

Modulation of Synaptic Processes in Cortical Neurons in Response to Painful Stimulation and Analgesia

Tamaz Sh Labakhua, Tinatin K Janashia, Gulnara I Gedevanishvili

Tamaz Sh Labakhua, Tinatin K Janashia, Gulnara I Gedevanishvili, LEPL Ivane Beritashvili Center of Experimental Biomedicine, Tbilisi, Georgia

Correspondence to: Tamaz Sh Labakhua, LEPL Ivane Beritashvili Center of Experimental Biomedicine, Tbilisi, Georgia.

Email: labakhuat@mail.ru

Telephone: +995-32-2375796

Received: December 19, 2014

Revised: February 8, 2015

Accepted: February 9, 2015

Published online: May 13, 2015

ABSTRACT

We studied effects of electrical stimulation of the substantia nigra (SN), locus coeruleus (LC), raphe nuclei (RN), substantia innominata (SIn), nucleus caudatus (NC) and central grey (CG) on postsynaptic processes evoked in neurons of the cat somatosensory cortex by excitation of nociceptive and non-nociceptive afferent inputs (intense stimulation of the dental pulp and moderate stimulation of the thalamic ventroposteromedial nucleus, VPMN, respectively). We analyzed intracellularly recorded activity of cortical cells activated exclusively by stimulation of nociceptors and cells activated by both nociceptive and non-nociceptive influences (“nociceptive” and “convergent” neurons). In neurons of both groups, stimulation of both nociceptive afferents and thalamic VPMN resulted in the development of successions of EPSP – action potential (AP) or their series – IPSP (IPSP duration 200–300 msec). Conditioning electrical stimulation of the above-mentioned nuclei induced suppression of synaptic reactions that occur in cortical neurons in response to stimulation of nociceptive inputs. The maximum decrease in the amplitude of the IPSP was observed at test intervals of 600 to 800 msec when stimulated nuclei containing biogenic amines and 100–150 msec at conditioning electrical stimulation of cholinergic structures. We observed certain parallelism between conditioning influent ions of CG activation and effects of systemic injections of morphine. Discusses is the physiological significance of presumably dendritic action potentials observed in our experiments. Decrease in

the amplitude or complete postsynaptic inhibition of IPSP in cortical neurons under different treatments associate with the occurrence of convulsive epileptic activity, and at the painful action with analgesic effect. Discussed are the mechanisms for modulatory influences exerted by conditioning stimulation of SN, LC, RN, NC, SIn and CG on the somatosensory neurons, activated upon excitation of high-threshold (nociceptive) afferent inputs. Such modulation is probably based on changes developing in both pre and post-synaptic intracortical mechanisms.

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Key words: Somatosensory cortex; Synaptic transmission; Nociception; Modulation of postsynaptic reactions; Analgesia

Labakhua TS, Janashia TK, Gedevanishvili GI. Modulation of Synaptic Processes in Cortical Neurons in Response to Painful Stimulation and Analgesia. *International Journal of Neurology Research* 2015; 1(2): 51-67 Available from: URL: <http://www.ghrnet.org/index.php/ijnr/article/view/983>

INTRODUCTION

The problems of extracellular and intracellular signaling and its participation in control of the living systems are recognized to be one of the major directions in modern fundamental sciences, which are also used in clinical medicine for treatment and correction of the certain CNS disorders.

Etiology of a number of psychic and neurological diseases (schizophrenia, mood disorders, anxiety disorders and oth.) is underlined by description of functioning in dysfunction of various ekstrathalamic stem-cortical neurochemical systems. One of the most up-to-date methods to study the role played by different neurotransmitter systems in the regulatory mechanisms of cortical processes is to address the synaptic processes in the cerebral cortex.

The process of synaptic transmission, including the potential-

evoked action of a neurotransmitter release from the presynaptic terminals and a subsequent stage of generating the postsynaptic potential, may be regulated or modulated at the pre- and postsynaptic level. Activation of presynaptic receptors by their own neurotransmitter (presynaptic auto regulation) provides maintenance of its concentration in the synaptic clefts within definite limits, i.e. creates conditions for an optimal functioning of the synapse. Receptors of other mediators too may be localized at the presynaptic terminals. The membrane of presynaptic terminal of central neurons is not a homogeneous structure, but contains a variety of receptors through which the neurotransmitters, as well as hormones regulate the synaptic transmission (presynaptic heteroregulation or presynaptic modulation). Another group of factors that has stirred up interest in studying the means and mechanisms governing synaptic transmission and which had not found their reflection in classical comprehension of a synapse had been the data on postsynaptic modulation of the effects of transmitters. In a number of cases the interaction of two postsynaptic potentials, evoked by activation of different synaptic inputs does not lead to the summation based on passive properties of the neuronal membrane, but is mediated by special intracellular second messenger of cyclic amino-mono-phosphate (cAMP) and cyclic guanosin monophosphate (cGMP) type.

The main synaptic inputs to a definite population of neurons in the somatosensory cortex are formed by ascending pathways of the system of nociception. Some neurons of this neocortical population are strictly selectively activated by nociceptive influences, while other cells of the mentioned population are excited upon stimulation of both high-threshold (nociceptive) and low-threshold (non-nociceptive) somatic afferents. Such neuronal groupings form a “higher floor” of the nociceptive sensory system and, according to the conventional classification, can be considered a cortical section of the nociceptive analyzer. Information on the morphological, electrophysiological, and neurochemical characteristics of such cortical neurons remains relatively limited^[1,2].

Noradrenergic and dopaminergic (NA- and DA-ergic, respectively) cerebral systems are important brainstem complexes exerting modulatory actions on the activities of practically all parts of the brain cortex. The locus coeruleus (LC) is a central structure in the NA-ergic brain system, while the substantia nigra (SN) occupies the corresponding position in the DA-ergic system. The modulatory effects of activation of neuronal systems of the LC and SN on neuronal activity in different functional subdivisions of the cortex have attracted considerable interest from experimenters^[3-6]. In this case, however, studies of the effects exerted by the LC and SN on neurons of the somatosensory cortex were mostly directed toward reactions evoked in cortical neurons by activation of relatively low-threshold afferents. At the same time, information on NA- and DA-ergic modulation of the cortical activity related to nociceptive synaptic influences is relatively limited. The question on the pattern of convergence of the effects of these systems on “nociceptive” neocortical neurons remains open. In addition, the amount of experimental material obtained in our earlier studies was relatively limited. This was explained, to a great extent, by the necessity to provide rather long-lasting recording from single cortical neurons; this circumstance resulted in the exclusion from the analyzed material of a significant proportion of the records that were insufficiently qualitative.

Recently, the leading role of serotonergic (5-HT-ergic) neurons localized in the dorsomedial structures of the gray matter of the brain in the induction of “central “analgesia^[7] and the involvement of the raphe nuclei (RN) containing such neurons in the phenomena of

analgesia and hyperalgesia of an opioid nature^[8] were demonstrated. At the same time, some aspects of the involvement of cortical neurons in the mechanisms of pain and analgesia remain little studied. The activity of cortical neurons belonging to the nociceptive system, similarly to the activity of neurons of other cerebral structures, underwent considerable modulatory influences coming from a number of the cerebral centers. The serotonergic (5-HT-ergic) brainstem system is one of the most important systems exerting modulatory actions on the activity of cortical neurons^[7-10]. Nerve cells of the RN form rather significant monosynaptic projections to the cortex^[11,12]. It is believed that they are capable of providing considerable modulation of the functioning of cortical neurons. The earlier obtained data on the RN-born effects on the activity of neurons of the somatosensory cortex concerned mostly reactions of these cells evoked by excitation of relatively low threshold afferents. Unfortunately, up to present there are only fragmentary data on modulation of the activity of cortical neurons initiated by incoming of nociceptive influences via high-threshold afferent inputs. Studies devoted to elucidation of physiological characteristics and topography of cortical neurons involved in the process of nociception, as well as of cellular and membrane mechanisms underlying functioning of the above units, are relatively scant^[13,14].

The cholinergic cerebral system affects the activity of practically all structures of the cerebral cortex. Results of histochemical and immunocytochemical studies demonstrated that fibers of the cholinergic system in the cortex are formed by two sources, intracortical bipolar radially oriented neurons localized in cortical layers II-IV and corticopetal fibers coming from the basal forebrain nuclei and Broka’s diagonal ligamentum^[11]. As is believed, ascending axons of neurons of the basal ganglia (nucleus caudatus (NC), globus pallidus, putamen, and substantia innominata (SIn) are the main source of acetylcholine (ACh) in the somatosensory cortex. In carnivores, the nucl. basalis magnocellularis, an analogue of the nucleus of Meynert in primates, contains cells localized in the ventral and medial parts of the globus pallidus and substantia innominata (SIn), and these neurons are cholinergic units^[15,16]. Results of the neurophysiological and behavioral experiments and clinical observations showed that the basal ganglia are involved in the processing of both pain-related and non nociception-related information. Most neurons of the basal ganglia are activated mostly by nociceptive stimulation and probably can encode the intensity of a pain stimulus; they are involved in sensory discrimination of the pain information, and translation of the corresponding information to motor cortical areas^[16,17]. At present, the effects of the basal nuclei on the activity of neurons of different functional cortical subdivisions are attracting considerable interest among researchers^[17-21].

As is shown, electrical stimulation of the cerebral central (periaqueductal) grey (CG) significantly modifies nociceptive stimulation-induced responses in neurons of the spinal cord and brainstem^[22] and also in thalamic neurons^[23]. Such stimulation noticeably influences the field electrical activity of mostly neocortical genesis, evoked potentials (EPs). At the same time, the effects of CG stimulation on synaptic processes in single neurons of the cerebral cortex, which, according to the characteristics of their synaptic inputs, belong to the system of nociception, remain little studied.

Introduction of micro doses of opiate alkaloids and opioid peptides in the CNS induces clearly expressed analgesic effects^[24]. Such experimental observations demonstrate that the CG is specifically related to the antinociceptive cerebral system whose functions are realized with involvement of opioid receptors. The distribution of such receptors in subcortical brain structures has been studied in

detail, but information on the effects of opioidergic cerebral systems on various neuronal populations of the cerebral cortex still remains fragmentary^[2].

Taking into account the above situation, we continued our examination of the above-listed aspects and tested modulatory effects exerted by stimulation of the SN, LC, RN, NC, SIn and CG on postsynaptic reactions in neurons of the cat somatosensory cortex receiving excitatory synaptic influences from nociceptive afferents^[25-29].

METHODS

Acute experiments were carried out on; the experimental technique was in accordance with requirements of the International Association for Studies of Pain. Surgical procedures (tracheotomy, cannulation of the femoral vein, pneumothorax, and trepanation of the skull above the pericruciate cortical area) were performed under ether inhalation anesthesia, and then the animals were injected with 40 mg/kg α -chloralose. According to the stereotaxic coordinates^[30], bipolar Constantan wire electrodes with standard insulation (interpolar distance 0.5 mm) were inserted into the regions corresponding to the LC, SN, RN, NC, SIn, CG and thalamic VPMN. Electrodes were fixed to the skull bones by a fast-hardening plastic. The accuracy of introduction of the stimulating electrode in the thalamic VPMN was additionally controlled by stimulation of this nucleus and mapping of the maximum activity focus (MAF) within the somatosensory cortex; evoked potentials (EPs) were recorded using a ball microelectrode.

Drillings were made in the upper fangs by a dental burr, and thin wire electrodes insulated except for their tips touching the dental pulp were introduced and fixed in the openings using dental cement. The animal was immobilized by i.v. injection of a myorelaxant (d-tubocurarine or arduane) and artificially ventilated. The edges of the surgical wounds and fixator pressing regions were thoroughly anesthetized with Novocain. Before immobilization, we tested effects of stimulation of the dental pulp and measured the pain threshold (PT) according to initiation of the mouth opening reflex.

