). (Bb), depolarization of DFN shorten the delay in APs. The intensity of stimulation is the one used for trace 2 from the panel a, RMP is the same for traces 2^s in both panels (indicated by dotted line). (C) Voltage dependence of the delay before first AP in DFN and ESNs (n = 5 in both groups). The curve for ESN is shifted in depolarized direction

from the values for ESNs mentioned above, $P > 0.5$, unpaired t-test).

The prominent feature of DFNs is the expression of high-amplitude K_A [7, 13, 18]. The properties of ESNs and DFNs were compared in this respect. Figure 2 (A and B) shows ionic currents evoked by voltage steps to between –55 and –35 mV delivered after a hyperpolarizing pre-pulse to –120 mV to remove inactivation. Both ESNs (A) and DFNs (B) had fast K_A activated at subthreshold voltages, i.e. before Na^+ current. Initial I-V curves are demonstrated on panel C, dotted and solid lines schematically show activation of Na^+ spike. Such presentation of only initial I-V curves was chosen to minimize the voltage error inevitable in recording of high-amplitude currents. As evident from the panel C, the K_A current was significantly larger in ESNs.

Morphology of neurobiotin-filled and reconstructed in parasagittal slices ESNs was studied using confocal microscopy and compared with that of DFNs [13]. The orientations of dendritic tree and axon trajectory are considered as the most important morphological criteria, which indicates directionality of information processing in the spinal dorsal horn [4, 5, 20]. Individual ESN could have quite variable appearance, e.g., shape and size of the soma, number and width of primary dendrites, their branching pattern, etc. However the overall orientation of their dendritic trees was predominantly vertical (6 out of 9 cells), as in DFNs (Fig. 3). Similarly, their axons went to lamina I and in 3 cases both to lamina I and III.

DISCUSSION

SG neurons are known for the large variability of their properties. Here I report the existence of previously unrecognized cell group in SG with remarkable electrical behavior, the neurons of which were unable to generate APs at RMP (ESNs). With membrane depolarization to > -60 mV, ESNs did generate APs. This suggests, first, that some ionic current is ac-

tive at RMP and effectively blocks APs, and second, preliminary membrane depolarization inactivates the current and unblocks the APs. Such voltage dependence of firing is known specifically for DFNs and is attributable to powerful K_A, which activates at subthreshold potentials and causes the delay in APs generation [7, 10, 13, 18]. In agreement, ESNs possessed even larger K_A than DFNs suggesting that it might cause not only the delay but also a block of APs generation. K_A is known to inactivate strongly at negative subthreshold potentials [10] and it matches well with our experiments, where ESNs started generating spikes after preliminary depolarization. However, to prove definitely the causative relation between the expression of K_A and block of APs, further experiments with selective pharmacological suppression of the current

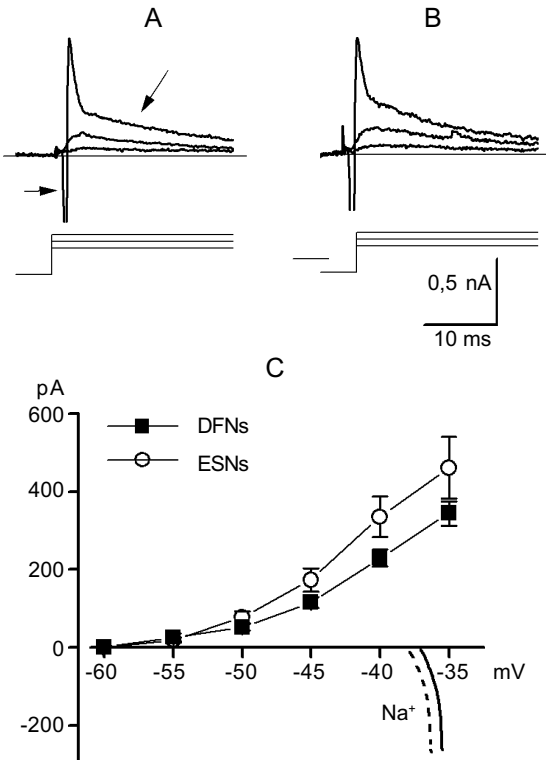

Fig. 2. Voltage-clamp recordings of subthreshold currents in delayed firing (DFNs, A) and electrically silent (ESNs, B) neurons. Voltage protocol is the same and shown below traces. Both cell types possessed outward A-type K^+ current (K_A) activating before inward Na^+ current (arrow). (C) Initial I-V curve of K_A in DFNs (filled) and ESNs (open symbols). Activation of Na^+ current is shown schematically for both groups by dashed and solid lines, respectively. ESNs had larger K_A ($P < 0.03$, unpaired t-test, $n = 6$)