The activity of cortical neurons was intracellularly recorded using standard techniques. Glass microelectrodes filled with 2.0 M potassium citrate were inserted into the somatosensory cortical zone under visual control. Neurons were identified as cells of the first group (nociceptive units) in the case when synaptic potentials were evoked in them exclusively by stimulation of afferents of the dental pulp with the supertreshold (2.0 threshold, or more) intensity. Weaker stimulations did not evoke any postsynaptic responses in such neurons. Neurons of the second group (convergent units) were excited by stimulation of both the dental pulp, as well as by stimulation of the thalamic VPMN with 3 to 5 T intensity.

After the experiment, electrical current providing electrocoagulation labeling of the stimulated sites in the LC, SN, RN, NC, SIn and CG was passed through the respective stimulating electrodes. Localization of the tips of the latter was histologically verified on frontal brain slices. Numerical data were treated statistically using standard techniques, in particular Student's *t*-test. Intergroup differences were considered to be significant in the cases with $P \leq 0.05$.

I. Responses of nociceptive neurons of the neocortical somatosensory zone and glial cell to stimulation of the tooth pulp

Intracellular recordings of satisfactory quality (membrane potential, MP, not less than 58-60 mV throughout the entire recording period) were obtained in our study from neurons localized within the primary

somatosensory cortical area. The depth of recording of most of the above neurons varied from 1.5 to 2.8 mm. Some cells were identified, according to the above-described criteria, as nociceptive neurons (responding exclusively to strong stimulation of the dental pulp exciting A δ and C afferent fibers), while others were convergent, possessing synaptic inputs from both pain and non-nociceptive afferents. In most nociceptive neurons, relatively weak (about 1.5 to 2.0 T with respect to initiation of the reflex of mouth opening in a non-immobilized animal) stimulation evoked small-amplitude EPSPs (Figure 1A, 1). An increase in the strength of a single stimulus resulted in an increase in the EPSP amplitude and arrival of a longer-latency hyperpolarization potential; the latter was probably also a postsynaptic event, i.e., an IPSP (Figure 1A, 2, 3).

Upon further intensification of stimulation, EPSP exceeded the threshold for generation of the action potential (AP), and the response to single stimulation of the dental pulp acquired a complex EPSP-AP-IPSP pattern (Figure 1A, 4).

In some nociceptive neurons characterized by a relatively low frequency of the background activity, suprathreshold stimulation of the dental pulp of both ipsi- and contralateral fangs evoked several APs arriving on the EPSP maximum, and this was followed by strong hyperpolarization (IPSP) (B). In neurons generating high-frequency background spiking, responses to stimulation of the dental pulp (EPSP-AP-IPSP complexes) were evoked with comparatively short latencies (C). The development of IPSP after synaptically evoked APs resulted in inhibition of background impulsion; the duration of such suppression periods can reach 100-200 msec (in the neuron whose activity is shown in figure 1D, such a period was about 170 msec long). There was no response of glial cells to painful stimulation (Figure 1D, 2-3). Intergroup differences were considered to be significant in the cases with $P \leq 0.05$

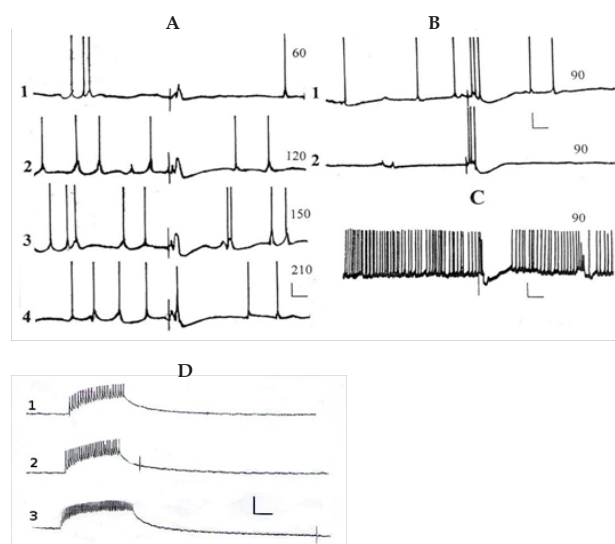


Figure 1 Responses of nociceptive neurons of the neocortical somatosensory zone to stimulation of the tooth pulp (intracellular records from three neurons, A-C and glial cell, D. A) Responses to single stimulation of the dental pulp of the contralateral fang with increasing intensity (1-4). The depth of localization of the neuron was 2.0 mm; membrane potential (MP) was -65 mV. B). Responses to stimulation of the pulp of the contra-(1) and ipsilateral (2) fangs. Depth 1.9 mm, MP= -60 mV. C). Response to stimulation of the contralateral tooth pulp. Intensity of stimulation (μ A) is shown above the traces in the right. Here and in the following figures calibration: 20 mV; 100 msec. D. Intracellular potential of glial cell during tetanic stimulation of thalamic VPMN. The reactions of glial cells in rhythmic stimulation of thalamic VPMN (1) - and lack of response to stimulation of the tooth pulp at different intervals (2-3). Depth 2.35 mm, MP= -90 mV. Calibration: 2 mV; 0.5 sec.

II. Modulation of postsynaptic responses of nociceptor-activated neurons of the cat somatosensory cortex by stimulation of the substantia nigra

In this study, we examined the effects of conditioning electrical stimulation of the SN on postsynaptic responses of neurons of the somatosensory cortex. Responses of the neurons to high-threshold (nociceptive) stimulation of the dental pulp was used as the test reaction (Figure 2, A and C); in convergent neurons, stimulation of the thalamic VPMN activating corticopetal pathways of both nociceptive and non-nociceptive neurons was also used (Figure 3, A and C). Intracellular recordings preserving satisfactory quality throughout a rather long period (the membrane potential, MP, did not drop below -58 mV within these periods) were obtained from 17 neurons localized within the borders of the primary somatosensory cortex; (such a rather strict criterion of the selection mode compelled us to ignore a significant part of the recorded units). The depth of localization of most the above neurons varied from 1.5 to 2.8 mm. Among the analyzed units, 7 cells were identified according to the above-described criteria as nociceptive (they responded exclusively to strong stimulation of the dental pulp, i.e., activation of the receptors connected only with A δ and C fibers). Ten neurons were qualified as convergent units activated by both nociceptive (pain) and relatively low-threshold afferents.

Intracellularly recorded responses of nociceptive cortical neurons to stimulation of the dental pulp appeared as a complex EPSP-action potential (AP)-IPSP (Figure 2A, 1; C,1). Responses of convergent cortical neurons to stimulation of the thalamic VPMN also included EPSP with one AP (Figure 3 C, 2-8) or a burst of APs generated with a high intraburst frequency (Figure 3 A, 2-7). After these excitatory components, postsynaptic hyperpolarization (IPSP) developed; it

lasted about 200 to 250 msec, and its amplitude reached, under our conditions of recording, 6 to 10 mV. This complex response was probably generated due to summation of the effects of nociceptive and non-nociceptive influences. For activation of the SN, we used short high-frequency (200-250 sec $^{-1}$) series of stimuli. Isolated stimulation of the SN by such high-frequency series evoked two types of responses in the examined cortical neurons. In a part of the convergent neurons (4 of cells out of 10), stimulation of the SN evoked long-lasting (several hundreds of milliseconds) EPSPs. These potentials were characterized by a complex time course, and APs were generated on their maxima. The frequency of these spikes exceeded the frequency of background spiking of these neurons. We cannot rule out that such impulses were dendritic APs (Figure 3A, 2-7).

In all nociceptive and remaining convergent neurons, hyperpolarizing IPSPs of a rather high amplitude (up to 8-10 mV) lasting 60 to 120 msec were primary reactions to stimulation of the SN. In the course of these potentials, generation of background impulse activity was blocked (Figure 2 A, recordings 2-7, 2C, recordings 2-8, and 3A, recordings 2-7). In the case where stimulation of the SN preceded test stimulation of the dental pulp or of the thalamic VPMN, postsynaptic inhibitory components in the responses to test stimulation (in complexes EPSP-AP-IPSP) were subjected to intense suppression at certain conditioning/test intervals. The latencies of such inhibitory effects were rather long and varied considerably from neuron to neuron. In some cells, this inhibition began at test intervals about 100 to 200 msec (Figure 2B). In other cortical neurons, the delay of such suppression can reach 200-300 msec (Figures 3B and 3D). In all cases, however, the maximum suppressive effect of conditioning stimulation of the SN was observed

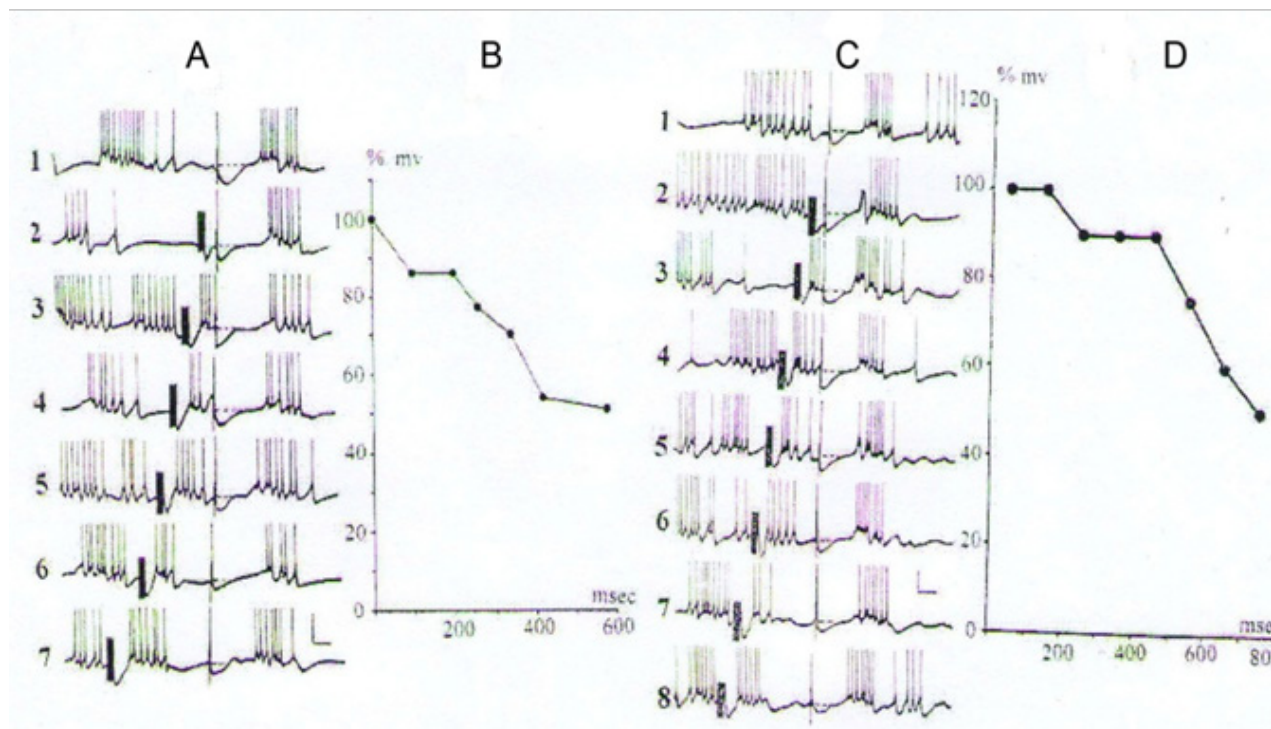


Figure 2 Effects of electrical stimulation of the substantia nigra (SN) on postsynaptic responses of two nociceptive neurons evoked by stimulation of the dental pulp. In A and C) responses to isolate stimulation of the dental pulp (1) and effects of conditioning stimulation of SN by a short high-frequency series on the response evoked by test stimulation of the dental pulp with different intrastimulation intervals. B and D) Dependence of the normalized amplitude of pulp stimulation-caused IPSP (%) on the interval between conditioning stimulation of the SN and test stimulation of the dental pulp (msec). The IPSP amplitude in the absence of stimulation to the CN was taken as 100%. Depth of localization of the neurons was 1.56 (A) and, 1.47 (C) mm; membrane potential was: -59 (A) and -61 (C) mV.

at durations of the test intervals about 350-700 msec (Figures 2B, 3B, 2D, and 3D). The total duration of the inhibitory effect reached at least several seconds (not illustrated). A relatively limited number of observations make difficult accurate statistical treatment, but we should note a somewhat greater intensity of suppression of IPSPs in cortical nociceptive neurons under the influence of conditioning stimulation of the SN, as compared with the corresponding effects in convergent neurons (this fact can be illustrated by a comparison of figure 2 with figure 3). Initial excitatory postsynaptic components in complex responses of cortical neurons to stimulations of the dental pulp, infraorbital nerve, or thalamic VPMN could also be somewhat inhibited after conditioning stimulation of the SN, but the intensity of inhibition of these components was rather moderate. Such effects can be detected only under conditions of weakening of test stimulation. We did not, however, examine these effects in detail. At least, they were much less intense than suppression of post activating IPSPs. Intergroup differences were considered to be significant in the cases with $P \leq 0.05$

III. Modulation of postsynaptic responses of nociceptor- activated neurons of the cat somatosensory cortex by stimulation of the locus coeruleus

Isolated stimulations of the LC by short high-frequency series of pulses induced two types of responses in the studied cortical neurons. We selected 22 neurons localized within the primary somatosensory cortex. Among them ten neurons were identified as nociceptive, while 12 neurons were qualified as convergent units. In some nociceptive and convergent neurons, stimulation of the LC by short bursts of stimuli resulted in the development of long-lasting (several hundreds of milliseconds) complex EPSPs. Action potentials were generated on the maxima of depolarization waves, and the AP frequency exceeded the frequency of background activity (Figures 4 A, 2-8, and 5A, 2-8).

In another part of nociceptive and convergent neurons, rather high-amplitude (up to 8-12 mV) hyperpolarization was the primary

response to stimulation of the LC; these IPSPs were 60 to 120 msec long. In the course of the development of such hyperpolarization potentials, generation of the background activity was blocked (Figures 4C, 3-8; 4C, 2-7).

In the case where stimulation of the LC preceded test stimulation of the dental pulp or of the thalamic VPMN, postsynaptic inhibitory components in the responses to test stimulation (in complexes EPSP-AP-IPSP) were subjected to intense suppression at certain conditioning/test intervals (Figures 4-5, panels B,D). Intergroup differences were considered to be significant in the cases with $P \leq 0.05$.

IV. Raphe stimulation-evoked modulation of postsynaptic responses by neurons of the cat somatosensory cortex activated by stimulation of nociceptors

Fifteen cells that responded to stimulation of the dental pulp and thalamic VPMN and corresponded to the above criteria of the analysis were selected from all the studied cortical neurons, whose activity was recorded intracellularly. The resting membrane potential (MP) of all these neurons throughout the entire recording period was -58 mV or higher. Among them, six cells were identified as selectively nociceptive, while nine units were classified as convergent. The depth of localization of most of the above neurons from the surface of the cortex varied from 1.23 to 2.50 mm. Short-lasting high-frequency series of stimuli were found to be an effective mode of RN stimulation. Such stimulation induced noticeable changes in the background synaptic and impulse activity of the examined cortical neurons. In some studied neurons, isolated stimulation of the RN caused discrete relatively short-latency responses in the form of complex EPSPs (Figure 6 A); against the background of such postsynaptic depolarization, one or several APs were generated. In other cells where IPSPs of a significant amplitude and duration (120 to 140 msec) appeared with nearly the same latency, generation of impulse activity was blocked (Figure 6 A and C, 2-8).

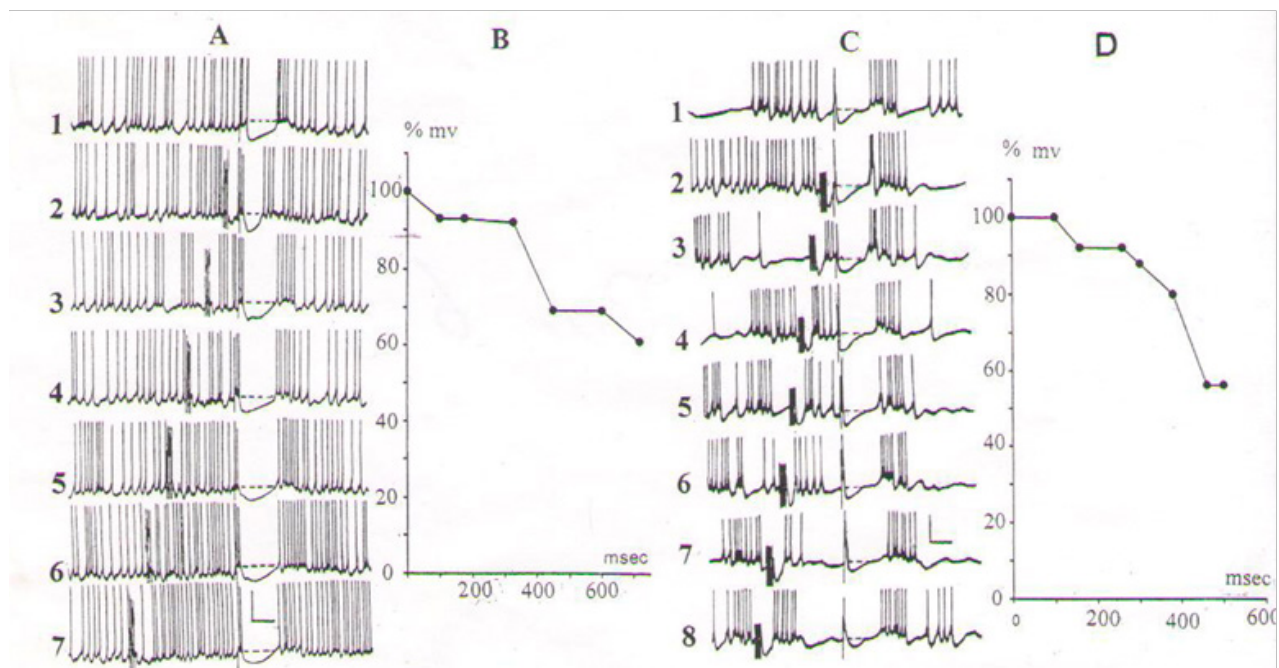


Figure 3 Effects of electrical stimulation of the substantia nigra (SN) on postsynaptic responses of two convergent neurons evoked by stimulation of the thalamic VPMN. In A and C) responses to isolate stimulation of the thalamic VPMN (1) and effects of conditioning stimulation of LC by a short high-frequency series on the response evoked by test stimulation of the thalamic VPMN with different intrastimulation intervals. B and D) Dependence of the normalized amplitude of pulp stimulation-caused IPSP (%) on the interval between conditioning stimulation of the SN and test stimulation of the dental pulp (msec). The IPSP amplitude in the absence of stimulation to the SN was taken as 100%. Depth of localization of the neurons was 2.1 (A) and 1.3 (C) mm; membrane potential was -58 (A) and -62 (C) mV.

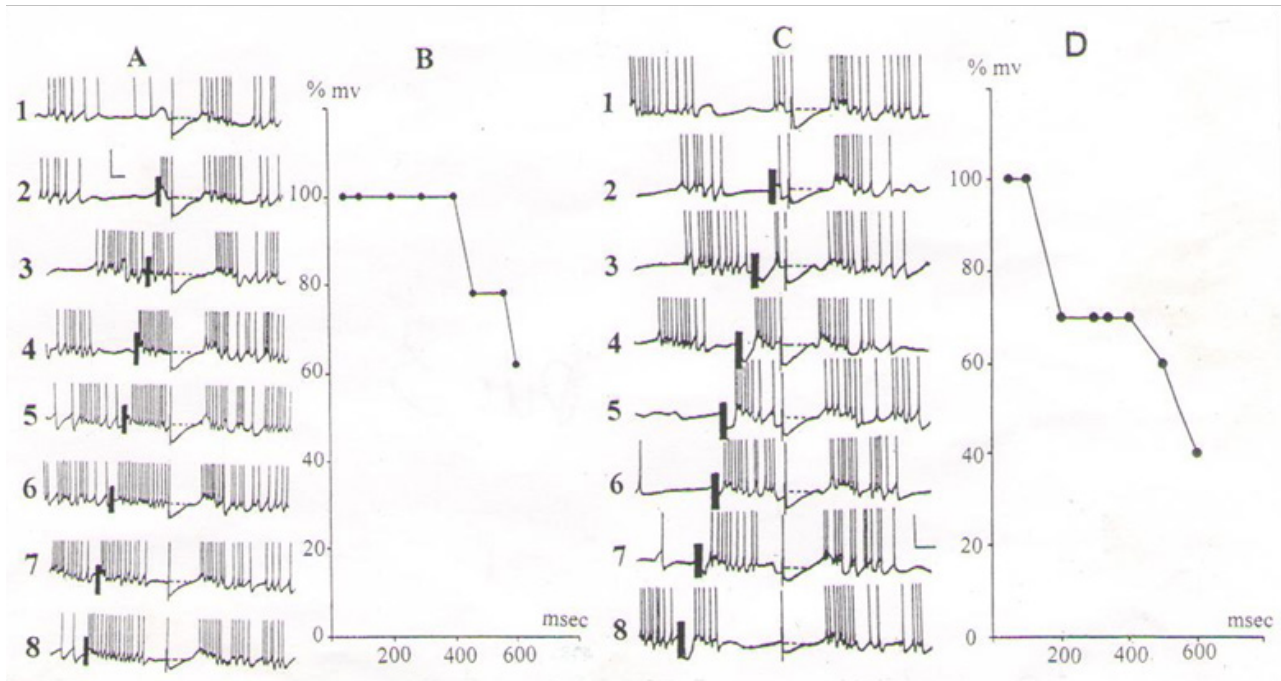


Figure 4 Effects of electrical stimulation of the locus coeruleus (LC) on postsynaptic responses of two nociceptive neurons evoked by stimulation of the dental pulp. In A and C) responses to isolate stimulation of the dental pulp (1) and effects of conditioning stimulation of LC by a short high-frequency series on the response evoked by test stimulation of the dental pulp with different intrastimulation intervals. B and D) Dependence of the normalized amplitude of pulp stimulation-caused IPSP (%) on the interval between conditioning stimulation of the LC and test stimulation of the dental pulp (msec). The IPSP amplitude in the absence of stimulation to the LC was taken as 100%. Depth of localization of the neuron was 2.4 (A) and 2.8 (C) mm; membrane potential was -56 (A) and -63 (C) mV.