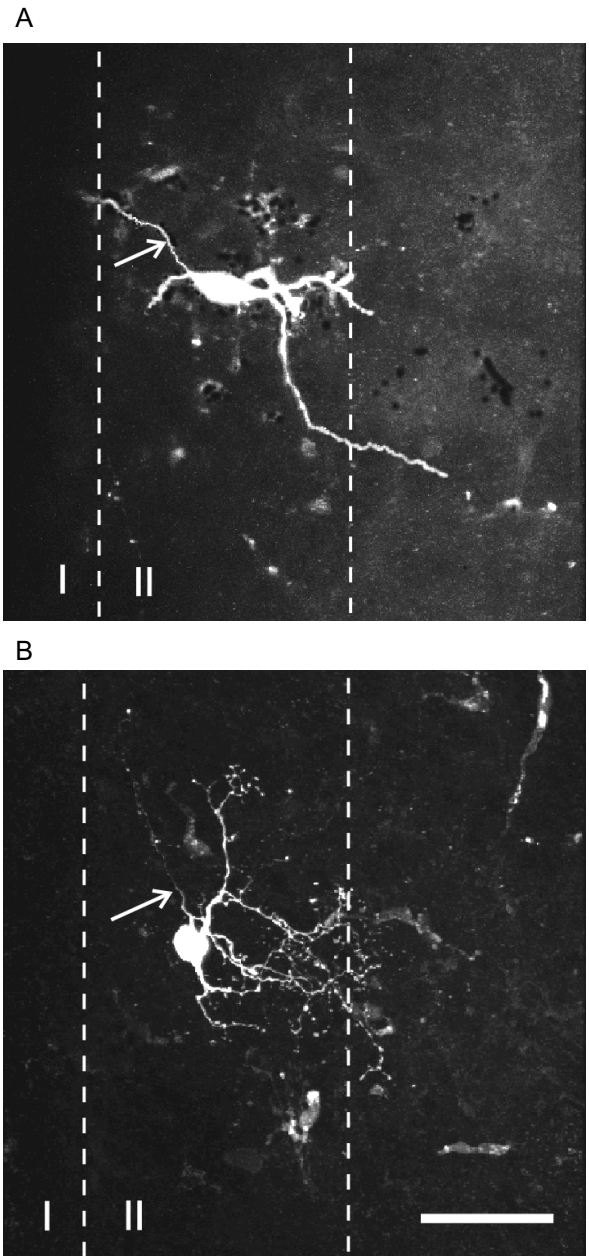

Fig. 3. Morphologic appearance of electrically silent (ESN, A) and delayed firing neuron (DFN, B) in parasagittal slices. Confocal images of cells are presented as summed Z-projections. Dashed lines indicate borders between laminas (numbered). Arrows point to axon-like processes. Calibration bar, 50 mm

are needed. All other tested electrophysiological and morphological properties of ESNs and DFNs were similar suggesting that they might be the same physiological type of neurons in dorsal horn networks; ESN might represent then a non-firing state, i.e. a silent mode, of DFNs due to up-regulation of K_A. If it is the case, the functional impact of ESNs on sensory processing can be much larger than if to judge based on their rare abundance; really, DFNs and ESNs together constitute about one third of SG neurons. Vertical orientation of their dendritic and axonal trees suggests that those neurons perform interlaminar integration by acquiring synaptic input from several spinal layers and transmitting it to projection cells, the axons of which form spinothalamic tract [17]. Their physiological features fit well with this idea. Really, the delay and high rheobase of APs in DFNs indicates that significant summation of afferent impulses (i.e., in frequency and amplitude) must happen before neuronal activation. The appearance and role of ESNs then can be hypothesized within following physiological scenario. Numerous descending fibre systems have been described in the dorsal spinal cord, which are supposed to be involved in antinociception, i.e. in regulation of pain neurotransmission [15]. These fibers might release various neuromodulators from their terminals (e.g., oxytocin, serotonin, noradrenalin, dopamine) [2, 3, 6, 9, 16], which might up-regulate the K_A in DFNs and thus "turn" them off; the latter would efficiently modulate sensory processing. In principle, the properties of the channels mediating K_A can be changed by phosphorylation via various protein kinases [8, 10]. Those kinases, in turn, can be activated in situ by different mechanisms, e.g., via β-adrenergic receptors, as was shown in hippocampal neurons [8]. To give more physiological weight to this hypothesis, the properties of K_A in DFNs of SG and its modulation be relevant neurotransmitters both in vitro and in situ have yet to be demonstrated.