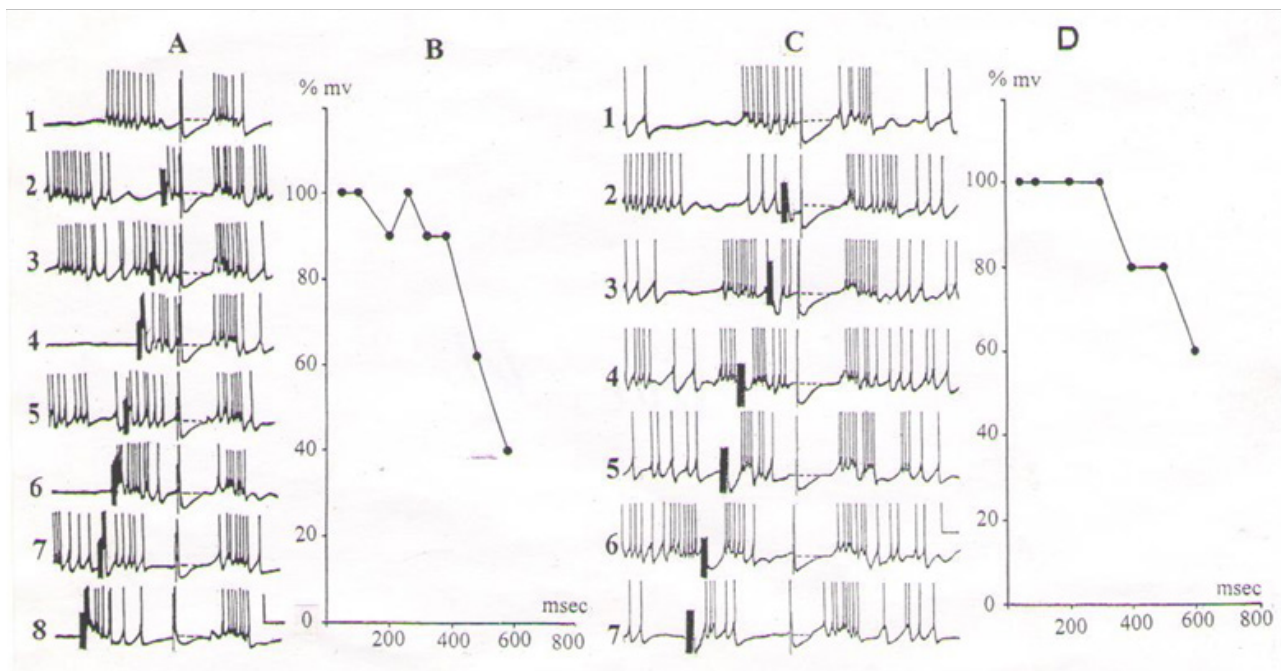


Figure 5 Effects of electrical stimulation of the locus coeruleus (LC) on postsynaptic responses of two convergent neurons evoked by stimulation of the thalamic VPMN. In A and C) responses to isolate stimulation of the thalamic VPMN (1) and effects of conditioning stimulation of LC by a short high-frequency series on the response evoked by test stimulation of the thalamic VPMN with different intrastimulation intervals. B and D) Dependence of the normalized amplitude of pulp stimulation-caused IPSP (%) on the interval between conditioning stimulation of the LC and test stimulation of the dental pulp (msec). The IPSP amplitude in the absence of stimulation to the RN was taken as 100%. Depth of localization of the neuron was 1.7 (A), 25 (C); membrane potential was -56 (A) and -63 (C) mV.

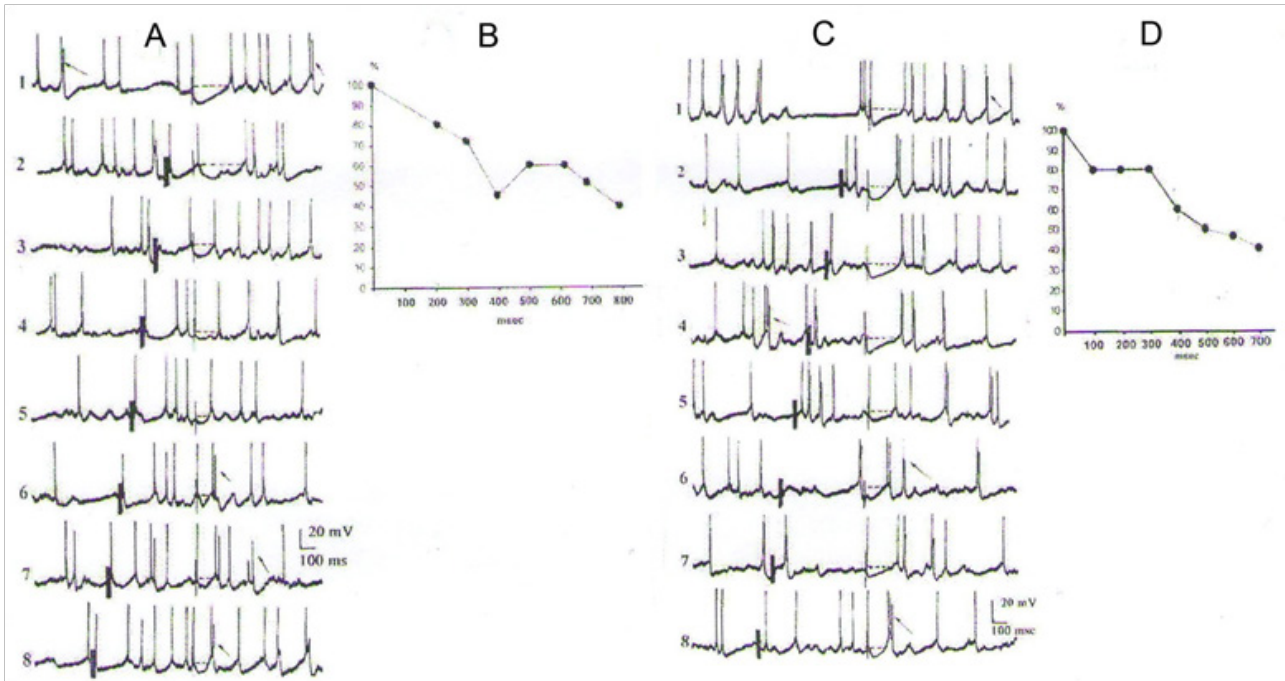


Figure 6 Effects of electrical stimulation of the raphe nuclei (RN) on postsynaptic responses of a nociceptive neuron of the somatosensory cortex caused by stimulation of the dental pulp (A, B) and postsynaptic responses of a neuron of the somatosensory cortex evoked by stimulation of the thalamic VPMN (C, D). In A: 1) effect of isolated stimulation of the dental pulp; 2-8) effects of conditioning stimulation of the RN (using short-lasting high-frequency series of stimuli) on the reaction evoked by test stimulation of the dental pulp with different interstimulation intervals. B) Dependence of the normalized amplitude of pulp stimulation-caused IPSP (%) on the interval between conditioning stimulation of the RN and test stimulation of the dental pulp (msec). The depth of localization of the neuron was 2.1 mm; the membrane potential was -59 mV. In C: 1) neuronal response to single stimulation of the thalamic VPMN 2-8) neuronal responses evoked by paired stimulation (conditioning stimulation of the raphe nuclei, RN, and test stimulation of the thalamic VPMN). B) Dependence of the normalized amplitude of IPSP (%), caused by stimulation of the thalamic VPMN, on the interval (msec) between conditioning stimulation of the RN and test stimulation of the thalamic VPMN. The depth of localization of the neuron was 2.4 mm; the membrane potential was -62 mV. The IPSP amplitude in the absence of stimulation to the RN was taken as 100%. Arrows indicate supposedly dendritic action potentials; arrows show such potentials generated separately, with no relation to preceding full-size somatodendritic peaks.

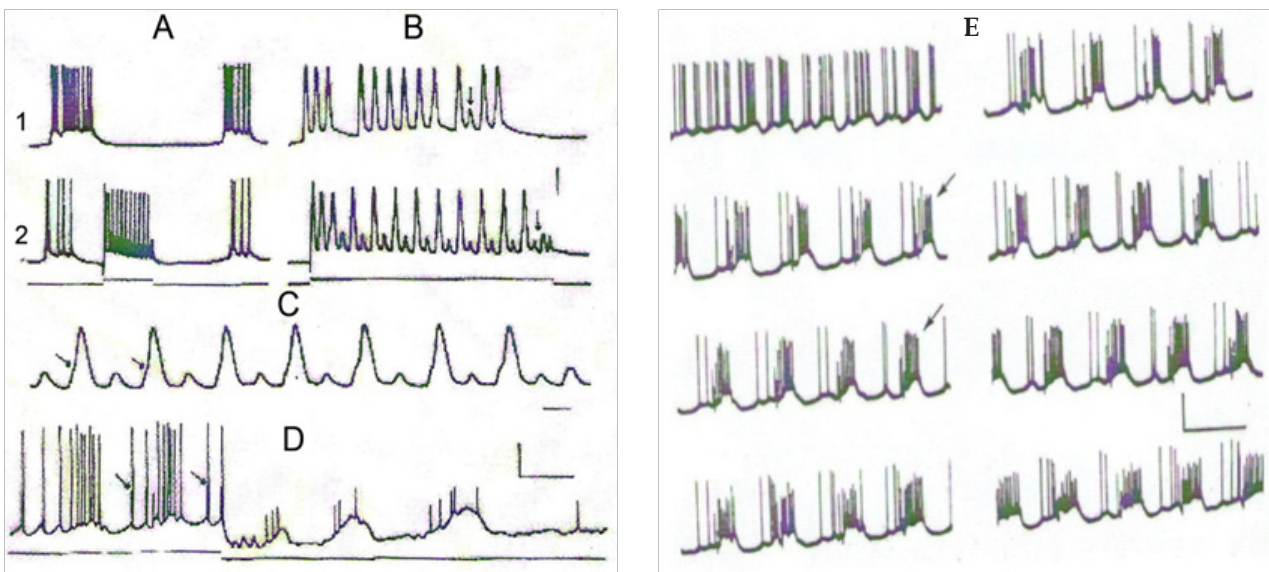


Figure 7 Intracellular recording of dendritic spikes of cortical pyramidal neurons during induced depolarization (A, B, C, the depth of localization of the neuron was 1.7 mm; the membrane potential was -60 mV), hyperpolarization (D, the depth of localization of the neuron was 2.8 mm; the membrane potential was -70 mV) and during 10/s stimulation of the thalamic VPMN (E, depth of localization of the neuron was 1.6 mm; the membrane potential was -70 mV). Arrows indicate supposedly dendritic action potentials.

However, conditioning stimuli applied to the RN caused, independently of the type of the initial response, long-lasting (several seconds) suppression of synaptic responses induced in cortical neurons by stimulation of nociceptive inputs. Intracellular reactions of nociceptive neurons responding to stimulation of the dental pulp and also to intense stimulations of the thalamic VPMN (which resulted in excitation of both nociceptive and nonnociceptive afferent pathways) corresponded to the EPSP–AP–IPSP sequence (Figure 6). In the case where conditioning stimulation of the RN preceded test stimulation of the dental pulp or thalamic VPMN, all synaptic components of the responses to test stimulation (EPSP–AP–IPSP) underwent intense suppression at certain intervals between conditioning and test stimulations. Test intervals that provided a maximum suppressing effect of conditioning stimulation of the RN were about 400 to 800 msec. It should be noted that the studied neurons of the somatosensory cortex also generated impulses of a noticeably (in some cases, significantly) smaller amplitude and duration in a parallel manner with “full-sized” (probably somatic) APs. Such impulses could be observed both within the background activity of nociceptive and convergent neurons of the studied cortical zone and in the responses induced by stimulation of nociceptive afferents of the dental pulp and thalamic VPMN. Such APs appeared more frequently after generation of “full-sized” APs with relatively short intervals (no more than 10 to 15 msec, usually 3 to 5 msec). In Figure 1 A and C, such APs are indicated by arrows. However, impulses of a reduced amplitude and duration could, in some cases, appear independently of full-sized APs or precede such APs. In Figure 1A and C, such situations are marked by arrows with letter “s” (“isolated”). The amplitudes of these APs demonstrated a high variability; the minimal values of the amplitudes of these peaks were observed in the case where such APs appeared after full-sized APs with the shortest intervals (Figure 6. A, C). Figure 7 shows intracellular activity from three cortical pyramidal neurons (Figure 7 A, D, E). Passage of depolarizing current through the recording microelectrode caused, alongside with somatic action potentials (AP), generation of dendritic APs of different amplitude (Figure 7A). Recording of activity at varying circular sweeps (Figure 7 B, C) testifies that the amplitude of the assumed dendritic APs significantly differed from the amplitude of APs of the initial segment of axon.

Figure 7 D presents intracellular activity of another pyramidal

neuron with simultaneous recording of somatic and assumed dendritic APs. Upon artificial hyperpolarization of a neuron disappeared only baseline somatic APs, whereas the assumed dendritic ones maintained steady amplitude. On figure 7E baseline activity (upper left) was presented only by somatic APs. When thalamic VPMN was stimulated with 10/sec frequency, alongside with APs, appeared evoked dendritic APs of different amplitude and high-frequency on the background of pronounced EPSPs, arising after delivery of each stimulus. Intergroup differences were considered to be significant in the cases with $P \leq 0.05$.

V Effects of stimulation of the *substantia innominata* and *caudate nucleus* on postsynaptic reactions of neurons of the cat somatosensory cortex activated by excitation of nociceptors

We examined the effects of conditioning electrical stimulation of the NC (Figure 8. A and B) and SIn (Figure 9.A and B) on postsynaptic reactions in neurons of the somatosensory cortex. Short high-frequency (200–250 sec⁻¹) series of stimuli were used for such stimulation.

Responses of the neurons to stimulation of the tooth pulp (in the case of nociceptive neurons) and of the pulp and thalamic VPMN, i.e., the effects of stimulation of both nociceptive and non-nociceptive corticopetal pathways (in convergent neurons), were used as test synaptic reactions. From the entire group of intracellularly recorded cortical cells, we selected 16 neurons localized within the primary somatosensory cortex; in these units, the membrane potential (MP) exceeding -58 mV was preserved during the entire long-lasting period of recording and testing. The depth of recording from the majority of such neurons varied from 1.4 to 2.7 mm. Among the examined cells selected according to the above-described criteria, seven neurons were identified as nociceptive, while nine neurons were qualified as convergent units. In the case of sufficiently high-quality intracellular recording, responses of nociceptive cortical neurons to pain-inducing stimulation of the dental pulp included EPSPs accompanied by single action potentials (APs) or bursts of several APs. This complex was followed by a hyperpolarizing MP inclination (IPSP) (Figs. 8 A and 9A). The IPSP amplitude in these cases could reach 6–8 mV, while the duration of these inhibitory potentials was 250–300 msec. In all nociceptive and convergent neurons, EPSPs were evoked primarily after isolated stimulations of the NC and SIn.

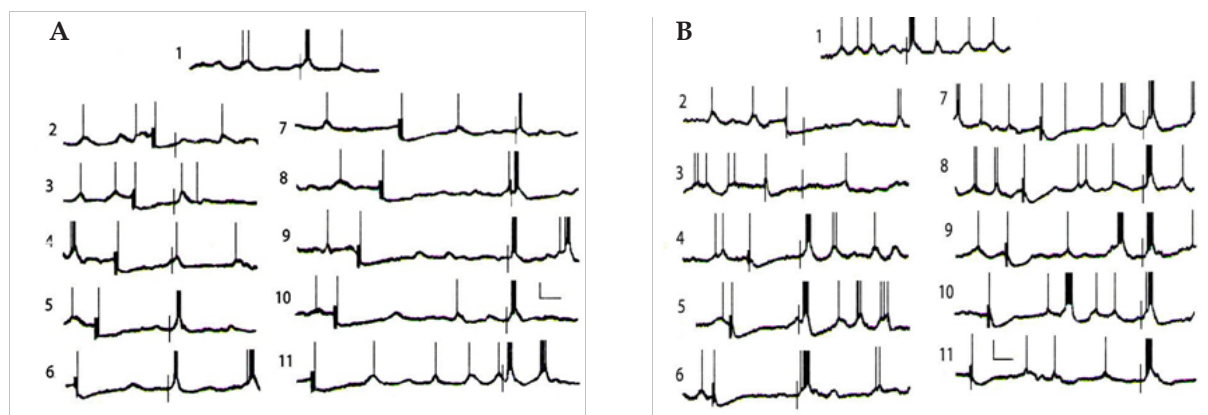


Figure 8 Effects of electrical stimulation of the nucleus caudatus (NC) on postsynaptic responses of a nociceptive neuron of the somatosensory cortex caused by stimulation of the dental pulp (A) and postsynaptic responses of a neuron of the somatosensory cortex evoked by stimulation of the thalamic VPMN (B). In A: 1) effect of isolated stimulation of the dental pulp; 2–11) effects of conditioning stimulation of the NC (using short-lasting high-frequency series of stimuli) on the reaction evoked by test stimulation of the dental pulp with different interstimulation intervals. The depth of localization of the neurons was 2.1 mm; the membrane potential was -59 mV. In B: 1) neuronal response to single stimulation of the thalamic VPMN; 2–11) neuronal responses evoked by paired stimulation (conditioning stimulation of the NC and test stimulation of the thalamic VPMN). Depth of localization of the neurons: 1.6 (A) and 1.8 (B) mm; membrane potential: -59 (A) and -61 (B) mV.

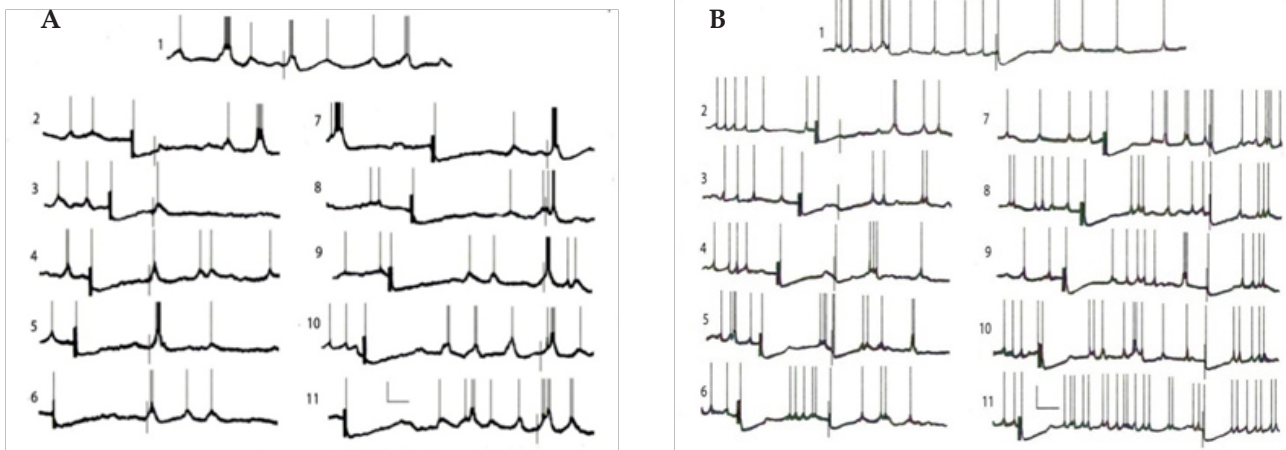


Figure 9 Effects of electrical stimulation of the substantia innominata (SIn) on postsynaptic responses of a nociceptive neuron of the somatosensory cortex caused by stimulation of the dental pulp (A) and postsynaptic responses of a neuron of the somatosensory cortex evoked by stimulation of the thalamic VPMN (B). In A: 1) effect of isolated stimulation of the dental pulp; 2-11) effects of conditioning stimulation of the SIn (using short-lasting high-frequency series of stimuli) on the reaction evoked by test stimulation of the dental pulp with different interstimulation intervals. In B: 1) neuronal response to single stimulation of the thalamic VPMN; 2-11) neuronal responses evoked by paired stimulation (conditioning stimulation of the SIn and test stimulation of the thalamic VPMN). Depth of localization of the neurons was 2.3 (A) and 1.9 (B) mm; membrane potential was -58 (A) and -61 (B) mV.

In all nociceptive and convergent neurons, EPSPs were evoked primarily after isolated stimulations of the NC and SIn. These excitatory synaptic effects provided generation of APs, and such complexes were followed by hyperpolarization PSPs (IPSPs). The amplitude of the latter was rather significant (8-10 mV), and their duration was 250-300 msec (Figures 8 and 9). Thus, synaptic effects on isolated stimulations of the NC and SIn were, in general, rather similar to those evoked by nociceptive stimulation of the tooth pulp. In the cases where stimulations of the NC and SIn preceded testing stimulation of the tooth pulp or that of the thalamic VPMN, synaptic components of the responses to test stimulation were intensely suppressed at definite intervals between conditioning and testing stimulations. If the duration of interstimulation intervals was equal to 100 to 200 msec, spike responses to test stimulations were completely suppressed in nearly all cases (Figures 8 and 9). With increases in the intervals between conditioning and testing stimulations, the amplitude of test EPSPs gradually increased and reached a critical level for AP generation but was still significantly lower than that in the control ($P < 0.05$). Thus, the test reaction partially recovered. The recovery was full or nearly full ($P > 0.05$) when the test reaction coincided with late phases of after-IPSPs in response to conditioning NC or SIn stimulation or at longer intervals (300-600 msec). Intergroup differences were considered to be significant in the cases with $P \leq 0.05$.

VI Modulation of postsynaptic responses of nociceptor-activated neurons of the cat somatosensory cortex by stimulation of the Central Grey

Twenty one cells that responded to stimulation of the dental pulp and thalamic VPMN and corresponded to the above criteria of the analysis were selected from all the studied cortical neurons, whose activity was recorded intracellularly. The resting membrane potential (MP) of all these neurons throughout the entire recording period was -58 mV or higher. Among them, nine cells were identified as selectively nociceptive, while twelve units were classified as convergent. Single stimulation of the ipsilateral CG locus evoked, as a rule, responses of nociceptive cortical neurons rather similar to those evoked by dental pulp stimulation; they also looked like EPSP-AP-IPSP complexes. The responses to CG stimulation, nonetheless, differed by shorter

latencies (Figure 10. A, 1, 2). It should be specially mentioned that a long period of inhibition of background impulse activity (at least several hundreds of milliseconds) was observed in these neurons after stimulation of the CG. If a test stimulation of the dental pulp was applied within this period, postsynaptic responses to such stimulation could be entirely suppressed; neither EPSPs (and, moreover, transsynaptically initiated APs) nor IPSPs were evoked (Figure 10. A, 3). Figure 2B illustrates the effect of conditioning stimulation of the CG on the response evoked by test stimulation of the thalamic VPMN in one of the convergent neurons. In this case, short high-frequency (200sec-1) series containing three to eight stimuli were used. This neuron was classified as a convergent unit because it generated postsynaptic responses not only to stimulation of the dental pulp but also to relatively low-intense stimulations of the infraorbital nerve and thalamic VPMN. As can be seen in panel B, 1, strong stimulation of the thalamic VPMN (probably activating both nociceptive and non-nociceptive pathways) evoked EPSP-AP-IPSP complexes in this neuron. The inhibitory postsynaptic reaction was followed by augmentation of spiking of this neuron (probably, a rebound effect). Burst stimulation of the CG resulted in the development of a complex synaptic response, containing both excitatory and inhibitory components. In the case where stimulation of the CG was used for conditioning and the effect of such stimulation was tested by stimulation of the thalamic VPMN, synaptic potentials evoked by such testing were significantly suppressed at definite interstimulation intervals. Afterspike IPSPs were suppressed especially intensely. Such inhibition was characterized by a rather long latency (at least 100 msec) and reached its maximum at test intervals about 600-800 msec. Under such conditions, the amplitude of these inhibitory potentials decreased by 50 to 70% (B).