CONCLUSIONS

1. Electrically silent neurons (ESNs) are found in rat Substantia Gelatinosa. They do not generate action potentials (APs) in response to stimulations at their resting membrane potential, but only with preliminary depolarization.

2. All electrophysiological and morphological properties of ESNs, except of APs block, are similar to those of delayed firing neurons (DFNs).

3. ESNs possessed significantly larger A-type K^+ current than DFNs, which is presumably a cause for APs block.

І.В. Мельник

ЕЛЕКТРИЧНО НЕЗБУДЛИВІ НЕЙРОНИ В ЖЕЛАТИНОЗНІЙ СУБСТАНЦІЇ СПИННОГО МОЗКУ ЩУРІВ

За типом генерації потенціалів дії (ПД) нейрони желатинозної субстанції спинного мозку звичайно підрозділяють на тонічні, з адаптацією та нейрони затриманої генерації (НЗГ). В експериментах на зрізах спинного мозку 3–5-тижневих щурів за допомогою методів patch-clamp-реєстрації та конфокальної мікроскопії була виявлена група електрично незбудливих нейронів (ЕНН, 9,3 %), які не генерували ПД у відповідь на стимуляцію при їх власному мембранному потенціалі (близько -69 мВ). ПД могли активуватися лише при деполяризації мембрани до -60 мВ, при цьому вони з'являлися, починаючи з кінця стимуляції – характерна риса для НЗГ. Були схожими також майже всі інші електрофізіологічні та морфологічні властивості ЕНН і НЗГ. На відміну від останніх, ЕНН мали більшу амплітуду калієвого струму А-типу (K_A), що, вірогідно, і блокувало ПД. Таким чином, припускається, що ЕНН і НЗГ належать до єдиної функціональної популяції нейронів желатинозної субстанції. Збільшення K_A у частини таких нейронів, наприклад під дією деяких нейромедіаторів чи модуляторів, призводить до їх електричного блоку, що може відігравати значну роль у контролі проведення больової інформації.
Ключові слова: спинний мозок, желатинозна субстанція, біль, потенціали дії, тип генерації, струм А-типу.

И.В. Мельник

ЭЛЕКТРИЧЕСКИ НЕВОЗБУДИМЫЕ НЕЙРОНЫ ЖЕЛАТИНОЗНОЙ СУБСТАНЦИИ СПИННОГО МОЗГА КРЫСЫ

По способу генерации потенциалов действия (ПД) нейроны желатинозной субстанции спинного мозга обычно подразделяют на тонические, адаптирующие и задержанной генерации (НЗГ). В экспериментах на срезах спинного мозга

3–5-недельных крыс при помощи методов patch-clamp-регистрации и конфокальной микроскопии была обнаружена группа электрически молчащих нейронов (ЭНН, 9,3 %), которые не генерировали ПД в ответ на стимуляцию при их естественном мембранном потенциале (примерно -69 мВ). ПД могли активироваться лишь при предварительной деполяризации мембраны до -60 мВ, при этом они появлялись с задержкой – характерная черта НЗГ. Были сходными также почти все другие электрофизиологические и морфологические свойства ЭНН и НЗГ. В отличие от последних, ЭНН имели большую амплитуду калиевого тока А-типа (K_A), что, вероятно, и блокировало ПД. Таким образом предполагается, что ЭНН и НЗГ принадлежат к единой физиологической популяции нейронов желатинозной субстанции. Увеличение K_A у части таких нейронов, например под действием некоторых медиаторов или модуляторов, приводит к их электрическому блоку, что может иметь важную роль в контроле проведения болевой информации.

Ключевые слова: спинной мозг, желатинозная субстанция, боль, потенциалы действия, тип генерации, ток А–типа.