Inhibitory effects evoked by stimulations of the CG in nociceptive and convergent somatosensory cortex neurons showed certain similarity to the effects of systemic injections of 0.3 mg/kg morphine. In nociceptive neurons, the amplitude of IPSP components in the EPSP-AP-IPSP complexes evoked by stimulation of the dental pulp (i.e., by nociceptive stimulation) demonstrated a severalfold drop as early as 1 min after i.v. injection of this agent (Figure 10 D, 1, 2). Simultaneously, a clear decrease in the frequency of background activity generated by this neuron was observed. Three minutes

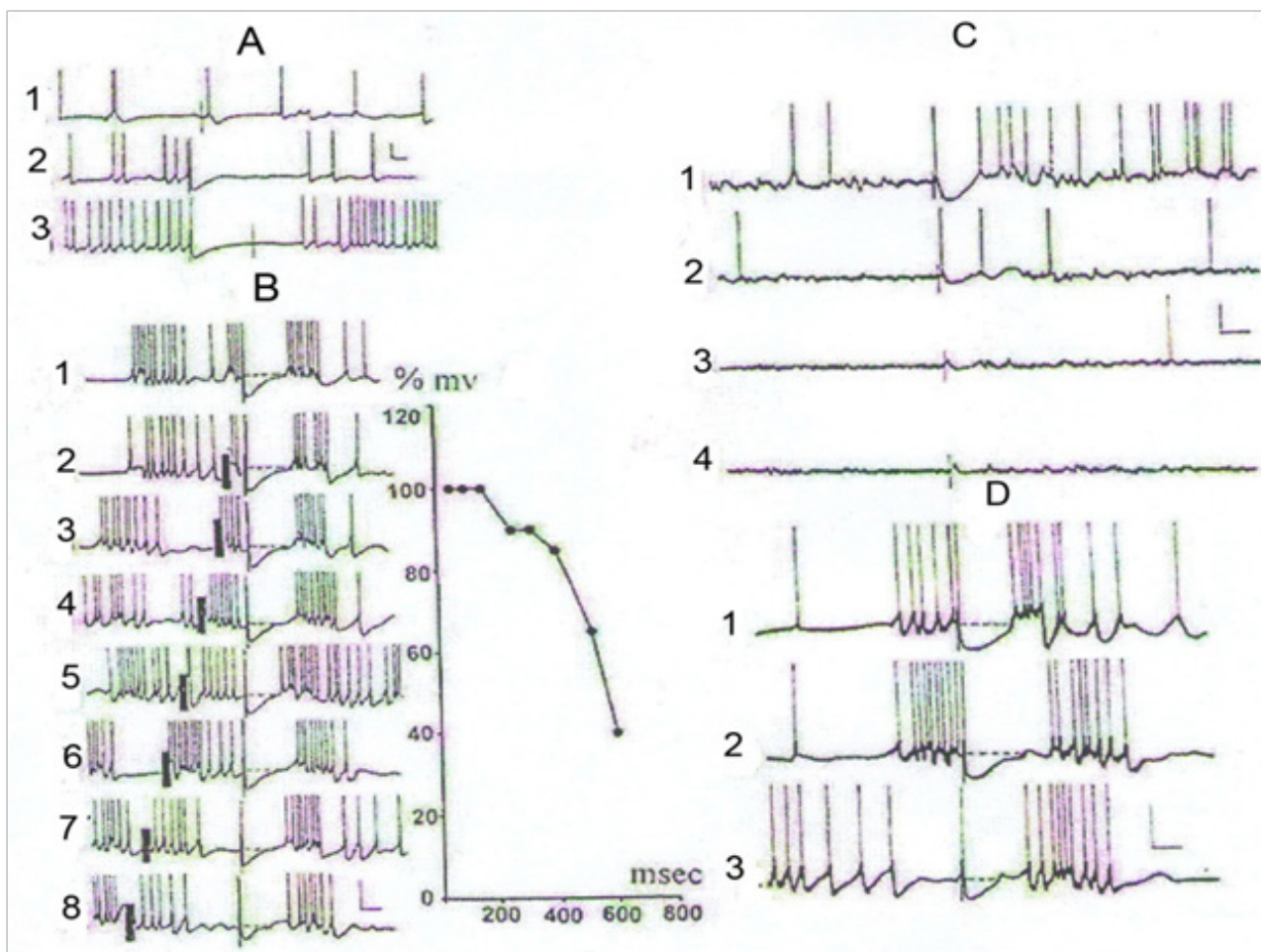


Figure 10 Effects of conditioning stimulation of the central grey (CG) and systemic introduction of morphine (0.3 mg/kg) on the activity of nociceptive (A, C) and convergent (B, D) neurons of the somatosensory cortex. A) Recording from a nociceptive neuron (depth 1.8 mm; membrane potential, MP, -65 mV); 1) response to stimulation of the dental pulp; 2) that to single stimulation of the CG; 3) effect of conditioning stimulation of the CG on the test response evoked by dental pulp stimulation. B) Recording from a convergent neuron (depth 1.9 mm; MP -62 mV); 1) response to single stimulation of the thalamic VPMN; 2-8) those to conditioning stimulations of the CG by short high-frequency series and test stimulations of the thalamic VPMN. At the right) Dependence of the normalized amplitude (%) of IPSPs evoked by thalamic VPM stimulations on the interval between conditioning stimulation of the CG and test stimulation of the thalamic VPMN (msec); the IPSP amplitude in the absence of CG stimulation is taken as 100%. C) Background activity and responses of a nociceptive neuron (depth 2.3 mm; MP -59 mV) to stimulation of the dental pulp before injection of morphine (1) and 1, 3, and 5 min after such injection (2-4, respectively). D) Background activity and responses of a convergent neuron (depth 1.8 mm, MP -64 mV) to stimulations of the thalamic VPMN before (1) and 2 and 5 min after (2 and 3, respectively) injection of morphine.

after injection of morphine, synaptic effects induced by dental pulp stimulation were completely reduced (D, 3). This suppression was not related to depolarization of the membrane of this neuron because the latter continued to generate background spikes (although low-frequency). The amplitude of APs constituting this background activity remained practically identical to that observed in the initial state (D, 1). Five minutes after injection of morphine, synaptic responses evoked by stimulation of the dental pulp remained completely suppressed. Within this period, background impulse activity in nociceptive neurons was usually absent, and only low-amplitude synaptic noise was noticeable. It should be emphasized that the MP showed no considerable changes within this period, as compared with the initial state (D, 4). Changes in the background activity and synaptic responses induced in convergent neurons by systemic introduction of morphine were rather specific. An example of such modulation is shown in Fig.10 E. This neuron generated comparatively high-frequency background activity and responded to stimulation of the thalamic VPMN by an EPSP-AP-IPSP complex. Injection of morphine resulted in a progressive decrease

in the amplitude of IPSP in the above reaction; on the 5th min after injection, such depression of IPSP was more than twofold. Injection of morphine, however, did not block generation of background synaptic activity and background APs in this neuron (E, 2, 3). Intergroup differences were considered to be significant in the cases with $P \leq 0.05$.

DISCUSSION

Intracellular records obtained in our study from neurons of the somatosensory cortex under conditions of the arrival of afferent volleys via ascending nociceptive pathways demonstrate that synaptic responses evoked in cortical neurons after corticopetal bursts of a specific pain modality are, in general, rather similar to responses of somatosensory cortex neurons to the influences of other sensory modalities. The magnitude of such nociceptive responses depends in a gradual manner on the intensity of stimulation of nociceptors of the dental pulp.

Results of our study show that responses of nociceptive and

convergent neurons of the sensorimotor cortex to sufficiently strong stimulation of the main afferent inputs, when recorded intracellularly, appear as EPSP-AP-IPSP complexes. Conditioning stimulation of the SN preceding test stimulation with certain (rather long) intervals induced intense selective suppression of late inhibitory postsynaptic components in these complex responses.

Isolated stimulation of the SN by short high-frequency pulse series could result in generation of two types of responses in the studied cortical neurons. In a noticeable part of convergent neurons, long-lasting (several hundreds of milliseconds) synaptic excitation with a complex polycomponent time course was observed after burst stimulation of the SN. The development of such EPSPs could result in generation of APs with a frequency exceeding the frequency of background activity. If we take into account the pattern of these APs, they can supposedly be considered dendritic spikes. At the same time, hyperpolarization (IPSPs) of rather high amplitude was the primary reaction to SN stimulation in all nociceptive neurons and the remaining part of the convergent units.

Two components should probably be differentiated in the effects induced by stimulation of the SN. These are immediate synaptic effects on the target cells and DA-ergic modulation of synaptic transmission provided by other neurotransmitters. In monkeys (Macaque), DA-containing corticopetal afferents coming from the SN form symmetric contacts on the somata and dendrites of pyramidal cells in the prefrontal, cingulate, and motor cortices. Such innervation provides direct modulating effects of DA released from terminals of nigrostriatal fibers on the excitability of cortical neurons^[31]. Intracellular recording from neurons in frontal slices of the anterior regions of the rat neocortex showed that DA exerts an excitatory effect on pyramidal cells and GABA-ergic neurons sending axons that terminate on pyramidal cells^[5]. At the same time, in slices of the guinea-pig hippocampus, applications of both DA and its agonist apomorphine evoked hyperpolarization mediated by changes in the Ca²⁺ activated potassium permeability of the membrane^[32]. In the SN neurons themselves, DA also induced hyperpolarization; it decreased the input resistance due to an increase in the potassium permeability^[33]. The DA-ergic cerebral system in neurons of the rat striatum is capable of exerting significant modulatory influences on the activity of cholinergic interneurons and effects of neurotransmitter amino acids^[34,35]. As was reported earlier, cholinergic responses in synapses formed by neurons in the rat retina are subjected to DA-ergic modulation^[36]. Such modulation of cholinergic responses in the medial prefrontal cortex of rats is mediated by D1 and D2 receptors^[37]. Mutual modulatory effects of such monoamines as DA and serotonin, when they act on neurons of the spinal ganglia of rats, is related to changes in the intracellular activity of secondary messengers^[38]. Iontophoretic application of DA to neurons of the striatum modified the capability of glutamate of activating postsynaptic neurons; this finding is one more proof of the neuromodulatory role of DA^[39].

The published data indicate that there are two systems responsible for generation and suppression of IPSPs; these systems function at the thalamic and cortical levels. Cortical IPSPs elicited by thalamic stimulation are of an intracortical nature, and their suppression is also realized at the cortical level.

Suppression of cortical IPSPs can reflect the development of inhibitory postsynaptic processes in the intracortical inhibitory interneuronal system, i.e., postsynaptic inhibition of inhibition. In this case, however, manifestations of “switching on” of the disinhibition mechanism should be observed practically from the very beginning of action of conditioning volleys. At the same time, the powerful

effect of conditioning stimulation of the SN was observed in our experiments at significant delays of test stimulation with respect to the conditioning one; it reached its maximum at intervals of 300 to 700 msec. In the context of our experiments, we prefer to interpret suppression of postactivating IPSPs in neurons of the somatosensory cortex as manifestation of generalized modulatory suppression of synaptic inputs evoked by activation of nociceptive inputs and not as a phenomenon of purely synaptic “inhibition of inhibition.”

As was mentioned above, the effects of the DA system, whose fibers monosynaptically project to the cerebral cortex, can evoke increases in the levels of cAMP and cGMP and also of calcium ions in the nerve cells. These effects are realized via the corresponding receptors. Activation of the latter results in activation of protein kinases, phosphorylation of membrane proteins, and an increase in the responsiveness of neurons in many cerebral regions.

A decrease in the amplitude of IPSPs in nociceptive neurons upon the conditioning influence of stimulation of the SN can be related to the action of DA on the system of secondary messengers. Analysis of two types of DA-initiated reactions in neurons of the spinal ganglia showed that depolarization responses are related to activation of D1 receptors and mediated by an increase in the conductivity of cAMP-dependent sodium channels. At the same time, hyperpolarization responses are initiated by activation of D2 receptors; this effect increases the conductivity of potassium channels^[38]. Application of DA and intracellular injection of cAMP evoked the appearance of an inward current related to an increase in the permeability for sodium ions^[40]. According to other data, DA reversibly decreases in a dose-dependent manner the amplitude and duration of afterhyperpolarization in pyramidal cells of the rat hippocampus related to an increase in the Ca-dependent potassium conductance^[41]. In convergent neurons, where IPSPs are generated due to the integral inflow of pain and non-pain afferent volleys, stimulation of the SN suppresses effects of only (or preferably) nociceptive afferent signals; this results in smaller drops in the IPSP amplitudes. Such suppression of IPSPs in cortical neurons induced by stimulation of the dental pulp and thalamic VPMN was also observed at activation of the noradrenergic (NA-ergic) cerebral system^[25]. Dopamine is reserved mostly in axonal vesicles of DA-ergic neurons. This monoamine is a metabolic precursor of NA and is released in the course of synaptic activation together with the above neurotransmitter^[42]. Dopamine can regulate the sensitivity of the NA-ergic system, in particular via influencing the state of β -adrenoreceptors, e.g., in the prefrontal cortex of rats^[43].

Studies of the mechanisms underlying development of postsynaptic effects of neuromodulators on systems of intracellular messengers and on functions of receptor-linked ion channels led to the conclusion that the effects of neurotransmitters undergo divergence on receptors of different subtypes and, vice versa, the effects of different neurotransmitters can converge on the same ion channels because the systems of secondary messengers are to a great extent common^[44].

Therefore, comparison of our findings and the data published earlier allows us to conclude that stimulation of the SN initiating DA-ergic influences on nociceptive synaptic effects in neurons of the cat somatosensory cortex (via acting on DA receptors of these neurons and the system of secondary messengers) provides intense modulation of the activity of the respective populations of cortical neurons. These effects should probably be interpreted as one of the aspects of analgesic influences resulting from activation of the SN.

Effects of stimulation of the LC and iontophoretic applications of its transmitter (noradrenaline) in neurons of different cortical regions can be either excitatory or inhibitory^[45,46]. The excitability of

neocortical neurons can be noticeably modified under the influence of NA because of suppression of slow potassium currents^[47]. According to observations on cerebral cortex slices^[48], NA and DA exert inhibitory influences on Ca^{2+} entries and glutamate release in synaptic connections. On the other hand, there are indications that the influences of NA and DA induce increases in the cAMP, cGMP, and Ca^{2+} levels in the nerve cells. This results in activation of protein kinases and phosphorylation of membrane proteins; finally, this increases the responsiveness of the neurons in many brain regions.

Stimulation of the LC resulting in activation of β adrenoreceptors on the membranes of target cells, an increase in the activity of adenylate cyclase, and intensification of cAMP production provides intensification of GABA-ergic inhibition. This was shown on Purkinje cerebellar cells; similar processes can, probably, be realized in cells of other cerebral systems^[45]. Dopamine-containing afferents coming from the SN form symmetric contacts on the somata and dendrites of pyramidal cells of the prefrontal, cingulate, and motor cortices in macaques; such connections provide direct modulatory effects of DA on the excitability of cortical projection neurons^[6]. Intracellular recording of neuronal activity in frontal slices of anterior regions of the rat neocortex showed that DA exerts excitatory effects on pyramidal cells and GABA-ergic neurons projecting to the above units^[5]. It was also demonstrated that DA-ergic neurons are involved in the control of sensitivity of β adrenoreceptors in the prefrontal cortex of rats^[49]. In slices of the hippocampus of guinea pigs, application of DA, similarly to that of its agonist apomorphine, evoked hyperpolarization mediated by Ca^{2+} activated potassium conductivity of the membrane^[50,51]. It was shown that the DA-ergic system modulates the activity of cholinergic neurons and efficacy of action of neuromodulatory amino acids in neurons of the rat striatum^[35]. Dopaminergic modulation of cholinergic responses in neurons of the rat prefrontal cortex is mediated by D1 and D2 receptors^[37]. Iontophoretic application of DA to striatal neurons modifies the ability of glutamic acid to postsynaptically activate neurons; this fact confirms the significant neuromodulatory role of DA^[39].

In convergent cortical neurons, where postactivation IPSPs represent an integral effect of the arrival of both pain and non-nociceptive afferent influences, stimulations of the LC and SN result in relatively selective suppression of pain afferent signals; this also results in decreases in the IPSP amplitudes, but such suppression is less intense than that in nociceptive neurons.

There is proof that NA and met-enkephalins increase the potassium conductivity in CNS neurons of the guinea pig; these effects are mediated by opioid receptors and β_2 adrenoreceptors conjugated with potassium channels by GI proteins^[52].

Nociceptive stimulation was shown to increase NA release from the terminals of NA-ergic neurons^[53]. Electrical and chemical stimulation of the LC induces antinociceptive effects realized at the spinal level^[54]. Our findings also demonstrate that stimulation of the LC and SN results in long-lasting suppression of synaptic responses evoked in somatosensory cortex neurons by stimulation of nociceptive afferents and ascending pathways. Probably, such effects should also be interpreted as one of the aspects of analgesic influences related to activation of the LC and SN, i.e., main structures of cerebral NA- and DA-ergic systems.

As is known, terminals of the axons of neurons of the LC, which exclusively and extensively project to many structures of the brain, form not only "classic" synaptic connections with target neurons but also a number of free NA-ergic terminal structures (varicosities). Noradrenaline is released from these structures in

the intracellular space and, in such a way, can exert its influence simultaneously on great populations of the neurons (providing the so-called volume transmission)^[55]. The temporal characteristics of LC stimulation-induced suppression of synaptic effects (in particular, of postactivation IPSPs) in somatosensory cortex neurons (long latencies and very long duration of such modulatory effects) probably correspond just to such a mode of the NA effects. In this case, modulatory influences of NA on the efficacy of synaptic transmission from thalamic corticopetal afferents plays a more significant role; at the same time, the importance of direct synaptic action of NA-ergic inputs formed by LC neurons on cortical neurons, as well as of NA-ergic modulation of the efficacy of functioning of intracortical neuron-to-neuron systems, should not be underestimated.

Our results agree with modern concepts on the nature of regulation of the pain sensitivity at the level of higher CNS structures and allow us to conclude that the modulatory effects induced by stimulation of the LC and SN with respect to nociception-related processes in the somatosensory cortex demonstrate a significant similarity to each other.

Our own experiments and studies of other authors^[27,56] showed that not only the neurons selectively activated from ascending nociceptive pathways but also the cells receiving both influences of a nociceptive character and influences from lower threshold pathways (convergent neurons) are present in the somatosensory cortex. The effects observed in such neurons after stimulation of the RN using short high-frequency series of stimuli demonstrated certain similarity to the responses caused by stimulation of the catecholaminergic brainstem systems^[27]. These responses could be easily divided into two groups. Relatively short-latency discrete EPSPs or IPSPs of rather great durations were one type of the observed effects. Since the number of our observations is relatively small, we have no way of relating the type of synaptic effects induced by stimulation of the RN to concrete functional peculiarities of one cortical neuron or another. It should only be noted that the number of cases of excitatory or inhibitory synaptic effect from the RN was comparable in the examined group. The development of late very long nonspecific suppression of all synaptic responses induced in such neurons by excitation of somatic afferent pathways (both nociceptive and low-threshold) was another kind of influences elicited by stimulation of the RN on the studied nociceptive and convergent neurons of the cat somatosensory cortex. Such inhibition was rather clearly pronounced with respect to IPSPs developing after the EPSP-AP complex. The duration of such inhibitory effects exceeded 700 or 800 msec. At these test intervals, we observed only the maximum of inhibition. Thus, the total duration of the latter can exceed 1,500 msec.

Interpretation of the mechanisms underlying postsynaptic effects induced by stimulation of the RN in nociceptive and convergent neurons of the somatosensory cortex and, in particular, those underlying long-lasting inhibitory modulation of synaptic responses of such neurons, which results from the activation of somatosensory inputs, is complicated. To a significant extent, it is determined by the fact that membrane receptors sensitive to 5-HT (5-HT receptors) are rather diverse and form a great number of subtypes and subgroups. The action of 5-HT, the main neurotransmitter released by neurons of the RN, includes a rise in the cAMP level, which is mediated by adenylate cyclase; in turn, cAMP activates cAMP-dependent protein kinase, which results in phosphorylation of a great number of substrate proteins, including proteins of potassium channels^[57,58]. Therefore, it is obvious that an increase in the potassium conductance through the membranes of nerve cells possessing 5-HT receptors is a basic effect determined by the action of 5-HT and mediated by cAMP.

This conclusion was confirmed by the data obtained in experiments on different objects (neurons of invertebrates and vertebrates) [38,59,60,61]. It is obvious that the increase in the conductance through just calcium-activated potassium channels dependent on 5-HT_{1A} receptors but not through voltage-directed ones plays the main role [38]. These effects are related to changes in the intracellular Ca²⁺ concentration, which was demonstrated on hippocampal neurons [62,63]. However, the action of 5-HT is, probably, more complicated; as is hypothesized, this neurotransmitter/modulator influences at least two different types of receptors; one of them provide opening of potassium channels, while others mediate a decrease in the potassium conductance [64].

The above phenomena did not limit the complexity of the effects induced by the release of 5-HT into the tissues of different cerebral structures, including the neocortex. Changes in the level of 5-HT exert a significant modulatory effect on cholinergic transmission, which was found in different objects [65-68] interaction of these agents is realized via both membrane receptors and systems of intracellular signaling. This monoamine actively influences the neuronal systems of GABA-ergic inhibition due, probably, to its direct action on GABA-ergic inhibitory interneurons (in any case, in the hippocampus) [69]. The 5-HT-ergic afferent inputs actively interact with neuronal chains where glutamatergic transmission is realized [60,70,71]. The data on "global" effect of 5-HT in the cortex are to a certain extent contradictory. It was demonstrated that application of 5-HT facilitates early and late impulse reactions of neurons of the somatosensory cortex, which are induced by tactile stimulation, and modulates responses of many cells to conditioning stimulation [15]. On the other hand, it was found that excitatory responses of neurons of the rat prefrontal cortex (evoked by pain stimulation of the tail or stimulation of the thalamic mediodorsal nuclei) were significantly inhibited after preliminary stimulation of the RN [72]. Our own observations agree with the data obtained in the cited study and indicate that not only excitatory effects observed in nociceptive and convergent cortical neurons but also postexcitatoryinhibition in these cells undergo long-lasting (in any case, 1 sec long) depression after stimulation of the RN using short high-frequency series of stimuli. The nonspecific pattern of such depression and pattern of its time course (significant latency and long duration) allows us to hypothesize that this phenomenon is, to the great extent, of a presynaptic nature. The presence of certain groups of 5-HT receptors on presynaptic terminals in the cortex has been convincingly evidenced; it should be taken into account that presynaptic 5-HT autoreceptors provide autoregulation of the effects of the latter by the feedback mechanism [73]. Therefore, stimulation of the RN results in the release of 5-HT and increase in the 5-HT level in the cortex. Serotonin influences target neurons via numerous and diverse 5-HT receptors and the system of secondary messengers and, in such a way, provides considerable modulation of the activity of the corresponding populations of cortical neurons. The most important effect is, in this case, general nonselective suppression of synaptic reactions of cortical neurons evoked by activation of nociceptive inputs