REFERENCES

1. Мельник И.В. Физиологические типы нейронов желатинозной субстанции спинного мозга крысы // Нейрофизиология. – 2008. – **40**. – С. 191–198.
2. Оксамитный В.Н., Тамарова З.А. Деполяризующее действие дофамина на терминали первичных афферентных волокон изолированного сегмента спинного мозга крысят // Там же. – 1987. – **19**. – P. 741–748.
3. Тамарова З.А. Влияние вазопрессина и окситоцина на потенциалы дорсального корешка изолированного перфузируемого спинного мозга крысят // Там же. – 1988. – **20**. – P. 757–763.
4. Gobel S. Golgi studies of the substantia gelatinosa neurons in the spinal trigeminal nucleus // J. Comp. Neurol. – 1975. – **162**. – P. 397–416.
5. Grudt T.J., Perl E.R. Correlations between neuronal morphology and electrophysiological features in the rodent superficial dorsal horn // J. Physiol. – 2002. – **540**. – P. 189–207.
6. Hasegawa Y., Ono H. Descending noradrenergic neurones tonically suppress spinal presynaptic inhibition in rats // Neuroreport. – 1995. – **7**. – P. 262–266.
7. Heinke B., Ruscheweyh R., Forsthuber L., Wunderbaldinger G., Sandkuhler J. Physiological, neurochemical and morphological properties of a subgroup of GABAergic spinal lamina II neurones identified by expression of green fluorescent protein in mice // J. Physiol. – 2004. – **560**. – P. 249–266.
8. Hoffman D.A., Johnston D. Downregulation of transient K^+ channels in dendrites of hippocampal CA1 pyramidal neurons by activation of PKA and PKC // J. Neurosci. – 1998. – **18**. – P. 3521–3528.
9. Holden J.E., Farah E.N., Jeong,Y. Stimulation of the lateral hypothalamus produces antinociception mediated by 5-HT1A, 5-HT1B and 5-HT3 receptors in the rat spinal cord dorsal horn // Neurosci. – 2005. – **135**. – P. 1255–1268.
10. Jerng H.H., Pfaffinger P.J., Covarubias M. Molecular physiology and modulation of somatodendritic A-type potassium channels // Mol. Cell. Neurosci. – 2004. – **27**. – P. 343–369.
11. Lopez-Garcia J.A., King A.E. Membrane properties of physiologically classified rat dorsal horn neurons in vitro: correlation with cutaneous sensory afferent input // Eur. J. Neurosci. – 1994. – **6**. – P. 998–1007.
12. Melnick I.V. Morphophysiologic properties of islet cells in substantia gelatinosa of the rat spinal cord // Neurosci. Lett. – 2008. – **446**. – P. 65–69.
13. Melnick I.V. Morphophysiologic properties of delayed firing neurons in substantia gelatinosa of the rat spinal cord // Фізіол. журн. – 2009. – **55**, № 2. – P. 44–49.
14. Melnick I.V., Santos S.F., Szocol K., Szucs P., Safronov B.V. Ionic basis of tonic firing in spinal substantia gelatinosa neurons of rat // J. Neurophysiol. – 2004. – **91**. – P. 646–655.
15. Millan M.J. Descending control of pain // Prog. Neurobiol. – 2002. – **66**. – P. 355–474.
16. Miranda-Cardenas Y., Rojas-Piloni G., Martinez-Lorenzana G., Rodriguez-Jimenez J., Lopez-Hidalgo M., Freund-Mercier M.J., Condes-Lara M. Oxytocin and electrical stimulation of the paraventricular hypothalamic nucleus produce antinociceptive effects that are reversed by an oxytocin antagonist // Pain. – 2006. – **122**. – P. 182–189.
17. Ruscheweyh R., Ikeda H., Heinke B., Sandkuhler J. Distinctive membrane and discharge properties of rat spinal lamina I projection neurones in vitro // J. Physiol. – 2004. – **555**. – P. 527–543.
18. Santos S., Melnick I.V., Safronov B.V. Selective postsynaptic inhibition of tonic-firing neurons in substantia gelatinosa by м-opioid agonist // Anesthesiology. – 2004. – **101**. – P. 1177–1183.
19. Thomson A.M., West D.C., Headley P.M. Membrane characteristics and synaptic responsiveness of superficial dorsal horn neurons in a slice preparation of adult rat spinal cord // Eur. J. Neurosci. – 1989. – **1**. – P. 479–488.
20. Todd A.J., Lewis S.G. The morphology of Golgi-stained neurons in lamina II of the rat spinal cord // J. Anat. – 1986. – **149**. – P. 113–119.
21. Yasaka T., Kato G., Furue H. , Rashid M.H., Sonohata M., Tamae A., Murata Y., Masuko S., Yoshimura M. Cell-type-specific excitatory and inhibitory circuits involving primary afferents in the substantia gelatinosa of the rat spinal dorsal horn in vitro // J. Physiol. – 2007. – **581**. – P. 603–618.
22. Yoshimura M., Jessell T.M. Membrane properties of rat substantia gelatinosa neurons in vitro // J. Neurophysiol. – 1989. – **62**. – P. 109–118.

O.O.Bogomoletz Institute of Physiology, National Academy of Sciences of Ukraine, Kyiv
E-mail: igorm@biph.kiev.ua

Received 18.01.20010