During the last years, certain success was achieved in the understanding of the mechanisms of pain perception and formation. At the same time, a number of insufficiently examined theoretical and practical aspects remained. At present, a number of channels transmitting modulatory influences on the cerebral cortex have been identified. These are pathways coming from the raphe nuclei, locus coeruleus, and nonspecific, associative, and relay thalamic nuclei. In addition, there is another extrathalamic channel transmitting nonspecific effects on the cortex; it originates from the

basal forebrain structures. These structures (NC, globus pallidus, putamen, and SIn) contain large cholinergic neurons and send topically organized projections to the cortex. Activation of caudate cortical mono- and oligosynaptic pathways evokes relatively short-latency responses in the cortex. The caudate nucleus, acting both directly and via nonspecific nuclei of the thalami optici and midbrain reticular formation, polysynaptically affects the activity of the brain cortex; these effects significantly modulate the parameters of this activity [74]. Glutamate is released from presynaptic terminals in various regions of the brain after any nociceptive impact. As is believed, realization of physiological pain-related reactions (e.g., a defensive reflex of the limb withdrawal) upon the action of glutamate is mediated via AMPA receptors; NMDA receptors provide in this case long-lasting hyperactivity of nociceptive neurons (under certain conditions, this activity is pathological) [75]. The activating effects of glutamate on nociceptive neurons are potentiated by substance P. This agent coexists as a modulator in more than 90% glutamate-containing terminals of high-threshold sensory fibers. Substance P, when interacting with NK1 (neurokinin-1) receptors not only increases the concentration of intracellular calcium by its mobilization from intracellular stores, but also intensifies the activity of NMDA receptors. At present, nitric oxide (NO), which plays the role of a no typical extrasynaptic mediator, is considered an important factor in the mechanisms providing sensitization of nociceptive neurons. Nitric oxide is produced from L-arginine in neurons containing NO-synthase. It is released from cells upon NMDA induced excitation and interacts with presynaptic terminals of C afferents (intensifies glutamate and neurokinin release from these structures) [76]. Pathological hyperalgesia is based on sensitization of nociceptors with respect to the action of injuring stimuli. Electrophysiologically, sensitization of nociceptors is manifested as decreases in the threshold of their activation and enhancement of the frequency and duration of discharges in nervous fibers of groups A δ and C; these events result in increase in the intensity of the afferent nociceptive drive. According to the concept of Kryzhanovskii [77], aggregates of interacting sensitized neurons with abnormally modified inhibitory mechanisms and increased excitability are the structural bases of neurogenic pain syndromes. Such aggregates can produce long-lasting self-maintained pathological activity; initiation of the latter does not necessarily require input of afferent impulsation from the periphery. An important role in the mechanisms of formation of neuronal hyperactivity in the CNS structures is attributed to suppression of glycine and GABA-mediated inhibitory responses. Under conditions of functional insufficiency of inhibitory mechanisms and increased excitability of neurons, neuron-to-neuron activating synaptic interactions are facilitated, the activity of "silent" inactive synapses is triggered, and neighboring sensitized neurons are united in an integral aggregate. Sensitization of nociceptive neurons can be related to injury of tissues resulting in increases in the excitability and responsiveness of nociceptive neurons in the abovementioned centers (including thalamic nuclei and somatic cortex of the hemispheres).

Acetylcholine exerts diverse postsynaptic actions on neurons of the CNS. Different types of responses to application of ACh are manifested in various CNS structures to different extents. Even within a single CNS structure, the effects of ACh of neurons of different types can be dissimilar. Depolarizing ACh-induced potentials in neurons of invertebrates (mollusks) are related to increased permeability's of the membrane to ions of Na⁺, Ca²⁺, and small amounts of Cl⁻. Activation of nicotinic receptors induces mostly sodium permeability, while that of muscarinic receptors

intensifies calcium permeability^[78]. The effects of ACh result in modulation of glutamatergic transmission. The depolarizing effect of glutamate on the membrane of motoneurons of the isolated frog spinal cord resulted from increases in the permeability to Na and K cations^[79]. The action of glutamate and aspartate on large nerve cells of the rat cerebellum evoked inward sodium currents^[80]. Glutamatergic influences induced increase in the content of Ca in neurons of cerebral cortex^[81]. The involvement of ACh in the mechanisms of suppression of IPSPs in pyramidal neurons of hippocampus was demonstrated. In this case, the effects are mostly mediated by muscarinic receptors; in particular, they induce suppression of recurrent inhibition. Acetylcholine also acts on a presynaptic level; it suppresses either the activity of inhibitory interneurons or GABA release from presynaptic terminals^[82]. Acetylcholine can influence the activity of hippocampal pyramidal cells via two pathways, postsynaptically (by modulation of the permeability of the somatic membrane to cations) and presynaptically (by modulating the efficacy of noncholinergic inputs)^[83]. According to some authors, the postsynaptic actions of ACh are manifested as slow muscarinic depolarization elicited due to decrease in the voltage-dependent potassium current, and, probably, intensification of sodium and calcium currents. The presynaptic action of ACh is either realized through direct effects of presynaptic terminals or mediated by interneurons. After examination of neuronal activity in the somatosensory cortex before and after ACh applications, it was concluded that the cholinergic system modulates sensory processes in the cortex by changes in the efficacy of influences of afferent inputs on neurons in all layers of the cortex^[84]. Acetylcholine induces “fast” excitation of neurons related to increase in the permeability to cations and mediated by nicotinic and muscarinic receptors. In a parallel manner it results in the development of slow excitation resulting from decrease in the potassium permeability and blocks the Ca-dependent potassium permeability^[85]. In pyramidal cells of cortical layer V, application of ACh evoked EPSP-IPSP complexes and suppressed slow afterhyperpolarization following generation of APs. Cholinergic inhibition in the cortex is mediated by fast excitation of nonpyramidal GABA-ergic cells^[86]. Studies of changes in the permeability of the membrane of nerve cells within different sections of after-potentials and postsynaptic hyperpolarization potentials and involvement of ion channels in their genesis allowed researchers to form a well based conclusion that such inhibitory potentials are multicomponent. Their initial component is mostly determined by increase in the membrane permeability to Cl⁻ ions and, in many cases, can be identified as a synaptic GABAA-mediated response. The second component is mostly determined by potassium current and should be interpreted as a synaptic GABAB-mediated response. The terminal component is related mostly to Ca-dependent potassium current. These components are manifested in nearly all CNS neurons. They are not sharply separated in time, partially overlap with each other, and form a long-lasting integral hyperpolarizing potential deviation^[87]. In our experiments, the most intense suppression of impulse responses and inhibitory synaptic effects in sensorimotor cortex neurons to test stimulation of the tooth pulp or thalamic VPMN was observed within the initial period of development of IPSPs after conditioning stimulations of the SIn and NC. Within this period, depolarizing potentials are suppressed, and such inhibition is related to the action of inward chloride current and a drop in the resistance of the postsynaptic membrane. Testing stimulations of the tooth pulp or thalamic VPMN in the course of the second IPSP component (i.e., within the period of functioning of GABAB-mediated transmission) evoke generation of partially suppressed slow depolarization whose

amplitude was in some cases sufficient for generation of single or several APs.

In other words, partial recovery of the test response was noticed within this time interval. Full or nearly full recovery of the responses to test stimulation of the tooth pulp and thalamic VPMN corresponded to the final part of the hyperpolarization potential and to cases where the intervals between conditioning and test stimulations exceeded the duration of the hyperpolarization potentials evoked by conditioning SIn and NC stimulations. Therefore, stimulations of the NC and SIn result in ACh release in the cortex; ACh acts upon target neurons via nicotinic and muscarinic receptors. This provides strong modulation of activity of the corresponding populations of nociceptive and nonnociceptive cortical neurons.

Our observations agree with the results of earlier studies where the development of clearly expressed analgesia was found under conditions of electrical stimulation of the CG or microinjections of opiate alkaloids or opioid peptides into this structure [88,89, 90]. It is known that neurons of the CG (at least, a considerable part of them) are enkephalin and dinorphinergic units. About half of these neurons are excited or inhibited upon nociceptive stimulation, and these cells possess relatively clearly delineated receptive fields. As is supposed, opioidergic neuronal systems play a crucial role in the processes determining processing of nociceptive information at the higher CNS levels. Opioids probably realize their modulatory function at a presynaptic level, via inhibition of neurotransmitter release into synaptic connections of nociceptive neuronal networks. It is believed that enkephalin-type endogenous opioids suppress the entry of Ca²⁺ into presynaptic terminals, i.e., the process necessary for exocytose of the neurotransmitter and its release into the synaptic cleft. Such effects of endogenous opioids are imitated by the action of their exogenous agonists, morphine-like alkaloids and their derivatives^[41]. The results of our experiments, where a certain similarity between the effects evoked in cortical nociceptive and convergent neurons by electrical stimulation of the CG and systemic introduction of morphine was demonstrated, can be interpreted as proof of the existence of the above mechanism. Both influences result in suppression of EPSPs and IPSPs, i.e., in general depression of the synaptic effects evoked by activation of nociceptors. These influences also induce decreases in synaptic potentials in convergent neurons. We should note that, nonetheless, the effects of stimulation of the CG and introduction of morphine manifest certain specificity with respect to the above cells. Our experiments demonstrate that there is certain parallelism between modulatory effects of stimulation of the CG, LC, and SN exerted on “nociceptive” processes in the somatosensory cortex. As was shown^[91,92], activation of both the LC (the main source of NA-ergic effect) and the SN (the source of DA-ergic projections) in many cases exert no considerable effects on the evoked activity generated by neurons in various cerebral centers. In other cases, the effects induced by stimulation of the above structures are rather significant. In these effects of stimulation of the LC and SN, two components should be differentiated. These are immediate synaptic effects on target cells and modulation of synaptic effects exerted by other neurotransmitters by LC- and SN-born influences. Actions of both types exert considerably weaker influences on those synaptic processes in convergent neurons, which are related to the arrival of impulsation via non-nociceptive pathways.

A great number of data indicating that spreading APs can be generated in dendrites of neurons of different CNS structures of vertebrates has been accumulated at present. Findings made in the course of presumably intradendritic microelectrode recordings indicate that depolarization of the membrane of many central neurons

can, in principle, evoke, in some cases, APs in dendrites according to the “all-or-nothing” mode and that these potentials can reach the soma of the cell and can be recorded as APs distinguished by a reduced amplitude and duration. Taking into account an increased variability of the amplitude of presumably dendritic APs observed in our experiments, the possibility that such potentials are initiated in the first axonal segment of the studied cortical neurons is ruled out. Based on the parameters (amplitude and T) of the putative dendritic APs recorded from one and the same neuron, we can hypothesize that multiple trigger zones of generation of APs exist in neocortical cells^[93,94]. According to the existing viewpoint, the putative dendritic APs are, to a considerable extent, provided by currents through calcium channels, which can be distributed along the membrane of both the soma and dendrites. In this relation, the data on significant effects of some neurotransmitters and neuropeptides on calcium currents attract specific interest. In particular, it was demonstrated that 5-HT increases the concentration of intracellular Ca²⁺ [75,95]. Therefore, rather intense stimulation of the RN used in our experiments could, to a certain extent, promote generation of dendritic APs. The ability of neuronal dendrites to generate APs independently of the events in the main trigger zone of the neuron (axon hillock) appreciably complicates the ideas of integrative processes in nerve cells as the simple algebraic summation of depolarizing and hyperpolarizing influences on the somatodendritic membrane. In particular, summation of excitatory effects to the generation threshold for APs can probably be realized not only in the axon hillock but also in multiple loci of the dendrites. The dendrite apparatus is considered to be a complex neurochemical integrator [94]. Different neurotransmitters act upon target neurons and this provides strong modulation of activity of the corresponding populations of nociceptive and nonnociceptive cortical neurons. Such modulation is probably based on changes developing in both pre and post-synaptic intracortical mechanisms.

Development of IPSP on the bodies of pyramidal cortical neurons is associated with activity of system of intracortical “recurrent inhibition”. In the presynaptic and postsynaptic inhibition are involving different transmitters. Postsynaptic inhibition removed by a number of pharmacological agents (strychnine *et al*) enhances presynaptic inhibition, whereas picrotoxin suppressing presynaptic inhibition has no effect on postsynaptic inhibition. Decrease in the amplitude or complete postsynaptic inhibition of IPSP in cortical neurons under different treatments associate with the occurrence of convulsive epileptic activity, and at the painful action with analgesic effect.

CONFLICT OF INTERESTS

The Author has no conflicts of interest to declare.

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Peer reviewer: Guo-Gang Xing, MD., Ph.D., Professor of Neurobiology, Department of Neurobiology, School of Basic Medical Sciences, Peking University Health Science Center, Neuroscience Research Institute, Peking University, 38 Xue-Yuan Road, Beijing 100191, P.R. China.